Ph.D. Thesis

Estudio del potencial de variedades de calabacín para ser transformadas a un producto IV Gama

Suitability of summer squash cultivars to be processed as fresh-cut products

María Teresa Blanco Díaz

2015

Departamento de Bromatología y Tecnología de los Alimentos
Universidad de Córdoba

Instituto de Investigación y Formación Agraria y Pesquera
CONSEJERÍA DE AGRICULTURA, PESCA Y DESARROLLO RURAL

UNIVERSIDAD D CÓRDOBA

TITULO: *Estudio del potencial de variedades de calabacín para ser transformadas a un producto IV Gama. (Suitability of summer squash cultivars to be processed as fresh-cut products)*

AUTOR: *María Teresa Blanco Díaz*

Campus de Rabanales
Ctra. Nacional IV, Km. 396 A
14071 Córdoba

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Departamento de Bromatología y Tecnología de los Alimentos

Programa de Doctorado

Ingeniería de Plantas Agroindustriales

Línea de Investigación

Tecnología Poscosecha y Sensores no Destructivos para el Control de la Calidad y Seguridad de Frutas y Hortalizas

Tesis Doctoral

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Autora:
María Teresa Blanco Díaz

Dirigida por:
Dr. D. Rafael Font Villa
Dra. Dña. Alicia Fayos Moltó

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Tesis Doctoral presentada por Dª. María Teresa Blanco Díaz, en satisfacción de los requisitos necesarios para optar al grado de Doctora Ingeniera Agrónoma con *Mención Internacional* y con *indicios de calidad*, dirigida por el Dr. D. Rafael Font Villa y la Dra. Dña. Alicia Fayos Moltó, del Institudo de Investigación y Formación Agraria y Pesquera de la Junta de Andalucía (IFAPA).

Los directores

La doctoranda

Fdo.: Dr. Rafael Font Villa

Fdo.: Dra. Alicia Fayos Moltó

Fdo.: Mª Teresa Blanco Díaz

TÍTULO DE LA TESIS:
Estudio del potencial de variedades de calabacín para ser transformadas a un producto IV Gama. - Suitability of summer squash cultivars to be processed as fresh-cut products.

DOCTORANDO/A:
Mª Teresa Blanco Díaz

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

La Tesis Doctoral de Dña. María Teresa Blanco Díaz se ha llevado a cabo en IFAPA-Centro La Mojonera de Almería, en el Área de Tecnología, Postcosecha e Industria Agroalimentaria.

La presente Tesis Doctoral ha supuesto un importante avance en el conocimiento de la tercera hortaliza más importante en volumen de producción de la principal zona exportadora de hortalizas a Europa, como es Almería.

El objetivo general de la presente Tesis Doctoral ha sido aumentar la competitividad y las posibilidades de mercado de calabacín fresco o I Gama, mediante el estudio del potencial de este producto para ser transformado a producto de IV Gama.

Para alcanzar dicha finalidad, se plantearon diversas líneas de trabajo. En un primer lugar, se ha realizado el estudio de la variabilidad genotípica/morfotípica en calabacín a partir de 27 accesiones (*Cucurbita pepo* spp. *pepo*) (pertenecientes tanto a la colección Europea de germoplasma de Cucurbitáceas, como a otros países), como paso previo a la selección de aquello/s morfotipos más óptimos para el procesado. Una vez definido qué morfotipo presentaba mayores ventajas desde el punto de vista nutricional, se estudió la influencia que tenía el estado de madurez y el momento de recolección sobre la calidad físico-química y nutricional de variedades comerciales de diferentes colores del morfotipo zucchini.

A continuación se han evaluado la influencia tanto de factores pre-cosecha (variedad y estado de madurez) como post-cosecha (formato de corte, temperatura de conservación y la atmósfera modificada) en la calidad fisicoquímica, nutricional, microbiológica y sensorial de calabacín IV Gama.

Como alternativa a la conservación en atmósfera modificada (MAP), se han estudiado sistemas activos emergentes en la conservación de los alimentos, como son los recubrimientos comestibles. Para ello, la doctoranda ha ensayado un total de 16 recubrimientos comestibles, siendo destacable la recomendación de 3 de ellos por mejorar el color, la firmeza y las características sensoriales del calabacín IV Gama tanto en crudo como tras su cocinado.

Finalmente, la doctoranda ha estudiado el potencial de la tecnología por reflectancia en el infrarrojo cercano NIRS, para la predicción de compuestos antioxidantes de calabacín a partir de 3 campañas de cultivo (desde 2009 a 2012).

Los resultados presentados muestran que además de alcanzarse con éxito los objetivos descritos, el calabacín presenta una excelente aptitud para su procesado en IV Gama, proporcionando esta Tesis Doctoral información muy útil y práctica que permite incrementar el valor añadido de esta hortaliza por parte del sector agroindustrial.

Asimismo la doctoranda ha completado su formación y desarrollo de la Tesis Doctoral en centros de reconocido prestigio internacional como son el Dipartimento di Scienze delle Produzioni e dell'Innovazione dei Sistemi Agroalimentari Mediterranei de la Università degli Studi di Foggia (Italia) (durante 3 meses, desde 01/09/2012 hasta el 30/11/2012) bajo la supervisión del Prof. Giancarlo Colelli, y el Departamento de Horticulture del United States Department of Agriculture (ARS-USDA) – Fort Pierce, Florida (Estados Unidos) (durante 6 meses, desde 01/09/2013 hasta el 30/11/2013 y desde el 01/01/2014 hasta el 31/03/2014) bajo la supervisión de la Dra. Elizabeth Baldwin.

Además, la doctoranda ha presentado sus resultados a través de numerosas contribuciones científicas, 8 de las cuales pertenecen a congresos nacionales y 13 a congresos internacionales. Es importante destacar, que fruto del trabajo realizado durante esta Tesis Doctoral, la doctoranda ha sido merecedora del premio a la mejor comunicación oral en una de las sesiones del VII Congreso Ibérico de Agroingeniería y Ciencias Hortícolas de la Sociedad Española de Ciencias Hortícolas (SECH), celebrado en Madrid del 26 al 29 de Agosto de 2013.

Como resultado de esta Tesis Doctoral, se destacan las publicaciones en **revistas SCI**:

Blanco-Díaz, M.T.; Del Río-Celestino, M.; Martínez-Valdivieso, D.; Font, R. 2014. Use of visible and near-infrared spectroscopy for predicting antioxidant compounds in summer squash (*Cucurbita pepo* ssp *pepo*). *Food Chemistry*, 164: 301–308 - DOI: 10.1016/j.foodchem.2014.05.019

Food Chemistry pertenece al primer cuartil de area de Agricultural and Biological Sciences (índice de impacto de 3,259 y posición 12/59).

Y otras publicaciones se encuentran en este momento bajo revisión:

Blanco-Díaz, M.T.; Pérez-Vicente, A.; Font, R. 2015. Quality of fresh cut zucchini as affected by cultivar, maturity at processing and packaging *(Enviada a Packaging Technology and Science).*

Packaging Technology and Science pertenece al primer cuartil del área de Mechanical Engineering (índice de impacto de 1,584 y posición 134/137).

Blanco-Díaz, M.T.; Font, R.; Martínez-Valdivieso, D.; Del Río-Celestino, M. 2015. Genotypic diversity of natural pigments and phytochemical compounds from exocarp and mesocarp of 27 *Cucurbita pepo* genotypes. *(Enviada a Scientia Horticulturae).*

Scientia Horticulturae pertenece al primer cuartil del área de Agricultural and Biological Sciences (índice de impacto de 1,504 y posición 11/19).

Los 4 restantes estudios incluidos en esta Tesis están en preparación para su publicación.

Además, la doctoranda ha colaborado en diferentes trabajos realizados en el Área de Tecnología, Postcosecha e Industria Agroalimentaria, en la que participa como coautora:

Martínez-Valdivieso, D.; Font, R.; Gómez, P.; **Blanco-Díaz, T.**; Del Río-Celestino, M. 2014. Determining the mineral composition in cucurbita pepo fruit using near infrared reflectance spectroscopy. *Journal of the Science of Food and Agriculture*, 94: 3171–3180. (Revista SCI del primer cuartil con índice de impacto 1,879).

Martínez-Valdivieso, D.; Font, R.; **Blanco-Díaz, M.T.**; Moreno-Rojas, J. M.; Gómez, P.; Alonso-Moraga, Á.; Del Río-Celestino, M. 2014. Application of near-infrared reflectance spectroscopy for predicting carotenoid content in summer squash fruit. *Computers and Electronics in Agriculture*, 108: 71–79. (Revista SCI del primer cuartil con índice de impacto 2,066).

Por todo ello, se autoriza la presentación de la Tesis Doctoral.

Córdoba, 1 de Junio de 2015

Firma de los directores

Fdo.: Dr. Rafael Font Villa

Fdo.: Dra. Alicia Fayos Moltó

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Mª Teresa Blanco Díaz

ESCRITO RAZONADO DEL RESPONSABLE DE LA LÍNEA DE INVESTIGACIÓN

La Tesis cuyo título se menciona arriba ha podido adaptarse, desde sus inicios, a la metodología y el diseño programados, derivando todo ello en la obtención de resultados de indudable relevancia científica y tecnológica.

Hay que destacar que del trabajo de esta Tesis se han establecido las bases científicotécnicas para el desarrollo de productos de IV Gama que tengan al calabacín como principal constituyente, llevándose a cabo la selección de variedades para tal fin, basándose en la determinación de parámetros de calidad tanto externa como interna de las mismas.

Asimismo, sirve este escrito para ratificar el informe emitido por los directores de la tesis.

Córdoba, 8 de Junio de 2015

Firma del responsable de línea de investigación

Fdo.: Mª Teresa Sánchez Pineda de las Infantas

Agradecimientos

Acknowledgements

El esfuerzo realizado durante esta Tesis Doctoral, habría dado escasos frutos sin la estimada ayuda que he recibido a lo largo de este tiempo, tanto de personas, como de instituciones.

Para empezar quisiera agradecer a mis directores de tesis, Dr. Rafael Font y Dra. Alicia Fayos, por darme la oportunidad de trabajar con vosotros, de compartir vuestros conocimientos durante este proceso de aprendizaje tanto profesional como personal.

Agradecer también al Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), por la concesión de la beca FPI-INIA, en el marco del proyecto RTA2009-00036-00-00, con la que he podido desarrollar esta Tesis Doctoral en el Instituto de Investigación y Formación Agraria y Pesquera (IFAPA). Al personal del IFAPA – Centro La Mojonera (Almería), por su inestimable ayuda en los ensayos a nivel de invernadero y a la Dra. Mercedes Del Río por su asesoramiento en las técnicas relativas al análisis de compuestos nutricionales. También al personal del Centro IFAPA Alameda del Obispo (Córdoba), por su siempre disposición en el uso y manejo del equipo NIRS.

Mi más sincero agradecimiento a los investigadores Dra. María Isabel Gil, Dra. Ana Allende y Dr. Juan Antonio Tudela del CEBAS-CSIC (Murcia), al Dr. Giancarlo Colelli y Dra. María Luisa Amodio de la Universidad de Foggia (Italia), así como a la Dra. Elizabeth Baldwin, Dra. Anne Plotto, Dra. Jan Narciso y Dr. Jinhe Bai del USDA – (Fort Pierce, Florida), por acogerme como una más y permitir desarrollar mi formación en vuestros grupos de investigación. Fue un placer aprender de vosotros, personas admirables humana y profesionalmente. Muchas gracias! Grazie mille! Thank you so much!

Dar gracias a todos mis amigos, es una pena no mencionaros a todos…Gracias a los del IFAPA por compartir risas en los almuerzos (Antonio, Almudena, Ana, Leti), por vuestros ánimos y hacer el trabajo más llevadero. A todos los amigos de Murcia, Italia, Florida con los que he compartido momentos inolvidables durante las estancias, por hacerme sentir en casa cuando me encontraba tan lejos de los míos. A mis amigos de Jerez, por estar siempre ahí aunque no nos viéramos durante largos períodos de tiempo, todo sigue igual…

Agradezco finalmente el ánimo recibido por mi familia. Os quiero, sin vuestro apoyo y comprensión, esto no hubiera sido posible.
Y gracias a Antonio, por tu apoyo, amor, paciencia… gracias por ser como eres, porque contigo los malos momentos se convierten en buenos…

A todos, gracias de corazón

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Note: This current PhD Thesis is written in a bilingual format as a requirement for the International Mention

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IV. RESULTS AND DISCUSSION

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Abreviaturas

Abbreviations

ABREVIATURAS

A = superficie total de intercambio de gases de la membrana
a* = rojo (+) a verde (-)
A.M.= atmósfera modificada
AA= ácido ascórbico
AATC= ácido tricloroacético
ACP = Análisis de Componentes Principales
AFHORLA= Asociación española de frutas y hortalizas lavadas listas para su empleo
AMG= monoglicérido acetilado
atm^{-1}= atmósfera^{-1}
b* = amarillo (+) a azul (-)
BIAS= media de los residuales
BW= cera de abeja
C* = croma
Ca= calcio
$CaCl_2$= cloruro cálcico
CC= caseinato de calcio
Ci = concentración del gas disuelto en la membrana.
CM= Virus del mosaico del pepino
cm: centímetro
CMC= carboximetilcelulosa
CO_2= dióxido de carbono
COEX= polímero biodegradable
CW= cera de carnauba
Cys= cisteína
D.O.U.E.= Diario Oficial de la Unión Europea
Di = coeficiente de difusión del componente i en la membrana.
día^{-1}= dia $^{-1}$
FAO= Food and Agriculture Organization of the United Nations
Fe= hierro
FEHRCAREM= Asociación Empresarial de Cadenas de Restauración Moderna
FEPEX= Federación Española de Asociaciones de Productores Exportadores de Frutas, Hortalizas, Flores y Plantas Vivas
G= gramo
Gly= glicerol
h^{-1}= hora^{-1}
H_2SO_4= ácido sulfúrico
h^{o}= tono
HDPE = polietileno de alta-densidad
HPMC= hydroxipropil-metilcelulosa
HR= humedad relativa
hr= hora
Ji = Flujo local del componente i en la membrana.
K= potasio
Kg= kilogramo
kg^{-1}= kilogramos^{-1}
Ki= coeficiente de solubilidad del componente i en la membrana.
KOH= hidróxido de potasio
Kpa= kilopascal
kPa^{-1}= Kilopascal^{-1}

L* = luminosidad
L = litros
LDPE = polietileno de baja-densidad
M = masa del producto.
m.m^{-2}= milímetro^{-2}
m^{-2}= metro^{-2}
MAGRAMA= Ministerio de Agricultura, Alimentación y Medio Ambiente
MC= metilcelulosa
mg= miligramos
Min= minutes
mL= mililitro
mm= milímetro
Mol= Mol
N_2= nitrógeno
Na= sodio
NaOH= hidróxido de sodio
NIRS=espectroscopia en el infrarrojo cercano
nm= nanómetro
O_2= oxígeno
ºC= grados celsius
ODEPA= Oficina de Estudios y Políticas Agrarias
OPP= polipropileno orientado
P= fósforo
PCO_2= permeabilidad al dióxido de carbono
PEG= polietilenglicol
P'i y P''i = Presiones parciales de los gases a uno y otro lado de la membrana.
Pi= presión parcial del componente i
PO_2= permeabilidad al oxígeno
PP = polipropileno de calibre-delgado
Ppm= partes por millon
PS= poliestireno
PS= sorbato de potasio
PVC= cloruro de polivinilo
RCO_2= tasa respiratoria de dióxido de carbono
RCP ó PCR= regresión por componentes principales
RLM ó MLR= regresión lineal múltiple
RMCP ó PLS= regresión mediante mínimos cuadrados parciales
RMCPM ó MPLS=regresión mediante mínimos cuadrados parciales modificada
RMSE= Error cuadrático medio
RO_2= tasa respiratoria de oxígeno
RPD= cociente entre el error estándar de la predicción y la desviación estándar de la muestra
RSQ= coeficiente de correlación múltiple
S.A.= Sociedad Anónima
S.A.T.= Sociedad Agraria de Transformación
S.L.= Sociedad Limitada
s= segundos
s^{-1}= segundo^{-1}
SB= benzoato de sodio
SCCL= sociedad cooperativa de trabajo asociado

SD= desviación estándar
SECV= error estándar de validación cruzada
SEP = error estándar de predicción
SPC= proteína de soja concentrada
SPI= proteína de soja aislada;
SqM.= Virus del mosaico de la calabaza
ß = PCO_2/PO_2
SWO= suero láctea en polvo
T^{a}= temperaura
Tn= Tonelada
ToLCNDV= Virus del rizado del tomate Nueva Delhi
W= vatio
WMV-2= Virus del mosaico de la sandía
WPC= proteína de suero lácteo concentrada
WPI= aislado de proteína de suero lácteo
X = dirección normal a la membrana
y cal i= valor de laboratorio
ZYMV= Virus del mosaico amarillo del calabacín
Δx = espesor de la membrana.
ŷ teo i,= valor predicho
%= porcentaje
µg^{-1}= microgramos^{-1}
µL= microlitro

ABBREVIATIONS

a* = red (+) to green (-)
AA= ascorbic acid
Ac = accession
ANOVA= analysis of variance
atm^{-1}= atmosphere^{-1}
b*= yellow (+) to blue (-)
BHT = butylated hydroxytoluene
C*= chroma
CAA= calcium ascorbate
$CaCl_2$ = Calcium chloride
CHIT= chitosan
CHIT+GLUC= chitosan + glucose
Chl-A= chlorophyll *a*
Chl-B= chlorophyll *b*
cm= centimeter
CMC= carboxymethyl cellulose
CO_2= carbon dioxide
CV = coefficient of variation
Cv = cultivar
CYS= cysteine
día^{-1} = day $^{-1}$
DM= dry matter
DNPH = 2, 4 – dinitrophenil hydrazine dye
DPPH = diphenylpicrylhydrazyl solution
DW= dry weight
EP1= endpoint
ET= ethanol
F = film packaging
FW =fresh weight
G= gram
GLM= General Linear Model
h^{-1} = hour^{-1}
H1 to H6 = harvest date 1 to 6
H_2SO_4 = sulfuric acid
h^{o} = hue
HPMC= hydroxypropyl methylcellulose
hr = hour
Kg = kilogram
kg^{-1} = kilograms^{-1}
KOH= Potassium hydroxide
Kpa= kilopascal
kPa^{-1} = Kilopascal^{-1}
L = liters
L* = lightness
log cfu g^{-1} = colony forming units
LSD = least significant difference
Ltd= limited company
M.A.P= modified atmosphere packaging
m.m^{-2} = milimiter^{-2}

m^{-2} = $meter^{-2}$
Mg = miligrams
Min = minutes
mL = mililiter
mm = milimeter
Mol = Mol
Ms1 or 1 = maturity stage 1
Ms2 or 2 = maturity stage 2
N = Newtons
n = number of samples
N_2= nitrogen
NaOH= Sodium hydroxide
NIRS = Near-infrared spectroscopy
nm = nanometer
nt = number of terms in the calibration model
O_2= oxygen
ºC = celsius grade
P= pumpkin
PAL = phenylalanine ammonia-lyase
PC = principal component
PCA = principal component analysis
PCO_2 = carbon dioxide permeability
PO_2 = oxygen permeability
Ppm= parts per million
R_2cal = coefficient of determination in the calibration.
$r_2$cval = coefficient of determination in the cross-validation
$r_2$ev = coefficient of determination in the external validation
RDA= recommended dietary allowance
RER = ratio of the range to standard error of prediction in the external validation.
RH= relative humedity
RPD_{cval} = ratio of the standard deviation to standard error of cross-validation
RPD_{ev} = ratio of the standard deviation to standard error of prediction in the external validation
Rpm = Revolutions per minute
$RRCO_2$ = carbon dioxide respiration rate
RRO_2 = oxygen respiration rate
Rs 1 = ripening stage 1
Rs 2 = ripening stage 2
S =storage time
s = seconds
s^{-1} = $second^{-1}$
SB= soy bean oil
SD = estándar deviation
SEC = standard error of calibration.
SECV = standard error of cross-validation
SEP = standard error of prediction in the external validation.
SNV+DT = standard normal variate plus de-trend transformations
Weighted+MSC= weighted plus multiplicative scatter correction
SPI= soy protein isolate
T = temperature

t_i = initial time (h)
TA = titrable acidity
T^a= temperaure
Tm= metric ton
TPC= total phenolic compounds
TSS = total soluble solids
Vf = free volume inside the glass jar (mL)
VM= vegetable marrow
W = watt
w= total weight of the product (kg)
WHO = World Health Organization
WPC= whey protein concentrate
y^i_{CO2}= % CO_2
y_{O2} = % O_2
Z= zucchini
% = percent
ß = PCO_2/PO_2
µg^{-1} = micrograms^{-1}
µL = microliter

Resumen

Summary

RESUMEN

Los cambios sociales y demográficos experimentados por la sociedad actual, la exigente demanda de productos naturales y fáciles de preparar que mantengan sus propiedades nutricionales y sensoriales, unido a la necesidad de incrementar el valor añadido de los productos hortofrutícolas frescos, hacen del calabacín un excelente material vegetal para ser transformado a un producto de IV Gama.

Así, el objetivo general de la presente Tesis Doctoral ha sido aumentar la competitividad y las posibilidades de mercado de calabacín fresco o I Gama, mediante el estudio del potencial de este producto para ser transformado a producto de IV Gama.

Para alcanzar dicha finalidad, se plantearon diversas líneas de trabajo consistentes en: 1) estudio de la variabilidad genotípica/morfotípica en calabacín, como paso previo a su posterior procesado, 2) influencia tanto de factores pre-cosecha como post-cosecha en la calidad fisicoquímica, nutricional, microbiológica y sensorial del producto IV Gama conservado en atmósfera modificada (MAP), 3) Diseño y aplicación de sistemas activos emergentes que permitan la conservación calabacín IV Gama como alternativa a la MAP tanto en procesado en fresco como tras su cocinado, 4) Aplicación de la tecnología en el infrarrojo cercano (NIRS) en la predicción de compuestos antioxidantes de calabacín.

El primer estudio realizado (*Capítulo IV, Estudio 4.1*), consistió en evaluar la variabilidad de compuestos antioxidantes (ácido ascórbico, contenido en polifenoles totales y clorofilas *a* y *b*) en 27 accesiones de calabacín (*Cucurbita pepo* spp. *pepo*) procedentes tanto de la colección Europea de germoplasma de Cucurbitáceas como otros de otros países y 13 híbridos comerciales (procedentes de empresas internacionales de semillas de calabacín). Una vez determinado qué morfotipo presentaba mayores ventajas desde el punto de vista nutricional (morfotipo zucchini), se estudió la influencia que tenía el estado de madurez y el momento de recolección sobre la calidad físico-química y nutricional de distintas variedades comerciales del morfotipo zucchini *(Estudio 4.2);* cubriéndose con éxito la primera línea de trabajo definida.

Los estudios posteriores (*Capítulo IV)* persiguieron evaluar la aptitud del fruto de calabacín para ser procesado a IV Gama. Para ello, inicialmente se estudiaron qué efecto tenían los factores precosecha (variedad y estado de madurez) y postcosecha (film de envasado) sobre la calidad físico-química, nutricional y sensorial a lo largo de la conservación bajo refrigeración y MAP (*Estudio 4.3*). A continuación, se establecieron diferentes estrategias comparativas consistentes en la modificación del

formato de corte, la temperatura de conservación y la atmósfera modificada, que permitieran alargar la vida útil del producto (*Estudios 4.4 y 4.5.*). Estos 3 estudios conforman la línea de trabajo 2.

También en el *Capítulo VI*, se desarrollaron y aplicaron un total de 16 recubrimientos comestibles (sistemas activos), obteniéndose los mejores resultados en 3 de ellos durante la conservación del calabacín IV Gama. El efecto de dichos recubrimientos sobre la calidad físico-química y sensorial de calabacín IV Gama tras ser conservado a diferentes temperaturas de refrigeración, se evaluó tanto en crudo como tras 5 métodos habituales de cocinado del calabacín (*Estudio 4.6)* (línea de trabajo 3).

Finalmente, el potencial de la tecnología NIRS en la predicción de compuestos antioxidantes de calabacín se muestra en el *Estudio 4.7* (línea de trabajo 4). Para ello se realizaron predicciones con éxito en los contenidos de ácido ascórbico, contenido en polifenoles totales y de clorofilas *a* y *b* en diversos genotipos de calabacín entre los años 2009 a 2012, siendo este hecho importante para la consecución de un modelo predictivo NIRS suficientemente robusto.

Las conclusiones y referencias bibliográficas de esta Tesis Doctoral se describen en el *Capítulo V* y *Capítulo VI*, respectivamente.

SUMMARY

Social and demographic changes experienced during the last decades, consumer's expectations for natural and ready-to-use products maintaining both sensory and nutritional properties linked to the need to increase the added value of vegetables, confers to summer squash as being an excellent raw material to be processed as a fresh cut product.

The general aim of this Doctoral Thesis was to increase the competitiveness and the opportunities of fresh summer squash in the market, by studying the potential of this product to be minimally processed.

For that, in this study we developed a high quality fresh-cut summer squash product carried out by four working lines: 1) to study the *Cucurbita pepo* genotypic/morphotypic diversity, as a first step to be minimally processed, 2) the influence of pre-harvest and post-harvest factors in the physicochemical, microbiological and sensory quality of the fresh-cut zucchini stored under modified atmosphere packaging conditions (MAP), 3) to design and develop new active packaging systems for preserving the quality of the freshly cut and cooked zucchini products, 4) the application of near infrared spectroscopy (NIRS) to predict nutritional compounds in summer squash fruits.

In the first study *(Chapter IV, Study 4.1)*, we aimed to evaluate the natural pigments (chlorophylls *a* and *b* and total chlorophylls) and phytochemical compounds (ascorbic acid and total phenolic compounds) in 27 *Cucurbita pepo* accessions belonging to 14 traditional genotypes (from the European Central Cucurbits germoplasm database collection developed at COMAV and other countries) and 13 commercial hybrids (from international seed companies). Once we had established more convenient minimally processed morphotype (zucchini) from a nutritional point of view, we studied the effect of fruit ripening stages and harvest dates on the physical properties, proximate composition and health promoting compounds on six coloured zucchini varieties *(Study 4.2);* covering successfully the first strategy defined in this Doctoral Thesis.

The following studies included (in *Chapter IV)* asses the suitability of zucchini fruits to be minimally processed. For that, firstly we studied the effect of both pre-harvest (variety and maturity at processing) and postharvest (film packaging) on the physicochemical, nutritional and sensory quality during the refrigeration storage and

MAP (*Study 4.3*). Then, we compared different strategies involved in the zucchini cutting size, storage temperature, modified atmosphere packaging, for extending the shelf-life in fresh-cut zucchini (*Study 4.4. and Study 4.5*). These three studies allowed the development of the working line 2.

In *Chapter IV* there were also designed and developed a total of 16 edible coating combinations (active systems) obtaining the best results in preserving fresh-cut zucchini quality in 3 of them. The effect in preserving physicochemical and sensory quality of freshly-cut zucchini under different storage temperatures was finally tested in raw fresh cut zucchini and after 5 different zucchini cooking methods *(Study 4.6)* (working line 3).

Finally, it was studied the potential of NIRS in predicting zucchini antioxidant compounds in the *Study 4.7* (working line 4). Successful predictions were developed for ascorbic acid, chlorophyll *a*, chlorophyll *b* as well as total phenolic compounds in several summer squash genotypes collected since 2009 to 2012, being this fact important for obtaining a sufficiently robust NIRS prediction model.

Conclusions and references are included in *Chapter V* and *Chapter VI*, respectively.

Capítulo I. Introducción

Chapter I. Introduction

I. INTRODUCCIÓN

I.1. EL CALABACÍN

I.1.1. Taxonomía

El calabacín (*Cucurbita pepo* spp *pepo*), originario de las zona tropical y subtropical de América, África y Asia (Sargent y Maynard, 2002), pertenece a la familia Cucurbitaceae, comúnmente denominadas cucurbitáceas. Esta familia está compuesta por 22 especies salvajes y 5 especies cultivadas (*C. maxima, C. moschata, C. pepo, C. ficifolia* y *C. argirosperma*) (Decker, 1988), siendo los géneros *Cucurbita maxima, Cucurbita moschata* y *Cucurbita pepo* los más importantes económicamente.

Se caracteriza por ser una planta anual de vegetación compacta y de crecimiento indeterminado. El calabacín posee un tallo principal con atrofia de brotaciones secundarias, teniendo el tallo un crecimiento en forma sinuosa, no erecto, alcanzando gran desarrollo: hasta 1 metro de longitud. Es áspero al tacto, cilíndrico, de superficie pelosa, grueso, consistente, con entrenudos cortos de donde parten hojas, flores, frutos y numerosos zarcillos de 10-20 cm de longitud, delgados y que nacen junto al pedúnculo del fruto **(Figura I.1.1a)**. Los frutos son de baya carnosa, unilocular, voluminosa, caracterizándose las flores por ser grandes, solitarias, vistosas, axilares, de color amarillo, acampanadas y con un largo pedúnculo, pudiendo ser masculinas o estaminadas (cinco pétalos y tres estambres, **Figura I.1.1b**) y femeninas o pistiladas (con un ovario inferior y tres carpelos, **Figura I.1.1b**). Ambos sexos coexisten en una misma planta monoica pero en flores distintas. La apertura de las mismas tiene lugar por las mañanas siendo la polinización entomófila (abejas principalmente) o polinización cruzada (**Figura I.1.1c).**

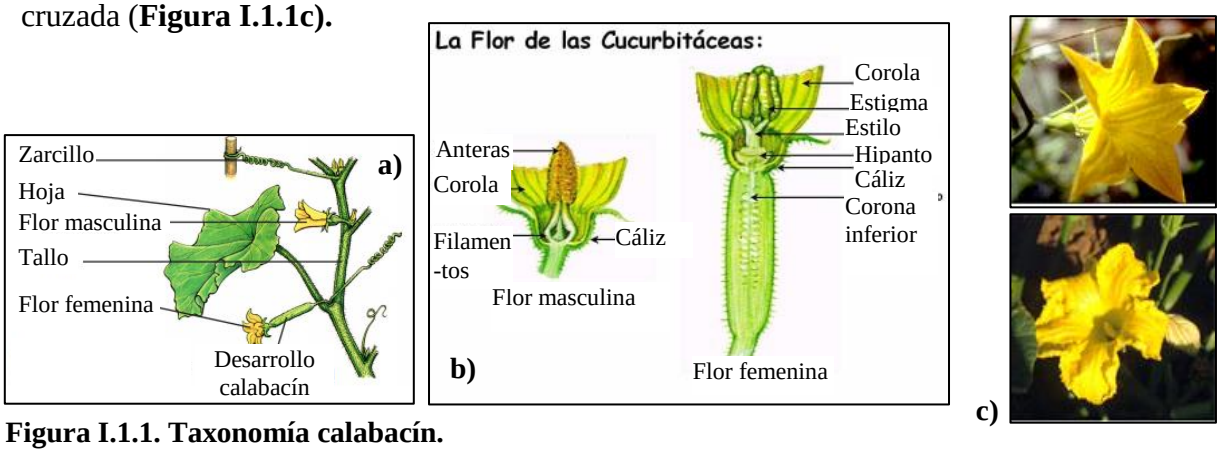


Figura I.1.1. Taxonomía calabacín.

Fuente: Paris, 1989.

Las principales características de los géneros más importantes económicamente se describen a continuación **(Tabla I.1.1).**

Tabla I.1.1. Características géneros *Cucurbitaceae.*

Especie	Semilla	Hoja	Tallo	Pedúnculo	Pulpa del fruto
C. maxima	blanca a marrón, hendidura oblicua	redonda, no lobulada, con espinas	blando, redondo y forma acampanada hacia el fruto	redondo, corto, poca intensidad anaranjada	fina, no fibrosa
C. moschata	blanca a marrón, extremo redondo, hendidura oblicua	lóbulos poco profundos y redondos, pubescencia suave	duro, puntiagudo en la zona próxima al fruto	duro, en ángulo, acampanado	fina, no fibrosa, color naranja intenso
C. pepo	marrón claro, margen liso prominente, hendidura redondeada	profundamente lobuladas, muy espinosas	duro y puntiagudo	duro, en ángulo, puntiagudo	grueso, anaranjada

Fuente: Paris, 1989.

Según las características externas del fruto (forma, color, tamaño) el género *Cucurbita pepo* se subdivide en 8 morfotipos (Paris, 2001): pumpkin, vegetable marrow, cocozelle, zucchini, incluidas en spp *pepo;* perteneciendo a spp. *ovifera* los morfotipos acorn, straightneck, scallop y crockneck **(Figura I.1.2).**

'Pumpkin' **'Zucchini'** **'Vegetable marrow'** **'Cocozelle'**

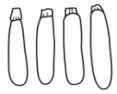
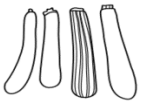
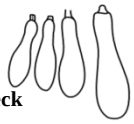
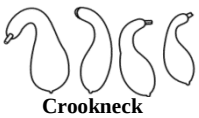
'Acorn' **'Straightneck'** **'Scallop'** **'Crookneck'**

Figura I.1.2. Morfotipos de *Cucurbitaceae*

Fuente: Paris, 2001.

A continuación se describen brevemente las características de cada morfotipo **(Tabla I.1.2):**

Tabla I.1.2. Principales caractersíticas de morfotipos *Cucurbitaceae*

Morfotipo	Principales características
 **Pumpkin**	Se consume en estado maduro. Incluye frutos esféricos u ovales, con extremos redondeados o planos, que pueden presentar surcos, costillas o verrugas y alcanzar 25 Kg. de peso. Debido a la gran variabilidad que presenta, se han establecido subgrupos dentro de este morfotipo
 **Zucchini**	Se consume en estado inmaduro. En la actualidad es el más importante económicamente y distribuido en el mundo. Incluye frutos denominados comúnmente calabacines, cilíndicos presentando un ratio longitude/anchura superior a 3,5. Los calabacines amarillentos han sido en la actualidad sustituidos por los verdes
 **Vegetable marrow**	Se consume en estado inmaduro. Muy utilizado en Oriente medio y en el norte de África. Sus frutos, de corteza lignificada, están ensanchados en la parte distal y son alargados, con un ratio longitud/anchura que varía de 2 a 3
 **Cocozelle**	Se consume en estado inmaduro. Frutos largos y bulbosos en el extremo distal, con un ratio longitud/anchura superior a 3,5
 **Acorn**	Se consume en estado maduro. Conocido también como *Table Queen* se compone de frutos ovoides o cónicos, con 10 surcos profundos. La mayoría de los cultivares modernos tienen frutos de color verde
 **Straightneck**	Se consume en estado inmaduro. Frutos cilíndricos, amarillentos, verrugosos y ensanchados en el extremo distal, con un cuello corto y estrecho en el extremo peduncular
 **Scallop**	Se consume en estado inmaduro. Frutos aplastados, lignificados, generalmente discoidales y con márgenes festoneados. En la actualidad, los colores amarillentos son preferidos a los blancos o verdes pálidos
 **Crookneck**	Se consume en estado inmaduro. Frutos alargados y presentan un cuello curvado, largo y fino, siendo la mayoría amarillos y verrugosos. Las plantas muestran mayoritariamente un hábito de crecimiento arbustivo

Fuente: Base de datos de European Central Cucurbits y patente US20120144515 A1.

1.1.2 Cultivo

1.1.2.1. Requerimientos edafoclimáticos

El calabacín, al igual que otras cucurbitáceas, es planta muy extendida por zonas con climas templados o cálidos. A continuación se resumen las necesidades edafoclimáticas para que el cultivo del calabacín se desarrolle con éxito (Reche, 2000):

❖ **Temperatura:** No es muy exigente en altas temperaturas, menos que el melón, pepino y sandía; siendo de mayor rusticidad que éstos cultivos. Sin embargo, es sensible a los fríos y las heladas, por lo que su cultivo al aire libre sólo es posible pasadas las épocas de heladas y a principios de la primavera **(Tabla I.1.3).**

Tabla I.1.3. Principales requisitos en temperatura en cultivo de calabacín.

Fases del cultivo	Tª óptima	Tª mínima	Tª máxima
Siembra (suelo)	20-25	15	40
Desarrollo vegetativo	25-30	10	35
Floración	20-25	10	35

Fuente: Adaptado de Reche, 2000.

❖ **Humedad:** el calabacín es exigente en humedad relativa del aire y del suelo, si bien excesos de humedad en el suelo pueden impiden la germinación **(Tabla I.1.4)**

Tabla I.1.4. Principales requisitos humedad en cultivo calabacín

Fases del cultivo	Humedad óptima (%)
Humedad del aire	65-80
Humedad del suelo	95

Fuente: Adaptado de Reche, 2000.

Con excesos de humedad en el suelo se puede impedir la germinación, mientras que un exceso de humedad ambiental aumenta la probabilidad de originar enfermedades y una deficiente fecundación. Si por el contrario la humedad es deficiente, puede producirse la deshidratación de los tejidos, disminución del desarrollo vegetativo y producción, retraso en el crecimiento y caída de flores.

❖ **Luminosidad:** para el calabacín no tiene excesiva repercusión la duración del día (planta de día neutro), no existiendo, en general, problemas de floración, por lo que el cultivo en invernadero puede realizarse en cualquier época.

❖ **Suelo:** el calabacín es medianamente tolerante a la salinidad del suelo y del agua de riego. Se adapta igualmente a terrenos con valores de pH entre 5 y 7, pero prefiere suelos algo ácidos, con valores medios entre 5,6-6,8. Los suelos alcalinos pueden provocar algunos síntomas de carencias.

A continuación se resume las etapas de cultivo hasta obtención de fruto de calabacín **(Figura I.1.3).**

Figura I.1.3. Etapas de cultivo del calabacín.

Fuente: Elaboración propia.

1.1.2.2. Períodos y calendarios

El ciclo de cultivo suele ser de 4-6 meses. El marco de plantación suele mantener distancias de 100-200 cm entre líneas y 60-150 cm entre plantas (Marín, 2013), siendo los más comunes: 2x 0,75; 1 x 1; 1,33 x 1; 1,5 x 0,75 m^2, a veces se sitúan

a tresbolillo. En cuanto a los ciclos de cultivo podemos diferenciar entre ciclos de otoño-invierno (transplante desde agosto hasta octubre) y ciclos de primavera (transplante desde diciembre hasta febrero) (Camacho, 2013). En la actualidad se pueden encontrar 149 variedades y 16 ecológicas, la mayoría de color verde con diferentes tonalidades (Marín, 2013). Los ciclos de cultivo pueden clasificarse en:

- Ciclo de otoño:
  - Extra temprano: fecha de siembra oscila del 1 al 15 de agosto.
  - Temprano: fecha de siembra oscila entre el 5 y 10 de septiembre.
  - Medio: fecha de siembra oscila entre el 5 y 10 de octubre.
  - Tardío: fecha de siembra oscila entre el 25 de octubre y 5 de noviembre.
- Ciclo de primavera:
  - Medio: fecha de siembra oscila entre el 1 y el 10 de diciembre.
  - Tardío: fecha de siembra se comprende entre enero y febrero.

1.1.2.3. Costes medios de producción

Los costes medios varían en función si el cultivo es de otoño o primavera. Si bien para ambas campañas, se deben tener en cuenta los siguientes los costes directos medios: insumos (semilla + semillero, fertilizantes, fitosanitarios, agua y energía, suministros), mano de obra asalariada, servicios externos contratados; costes indirectos medios (amortizaciones); gastos generales medios (gastos generales, gastos financieros); mano de obra familiar. A continuación se resumen los conceptos para ambas campañas **(Tabla I.1.5).**

Tabla I.1.5. Costes aproximados de cultivo de calabacín

Costes	Otoño			Primavera		
	Importe (€/m^2)	**Rendimiento medio (kg/m^2)**	**Ingresos (€/m^2)**	**Importe (€/m^2)**	**Rendimiento medio (kg/m^2)**	**Ingresos (€/ha)**
Directos	0,99	5,0		1,49	9,5	
Indirectos	0,22	5,0		0,29	9,5	
Generales	0,13	5,0		0,18	9,5	
Mano de obra familiar	0,51	5,0		0,61	9,5	
Sin mano de obra familiar	1,44	5,0		2,08	9,5	
Total	1,85	5,0	2,9	2,57	9,5	2,85

Precio medio calabacín en campaña Otoño: 0,58 €/kg; campaña Primavera: 0,30 €/kg *Fuente:* Consejería de Agricultura, Pesca y Medio Ambiente, 2013 (Campaña 2011/2012).

Los costes medios de una hectárea invernada de calabacín verde en la campaña de otoño suponen entre 14 400 y 18 500 €/ha, contabilizando en este último caso la mano de obra familiar. Mientras que los ingresos ascienden a 29 000 €/ha. Luego el beneficio durante el ciclo de otoño es de 10 500 €/ha.

Por otro lado, los costes medios de una hectárea invernada de calabacín verde durante la campaña de primavera suponen entre 20 800 y 25 700 €/ha, contabilizando en este último caso la mano de obra familiar. Mientras que los ingresos ascienden a 28 500 €/ha. Luego el beneficio durante el ciclo de primavera es inferior, próximo a 2 800 €/ha.

1.1.2.4. Plagas y enfermedades

Las condiciones agroclimáticas que se dan en los invernaderos favorecen el desarrollo de numerosas plagas y enfermedades, las principales enfermedades y los daños causados en calabacín (Reche, 2000).

- **Plagas:**

Pulgones: son insectos homópteros pertenecientes a la familia *Aphididae,* comúnmente conocido con el nombre de "piojillos", siendo *Aphis gossypii* (pulgón del melón), *Aphis fabae* (pulgón negro de las habas), y *Myzus persicae* (pulgón verde del melocotonero) los causantes de los principales daños al calabacín. No obstante, últimamente es *Aphis gossypii* el que se observa más frecuentemente en este cultivo.

*Daños producidos: c*omienzan los daños por focos, produciendo, con sus picaduras, salida de savia y paralización del crecimiento, rizado, abarquillando las hojas, deformándolas y debilitándolas. Como causa indirecta los pulgones pueden ser vectores de virosis.

Mosca blanca: es una plaga polífaga muy conocida por los agricultores que se desarrolla principalmente en los invernaderos, pudiendo observarse, en muchos casos, durante todo el ciclo vegetativo del cultivo. Hay dos especies de mosca blanca que parasitan al calabacín: *Trialeurodes vaporarioum*, conocida por mosca de los invernaderos y considerada como una de las más importantes plagas de cultivos protegidos; *Bemisia tabaci,* mosca blanca del tabaco y algodonero que desde hace unos años se observa junto con la anterior y que va convirtiéndose en otra importante plaga.

Daños producidos: existen 3 tipos de daños causados por mosca blanca.

a) Los adultos y larvas se alimentan del tejido celular, ocasionando más o menos daño dependiendo, fundamentalmente, del estado fenológico de la planta y de la infestación existente.

b) Las larvas segregan sustancias azucaradas sobre las que suelen desarrollarse diversos hongos (negrilla), los cuales no afectan directamente a los tejidos de las plantas, pero si reducen la superficie útil de las hojas para realiza la fotosíntesis.

c) Tanto *Bemisia tabaci* como *Trialeurodes vaporarioum* son vectores de diversas virosis.

Trips: los daños son producidos por una especie identiticada como *Frankliniella occidentalis.* Es plaga muy dañina en otras hortalizas (pimiento, tomate), pero en calabacín no es tan grave.

Daños producidos: en las hojas dañadas se observan unas placas de color grisáceo o plateado en las que están insertados diversos puntos negros (excrementos).

Posteriormente, estas zonas se necrosan totalmente. Si la infestación es alta y la superficie foliar dañada grande, las hojas de calabacín presentan muchas zonas de color pardo, pudiendo confundirse el aspecto general del cultivo al de una fitotoxicidad.

Minadora de hojas o submarino: es una plaga muy extendida por todo el litoral mediterráneo, que daña a gran número de plantas hortícolas y ornamentales. Al calabacín le ataca desde las primeras fases del cultivo.

Daños producidos: Se observa el inicio del daño por las picaduras y por las galerías realizadas por las larvas. A consecuencia de estas galerías, las funciones de las hojas se reducen al destruirse parte del parénquima foliar.

Ácaros: dentro de este nombre genérico y común, se suelen englobar a diferentes ácaros fitoparásitos. Sin embargo, es la araña roja, *Tetranichus urticae*, la que se encuentra con mayor frecuencia en calabacín.

Daños producidos: Las larvas y adultos de *Tetranichus urticae* se alimentan de los jugos celulares, causando decoloraciones y deformaciones a las hojas. Las hojas atacadas presentan un color bronceado por el haz, correspondiéndose con la presencia, en el envés, de adultos, larvas y puestas.

- **Enfermedades:**

Oídio: la enfermedad es producida por los hongos *Erysiphe cichoracearum y Sphaerotheca fuliginia,* es una enfermedad muy extendida entre los cultivos hortícolas y de fácil diagnóstico. Afecta, generalmente, a toda la planta y muy particularmente a las hojas tanto en el haz como en el envés.

Daños producidos: manchas aisladas y circulares en las hojas que se recubren con un micelio blanco de aspecto pulverulento por ambas caras, principalmente por el haz. Con ataques intensos las hojas amarillean, se secan y caen.

Mildiu: enfermedad producida por hongos del Grupo Ficomicetos, siendo *Pseudoperonospora cubensis* el hongo que ataca al calabacín.

*Daños producidos: e*n hojas adultas, se observan, por el haz, manchas internerviales, irregulares o poligonales, traslúcidas, de aspecto oleoso, que se tornan amarillentas, terminando por necrosarse y secarse; apreciándose igualmente en los bordes. Por el envés se recubre con unas eflorescencias de color grisáceo-violáceo constituidas por los esporangióforos del hongo.

Cladosporiosis: esta enfermedad, que ataca a numerosos cultivos, se propaga en los invernaderos en ausencia de agua sobre las plantas, aunque sí precisa humedad relativa alta, superior al 80 %. El hongo causante de la enfermedad pertenece al género *Cladosporium,* atacando diferentes cultivos. La especie que ataca al calabacín es *Cladosporiumcucumerinum.*

*Daños producidos: e*l hongo se refugia en los restos de plantas enfermas propagándose por las semillas, restos vegetales, etc. La sintomatología es la siguiente:

a) Frutos: se producen manchas deprimidas y con presencia de exudación que se recubre con una pelusilla grisácea formada por los órganos reproductores del hongo. Estas lesiones son parecidas a las ocasionadas por antracnosis, pero más pequeñas. Ataca a los frutos en cualquier fase de desarrollo, siendo más sensibles a la enfermedad los aún no formados. Estas lesiones, en calabacín, al contrario de otras cucurbitáceas, la cicatrización de las heridas no se produce.

b) Hojas: se observan manchas angulosas de color pardo-grisáceo que acaban necrosándose.

Botrytis o podredumbre gris: producida por el hongo *Botrytis cinerea* que presenta un aspecto de enmohecimiento gris. El desarrollo de la enfermedad se favorece con humedad relativa alta (80%), deficiente ventilación, abundante masa vegetal, marcos de plantación estrechos y exceso de abono nitrogenado. La infección se produce a través de los cortes producidos en la recolección, por la poda de hojas, en el ápice del fruto por permanecer los pétalos de las flores adheridos a los mismos y en los pecíolos de las hojas.

Daños producidos: este hongo puede causar al calabacín en invernadero importantes daños en cualquier fase de desarrollo. En la mayoría de los casos, el daño empieza a partir de la flor marchita que no se ha desprendido del fruto, iniciándose las lesiones en su extremidad, así como en el pedúnculo, observándose necrosis blanda en frutos y pudrición en el tallo, pecíolos y flores. Igualmente, a partir de las heridas producidas en la poda de hojas. Los frutos atacados desprenden grandes cantidades de esporas que propagan la enfermedad.

- **Virosis:**

Los principales virus que afectan al calabacín son (Reche, 2000; Consejería de Agricultura y Agua, 2015):

- Virus del mosaico amarillo del calabacín (ZYMV).
- Virus del mosaico de la sandía (WMV-2).
- Virus del mosaico de la calabaza (SqMV).
- Virus del mosaico del pepino (CMV).
- Virus del rizado del tomate Nueva Delhi (ToLCNDV) (introducido en 2013).

La transmisión y penetración en la planta puede ser de diferentes formas: por vectores aéreos, por contacto y a través de las semillas.

Daños producidos: reducción del crecimiento y deformación de hojas y frutos, modificación del color de la hoja (moteados, mosaicos, amarilleamientos, etc.) y abullonado de los frutos.

Al objeto de evitar o disminuir los daños descritos anteriormente y mejorar el cuajado del fruto del calabacín, en la actualidad se emplean diversos tratamientos **(Tabla I.1.6):**

Tabla I.1.6. Tratamientos empleados en el cultivo de calabacín.

Tratamiento	Principio activo	Nombre comercial	Tratamiento	Principio activo	Nombre comercial
Insecticida	Abamectina (EW)	Agrimec	Fungicida	Azufre mojable	Azufre
	Flonicamid	Teppeki		Azoxystrobin	Ortiva
	Imidacloprid	Confidor 20 ls		Ciproconazol	Caddy 10 pepite
	Jabón potásico	Jabón potásico		Clorantraniliprol	Altacor 35 WG
	Pimetrozina	Plenum		Fenhexamida	Teldor
	Piridaben	Sanmite		Triadimenol	Bayfidan
	Spinosad	Spintor 480 sc	Hormona de cuaje	AATC 5% + Ácido fólico	Ergostin
	Spiromesifen	Oberon			
	Tiacloprid	Calypso		Auxina	Fruitone

Fuente: IFAPA – Centro la Mojonera (campañas 2010 a 2014).

1.1.3. Producción y consumo

1.1.3.1. Situación mundial

A nivel mundial, España es uno de los principales países en la producción de calabacín, tal y como puede observarse durante el año 2012 **(Figura I.1.4).**

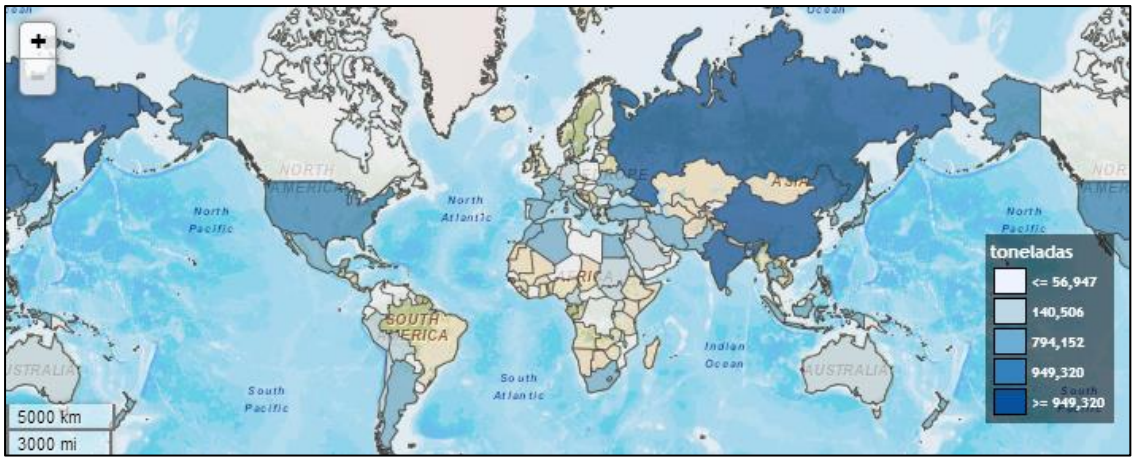


Figura I.1.4. Principales países productores de calabacín.

Fuente: FAO, 2015.

A nivel mundial España es el décimo país productor de calabacín, ocupando los primeros puestos China (7 000 000 Tn), India (4 900 000 Tn) y la Federación Rusa (1 080 845 Tn) (F.A.O., 2015). Mientras que a nivel europeo, España se sitúa como tercer país más importante en producción, por detrás de Ucrania (587 800 Tn) e Italia (520 000 Tn) **(Figura I.1.5).**

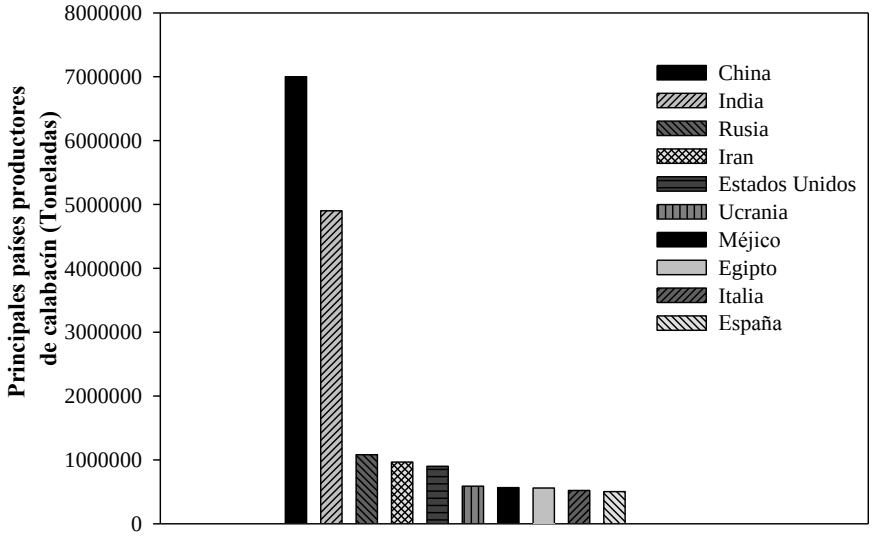


Figura I.1.5. Principales países productores de calabacín.
Fuente: Elaboración propia a partir datos FAO, 2015.

Con respecto a las exportaciones mundiales de calabacín en 2011, España destacó por ser el principal país exportador (270 919 Tn), seguido de Nueva Zelanda (86 782 Tn), Méjico (48 994 Tn) y Marruecos (41 782 Tn) **(Figura I.1.6a)**; mientras que los principales países importadores fueron Estados Unidos (365 519 Tn), Francia (143 257 Tn), Japón (114 574 Tn) y Alemania (69 405 Tn) **(Figura I.1.6b).**

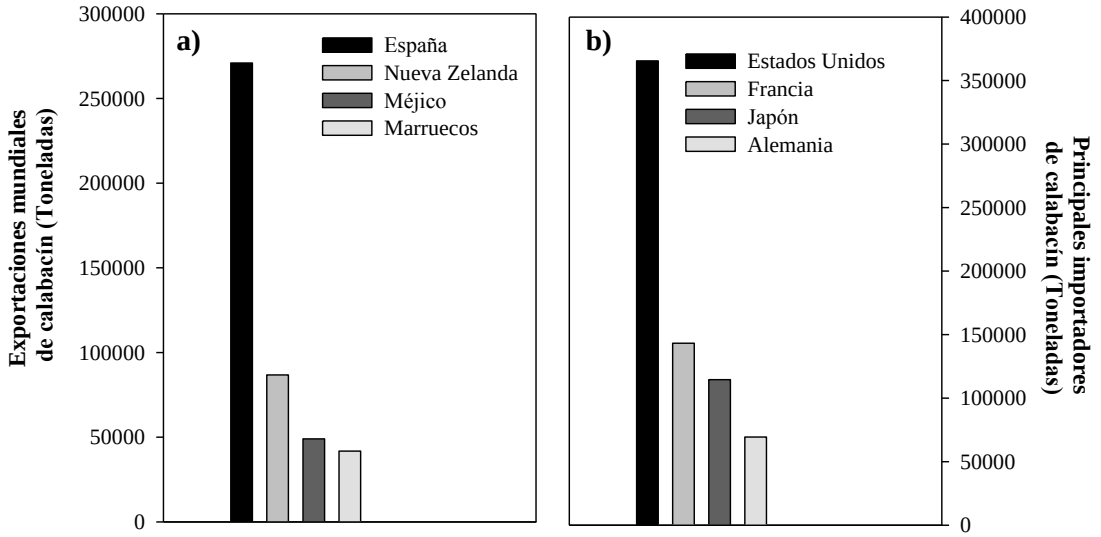


Figura I.1.6. Principales países exportadores e importadores de calabacín.
Fuente: Elaboración propia a partir datos FAO, 2015.

1.1.3.2. Situación en España y Almería

En España la producción de calabacín (*Cucurbita pepo* morfotipo zucchini) ha aumentado progresivamente durante los últimos 10 años, alcanzándose los máximos históricos tanto en superficie (8 900 has) como en producción media (465 000 Tn) durante el año 2012 **(Figura I.1.7)** (M.A.G.R.A.M.A., 2015).

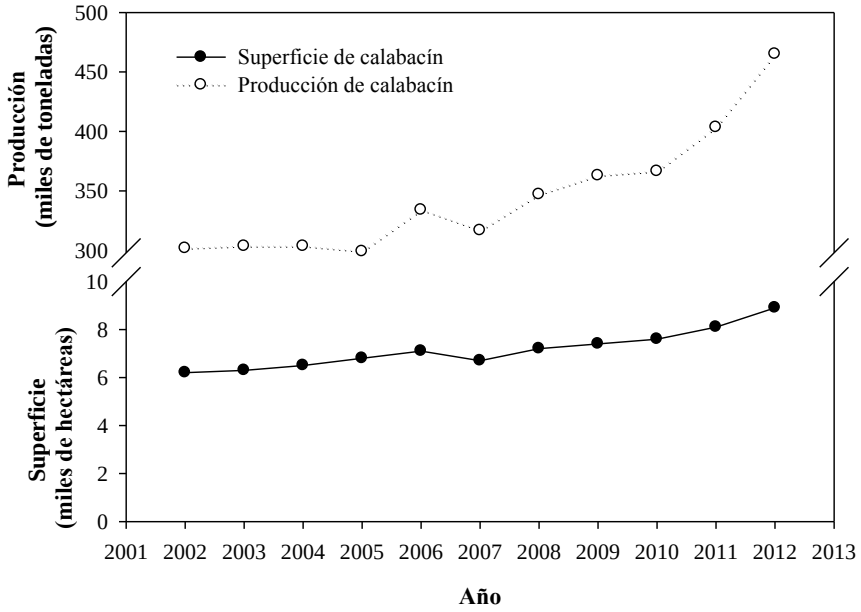


Figura I.1.7. Evolución de la superficie y la producción de calabacín en España.

Fuente: Elaboración propia a partir datos MAGRAMA, 2015.

En particular, es la Comunidad Autónoma de Andalucía **(Figura I.1.8)**, donde se concentra la mayor parte de calabacín cultivado en España, con un total de 6 680 has, dando lugar a 393 964 Tn de producción de calabacín (M.A.G.R.A.M.A, 2015).

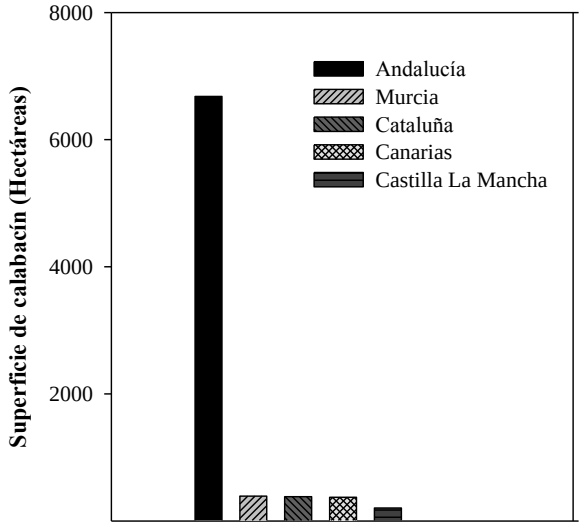


Figura I.1.8. Principales Comunidades Autónomas productoras de calabacín.

Fuente: Elaboración propia a partir datos MAGRAMA, 2015.

Dentro de la Comunidad Andaluza, Almería es la provincia líder en la producción de hortalizas, siendo el calabacín uno de los principales cultivos hortícolas explotados, con un total de 5 789 has que dan lugar a unas 354 156 Tn, ocupando el tercer lugar tras el tomate y el pimiento **(Figura I.1.9)** (Consejería de Agricultura, 2015).

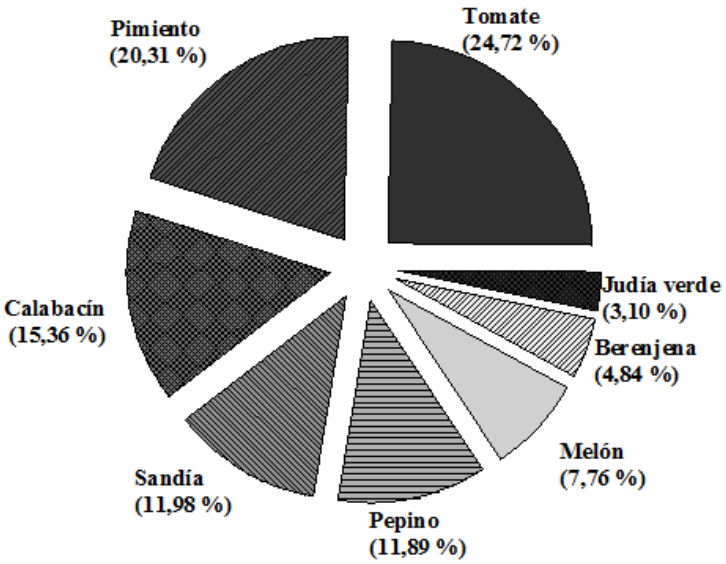


Figura I.1.9. Principales cultivos explotados en Almería.

Fuente: Elaboración propia a partir datos MAGRAMA, 2015.

Es importante destacar que la mayoría del calabacín nacional se produce bajo invernadero (cultivo protegido), posicionándose la provincia de Almería como líder en este sistema de cultivo con el 98 % **(Tabla I.1.7).**

Tabla I.1.7. Superficie de calabacín en diferentes sistemas de cultivo.

	Superficie de calabacín (hectáreas)		
	Aire libre	**Protegido**	**Total**
Almería	90	5 699	5 789
Cádiz	204	11	215
Córdoba	79	-	79
Granada	193	100	293
Huelva	20	-	20
Jaén	-	-	-
Málaga	-	190	190
Sevilla	35	30	65
Total	621	6 030	6 680

Fuente: Elaboración propia a partir datos MAGRAMA, 2015.

En resumen, es Almería la principal provincia productora de calabacín en España, con el 65 % de la superficie y el 76 % de la producción nacional (M.A.G.R.A.M.A, 2015).

Sin embargo, y a pesar de la importancia de este cultivo, el precio del calabacín en el mercado no es siempre constante **(Figura I.1.10)**, sufriendo notables variaciones anuales y siendo muy sensible a la aparición de alertas alimentarias (como las ocurridas en pepino durante el 2011).

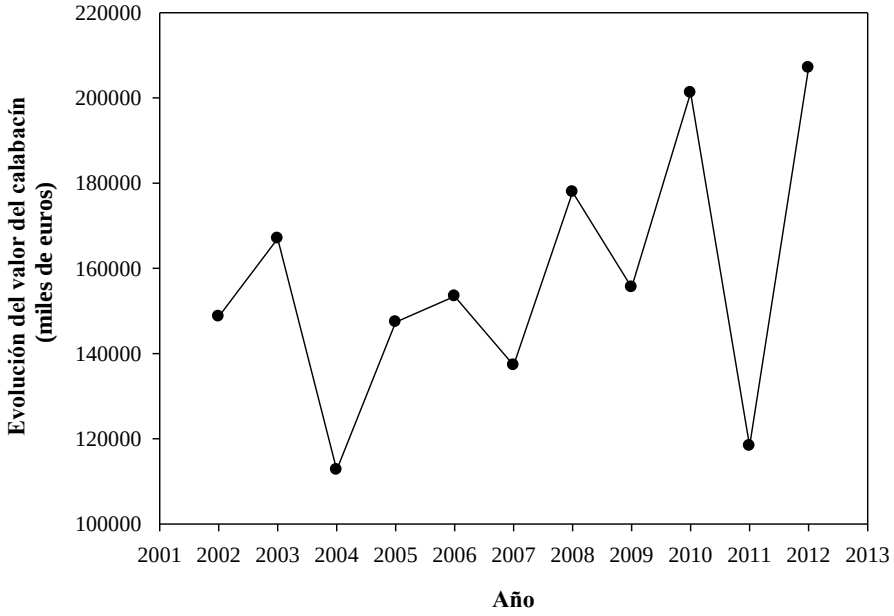


Figura I.1.10. Variaciones en el valor del calabacín en España.
Fuente: Elaboración propia a partir datos MAGRAMA, 2015.

Por este motivo, otras alternativas de procesado tales como la transformación a un producto mínimamente procesado o de IV Gama (descritos en el próximo apartado de Tesis) ofrecerían una alternativa de mercado en esta hortaliza, permitiendo además un incremento del valor añadido en dicho producto.

1.1.4. Composición nutricional

Como se puede observar en la **Tabla I.1.8** el agua es el principal componente en calabacín (a excepción de las semillas), variando entre el 88 % (calabacín morfotipo acorn) y el 96 % (calabacín morfotipo zucchini). Los frutos destacan también por ser bajos en calorías, proteínas, lípidos y carbohidratos, haciéndolos una buena elección en dietas de personas con exceso de peso.

Tabla I.1.8. Composición nutricional de morfotipos y partes comestibles de la familia *Cucurbitaceae*.

Cucurbitácea	Agua (%)	Energía (kcal)	Proteina (g)	Lípidos (g)	Carbohi-dratos (g)	Fibra (g)	Ca (mg)	P (mg)	Fe (mg)	Na (mg)	K (mg)	Vitamina A (IU)	Tia-mina (mg)	Ribofla-vina (mg)	Niaci-na (mg)	Ácido ascórbico (mg)	Vitamina B (IU)
Morfotipo pumpkin	92	26	1,0	0,1	6,5	1,1	21	44	0,8	1	340	1600	0,05	0,11	0,60	9	-
Flores de pumpkin (flowers)	95	15	1,0	0,1	3,3	-	39	49	0,7	5	173	1947	0,04	0,08	0,69	-	-
Hojas de pumpkin (leaves)	93	19	3,2	0,4	2,3	-	39	104	2,2	11	436	1947	0,09	0,13	0,92	11	0,21
Semillas de pumpkin (seeds)[1]	7	541	24,5	45,9	17,8	3,9	43	807	15,0	18	535	380	0,21	0,32	1,75	-	0,22
Morfotipo acorn	88	40	0,8	0,1	10,4	1,5	33	36	0,7	3	347	340	0,14	0,01	0,70	-	0,15
Morfotipo scallop	94	18	1,2	0,2	3,8	0,6	19	36	0,4	1	182	110	0,07	0,03	0,60	18	0,11
Morfotipo zucchini	96	14	1,2	0,1	2,9	0,5	15	32	0,4	3	248	340	0,07	0,03	0,40	9	0,09
Calabacín (summer squash)	94	20	1,2	0,2	4,4	0,6	20	35	0,5	2	195	196	0,06	0,04	0,55	14,8	0,11
Calabaza (winter squash)	89	37	1,5	0,2	8,8	1,4	31	32	0,6	4	350	4060	0,10	0,03	0,80	12,3	0,08

[1] Peso seco.

Fuente: US Department of Agriculture, 2002.

En cuanto al contenido en minerales, el calabacín destaca por ser una buena fuente de potasio, y presentar menores cantidades de calcio, fósforo, hierro y sodio. El potasio es un mineral necesario para la transmisión, generación del impulso nervioso y para la actividad muscular, actuando dentro y fuera de la célula. Por otro lado, el fósforo juega un papel importante en la formación de huesos y dientes, además de favorecer el buen funcionamiento del intestino, nervios y músculos, y junto con la fibra, posee una acción diurética.

Además, el calabacín posee beta-caroteno, un pigmento natural que le confiere el color amarillo-anaranjado-rojizo a los vegetales y que el organismo lo transforma en vitamina A. Dentro de las cucurbitáceas destacan las calabazas (winter squash), con pulpa de color anaranjado, por ser una excelente fuente de vitamina A **(Tabla I.1.8)**. La vitamina A posee propiedades antioxidantes y es esencial para la visión, el buen estado de la piel, el cabello, las mucosas, los huesos y además facilita el buen funcionamiento del sistema inmunológico. También participa en la elaboración de enzimas en el hígado y de hormonas sexuales y suprarrenales.

Al igual que vitamina A, el calabacín contiene vitaminas del tipo B, como la tiamina (vitamina B1) presente en mayores cantidades en el calabacín morfotipo acorn y la calabaza **(Tabla I.1.8)**; mientras que las semillas destacan por sus altas concentraciones en riboflavina (vitamina B2) **(Tabla I.1.8)**. Entre las principales funciones de la tiamina está la desintegración de los hidratos de carbono y el aprovechamiento de los principios nutritivos; permitiendo por su lado la riboflavina los procesos de respiración celular, desintoxicación hepática, desarrollo embrionario, mantenimiento de la envoltura de los nervios y mejoramiento del estado de la piel, las uñas y el cabello. Finalmente la niacina (o vitamina B3) mayoritariamente en las semillas **(Tabla I.1.8)**, actúa en el funcionamiento de los sistemas digestivo y nervioso, el buen estado de la piel y la conversión de los alimentos en energía.

También habría que destacar del calabacín la presencia de vitamina C (principalmente en forma de ácido ascórbico) que participa como antioxidante, interviniendo en la formación de colágeno, huesos, dientes y glóbulos rojos, ofreciendo una mayor resistencia frente a infecciones y favoreciendo la absorción de hierro de los alimentos.

1.2. LOS PRODUCTOS DE IV GAMA

En el mercado actual de frutas y hortalizas coexisten distintas formas de presentación del producto final, siendo como constante objetivo el aumento de la vida útil y el incremento del valor añadido de los productos finales. Dichas formas de presentación poseen una gran heterogeneidad en cuanto al grado de procesado (I Gama,

II Gama, III Gama, IV Gama y V Gama), teniendo todas ellas como principal finalidad conservar las características de los productos hortofrutícolas.

1.2.1. Clasificación según el grado de procesado industrial

Los principales métodos de procesado llevados a cabo en los productos hortofrutícolas se resumen a continuación **(Figura I.2.1)** (Blanco-Díaz, 2009).

I Gama

Se define como I Gama aquellas frutas u hortalizas frescas enteras cuya presentación no difiere significativamente de la que inicialmente poseían, aunque hayan sido sometidas a un breve procesado para mejorar las características físicas tales como el lavado, el encerado, enjuagado, etc. **(Figura I.2.1a).**

II Gama

Las frutas y hortalizas de II Gama están formadas por frutas y hortalizas que han sido seleccionadas, lavadas, cortadas, envasadas y sometidas a un tratamiento térmico (esterilización) al objeto de eliminar de ellas toda forma de vida, incluidas las esporas. La esterilización, dependiendo de las hortalizas y frutas a envasar, se lleva a cabo mediante la aplicación de temperaturas altas (120-140 ºC) durante un corto periodo de tiempo. De esta manera, se consiguen mantener las propiedades nutricionales y organolépticas (color, textura, olor, sabor, etc) durante un periodo largo de tiempo (años) **(Figura I.2.1b).**

III Gama

Las frutas y hortalizas de III Gama se caracterizan por haber sido seleccionadas, lavadas, cortadas, envasadas y sometidas a un tratamiento térmico (congelación, si las temperaturas alcanzadas son próximas a los -20 ºC, u ultracongelación, si las temperaturas son cercanas a los -40 ºC) al objeto de mantener las propiedades nutricionales y organolépticas. Al igual que la II Gama, las frutas y hortalizas que han sido sometidas a este tipo de procesado poseen mayor vida útil (años). En la **Figura I.2.1c** se muestran dos productos de III Gama (espárragos y arroz).

IV Gama

Formada por frutas y hortalizas frescas que han sido seleccionadas, lavadas, cortadas y envasadas, sin ser sometidas a tratamiento térmico. El envasado se realiza mediante películas plásticas, consistente por lo general en disminuir el contenido de O_2 y en el aumento de las concentraciones de N_2 y CO_2. Es un producto conservado bajo refrigeración siendo la vida útil del producto próxima a 7 días **(Figura I.2.1d).**

V Gama

Están formados por frutas y hortalizas que han sido seleccionadas, lavadas, cortadas, envasadas y sometidas a tratamiento térmico suaves (entre 65-85 ºC durante pocos minutos), ya que determinadas características tales como el color, la textura y el

sabor, se alteran en caso de ser sometidos a tratamientos térmicos más fuertes como la esterilización. Además se emplean otros métodos de conservación como el envasado al vacío y la posterior refrigeración. Sin embargo en ocasiones, al tratarse de platos precocinados, es necesario su calentamiento previo en horno microondas **(Figura I.2.1e).**

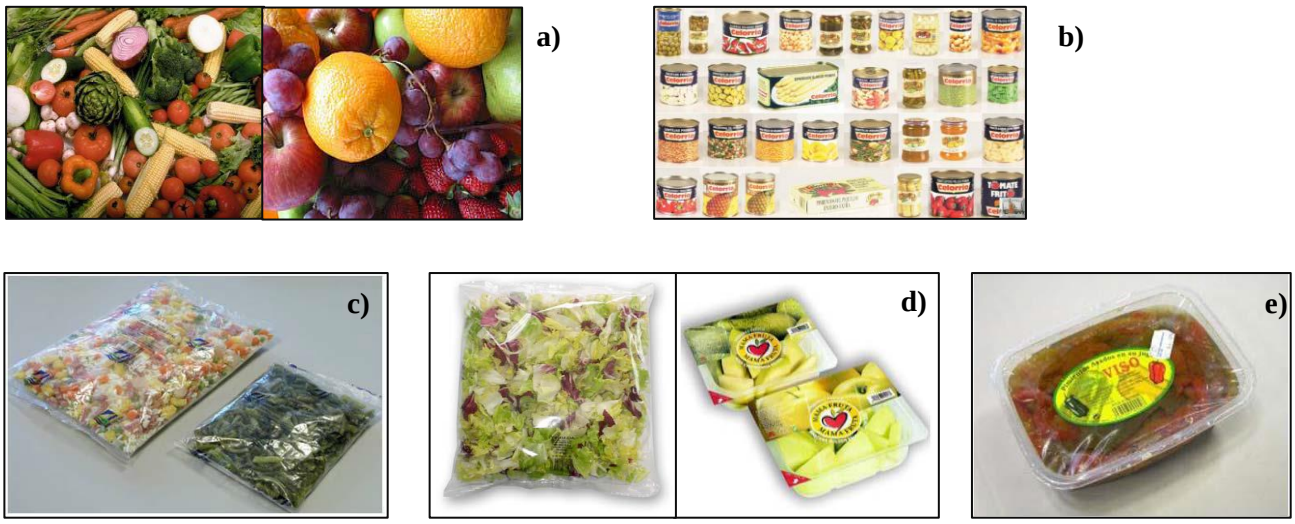


Figura I.2.1. Gamas de frutas y hortalizas, a) I Gama; b) II Gama; c) III Gama; d) IV Gama; e) V Gama

En cuanto a terminología empleada para hacer referencia a estos productos es amplia:

- Productos mínimamente procesados (minimally processed).
- Parcialmente procesados (partially processed).
- Preparados para consumir (ready to eat).
- Preparados para cocinar (ready to cook).
- Pre-cortados (pre-cut).
- Pre-preparados (pre-prepared).
- Frescos cortados (fresh-cut).

Analizando de manera exhaustiva la terminología empleada comúnmente para designar a este tipo de productos ‘mínimamente procesado’ o ‘de IV Gama’ existe una pequeña distinción, ya que el término ‘producto mínimamente procesado’ incluye tres variedades de productos: IV gama, V gama y otros productos como los que contienen liquido de gobierno pero con vida útil muy corta. Sin embargo, a nivel europeo no se establece ninguna distinción entre productos mínimamente procesados y de IV Gama (Segura y Díaz, 2001). Por este motivo, en el presente Tesis no se establecerá distinción alguna entre los términos mínimamente procesados y de IV Gama.

1.2.2. Producción y mercado de IV Gama

Los productos de IV Gama se desarrollaron a mediados de los años 70 en los Estados Unidos, siendo introducidos en Europa con posterioridad, en 1980. Los

primeros países Europeos en comercializar la IV Gama fueron Suiza y Alemania, a los que le siguieron Inglaterra, Francia, Países Bajos e Italia. Finalmente estos productos llegaron a España en 1989, situándose las primeras industrias de IV Gama en Navarra y extendiéndose hacia el sur y este del país (Andalucía, Murcia, Comunidad Valenciana, Cataluña, etc.).

Con el fin de consolidarse la IV Gama como una categoría específica dentro de las frutas y hortalizas, se creó la Asociación Española de Frutas y Hortalizas Lavadas, listas para su empleo (AFHORLA), que tras asociarse con FEPEX (Federación Española de Asociaciones de Productores y Exportadores de Frutas, Hortalizas, Flores y Plantas Vivas) velan por la defensa de los intereses de las principales empresas españolas en este sector (Vega Mayor, S.A., Verdifresh, S.L., Sogesol, S.A., Kernel Export, S.L., Primaflor, S.A.T., Tallo Verde, S.L. y Actel, S.C.C.L.).

En cuanto a la venta de productos de IV Gama, el crecimiento en España ha sido continuado incluso en épocas de crisis. El aumento de ventas ha crecido a raíz del 123% desde el 2004 (FEHRCAREM, 2015). Así, en el año 2014 el volumen comercializado de frutas y hortalizas de IV Gama en España ascendió a 81,5 millones de kilos (con un incremento del 4,9% frente al año anterior), de los cuales 79 millones de kilos corresponden a hortalizas y 2,5 millones de kilos a frutas **(Figura I.2.2).**

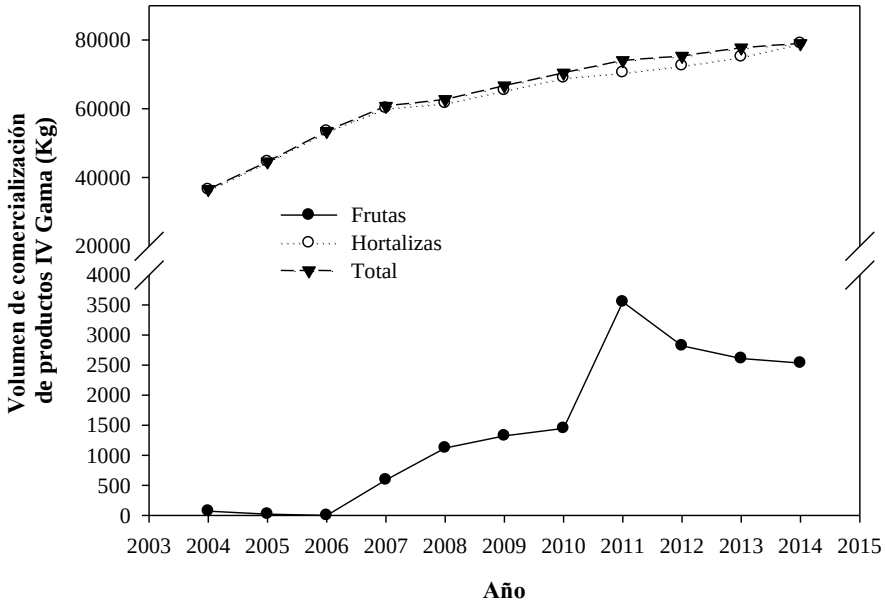


Figura I.2.2. Evolución en ventas de productos IV Gama.

Fuente: Elaboración propia a partir de datos FEPEX, MAGRAMA

Del volumen comercializado de productos IV Gama en España durante 2013 (77 740 Tn), el 81,85 % (63 633 Tn) correspondió a distribución, siendo el 18,15 % restante (14 108 Tn) a restauración, alcanzando un volumen de negocio cercano a los 200 millones de euros (FEPEX, 2015).

Además como puede observase **(Figura I.2.3)**, el volumen de productos de IV Gama exportado durante el año 2011 en España aumentó en 1 523 Tn (de 11 474 Tn a 12 997 Tn) (+ 13,27 %), disminuyéndose en toneladas la importación de los productos IV Gama en 150 Tn (de 195 Tn a 43 Tn) (- 450 %).

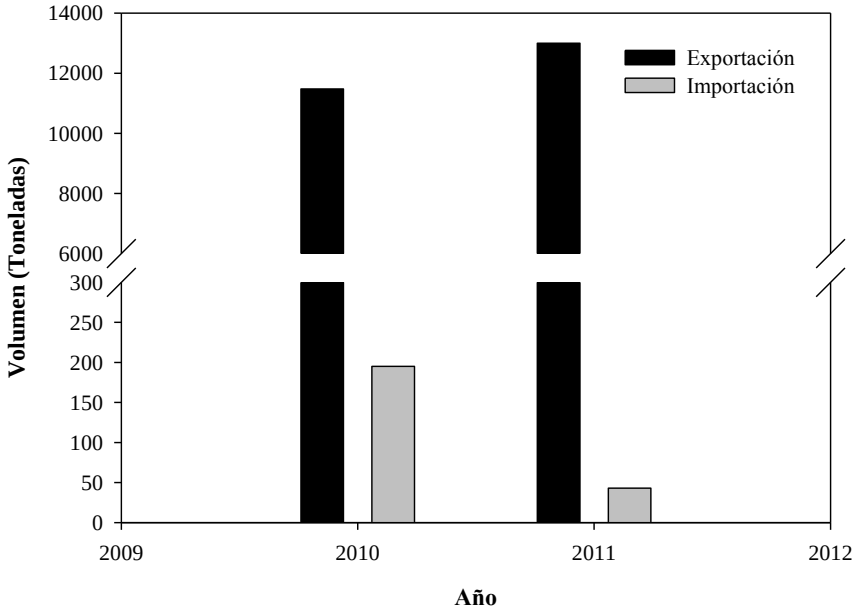


Figura I.2.3. Volumen de exportación e importación de productos IV Gama en España.

Fuente: Elaboración propia a partir de datos de FEPEX y MAGRAMA, 2013.

Es importante destacar, que a diferencia de Asia donde se consume mayoritariamente frutas de IV Gama, en Europa y América Latina predomina el consumo de hortalizas de IV Gama (Alimarket, 2015), siendo las lechugas y mezclas de ensaladas las más empleadas por la población española **(Tabla I.2.1).**

Tabla I.2.1. Consumo de IV Gama en España.

	2013		**2014**	
	Volumen (miles unidades)	**Valor (Millones de €)**	**Volumen (miles unidades)**	**Valor (Millones de €)**
Ensaladas IV Gama	58,7 %	58,7 %	59,6 %	60,6 %
Verduras y frutas IV Gama	41,3 %	41,3 %	40,4 %	39,4 %
Total	275,33	318,8	277,21	323,03

Fuente: Elaboración propia a partir de datos Alimarket, 2015.

En general para la elaboración de hortalizas IV Gama, se pueden utilizar como materia prima: hortalizas de hoja (lechuga, espinaca, escarola, canónigos, baby leaf); hortalizas de fruto (berenjena, melón, tomate, pepino, pimiento); hortalizas con raíces, bulbos e inflorescencias (zanahoria, cebolla y brassicas); siendo después de la lechuga

la espinaca (10 %), junto con la acelga (4 %), la zanahoria (3 %), la cebolla, el puerro y el apio (1 %) las verduras más empleadas en IV Gama (Artés et al., 2005).

A nivel mundial, Estados Unidos con 30 kg per cápita ocupa el primer lugar en consumo de productos IV Gama. España ocupa el séptimo lugar a nivel europeo en consumo de productos de IV Gama, posicionándose en primer lugar Reino Unido, seguido de Francia, Italia, Alemana, Países Bajos y Bélgica (ODEPA, 2014). Durante el 2013 en España, las hortalizas y frutas de IV Gama representaron el 5 y 2 % de lo consumido en fresco (I Gama), respectivamente **(Tabla I.2.2).**

Tabla I.2.2. Evolución del consumo en hogares de España productos de I vs. IV Gama.

Verduras y hortalizas (Tn)		**2010**	**2011**	**2012**	**2013**
	Frescas (I Gama)	2 781,0	2 884,4	2 870,6	2 923,5
	IV Gama	153,9	152,9	137,1	132,6
	Ecológicas	329,0	339,7	358,6	375,3
Total		3 263,9	3 377,1	3 366,3	3 431,5
Frutas (Tn)					
	Frescas (I Gama)	4 694,9	4 656,1	4 781,0	4 676,6
	IV Gama	136,2	127,6	98,3	94,0
	Ecológicas	376,8	370,9	421,2	404,5
Total		5 207, 5	5 154,6	5 300,5	5 175,1

El consumo per cápita nacional en productos de IV Gama en España fue de 11,1 kg, siendo el País Vasco con 15,45 kg (+ 39,18 %) la comunidad autónoma con mayor consumo **(Figura I.2.4).**

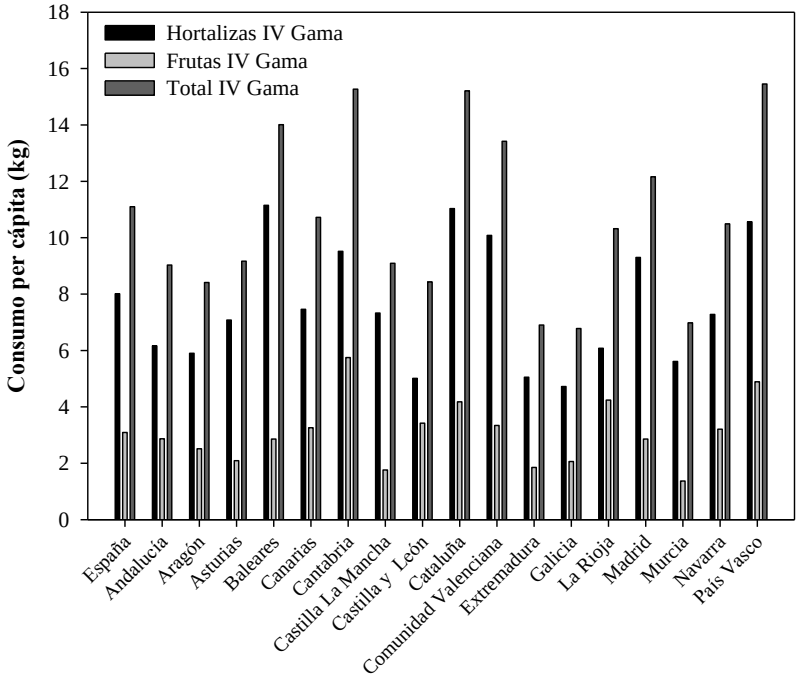


Figura I.2.4. Consumo de IV Gama por Comunidades Autónomas.

Fuente: Elaboración propia a partir de datos MAGRAMA, 2015.

También destacaron otras comunidades autónomas con elevado consumo per cápita de productos de IV Gama como Cantabria con 15,27 kg (+ 37,56 %), Cataluña con 15,21 kg (+ 37,02 %), Baleares con 14,01 kg (+ 26,21 %) y Comunidad Valenciana con 13,42 kg (+ 20,90 %). Andalucía se situó con un consumo medio per cápita de 9,03 kg (- 18,35 %), mientras que Galicia fue la comunidad autónoma con menor consumo per cápita en este tipo de productos (6,78 kg) (- 38,92 %) **(Figura I.2.4).**

1.2.3. Ingeniería de proceso en la elaboración de productos IV Gama

Las operaciones unitarias englobadas en el procesado mínimo, se sintetizan en las siguientes: selección de la variedad a procesar, elección del grado de madurez óptimo, recolección , acondicionamiento de la materia prima, lavado del producto entero para la eliminación de residuos, pelado/cortado, lavado y desinfección, enjuagado, secado, envasado y expedición **(Figura I.2.5).**

Tras el procesado, los productos se envasan mediante el empleo de bolsas o bandejas en atmosfera modificada para, posteriormente, ser transportados y conservados bajo refrigeración.

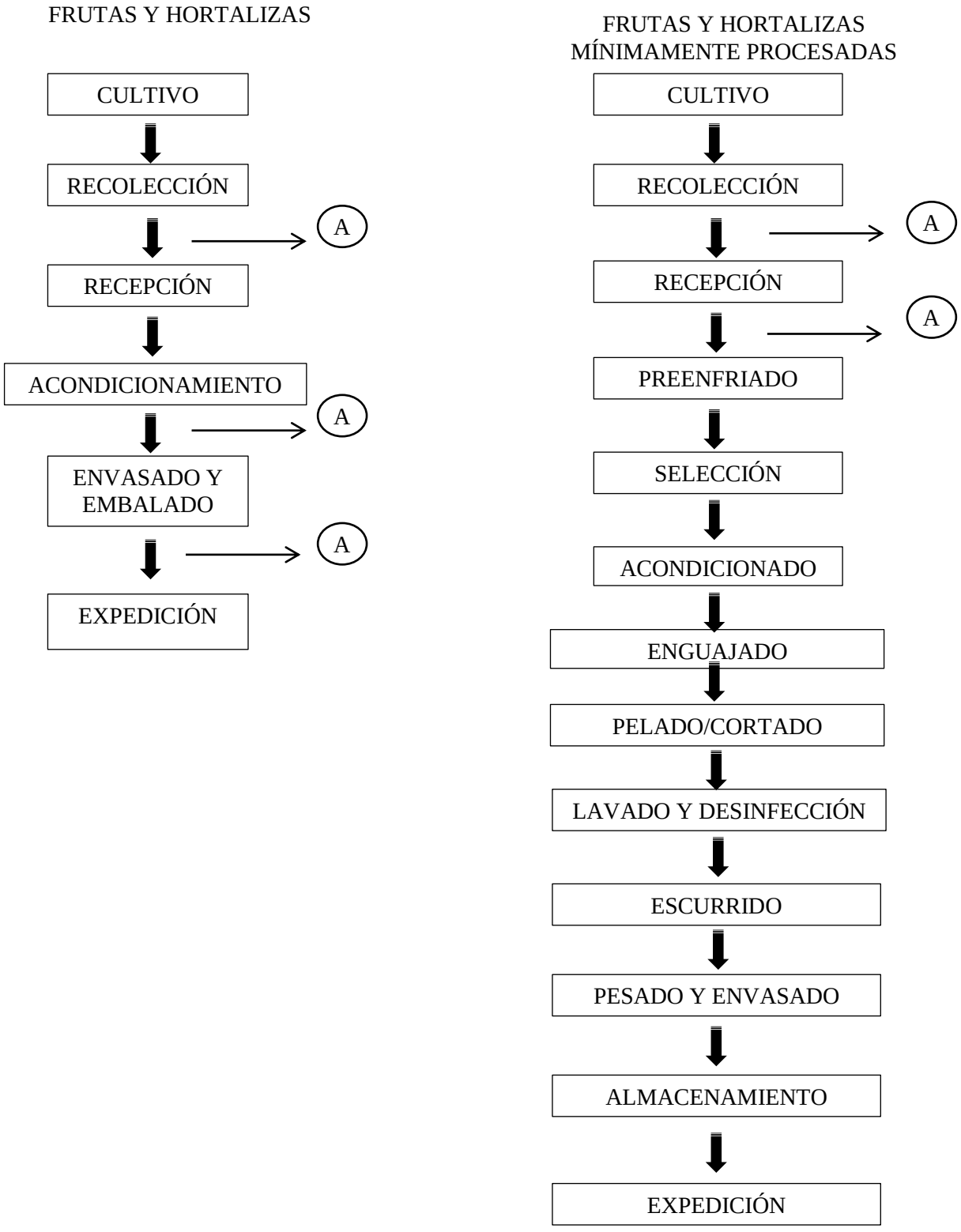


Figura I.2.5. Operaciones básicas en la elaboración de productos de I y IV Gama.

Fuente: Elaboración propia.

1.2.3.1. Recolección

La obtención de un producto mínimamente procesado de calidad radica en la adecuada selección de la materia prima y en la optimización de todas las etapas tecnológicas necesarias para su elaboración y mantenimiento de la calidad sensorial, higiénico-sanitaria y nutricional.

La materia prima ha de seleccionarse teniendo en cuenta dos aspectos: la variedad y el grado de madurez. La correcta selección de la variedad es de importancia crucial ya que puede simplificar las etapas y tratamientos de conservación que han de ser aplicados con posterioridad (Gil et al., 2012; Giné Bordonaba et al., 2014). De la misma manera es importante la temperatura a la que se realice el proceso de recolección, ya que si esta es lo más baja posible (durante la noche o primeras horas de la mañana) es más ventajosa para mantener la calidad de la fruta y hortaliza durante la manipulación y el almacenamiento (Wiley, 1997).

En particular, la recolección de calabacín se realiza de forma manual, dada la delicada naturaleza de dicha hortaliza requiriendo una manipulación cuidadosa debido a la fragilidad del exocarpo. Existe un reglamento comunitario por el que se establecen las normas de comercialización de los frutos de calabacín, el Reglamento (CE) Nº 1757/2003 de la Comisión de 3 de octubre de 2003 (D.O.U.E., 2003). En este reglamento se incluyen las siguientes especificaciones y exigencias para el calabacín fresco, siendo los requisitos mínimos de los frutos que lleguen a la industria:

- Deben ser enteros y provistos de pedúnculo, que podrá estar ligeramente dañado.
- Sanos, quedando excluidos los productos que presenten podredumbre u otras alteraciones que los hagan impropios para el consumo humano.
- Limpios, es decir, prácticamente exentos de materias extrañas visibles.
- Prácticamente exentos de parásitos y daños causados por los mismos.
- Exentos de un grado anormal de humedad.
- Exentos de olores y sabores extraños.

Además los frutos de calabacín deben ser:

- Firmes, exentos de cavidades y grietas.
- En un estado de desarrollo suficiente, antes de que sus semillas hayan adquirido firmeza.

Todos los calabacines se hallaran en un estado y una fase de desarrollo que les permitan:

  - Conservarse bien durante su transporte y manipulación.
  - Llegar en condiciones satisfactorias a su destino.

Siempre que se conserven sus características esenciales de calidad, conservación y presentación estos calabacines podrán tener los siguientes defectos:

  - Malformaciones.
  - Defectos de coloración.

- Ligeras quemaduras de sol.
- Ligeros defectos de la epidermis.
- Ligeros defectos debidos a enfermedades siempre y cuando no sean evolutivos ni afecten a la carne.

A continuación se definen las principales malformaciones o defectos en frutos de calabacín:

- ***Plateado:*** el limbo de las hojas adquiere un aspecto plateado. Los frutos cuajados se permanecen pequeños con color verde claro y aspecto plateado. Existe una estrecha relación entre este desorden y el ataque de la mosca blanca *Bemisia tabaci*, como consecuencia de la existencia de un factor toxicogénico asociado con la alimentación de las ninfas de dicho insecto.
- ***Frutos chupados:*** son frutos que no se desarrollan uniformemente y se quedan chupados generalmente por la extremidad apical. Existen diversas causas por las que se produce, tales como cambios bruscos de temperatura y humead ambiental, falta de agua en el suelo, estrés hídrico o tratamientos fitosanitarios.
- ***Frutos ennieblados:*** son frutos que detienen su desarrollo en un estado muy precoz y que finalmente se abortan. Las posibles causas por las que se producen son el agotamiento de la planta, la falta de vigor vegetativo o el empleo de tratamientos fitosanitarios.
- ***Frutos torcidos:*** son frutos que se doblan por el centro debido a un mal cuajado.
- ***Cogollos partidos:*** se producen por un exceso de vigor del cultivo.
- ***Frutos con golpes de sol (asoleamiento):*** consiste en un acorchamiento de la piel que deprecia al fruto como consecuencia del exceso de radiación en la cara más expuesta del fruto.
- ***Necrosis apical****:* produce desecaciones en la parte inferior del calabacín. Esta anomalía está provocada por una falta de calcio en la planta.

1.2.3.2. Transporte

Tras la recolección se ha de realizar el transporte lo más rápidamente posible para evitar que mermas por contaminación o deterioro del producto recién recolectado. Los vehículos empleados deberán estar limpios, desinfectados, secos y habilitados para el transporte exclusivo de sustancias alimenticias. Se deberá asegurar una adecuada ventilación en el caso de que no se empleen camiones refrigerados, siendo obligatorias la refrigeración próxima a los 10 ºC cuando la distancia desde campo a industria sea superior a 6 horas según las buenas prácticas de producción (AFHORLA, 2010) y una humedad próxima al 95 % en el caso del calabacín (Kader et al., 1992).

1.2.3.3. Pre-enfriado y conservación bajo refrigeración

La primera operación tecnológica que se realiza una vez que llegan los productos a la planta de procesado o bien en el mismo campo es la pre-refrigeración o pre-enfriado, que debe de realizarse para eliminarse el calor de campo. El objetivo es la obtención de un producto final de calidad, ya que permite ralentizar los procesos metabólicos como la tasa metabólica y el desarrollo y multiplicación de microorganismos alterantes. El pre-enfriamiento se puede realizar de tres sistemas diferentes: con agua fría (hidrocooling), aire frío a presión (air cooling) o mediante vacío (vacuum cooling) (Garrido et al., 2015). El primero de ellos, consiste en hacer pasar el agua (que puede contener bajas dosis de hipoclorito sódico) a través de los la parte superior e inferior de las cajas con el producto a través de un túnel con cinta transportadora. El sistema air cooling consiste en hacer pasar aire a través de los orificios de las cajas con los frutos apilados. Y finalmente el método vacuum cooling consiste en agregar una pequeña cantidad de agua que posteriormente se eliminará de la superficie del producto a través de someter éste a vacío, bajando rápidamente la temperatura del producto a procesar.

En el caso particular del calabacín, al tratarse de una hortaliza sensible a daños por frío (Grupo 1) **(Tabla I.2.3)**, es necesario establecer una especial atención a la temperatura de conservación previa al procesado en fresco.

Tabla I.2.3. Grupos de frutas y hortalizas susceptibles a daños por frío.

Grupo 1 **Los más susceptibles**	**Grupo 2** **Moderadamente susceptibles**	**Grupo 3** **Los menos susceptibles**
Aguacates	Arándanos americanos	Chirivías
Albaricoques	Apio, Brócolis (brotes)	Coles antiguas y de Saboya
Batatas	Calabazas	Coles de Bruselas
Bayas (excepto arándanos americanos)	Cebollas (desecadas)	Colinabo
	Coliflores	Col rizada
Berenjenas	Espinacas	Dátiles
Calabacín	Guisantes	Nabos
Ciruelas	Manzanas	Nabos suecos
Espárragos	Naranjas	Remolachas
Judía común	Peras	
Lechuga	Perejil	
Limas	Pomelos	
Limones	Rábanos	
Melocotones	Uvas	
Okra	Zanahorias	
Patatas		
Pepinos		
Pimientos dulces		
Plátanos		
Tomates		

Fuente: Adaptado de Hardenburg, Watada y Wang, 1986.

A la hora de la conservación bajo refrigeración es importante establecer qué características tienen las frutas u hortalizas que se van a almacenar juntas, al objeto de que las condiciones de unas no perjudiquen el estado de las otras. En la **Tabla I.2.4** se analizan por grupos la compatibilidad en almacenamiento entre frutas y hortalizas,

siendo para el caso de calabacín 10 ºC el óptimo de refrigeración, junto con una humedad relativa entre el 85-90 % (Grupo 5).

Tabla I.2.4. Condiciones adecuadas durante el almacenamiento de frutas y hortalizas.

Grupos de compatibilidad	Frutas y hortalizas compatibles	Tª (º C)	HR (%)	Características
Grupo 1	Albaricoques, cerezas, ciruelas, cocos, colinabo, melocotón, frambuesa, granada, higos, caqui, manzanas melocotón, membrillo, nabo, naranjas, níspero, peras, puerro, rábanos, remolacha sin hojas	0-2	90-95	Muchos de estos productos producen etileno
Grupo 2	Cereza, col de Bruselas, coliflor, endivia, escarola, esparrago, espinaca, granada, kiwi, lechuga, maíz dulce, nabo, perejil, puerro (no con higos o uvas), rábano, remolacha, repollo, uva, hortalizas sin hojas, zanahoria, alcachofa, apio, brócoli, berro, cebolla verde (no con higos, uvas o maíz dulce)	0-2	90-95	Muchos de estos productos son sensibles al etileno
Grupo 3	Ajos, cebollas secas	0-2	65-75	La humedad causa daños a estos productos
Grupo 4	Arándano, melón, clementina, lichi, mandarina, naranjas, tanguelos, tangerinas, yuca	4-5	90-95	---
Grupo 5	Aceituna, berenjena, calabacín, judías verdes, okra, patatas, pepino, pimiento, pomelo, tamarindo, taro	10	85-90	Muchos de estos productos son sensibles al etileno y al frío
Grupo 6	Aguacates, bananas, boniato, calabaza, melón, coco, chirimoya, granadilla, guayaba, limón, mango, maracuyá papaya, pina, plátano, tomates maduros, toronja	13-15	85-90	Muchos de estos productos son sensibles al etileno y al frío

Fuente: Adaptado de Pérez, 2000.

1.2.3.4. Selección y acondicionamiento

Durante la fase de selección se preparara la materia prima eliminando los desechos y dejando solo la parte útil para ser transformada. Los factores más importantes a tener en cuenta para clasificar la materia prima son tamaño, forma, color, firmeza, olor, magulladuras, superficies cortadas y posibles alteraciones. Aunque la clasificación automática permite mayor rapidez, fiabilidad y menor coste de mano de obra; lo más habitual (y común en calabacín) es la clasificación manual por personas entrenadas, capaces de comprobar los distintos estándares de calidad simultáneamente.

Por su lado, el acondicionamiento es una etapa de preparación de la materia prima que consiste en la separación de las partes no comestibles de forma manual sobre tablas y cuchillos habilitados a tal efecto. En el caso particular del calabacín, se eliminarán los extremos de los frutos de calabacín, ya que son partes no comestibles y por tanto se pueden gestionar como residuos para alimentación animal. El rendimiento

de la operación depende del tipo de producto y de la habilidad del personal, pudiendo estimarse de forma aproximada una media de 0,9 a 1,0 kg de materia prima por minuto y persona, cuantificándose en condiciones normales unas pérdidas del 35-40%. (Sánchez, 2003).

El personal deberá respetar todas las normas de higiene, tanto en lo que respecta a la manipulación del producto como a su indumentaria, que será exclusiva para el trabajo, estando compuesta por: ropa de abrigo, botas, guantes, cofia y mascarilla.

1.2.3.5. Enjuagado y pelado/cortado

La suciedad del producto (tierra, barro o arena) se elimina mediante el proceso de enjuagado. A nivel industrial, para las hortalizas con raíces ó bulbos como zanahorias, puerro, etc., el enjuagado se debe realizar mediante túnel cilíndrico rotativo constituido por placas metálicas o mallas metálicas; si bien para otros productos como las lechugas y espinacas suele emplearse otros sistemas de lavado de diseño más especiales. A nivel industrial, la forma más empleada consiste en sumergir el producto en un baño donde se mantiene en borboteo de aire. Esta turbulencia permite la eliminación prácticamente de todas las trazas de tierra y sustancias extrañas sin producir magulladuras del producto. El agua de enjuagado deberá estar fría (4-6 ºC) y si no se va a realizar un lavado y desinfección posterior, se puede incluir en esta etapa la dosificación de desinfectante que se debería de incluir en la etapa de lavado y desinfección tras el cortado (Blanco-Díaz, 2009).

La siguiente operación básica consiste en el pelado y/o cortado, dependiendo de la fruta u hortaliza a procesar. El pelado también denominado raspado, despellejado, descortezado o descascarillado consiste en la eliminación de la capa más externa y es necesario en patatas, zanahorias o naranjas. Sin embargo, esta etapa no se recomienda para el caso del calabacín, ya que aumenta la superficie dañada desencadenando un incremento de la tasa respiratoria del producto, además de disminuir el aporte nutricional tras su consumo.

El pelado puede hacerse de forma manual, con vapor o agua caliente, con lejía o alcalis (NaOH, KOH), mediante pelado caustico seco con calentamiento por infrarrojos, con llama, por medios mecánicos, con vapor a presión elevada, por congelación y mediante ácidos (Wiley, 1997). Por ejemplo, los tubérculos tales como patatas, remolachas, zanahorias, nabos y cebollas pueden pelarse por medios mecánicos o con lejía. Sin embargo, el pelado con lejía de productos como melocotones, peras, albaricoques y tomates origina menores pérdidas permitiendo una manipulación más rápida (Wiley, 1997). En la actualidad el método más habitual es el mecánico, llevando se a cabo mediante cuchillas u hojas de afeitado lo más afiladas posible y construidas en

acero inoxidable, siendo éstas lavadas continuamente con agua fría y clorada (Sánchez, 2004).

Por su lado el cortado de la materia prima acelera la respiración, provocando daños mecánicos y ablandamiento del tejido vegetal. Los tejidos cortados constituyen barreras menos eficaces a la difusión de los gases y toleran concentraciones más elevadas de O_2 y niveles inferiores de CO_2 que los productos intactos. De esta manera se llega a duplicar y hasta cuadruplicar su intensidad respiratoria como respuesta al 'stress' de corte (Sánchez, 2003).

1.2.3.6. Lavado y desinfección

Al objeto de disminuir el riesgo que supone la formación de compuestos halogenados a partir de dosis en exceso de derivados clorados al entrar éstos en contacto con materia orgánica (Ölmez y Kretzschmar, 2009; López-Gálvez et al., 2010; Tan et al., 2015), se está promoviendo realizar el enjuagado con la adición de desinfectantes (lavado y desinfección) como etapa previa al corte de la materia prima a procesar (Gómez-López et al., 2014) (eliminando de esta forma el lavado con desinfectante directamente sobre mayor superficie del producto cuando éste ya está cortado). Por este motivo, las indicaciones descritas en este apartado de Tesis, podrían completarse con las descritas en la etapa de enjuagado, y por tanto llevar a cabo un enjuagado con desinfectante antes del corte.

El agua de lavado deben tener una excelente calidad y la temperatura debe ser inferior a los 5 ºC, siendo la cantidad de agua a utilizar entre 5-10 l/kg de producto si éste se lava antes de pelar y cortar, y 3 l/kg si el lavado tiene lugar después del cortado (Sánchez, 2004). Con respecto a los agentes higienizantes, lo más extendido para la desinfección es el uso de dosis controladas de hipocloritos. Si bien, la actividad germicida de los hipocloritos disminuye con la concentración, especialmente en agua alcalina (pH > 8,5), de ahí la importancia de regular el pH del agua de lavado y mantenerlo entre 6,5 y 7,5 mediante ácido cítrico (Davidson et al., 2013).

Higienizantes deben usarse en las aguas de lavado para la reducción del número de microorganismos y retrasar la actividad enzimática al mismo tiempo que para incrementar el tiempo de conservación. Una cantidad de 100-200 mg de cloro libre es efectiva en el agua de lavado antes o después del pelado y/o cortado para incrementar el tiempo de conservación. Cuando se utilicen derivados del cloro, el producto deberá ser aclarado (Wiley, 1997).

Como valores orientativos de los niveles en cloro disponible en diversas hortalizas según la Universidad de Davis (2003), las hortalizas que más cantidad de cloro necesitan serían el chile dulce y picante (300 a 400 ppm), tomates (200-350 ppm),

brócoli, coliflor, lechuga, melones, pepinos, repollo y zanahoria (100-150 ppm). En concreto para el calabacín se ha podido comprobar que concentraciones de 150 ppm son las más adecuadas por disminuir en 2, 5 y 4 log ufc/g la concentración de microorganismos alterantes de la calidad en calabacín IV Gama como son las enterobacterias, los psicrótrofos y los mesófilos, respectivamente (Fayos et al., 2012).

Además de los comentados, existen otros higienizantes utilizados en la industria de IV Gama, de los cuales destacan los que se indican en la **Tabla I.2.5.**

Tabla I.2.5. Principales higienizantes utilizados en la industria de IV Gama.

Higienizante	Nombre comercial	Producto de IV Gama	Dosis (concentraciones* tiempo)	Tª lavado/ características	Cita
Acido láctico	Purac	Endibia	2 % * 1,5 min	22 ºC	Casa Comercial Purac
Hipoclorito de sodio		Lechuga	100 mg/L- 30 s, 2 y 5 min	4 ºC	Behrsing et al, 2000
Hipoclorito de sodio		Brócoli	50 mg/L- 30 s, 2 y 5 min	4 ºC	Behrsing et al., 2000
Clorito de sodio	Sanova	Col china	500 mg/L * 15 min	25 ºC	Inatsu et al., 2005
Dióxido de cloro estabilizado	Oxine	Lechuga	5 min	22 ºC, Tres lavados consecutivos	Singh et al., 2002
Ácido perioxiacético	Tsunami	Zanahoria	80 mg/L * 2 min	25 ºC	González, 2004
Perióxido de hidrogeno		Cantaloupe	5% * 2 min	25 ºC	Mendonca, 2003
Ozono en agua		Patata bastones	4ppm * 3 – 7 min	8 ºC Segundo lavado: 300mg/L	Beltrán,2005
UV-C		Lechuga	30 W * 15 min	50 cm por ambos lados	Singh, 2002

El método de lavado difiere enormemente en función de la fruta u hortaliza de la que se trate, pudiéndose realizar mediante remojo en agua estática o en circulación o bien en salmueras, utilizando pulverizadores de agua, lavadoras de tambor giratorio o cepillos giratorios. También pueden aplicarse los métodos secos de limpieza tales como cribado, cepillado, aspiración, abrasión y separación magnética. Por ejemplo, algunos productos como las cebollas maduras, champiñones, patatas y batatas no deben lavarse nunca o hacerlo después del almacenamiento ya que no es conveniente aumentar la humedad de estos productos (Wiley, 1997). Si bien un aspecto común a ellos es el control del agua de forma periódica para saber si su uso es apto o no.

1.2.3.7. Escurrido

La humedad residual y el exudado celular en la superficie de las hortalizas tienden a estimular el crecimiento de levaduras, mohos y bacterias. De ahí que después

del lavado se utilicen muchos tipos de maquinarias que eliminen el exceso de agua, tales como escurridores, centrifugas, tamizadores o deshumidificadores.
Según el método a seguir, las técnicas de secado difieren, siendo el centrifugado la más común y el secado por aire frío o las bandejas vibrantes los más novedosos. Por otro lado, es importante tener en cuenta que someter el producto a un secado con excesiva rapidez también puede dañar el material a secar, por lo que se debe realizar de forma controlada (Sánchez, 2004).

1.2.3.8. Pesado y envasado

El sistema de pesado más empleado en la industria agroalimentaria es el sistema de pesado asociativo, en el cual se disponen una serie de recipientes o células de pesado que se llenan simultáneamente hasta un peso parcial predefinido, de esta forma un microprocesador calcula en cada momento la combinación optima de un determinado número de ellas aproximándolo lo máximo posible al peso final deseado. Este es un sistema limpio, que alcanza una capacidad elevada, con una gran precisión de pesada y que permite abarcar una amplia gama de pesos.

Después de la disminución de temperatura de los productos, el envasado en atmósfera modificada (AM) o atmósfera protectora, se considera que es el segundo método más eficaz para prolongar la vida útil de los productos tanto frescos como mínimamente procesados (Álvarez et al., 2015); si bien no es un mecanismo de sustitución del propio control de la temperatura (Martínez-Hernández et al., 2015).

Existen dos modalidades de envasado en atmósfera modificada (AM) (Day, 2000):

- Envasado en atmósfera modificada pasivo: consiste en la colocación del producto mínimamente procesado en un envase sellado con un plástico con diferente permeabilidad a los gases (O_2, CO_2 y N_2) permitiendo que la respiración y la actividad bioquímica del producto origine una reducción de la concentración de O_2 y aumento de CO_2 en el interior del envase, hasta que se alcance el adecuado estado de equilibrio. Este sistema es el más económico y el llevado a cabo durante la experimentación de la presente Tesis.
- Envasado en atmósfera modificada activo: consiste en la colocación del producto mínimamente procesado en un envase, sustitución mecánica del aire que rodea al producto por una mezcla previamente seleccionada y adecuada de gases de gases (O_2, CO_2 y N_2) y sellado con plástico permeable.

En el apartado 1.3. (Envasado en atmósfera modificada) de la presente Tesis se describirá con mayor profundidad las principales características de ambos sistemas de envasado y cómo realizar el diseño del envase, aplicable a ambos casos.

En cuanto a los envases para los productos de IV Gama, por lo general se establecen pesos de 150 g y de 250 g (siendo esta última la referencia básica); mientras que con vistas al mercado de la restauración y las grandes colectividades se fijan pesos superiores: 500 g, 1.000 g y hasta 2.000 g (Sánchez, 2003).

La venta de los productos de IV Gama se realiza necesariamente en envases tales como bolsas de plástico cerradas (caracterizadas por poseer bajo coste), bandejas o tarrinas recubiertas por una película de plástico, cerradas también en su parte superior con una película de polímero, tales como polietilieno o polipropileno. En la etiqueta del envase del producto de IV Gama deberá indicar: denominación de venta, lista de ingredientes, peso neto, fecha límite de consumo, temperatura de conservación, modo de empleo, nombre o razón social, dirección y número de registro del fabricante.

1.2.3.9. Expedición

El producto elaborado se debe conservar en cámara frigorífica hasta el momento de su expedición ya que permite alcanzar una temperatura óptima para prolongar el tiempo de vida de un producto determinado. Dicha cámara estará a una temperatura comprendida entre próxima a los 4 ºC (en función de las características del producto procesado) para disminuir la actividad enzimática y el crecimiento microbiano. El tiempo de permanencia en estas condiciones debe minimizarse para evitar acortar el periodo de consumo. Por tanto, el ritmo de elaboración debe estar sujeto a las expectativas de comercialización.

Al igual que la cámara de conservación, el área de expedición debe estar en refrigeración a una temperatura próxima a los 10 ºC. La carga de los productos elaborados se efectuara de forma rápida en el muelle de carga de expedición, protegido con un sistema de abrigo para los camiones.

1.2.4. Factores que afectan a la de calidad de hortalizas IV Gama

El propósito de los productos mínimamente procesados es proporcionar al consumidor un producto fresco, con una vida útil prolongada y, al mismo tiempo, garantizar la inocuidad de los mismos, manteniendo una alta calidad nutritiva y sensorial.

Los principales factores precosecha que afectan la calidad de frutas y hortalizas son el genotipo/morfotipo (Chiesa et al., 2014; Stommel et al., 2015) y la variedad (Cabezas-Serrano et al., 2009; Nogales-Delgado et al., 2014) las prácticas agronómicas como la elección del momento de recolección (Ricci et al., 2013; Witkowska y Woltering, 2014), las condiciones fisiológicas y el estado de madurez de la materia prima (Luna et al., 2012; Giné-Bordonaba et al., 2014). Durante la postcosecha influye en la calidad final del producto IV Gama las técnicas de procesamiento relativas a los

formatos de corte del producto (Pirovani et al., 2015), el envasado o selección del film plástico (Finnegan y O'Beirne, 2015) y el manejo de las temperaturas durante la conservación del producto (Latifah et al., 2013; Cocetta et al., 2014). A continuación se definen los efectos de algunos de los factores comentados anteriormente en calabacín IV Gama.

1.2.4.1. Factores precosecha

1.2.4.1.1. Morfotipo y variedad

Como ya se ha descrito en el capítulo 1.1. (El cultivo), la familia *Cucurbita pepo* L. es una familia con una enorme variabilidad morfotípica, incluyendo a 8 morfotipos diferentes (pumpkin, acorn, vegetable marrow, cocozelle, zucchini, straightneck, scallop, crockneck) (Paris et al., 2001); siendo el morfotipo zucchini el más comercializado en el mundo debido a su ventajas en tamaño y forma (Loy, 2011).

Concluyéndose además, que es el morfotipo zucchini el que menor tasa respiratoria y menor producción de etileno posee **(Tabla I.2.6)**, convirtiéndolo en un excelente material vegetal para el procesado en IV Gama.

Tabla I.2.6. Tasas respiratorias calabacín IV Gama.

	Temperatura (ºC)	**Tasa respiratoria**	**Producción de etileno**
Cucurbita pepo (genérico)	10	34,2 a 35,8 ml/kg*hr	< 1,0 µL/kg*hr (a 20 ºC)
Cucurbita pepo (zucchini)	10	35 ml/kg*hr	< 0,5 µL/kg*hr (a 20 ºC)

Fuente: Sargent y Maynard, 2002.

Por otro lado, diversos autores (Lucera et al., 2010) han observado que determinadas variedades italianas de calabacín (morfotipo zucchini) como la 'Diamante' presentan mejores características para el procesado que la 'Sofia'. Las diferencias entre ambas radicaron en la calidad sensorial (mayor firmeza y valoración global) y calidad microbiológica (menor carga microbiana de *Enterobacteriaceae*, levaduras, bacterias ácido lácticas y *Pseudomonas* spp.).

La variedad seleccionada para el procesado también posee una enorme importancia en el perfil nutricional, llegando ciertas variedades de tomate a duplicar el contenido de licopeno que otras (Odriozola-Serrano et al., 2007). Este es el caso de la variedad 'Bodar' (80,5 mg/kg) si la comparamos con otras variedades como 'Rambo', 'Durinta', 'Pitenza', 'Cencara' o 'Bola' (20-43,1 mg/kg). También en patata se han descrito diferencias en vitamina C, compuestos fenólicos, capacidad antioxidante, azúcares, etc. entre las variedades 'Agata', 'Agria', 'Almera', 'Marabel' y 'Vivaldi' (Cabezas-Serrano et al., 2009). Por ejemplo, la variedad 'Almera' fue la más adecuada para preservar los contenidos en ácido ascórbico, polifenoles y actividad antioxidante, mientras que la variedad 'Marabel' destacó por poseer mayor contenido en azúcares.

Por tanto, para poder tomar una decisión de qué variedades de calabacín son las más adecuadas, es necesario conocer qué variedades fueron las más comercializadas por las casas de semillas durante el desarrollo de esta Tesis (período 2010 a 2014) y posteriormente analizar mediante ensayos las mismas. A continuación se describen las principales características de 6 de las variedades de calabacín morfotipo zucchini (con diferente aspecto exterior: amarillo, verde claro y verde oscuro) más comercializadas por las casas de semillas **(Tabla I.2.7)**.

Tabla I.2.7. Principales variedades comerciales de calabacín comercializadas.

Variedad	Descripción	Casa Comercial	Color externo	Fotografía
'Amalthée'	Buen vigor y permite ciclo largo, con alto rendimiento y frutos de gran calidad	Gautier Semences	Verde claro	
'Cassiope'	Variedad precoz y de alta producción, porte erguido y entrenudos cortos	Gautier Semences	Verde oscuro	
'Cronos'	Alto rendimiento y calidad post producción.Adecua-da recolección en estado baby por desprender bien la flor	Syngenta	Verde oscuro	
'Natura'	Planta vigorosa y fuerte. Frutos con mayor brillo. Variedad disponible en ecológico	Enza Zaden	Verde oscuro	
'Parador'	Variedad original, con un bonito color amarillo vivo. La planta es vigorosa y productiva.	Gautier Semences	Amarillo vivo	
'Sinatra'	Variedad de ciclo productivo largo. Alta producción en los meses de frío. Frutos con caras ortogonales lisos	Clause	Verde oscuro	

1.2.4.1.2. Estado de madurez

Los calabacines comercializados, sean mini o normales **(Figura I.2.6)** presentan todos inmadurez fisiológica. Sin embargo, el hecho de que sean comercializados en cualquier tamaño, les confiere madurez comercial.

Figura I.2.6. Estados de madurez en calabacín.

Tabla I.2.8. Categorías comerciales de calabacín según su estado de madurez (longitud y peso).

Categoría	Características	Requisitos	Calibre
Extra	Calidad superior	- Bien desarrollados y formados - Pedúnculo con corte limpio y longitud máxima de 3 cm. - Sin defectos, salvo ligerísimas alteraciones superficiales que no afecten al aspecto general del producto ni a su calidad de conservación.	Determinado por: su longitud y su peso. ➢ Longitud (7 a 35 cm): - De 7 cm a 14 cm, inclusive. - De 14 cm, exclusive, a 21 cm, inclusive. - De 21 cm, exclusive, a 35 cm. ➢ Peso (50 a 450 g): - De 50 g a 100 g, inclusive. - De 100 g, exclusive, a 225 g, inclusive. - De 225 g, exclusive, a 450 g.
I	Buena calidad	Con defectos, siempre que no afecten a su aspecto general y calidad, tales como: - Ligeras malformaciones y ligeros defectos de coloración. - Muy ligeros defectos de la epidermis o debidos a enfermedades siempre y cuando no sean evolutivos ni afecten al mesocarpo.	
II	Cumplan los requisitos mínimos	Los que no puedan clasificarse en las categorías superiores	

Fuente: Elaboración propia a partir del Reglamento (CE) Nº 1757/2003 de la Comisión de 3 de octubre de 2003 (D.O.U.E., 2003).

Aunque en España se comercializan calabacines cuya longitud es próxima a los 20 cm **(Tabla I.2.8)** por ser los más demandados por parte del consumidor, en el mercado de la exportación, sí que existen dos tamaños de calabacín bien diferenciados: mini o baby y medio **(Tabla I.2.9).**

Tabla I.2.9. Criterios en la clasificación de estados de madurez de calabacín a nivel mundial.

Categoría	Estado de madurez	Longitud	Diámetro
Baby o mini	Pequeño	120-150 mm	25-45 mm
Medio	Mediano	150-200 mm	35-50 mm
	Grande	200-250 mm	45-50 mm
	Muy grande	200-270 mm	45-60 mm

Fuente: Woolworths, Ltd.

Al igual que la variedad, el estado de madurez afecta a la calidad postcosecha del producto. Por ejemplo se ha observado que a 20 ºC, si el tomate se encuentra en pleno proceso de maduración posee una mayor tasa respiratoria y produce mayor concentración de etileno (hasta 5 veces más) que si se encuentra en estado verde o si ya ha finalizado la maduración (Cantwell, 1998). Otro ejemplo es la col rizada, en la cual los tejidos jóvenes a una temperatura de conservación de 5 ºC presentaron una tasa respiratoria próxima a 21 mg CO_2 kg h^{-1}, siendo la tasa respiratorias a esas mismas condiciones de conservación de 12 mg CO_2 kg h^{-1} en el caso de ser maduro (Kader, 2007).

Martínez-Sánchez et al., (2012) observaron en lechuga que el estado fresco los cogollos de mayor madurez poseían mayor contenido en materia seca y textura. Tras el procesado se observó que las lechugas baby o mini tenían mayor tasa respiratoria que las maduras. Durante la conservación en el envase se pudo observar que los estados inmaduros poseían concentraciones menores de O_2 y mayores de CO_2 que los estadios más maduros, influyendo este acelerado metabolismo en el decremento de la calidad visual del producto al final del período de conservación. Con respecto al contenido nutricional estos mismos autores observaron claramente que el estadio baby o mini era más rico en fenólicos, mientras que para la vitamina C no existieron diferencias significativas entre ambos estadios de madurez.

También el estado de madurez afectó a los parámetros fisicoquímicos en melón (perteneciente a la familia Cucurbitaceae), observándose menores valores en el parámetro L* (luminosidad) cuando los frutos eran inmaduros (Beaulieu y Jeanne, 2007).

Sin embargo para calabacín en IV no existen en la actualidad ningún estudio que establezca las principales diferencias entre estados de madurez, aspecto que se ha estudiado en la presente Tesis.

1.2.4.1.3. Momento de recolección

Al igual que el estado de madurez, el momento de recolección tiene una enorme importancia en las características fisicoquímicas, nutricionales, sensoriales (Gil et al. 2012; Valero y Serrano 2013). En la actualidad existe una gran controversia a la hora de relacionar el momento de recolección con una respuesta evidente en la calidad postcosecha, ya que no existe una tendencia lineal y homogénea, siendo cada fruta u hortaliza particular según sus características.

Basándose en el calidad física de las hortalizas, se ha observado que el momento de recolección afecta al color en calabacín (Paris y Nerson, 1986); mientras que la deshidratación o estrés hídrico propios de las recolecciones tardías afectan a la turgencia (Sams, 1999), incrementándose la textura en el caso de la lechuga recogida al final del ciclo (Luna et al., 2012). De igual forma se ha podido comprobar que una recolección temprana de la alcachofa (durante los meses con menores temperaturas) (Ricci et al., 2013) contribuye a una mejora en el color de la alcachofa tras el procesado en IV Gama.

Con respecto a los parámetros químicos de calidad en hortalizas, diversos autores observaron en brassica (Biesiada et al., 2007) y pimiento (Valero et al., 2014) que los frutos recogidos a final del ciclo productivo presentaron un incremento en los valores de sólidos solubles y acidez titulable, el cual podría estar relacionado con la concentración de estos compuestos tras estar expuestos los frutos a las elevadas

temperaturas veraniegas (coincidentes con el fin del ciclo de ambos cultivos) (Kader y Rolle, 2004).

Con respecto a la calidad nutricional, se ha observado que recolecciones tempranas en alcachofa mejoraron los contenidos en polifenoles y capacidad antioxidante del producto IV Gama y conservado bajo refrigeración) (Ricci et al., 2013). Por su parte, en pimiento un incremento en compuestos fenólicos estuvo estrechamente relacionado con las cosechas tardías (Marín et al., 2008).

Con respecto al perfil nutricional del calabacín, únicamente se han descrito correlaciones entre en el contenido de carbohidratos totales y el momento de recolección (Rouphael y Colla, 2005). En este estudio se observó que el contenido en carbohidratos totales de los frutos procedentes de recolecciones durante la primavera era mayor que y si se retrasaba la recolección hasta el verano.

Tampoco en la actualidad existe una conclusión clara sobre cómo afecta el momento de recolección a la calidad postcosecha del calabacín, siendo este aspecto desarrollado en la presente Tesis.

1.2.4.2. Factores postcosecha

1.2.4.2.1. Formato de corte

Debido a que los productos de IV Gama son organismos vivos, cualquier operación durante su elaboración produce un impacto fisiológico, siendo éste más grande cuanto mayor es el grado de procesado, aumentando su velocidad de deterioro y reduciendo, por ende, su periodo de vida útil.

El corte de los tejidos vegetales provoca también la descompartimentación celular, permitiendo la entrada en contacto de enzimas de localización citoplasmática con substratos de localización vacuolar. Igualmente se produce una fuerte pérdida de líquido celular, incrementándose el área de superficie por unidad de volumen. Esto puede acelerar la pérdida de agua y, además, el exudado constituye un medio muy favorable para el desarrollo de microorganismos (Krasaekoopt y Bhandari, 2011).

Asimismo, las operaciones de preparación de productos mínimamente procesados incrementan la contaminación microbiológica, debido a que transfieren la microflora de la piel y de los utensilios utilizados en los tejidos del fruto, disminuyendo la estabilidad del mismo (Pereira et al., 2013).

Tras el corte del material vegetal se produce un incremento en la tasa respiratoria, la cual consiste en la oxidación de los hidratos de carbono, originando la pérdida de materia seca y el sabor azucarado (siendo una reacción inversa a la fotosíntesis). De forma resumida la respiración conlleva la oxidación de glucosa, ácidos carboxílicos u ácidos grasos para formar CO_2 y agua. La diferencias entre éstos metabolismos es que: para la glucosa la cantidad de O_2 consumido es igual que la de CO_2 emitido; mientras que para los ácidos carboxílicos se genera más cantidad de CO_2

que el consumido en O_2; y finalmente para los ácidos grasos la cantidad de CO_2 emitido es menor que la de O_2 consumido. De esta forma, el coeficiente respiratorio (CR), se define como:

Cociente respiratorio = Volumen CO_2 producido / Volumen O_2 consumido

En el caso de la respiración por la oxidación de glucosa, el cociente respiratorio es 1, en el de los ácidos orgánicos es mayor que 1, y en el de los ácidos grasos es menor que 1.

Así, las mayores tasas de respiración indican un metabolismo más activo y usualmente una tasa de deterioro más acelerada ya que ocurre una pérdida más rápida de ácidos, azúcares que junto con las alteraciones de otros componentes determinan la calidad gustativa y el valor nutritivo del producto (Kader, 2007).

Diversos estudios confirman que el procesado afecta a la tasa respiratoria. En col rizada (Cantwell, 1998) piezas maduras cortadas (2 x 2 cm) conservadas a 10 ºC duplicaron las tasas de respiración correspondientes a de hojas intactas (29 mg CO_2 kg h^{-1} y 59 mg CO_2 kg h^{-1}, respectivamente) (Kader, 2007). En rábano (Saavedra del Águila et al., 2006), se incrementó la tasa respiratoria del formato rallado (~8 µg kg^{-1} s^{-1}) con respecto al formato entero (~4 µg kg^{-1} s^{-1}); produciéndose además una mayor oscilación de la respiración como consecuencia de la inestabilidad sufrida por el material vegetal tras el daño celular.

Como es de esperar, al influir el formato de corte en la respiración, también influye en la calidad fisicoquímica y sensorial del producto. Tal es el caso de las zanahorias mínimamente procesadas, observándose cambios en el parámetro de luminosidad (L*) debido al proceso de lignificación realizado en la reparación de la superficie cortada (Bolin y Huxsoll, 1991; Howard y Griffin, 1993). Asimismo, la agresividad del formato de corte estuvo directamente relacionada con el incremento del área de la superficie de corte (cubos > rodajas), promoviendo el amarillamento (modificación parámetro b*) de las cebollas cortadas (Dallocca-Berno et al., 2014).

Por otro lado, el procesado mínimo de IV Gama (pelado y/o cortado) puede afectar al contenido, composición, actividad y biodisponibilidad de los antioxidantes (Robles-Sánchez et al., 2007); variando su efecto según las características intrínsecas del producto a procesar. En lechuga el procesado favoreció un rápido estrés oxidativo que tuvo como consecuencia el incremento de la actividad enzimática fenilalanina amonio-liasa PAL en la síntesis de compuestos fenólicos (Ke y Salveit, 1989). Otro compuesto nutricional alterado por la acción del corte es la vitamina C, que en el caso de calabacín rallado se vio disminuido en un 53 % con respecto al entero (Reyes et al., 2007).

La importancia de la selección del formato de corte, también ha afectado a la proliferación de microorganismos alterantes del producto IV Gama. De este modo mientras que en melón no se observaron diferencias significativas entre aerobios mesófilos, psicrófilos y levaduras y mohos para los formatos rodajas, trapecios y cilindros conservados a 5 ºC (Silveira et al., 2013); en el caso del tomate IV Gama sí que se registraron diferencias significativas entre los formatos rodajas y gajos (incrementándose en rodajas en 1 log/ufc el crecimiento microbiano de mesófilos y levaduras) (Aguayo et al., 2004).

Con respecto al calabacín, no existe información relativa al efecto del formato de corte en parámetros de calidad, por ese motivo dicho aspecto se estudia en este trabajo de Tesis.

1.2.4.2.2. Film plástico

La elección de la película plástico de envasado posee una enorme importancia ya que dependiendo de la permeabilidad de ésta se alcanzarán las adecuadas concentraciones de O_2 y CO_2 en el interior del envase (desarrollo de la atmósfera modificada), afectando por tanto a la vida útil del producto de IV Gama.

En general, plásticos con alta permeabilidad inducen a que los niveles de O_2 en el interior del envase sean más altos y los de CO_2 sean más bajos. Por lo contrario, si se realiza el envasado con plásticos de menor permeabilidad a los gases, se contribuye que bajen más rápidamente y a menores valores los niveles de O_2, mientras que los niveles de CO_2 tienden a ser mayores (Kim et al., 2005; Kartal et al., 2012).

Es importante destacar, que aunque interese disminuir los niveles de O_2, es necesario que en el envase exista O_2, ya que de lo contrario se va a predisponer a la anaerobiosis (con la producción de etanol que además de ser tóxico para los tejidos es desagradable desde el punto de vista gustativo). Por otro lado, todo el conjunto de reacciones fisiológicas que ocurren en el interior del envase van a contribuir a que se que se desprenda agua del producto, promoviendo la condensación de agua dentro del envas y favoreciendo, por tanto, el desarrollo de microorganismos.

Por este motivo, y debido a que no existe un único plástico con máxima efectividad en preservar la frescura del producto recién cortado, es necesario realizar diferentes ensayos para cada producto en particular. En el caso de calabacín IV Gama, la calidad sensorial y microbiológica se vio mermada al envasar con el polímero biodegradable (COEX) en comparación con el polipropileno orientado (OPP) (Lucera et al., 2010).

A nivel fisicoquímico, cambios indeseables en color (especialmente en el parámetro L*, luminosidad) se produjeron como consecuencia de envasar el producto de IV Gama con películas plásticas con baja permeabilidad al CO_2 (promoviendo el

aumento de los niveles de CO_2 en el interior del envase) tanto en patatas (Gunes y Lee, 1997) o como en champiñón (Villaescuesa y Gil, 2003).

Por lo general, a nivel nutricional aquellos plásticos que permiten un mayor contenido de CO_2 en el interior del envase contribuyen a la existencia de mayores mermas en numerosos vegetales (De Ancos et al., 2010). Tal es el caso del tomate IV Gama, que al poseer altas concentraciones de CO_2 se vieron disminuidas en más de la mitad los contenidos en licopeno y vitamina C (Odriozola-Serrano et al., 2008). Por este motivo, el uso de películas microperforadas es recomendable en diversas hortalizas mínimamente procesadas tales como espárrago (Chinsirikul et al., 2013), champiñón (Villaescusa y Gil, 2003), brócoli y ají (Zhuang et al., 2013) o en mezcla de hortalizas de IV Gama incluyendo zanahoria, cebolla, pimiento y apio (Maltais y Laakel, 2012).

Con respecto al calabacín, no existe información relativa a la influencia que tiene la película de envasado en la calidad del producto, por ese motivo dicho aspecto se estudia en esta Tesis.

1.2.4.2.3. Temperatura de conservación

Al objeto de minimizar la actividad metabólica y por tanto las tasas respiratorias del producto tras el cortado, el control de la temperatura es un aspecto imprescindible para garantizar la calidad del producto. De esta forma, un adecuado manejo de la temperatura puede producir reducciones en la actividad respiratoria entre 2-3 (Q_{10}= 2-3) (O'Beirne, 1990). Por ejemplo, en rodadas de tomate se ha observado que la tasa respiratoria se incrementaba en 3,5 cuando la temperatura de conservación de las mismas se duplicaba (pasando de 10 a 20 ºC) (Watada et al., 1996).

La temperatura, además de a la respiración, afecta a otros parámetros físico-químicos del producto mínimamente procesado. Por lo general, la pérdida de agua suele ser directamente proporcional a la temperatura de conservación tal y como se ha descrito en calabacín fresco (Carvajal et al., 2011) siendo apreciablemente visibles dichas pérdidas incluso a niveles próximos al 3 % (McCollum, 2004) y estando éstas además inversamente correlacionadas con la firmeza del producto (Martínez et al., 2008). Con respecto a la evaluación del color, el principal efecto de la temperatura sobre el producto cortado es el pardeamiento (o amarillamento en caso del calabacín), viéndose reflejado en el incremento de los valores del tono amarillo (b*) así como en la tonalidad general (hue, hº) (Dalloca-Berno et al., 2014).

De igual manera, se han observado pérdidas en los compuestos bioactivos como consecuencia del aumento de la temperatura de conservación. De esta forma, un incremento en la temperatura de conservación hasta los 10 ºC produjo significativas pérdidas en ácido ascórbico en rábano procesado (Saavedra del Aguila et al., 2006). También se cuantificaron pérdidas en el contenido de vitamina C (entre el 56 y 98 %) en

seis de las variedades de brócoli estudiadas tras su conservación a 2 ºC durante dos semanas (Albrecht el al., 1990), mientras que por lo contrario otros autores no detectaron pérdidas en esta hortaliza tras su conservación durante tres semanas a 5 ºC (Esteve et al., 1995). Por otro lado, un incremento en la síntesis de polifenoles se registraron en cebolla procesada en IV Gama cuando los formatos fueron conservados a mayor temperatura (15 ºC) (Dalloca-Berno et al., 2014).

Como cabe esperar, la temperatura también influye en el contenido microbiano de los productos procesados. En este sentido, en lechuga IV Gama la población de *Escherichia coli* O157:H7 y *Listeria monocytogenes* a 5 ºC fue entre 3 y 3,5 log ufc/g menor para ambos patógenos que cuando el producto se conservó a 25 ºC (Oliveira et al., 2010). De igual forma, incrementos de temperatura en 5 ºC en pimiento IV Gama (de 5 a 10 ºC) produjo aumentos significativos en más de 3 log ufc/g en la población microbiana en diversos microorganismos alterantes (coliformes totales, bacterias lácticas, levaduras y mohos) (González-Aguilar et al., 2004).

Sin embargo, en el caso del calabacín IV, no existe información sobre el efecto de la temperatura tiene sobre la calidad del producto conservado, siendo un aspecto a cubrir en la presente Tesis.

1.3. ENVASADO EN ATMÓSFERA MODIFICADA

1.3.1. Principales alteraciones tras el procesado en IV Gama: importancia del envasado en atmósfera modificada.

Las operaciones de corte o pelado necesarias para el procesado mínimo producen daños al tejido estimulando la actividad respiratoria y la producción de etileno, lo que induce la biosíntesis de enzimas produciendo pérdidas de sabor y aroma, decoloración de superficies, deterioro del tejido, incremento de la velocidad de pérdida de vitaminas, ablandamiento del tejido y pérdidas de la turgencia y volumen, lo cual conduce a una menor vida útil del producto (Cantwell, 1992).

Debido a que cada material vegetal posee unas características propias, el procesado mínimo afecta de forma diferente y particular según de que fruta u hortaliza se trate. Por este motivo, es necesario resumir cuáles son los principales defectos en las diferentes frutas y hortalizas de IV Gama, entre ellas el calabacín, al objeto de disminuir los mismos en el mayor grado posible mediante el envasado en atmósfera modificada **(Tabla I.3.1).**

Tabla I.3.1. Principales defectos producidos tras el procesado en IV Gama de frutas y hortalizas.

Producto	Presentación del producto cortado	Tasa de respiración en aire a 5 ºC (mL CO_2 kg h^{-1})	Defectos comunes de calidad
Ajo	Dientes pelados	20	Crecimiento de brotes, decoloración
Apio	En rajas	2-3 (2,5 ºC)	Pardeamiento, deshidratado superficial
Brócoli	Inflorescencias	20-35	Amarilleamiento, malos olores
Calabacín	Cortada en cubos y rodajas (5 mm)	12-24	Oscurecimiento, jugo drenado
Caqui	En rebanadas	---	Pardeamiento, translucidez
Cebolla, bulbo	Rebanadas, cortadas en cubos	8-12	Pérdida de textura y jugos, decoloración
Cebollín	Picado	25-30	Decoloración, crecimiento de brotes, jugo drenado
Champiñones	Rebanadas (5 mm)	20-45	Pardeamiento
Col repollo	Tiras	13-20	Pardeamiento
Coliflor	Inflorescencias		Decoloración, malos olores
Espinaca	Cortada	6-12	Malos olores, rápida descomposición de piezas pequeñas
Fresa	En rebanadas, sin cáliz	12	Jugo drenado, pérdida de textura
Granada	Granos	2	Pardeamiento de la zona cortada
Jícama	En rajas	5-10	Pardeamiento, pérdida de textura
Judía	Cortados	15-18	Pardeamiento
Kiwi	Rodajas	1-3 (0 ºC)	Jugo drenado, pérdida de textura
Lechuga			
Cabeza	Picada	6-10	Pardeamiento de bordes cortados
Otras (no incluyen cabeza)	Picadas	10-13	Pardeamiento de bordes cortados
Manzana	En rebanadas	3-7 (2 ºC)	Pardeamiento
Melocotón	En rebanadas	6	Pardeamiento, daño mecánico
Melón			
Cantaloupe	Cortados en cubo	5-8	Jugo drenado, ablandamiento, translucidez
Gota de miel	Cortados en cubos	2-4	Jugo drenado, ablandamiento, translucidez
Nabo	Cortados en cubos	10	Jugo drenado, ablandamiento
Naranja	Pelada, en gajos	3	Jugo drenado, malos olores
Patata	Pelada	4-8	Pardeamiento, secado
Pera	En rebanadas	6-8 (2,5 ºC)	Pardeamiento, daño mecánico
Pimiento	En rebanadas, cortados en cuadritos	3-6	Pérdida de textura, oscurecimiento
Piña	Cortada en cubos	3-7	Jugo drenado, decoloración
Puerro	Brotes recortados	25	Decoloración
Puntas espárragos	Cortada en cubos	40	Pardeamiento, secado
Remolacha	Cortado en cubos	6	Jugo drenado, pérdida de color
Sandía	En rebanadas	2-4	Jugo drenado
Tomate	En rebanadas	3	Deshidratado superficial
Zanahoria	En rajas	7-10; 12-15	Pérdida de textura, Pardeamiento

Fuente: Cantwell, M., 1992., Watada et al., 1996, Avena Bustillos et al., 1997.

1.3.2. Definición y gases de envasado: recomendaciones en IV Gama

Como se ha indicado, la modificación pasiva consiste en el empleo de películas plásticas de diferente permeabilidad a los gases, para crear una atmósfera modificada favorable por efecto de la permeabilidad del envase, la respiración y actividad bioquímica del producto, entre otros factores (Day, 2000).

No obstante, cuando la atmósfera modificada en equilibrio no se consigue antes que se activen reacciones que llevan al deterioro del producto, tales como el pardeamiento enzimático o la pérdida de textura, se puede modificar activamente la atmósfera de envasado. En este último caso, la atmósfera modificada se consigue mediante la sustitución mecánica del aire que rodea al producto por una mezcla adecuada de gases, de tal manera que la atmósfera en el envase va variando con el paso del tiempo en función de las necesidades y respuesta del producto (Day, 2000).

El empleo de la atmósfera modificada en el envasado (ya sea activa o pasiva) presenta una serie de ventajas e inconvenientes resumidos en la **Tabla I.3.2.**

Tabla I.3.2. Ventajas e inconvenientes del envasado en atmósfera modificada.

Ventajas	Inconvenientes
- Incremento del período de vida útil (50-400 %) debido a: - Reducción en la respiración y en la producción de etanol - El retraso en la maduración - La reducción en la degradación de clorofila, la biosíntesis de carotenoides y antocianinas - La reducción del pardeamiento enzimático, mantenimiento del color	- Elevado coste debido a: - Maquinaria de envasado con incorporación de gases - Equipo analítico para controlar que se utilizan las mezclas gaseosas adecuadas - Sistema de control de calidad - Aumento del coste de transporte al aumentar el volumen ya que se trata de productos envasados
- Mejoría en los desórdenes fisiológicos y daños por refrigeración - Conservación de las vitaminas del producto mínimamente procesado - Reducción de costes de producción y almacenamiento debido a la mejor utilización de la mano de obra, equipos y espacio - Posibilidad de distribución a grandes distancias - Mejora la presentación al utilizarse envases plásticos donde se ve el producto a través del envase transparente - El envase es termosellado que lo hace más higiénico y evita contaminaciones posteriores, facilitando su transporte y almacenamiento. al estar sellado, el producto no gotea ni desprende olores	- Necesidad de temperatura controlada, debido a: - Respiración anaerobia con la consecuente producción de olores y sabores desagradables - Crecimiento potencial de gérmenes patógenos por elevación de la temperatura - Posibilidad de pérdida de defectos del envase de las características de la atmósfera modificada - Diferentes mezclas gaseosas para diferentes productos, lo que provoca diseños especiales de equipos

Fuente: Elaboración propia.

Antes de seleccionar la mezcla de gases correcta para cada producto, es necesario conocer las funciones básicas de los gases empleados, sus propiedades más importantes, cómo pueden afectar a la alteración y los límites exactos de su acción.

En términos generales, son tres los gases que actualmente se emplean en el envasado de productos mínimamente procesados en atmósfera modificada: nitrógeno

(N_2) dióxido de carbono (CO_2) y oxígeno (O_2) (Parry, 1993; Toivonen et al., 2009; Gammariello et al., 2015).

Si bien, las investigaciones relativas a los gases protectores se estructuran en dos líneas básicas: por un lado nuevos sistemas de aplicación de los gases convencionales (oxígeno, dióxido de carbono y nitrógeno) y por otro lado, el empleo de otros gases de interés en el envasado de alimentos. En la primera línea se lleva a cabo la realización de ambientes muy pobres en O_2 y de elevadas concentraciones del mismo (choque de oxígeno) para inhibir el crecimiento de microorganismos y otras alteraciones responsables del deterioro de estos productos (Pan et al., 2015). En segundo lugar, se investigan diversos gases (monóxido de carbono, argón, helio, hidrógeno y óxido nitroso) como alternativa a los empleados habitualmente en las tecnologías de envasado en atmósfera modificada (García et al., 2006; Han, 2014a).

A continuación se analizan brevemente las características, ventajas e inconvenientes de los principales gases de envasado **(Tabla I.3.3).**

Tabla I.3.3. Ventajas e inconvenientes de los principales gases de envasado en atmósfera modificada.

Gas	Propiedades físicas	Ventajas	Inconvenientes
Oxígeno	Incoloro	Soporta el metabolismo de los vegetales	Favorece la oxidación
	Inodoro	Inhibe microorganismos anaerobios	Favorece el crecimiento de microorganismos aerobios
	Insípido		
	Inflamatorio		
Dióxido de carbono	Incoloro	Bacteriostático	Produce el colapso del envase
	Inodoro	Fungistático	Produce exudado
	Ligero sabor ácido	Insecticida	Difunde rápidamente a través del envase
	Soluble en agua y grasa	Mayor acción a baja temperatura	
Nitrógeno	Incoloro	Inerte	Favorece el crecimiento de anaerobios (100 % nitrógeno)
	Inodoro	Desplaza al oxígeno	
	Insípido	Inhibe microorganismos aerobios	
	Insoluble	Evita el colapso del envase	

Fuente: Elaboración propia a partir de Rodríguez, 1998.

El envasado en atmósfera modificada del producto IV Gama es un proceso dinámico, donde el envase cerrado interactúa con el producto envasado para finalmente alcanzar un equilibrio en la atmósfera gaseosa interna que reducirá la velocidad de respiración, la sensibilidad al etileno y la pérdida de humedad (por transpiración) así como aumentará la fase de latencia del desarrollo microbiano e incrementará el tiempo de generación de microflora (Hotchkiss, 1988).

Debido a que las tasas de respiración de productos mínimamente procesados bajo atmósferas modificadas, son muy variadas, es necesario definir de forma específica para cada producto que atmósfera es la óptima para preservar la frescura y las características iniciales de la materia prima, entre ellas el calabacín **(Tabla I.3.4).**

Tabla I.3.4. Atmósferas modificadas beneficiosas en frutas y hortalizas de IV Gama.

Producto	Presentación del producto cortado	Defectos comunes de calidad	Atmósfera beneficiosa	
			kPa O_2	kPa CO_2
Ajo	Dientes pelados	Crecimiento de brotes, decoloración	3	5-10 ±
Apio	En rajas	Pardeamiento, deshidratado superficial	----	----
Brócoli	Inflorescencias	Amarilleamiento, malos olores	3-10	5-10
Calabacín	Cortada en cubos y rodajas (5 mm)	Oscurecimiento, jugo drenado	1	----
Caqui	En rebanadas	Pardeamiento, translucidez	2	12
Cebolla, bulbo	Rebanadas, cortadas en cubos	Pérdida de textura y jugos, decoloración	2-5	10-15
Cebollín	Picado	Decoloración, crecimiento de brotes, jugo drenado	----	----
Champiñones	Rebanadas (5 mm)	Pardeamiento	3	10*
Col repollo	Tiras	Pardeamiento	3-7	5-15
Coliflor	Inflorescencias	Decoloración, malos olores	5-10	< 5 ±
Espinaca	Cortada	Malos olores, rápida descomposición de piezas pequeñas	1-3	8-10
Fresa	En rebanadas, sin cáliz	Jugo drenado, pérdida de textura	1-2	5-10
Granada	Granos	Pardeamiento de la zona cortada	21	15-20
Jícama	En rajas	Pardeamiento, pérdida de textura	3	10 ±
Judía	Cortados	Pardeamiento	2-5	3-12
Kiwi	Rodajas	Jugo drenado, pérdida de textura	2-4	5-10
Lechuga				
Cabeza	Picada	Pardeamiento de bordes cortados	< 0,5-3	10-15
Otras (no incluyen cabeza)	Picadas	Pardeamiento de bordes cortados	1-3	5-10
Manzana	En rebanadas	Pardeamiento	< 1	----
Melocotón	En rebanadas	Pardeamiento, daño mecánico	1-2	5-12
Melones				
Cantaloupe	Cortados en cubo	Jugo drenado, ablandamiento, translucidez	3-5	5-15
Gota de miel	Cortados en cubos	Jugo drenado, ablandamiento, translucidez	2-3	5-15
Patata	Pelada	Pardeamiento, secado	1-3	6-9
Pepino	Rebanada	Jugo drenado	----	----
Pera	En rebanadas	Pardeamiento, daño mecánico	0,5	< 10
Pimiento	En rebanadas, cortados en cuadritos	Pérdida de textura, oscurecimiento	3	5-10
Piña	Cortada en cubos	Jugo drenado, decoloración	3	10
Puerro	Brotes recortados	Decoloración	5	5
Puntas espárragos	Cortada en cubos	Pardeamiento, secado	10-20	10-15
Remolacha	Cortado en cubos	Jugo drenado, pérdida de color	5	5
Sandía	En rebanadas	Jugo drenado	3-5	5-15
Tomate	En rebanadas	Deshidratado superficial	3	3
Zanahoria	En rajas	Pérdida de textura, pardeamiento	0,5-5	10

Modificado de Gorny, 1997. ± indica resultados no publicados. * no utilizado debido al riesgo de *C. botulinum*
Fuente: Elaboración propia a partir de Cantwell, M, 1992, Watada et al., 1996., Avena Bustillos et al., 1997.

1.3.3. El envase

1.3.3.1. Clasificación de envases según consistencia

Los envases empleados en el envasado de frutas y hortalizas mínimamente procesadas constituidos por materiales poliméricos se pueden clasificar en dos categorías (Blanco-Díaz, 2009; Toivonen et al., 2009):

- **Envases flexibles**. A este grupo pertenecen los envases tipo bolsas, los cuales tienen una soldadura longitudinal y dos transversales en los extremos.
  - ✓ **Bolsas:** Muy utilizadas en IV Gama debido a su uso práctico y bajo coste, destacando la naturalidad del producto. Su empleo suele estar sujeto a hortalizas más que a frutas, tales como ensaladas, monoproductos, con bajo peso, sin necesidad de tener un soporte más rígido que sujete **(Figura I.3.1a).**
- **Envases rígidos.** A esta categoría pertenecen los envases compuestos por dos partes. La parte inferior presenta distintas formas como copa, cuenco, etc., mientras que el otro componente es una película flexible que sirve para cubrirlo. A este grupo pertenecen los envases tipo bandeja y tarrina.
  - ✓ **Bandejas:** envase destinado al almacenamiento en atmósfera modificada de hortalizas mínimamente procesadas, tales como calabacín, pimiento, ajo, etc. En la actualidad se comercializa calabacín de IV Gama (en rodajas) en diversos supermercados de los Estados Unidos **(Figura I.3.1b).**
  - ✓ **Tarrinas:** destinadas habitualmente al almacenamiento de cocktails de frutas mínimamente procesadas (con o sin líquido de gobierno) o salsas de frutas (productos V Gama) **(Figura I.3.1c).**

Figura I.3.1. Envases para productos de IV Gama; a) bolsa, b), bandeja, c) tarrina.

1.3.3.2. Materiales de envasado en atmósfera modificada

Los polímeros son una clase de moléculas orgánicas de gran tamaño y elevado pesomolecular (hasta 10^6) que son capaces de ser sintetizadas o despolimerizadas en un númerode unidades, identificables químicamente, denominadas monómeros (Oswin, 1975). Más concretamente, los plásticos (incluyendo las películas utilizadas en el envasado en atmósfera modificada del calabacín IV Gama) son formulaciones que contienen uno o más polímeros de elevado peso molecular en combinación con varios

aditivos tales como plástificantes, obturadores, estabilizantes, etc., (Byun y Zhang, 2014). Se trata de materiales sintéticos obtenidos por aplicación de calor o presión y a pesar de que son sólidos en el estadio final, durante su fabricación pasan por el estado líquido pudiendo igualmente moldearse de distintas formas (Hun et al., 2014b).

Las películas plásticas empleadas para el envasado en IV Gama, son materiales flexibles caracterizadas por tener un espesor de 0,254 mm o menos (Sacharow, 1976), esto es, con grosor suficiente para sostenerse pero lo bastante delgado para que sea flexible, se pueda doblar o arrugar sin agrietarse.

Por otro lado, la copolimerización es un proceso en el cual reaccionan más de un tipo de monómeros para formar una macromolécula que contiene los nuevos monómeros en una proporción aproximadamente equivalente a la proporción de monómeros originales. De forma inversa, un plástico homopolímero está formado por la polimerización de un solo tipo de monómero.

En general, los polímeros plásticos destinados a envases de IV Gama deben reunir las características siguientes (Greengras, 1995; Artés, 2000; Kim et al., 2014):

- Permeabilidad requerida y, en su caso, selectiva para los distintos gases.
- Baja permeabilidad al vapor de agua.
- Elevada transparencia, claridad y brillo.
- Peso reducido.
- Espesor idóneo.
- No tóxico.
- Resistencia a la rotura y al estiramiento.
- Facilidad para sellarse por calor a temperatura relativamente baja (30-40 ºC).
- Inocuo y que no reaccione con el producto vegetal.
- Buena resistencia térmica y al ozono.
- Buena transmisión del calor.
- Adecuado para uso alimentario y comercial.
- Fácil manejo en el envasado y etiquetado mecanizado a elevada velocidad.
- Fácil de imprimir.
- Que no se empañe (que admita bien un tratamiento antivaho).
- Precio asequible.

De entre ellas, la esencial es que disponga de una permeabilidad idónea para mantener la atmósfera adecuada a la temperatura de conservación óptima, además de favorecer la calidad, higiene y seguridad alimentaria del producto que contiene y que se convierta en un elemento positivo clave en la comercialización (sobre todo que resulte atractivo al consumidor).

Los recientes avances en el sector de la IV Gama se deben en buena medida a la aparición en el mercado de nuevos polímeros y envases, con un amplio rango de difusión a los gases, aunque solo unos pocos presentan las características para generar

atmósferas modificadas idóneas en productos vegetales (González et al., 1998; Martínez y Artés, 2005).

Como envases de productos de IV Gama se han utilizado diferentes películas plásticas que incluyen polietileno de baja-densidad (LDPE), polietileno de alta-densidad (HDPE), polipropileno de calibre-delgado (PP), poliestireno (PS), varias clases de cloruro de polivinilo (PVC) (Ben-Yehoshua et al., 1983; O'Beirne, 1990; Zhuang, 2011), siendo de todos ellos el polipropileno el más común y utilizado. A continuación **Tabla I.3.5** se muestran las velocidades de transmisión a los gases de los polímeros citados.

Tabla I.3.5. Permeabilidades de envasado de películas plásticas.

Tipo de plástico	**Velocidades de transmisión (gm^{-2} dia^{-1}) (37,8 ºC y 90 % HR)**			PCO_2/PO_2 (ß)
	O_2	**CO_2**	**Vapor de agua**	
Polietileno de baja densidad (LPDE)	3 900 – 13 000	7 700 – 77 000	6 – 23,2	1,97 – 5,92
Polietileno de baja densidad lineal (LLDPE)	7 000 – 9 300	----	16 – 31	----
Polietileno de media densidad (MDPE)	2 600 – 8 293	7 700 – 38 750	8 – 15	2,96 – 4,67
Polietileno de alta densidad (HDPE)	520 – 4 000	3 900 – 10 000	4 – 10	2,5 – 7,5
Polipropileno (PP)	1 300 – 6 400	7 700 – 21 000	4 – 10,8	3,2 – 5,92
Cloruro de polivinilo (PVC)	620 – 2 248	4 263 – 8 138	----	3,62 – 6,87
Poliestireno (PS)	2 000 – 7 700	10 000 – 26 000	108,5 – 155	3,37 - 5

Fuente: Adaptado de Sacharow, 1976; Anon, 1979; Anon, 1982; Brydson, 1982; Ballantyne, 1986; Zagory y Kader, 1988; Ballantyne et al., 1988; Crosby, 1991.

Es importante que las películas plásticas utilizadas tengan una relación de permeabilidad de CO_2 a O_2, definido como ß, relativamente alta. De esta forma se permite que disminuya la concentración de O_2 sin que sea excesiva la acumulación de CO_2 dentro del envase (Kader et al., 1989). Aunque para la permeabilidad, los polímeros a los gases usados en IV Gama muestran diferencias sustanciales, la relación de permeabilidad del CO_2 respecto del O_2 normalmente se encuentra entre 3 y 6 (Kader 2002, Toivonen et al., 2009), siendo el ratio próximo a 1 en el caso de los plásticos perforados (Mir y Beaudry, 2003; Villaescusa y Gil, 2003; Brody, 2005). De esta manera, la mayoría de las películas poliméricas tienen mayor permeabilidad al CO_2 que al O_2, en parte debido a que la solubilidad del CO_2 en el polímero es mayor (Kader et al., 1989).

Es aceptado que una pequeña reducción en la concentración del O_2 por debajo del 21 % tiene poco efecto sobre la velocidad de la respiración de los productos envasados, siendo necesario alcanzar concentraciones próximas al 8-12 kPa de concentración de O_2 para producirse resultados significativos (Kader et al., 1989).

En la práctica, la cantidad de O_2 en un producto envasado en atmósfera modificada se reduce normalmente desde el 21 kPa al 2-5 kPa y el potencial del CO_2 se incrementa desde el 0,03 kPa hasta tan elevado como el 16-19 kPa (Zagory y Kader, 1988; Zhang et al., 2013) como en calabacín (Lucera et al., 2010).

Por otro lado, en la mayoría de los casos se señalan amplios márgenes de variación en las velocidades de transmisión de los gases, ya que, entre otros factores, existe variabilidad en función del procedimiento de comprobación. Si bien hay que tener en cuenta que las características de permeabilidad de una película plástica dada son aproximadamente proporcionales al espesor de la misma (Gibbons, 1973), que a su vez es también variable dentro de los límites de tolerancia de su fabricación.

Finalmente, existen ciertos aditivos que permiten modificar las características de las películas plásticas. Tal es el caso de las resinas plásticas, mejoradores de la plasticidad, estabilizadores, modificadores del impacto, obturadores, lubricantes y agentes frente al empañado, etc. que hacen que se puedan preparar materiales a medida para aplicaciones específicas cuyos aditivos pueden afectar a las características de permeabilidad (Byun y Zhang, 2014).

1.3.4. Diseño de un envase destinado a la IV Gama

El diseño de un envase en atmosfera modificada depende de variables como las características del producto, su peso, la composición gaseosa recomendada, la permeabilidad a los gases del material de envasado, su dependencia de la temperatura, y la actividad respiratoria del producto que variara en función de la composición gaseosa y la temperatura (Fonseca et al., 2002). En cuanto a las propiedades de permeabilidad de las películas poliméricas dependen de diversos factores: el tipo de material, de la temperatura ambiente, del grosor del plástico, de la permeabilidad del gas y de la diferencia de concentración del gas a través del plástico (Al-Ati y Hotchkiss, 2002).

1.3.4.1. Cálculo de la permeabilidad del plástico de envasado

Si las características de respiración del producto están adecuadamente ajustadas a los valores de permeabilidad del film, se puede crear pasivamente una beneficiosa atmósfera modificada en el interior del envase. Si se elige un film de una adecuada permeabilidad intermedia, se establecerá una atmósfera modificada de equilibrio cuando las intensidades de transmisión del O_2 y del CO_2 a través del envase sean igual a la intensidad de respiración del producto. Es importante no seleccionar films de insuficiente permeabilidad por los riesgos de crear condiciones anaerobias y/o niveles excesivamente elevados de CO_2.

La modelización es un procedimiento muy útil en el diseño de envases de atmósfera modificada, ocurriendo la permeación o paso de un gas a través de una membrana polimérica tiene lugar en tres etapas sucesivas: difusión del gas en la interfase

membrana-disolución, disolución del gas en el seno de la membrana y, finalmente, transferencia del gas al ambiente en la interfase opuesta de la membrana.

Es por tanto necesario tener en cuenta los factores que influyen en la difusión del gas a través de la membrana del envase (Aguayo, 2003; Martínez y Artés, 2005):

- Estructura del polímero.
- Permeabilidad a los gases.
- Espesor.
- Superficie total de intercambio gaseoso.
- Gradiente de presiones parciales o concentraciones del gas en cuestión a un lado y otro de la membrana.
- Temperatura.
- Humedad relativa.
- Existencia de condensaciones.

La permeación se puede definir, en términos de la Ley de Fick, como:

$$Ji = \frac{DidCi}{dx} \qquad [1]$$

siendo:

Ji = Flujo local del componente i en la membrana.

Di = coeficiente de difusión del componente i en la membrana.

Ci = concentración del gas disuelto en la membrana.

X = dirección normal a la membrana.

Por otro lado, la Ley de Henry establece que:

$$Ci = KiPi \qquad [2]$$

siendo:

Ki: coeficiente de solubilidad del componente i en la membrana.

Pi: presión parcial del componente i.

Integrando las ecuaciones [1] y [2], se obtiene:

$$Ji = \frac{DiKi\ (P'i - P''i)}{\Delta x} \qquad [3]$$

siendo:

Δx = espesor de la membrana.

P'i y P"i = Presiones parciales de los gases a uno y otro lado de la membrana.

Particularmente en [3], el flujo de O_2 a través de la membrana de superficie A es:

$$J_{O2} = \frac{P_{O2}\ A\ ([O_2]atm - [O2]_{envase}}{x} \qquad [4]$$

dónde:

A = superficie total de intercambio de gases de la membrana.

P_{O2} = permeabilidad o constante de permeabilidad de la membrana al O_2, siendo Pi = DiKi.

Se denomina permeabilidad a la velocidad de permeación, o cantidad de gas que atraviesa una membrana de sección unidad, en la unidad de tiempo. La permeabilidad propiamente dicha de la membrana, se define como la constante de permeabilidad dividida por el espesor.

De forma análoga a [4], el flujo de CO_2 a través de la membrana que constituye el envase, cuya superficie total de intercambio de gases es A, será:

$$J_{CO2} = \frac{P_{CO2}\, A\, ([CO_2]envase - [CO_2]atm}{x} \qquad [5]$$

Por otro lado, el flujo de O_2 consumido por el/los órganos vegetales alojados en el interior de un envase cerrado herméticamente y fabricado con la membrana de referencia es:

$$J_{O2} = R_{O2}\, ([O_2]_{envase})\, M \qquad [6]$$

dónde:

M = masa del producto.

Análogamente a [6], el flujo de CO_2 emitido por los órganos vegetales es:

$$J_{CO2} = R_{CO2}\, ([CO_2]_{envase})\, M \qquad [7]$$

Una vez alcanzada la atmósfera de equilibrio en el interior del envase, para lo que es preciso que la tasa respiratoria del órgano vegetal se haya estabilizado, ocurrirá que:

$$J_{O2\ membrana} = J_{O2\ vegetal} \qquad [8]$$

y análogamente,

$$J_{CO2\ membrana} = J_{CO2\ vegetal} \qquad [9]$$

En consecuencia, sustituyendo valores en [8] según las igualdades [4] y [6], se puede escribir

$$P_{O2}\, A\, ([O_2]_{atm} - [O_2]_{envase} = R_{O2}\, Mx \qquad [10]$$

y también, sustituyendo valores en [9] según las igualdades [5] y [7] se puede escribir

$$P_{CO2}\, A\, ([CO_2]_{atm} - [CO_2]_{envase} = R_{CO2}\, Mx \qquad [11]$$

Las ecuaciones que ponen en relación parámetros fisiológicos o biológicos (RO_2, RCO_2 y M) del órgano vegetal con parámetros físicos de la membrana del polímero.

Estas ecuaciones permiten calcular las características exigibles al polímero conociendo los parámetros biológicos.

De esta manera, pueden ser utilizadas para el cálculo de la permeabilidad al O_2 y CO_2 exigible al polímero como:

$$P_{O2} = \frac{R_{O2}\, Mx}{A\, ([O_2]atm - [O_2]envase} \qquad [12]$$

$$P_{CO2} = \frac{R_{CO2}\, Mx}{A\, ([CO_2]envase - [CO_2]atm} \qquad [13]$$

dónde (en unidades del Sistema Internacional):

R_{CO2} y R_{O2} se expresan en mol. kg $^{-1}$ s^{-1}; M en kg; x en mm; A en m^2 ; $[O_2]$ y $[CO_2]$ en kPa y P_{CO2} y P_{O2} en mol.m.m^{-2} s^{-1} kPa^{-1}.

Al diseñar un envase para el caso del calabacín, en [12] y [13] el espesor (x) se debe fijar por criterios mecánicos (soportar el peso, evitar desgarros, facilitar la manipulación, etc.) y la superficie (A) a albergar dentro del envase en volumen corresponde a la masa (M) del calabacín IV Gama (Artés, 1976, 1993). La temperatura y los niveles óptimos de O_2 y CO_2 a generar y estabilizar en el interior del envase se obtienen del apartado 1.3.2 (Definición y gases de envasado: recomendaciones en IV Gama) donde también se encuentran los valores de las tasas respiratorias (RO_2 y RCO_2) bajo dichas condiciones óptimas de almacenamiento.

Por otro lado, en el exterior del envase se considera que existe aire, por lo que $[O_2]_{atm}$ tomará el valor de 21 kPa y $[CO_2]_{atm}$ se fijará en 0,03 kPa. En consecuencia, hay que determinar en cada caso particular si se cumplen o no suficientemente ambas igualdades fundamentales [12] y [13] y solo cuando lo hagan, se tendrá éxito en el almacenamiento bajo en atmósfera modificada.

1.3.4.2. Principales problemas en el cálculo de la permeabilidad

Como se ha podido ver, las permeabilidades de los polímeros requeridas para el envasado en atmósfera modificada varían con el producto, la atmósfera a estabilizar y la temperatura de envasado. De este modo, puede existir un amplio rango desde 10 mL m^{-2} día^{-1} atm^{-1} para productos que exigen muy poco O_2 y/o mucho CO_2 (patata pelada o manzana cortada), hasta 200.000 mL m^{-2}día^{-1}atm^{-1} para productos de muy elevada intensidad respiratoria (brócoli o champiñón) (Artés et al., 1996).

Por otro lado, la permeabilidad a cada gas de un determinado polímero depende básicamente de la temperatura a la que se encuentre y su evolución difiere para cada gas (Artés et al., 1998).

Existiendo un problema en la actualidad ya que el valor de la permeabilidad a los gases de los polímeros conservados a baja temperatura (habitual necesaria en la ingeniería de proceso de un producto de IV Gama, ~ 6 ºC) no suele ser suministrado por el proveedor, facilitándose por lo general el valor de la permeabilidad a una temperatura muy diferente ($\geq$ 20 ºC); y por tanto un valor mayor de permeabilidad que lo que realmente posee el plástico a esa determinada temperatura de conservación.

En el caso de la P_{O2} (mL m^{-1} dia^{-1} atm^{-1}) se ha podido comprobar que dichas variaciones son altas, entre el 44 al 87 % (Martínez y Artés, 2005). Por ejemplo, para el caso del polipropileno sin orientar de 25 µm la permeabilidad difiere un 87% ($P_{O2 \text{ a } 2ºC}$ =

1063 ± 409 vs. $P_{O2\ a\ 20\ ºC}$ = 8 333 ± 333 mL m^{-1} dia^{-1} atm^{-1}); para el polipropileno orientado de 40 µm un 81 % ($P_{O2\ a\ 2ºC}$ = 1 475 ± 57 vs. $P_{O2\ a\ 20\ ºC}$ = 7 932 ± 422 mL m^{-1} dia^{-1} atm^{-1}); para el polipropileno de 40 µm un 44 % ($P_{O2\ a\ 5ºC}$ = 290 ± 14 vs. $P_{O2\ a\ 15\ ºC}$ = 517 ± 10 mL m^{-1} dia^{-1} atm^{-1}) y para el polietileno de baja densidad de 14 µm un 78% ($P_{O2\ a\ 0ºC}$ = 6 577 ± 4 002 vs. $P_{O2\ a\ 20\ ºC}$ = 30 133 ± 1 704 mL m^{-1} dia^{-1} atm^{-1}).

De igual forma, en el caso de P_{CO2} (mL m^{-1} dia^{-1} atm^{-1}) existen variaciones muy altas (Martínez y Artés, 2005). En referencia a los plásticos citados anteriormente, en el polipropileno sin orientar de 25 µm la permeabilidad difiere un 72 % ($P_{CO2\ a\ 2ºC}$ = 4 650 ± 875 vs. $P_{CO2\ a\ 20\ ºC}$ = 16 667 ± 1 667 mL m^{-1} dia^{-1} atm^{-1}); para el polipropileno orientado de 40 µm un 68 % ($P_{CO2\ a\ 2ºC}$ = 6 165 ± 966 vs. $P_{CO2\ a\ 20\ ºC}$ = 19 092 ± 7 272 mL m^{-1} dia^{-1} atm^{-1}); para el polipropileno de 40 µm un 36 % ($P_{CO2\ a\ 5ºC}$ = 1 112 ± 23 vs. $P_{CO2\ a\ 15\ ºC}$ = 1 747 ± 15 mL m^{-1} dia^{-1} atm^{-1}) y para el polietileno de baja densidad de 14 µm un 84 % ($P_{CO2\ a\ 0ºC}$ = 20 687 ± 7 497 vs. $P_{CO2\ a\ 20\ ºC}$ = 130 338 ± 1 885 mL m^{-1} dia^{-1} atm^{-1}).

Asimismo, a este hecho hay que añadirle la habitual variación de permeabilidad dentro de una misma bobina de cualquier polímero, debido a la falta de uniformidad en el proceso de fabricación de éstos. Por tanto, la determinación de la permeabilidad a los gases de las membranas de polímeros es un problema complejo, además de que el empleo de éstos en el envasado en atmósfera modificada puede verse afectada por el uso de barnices, pinturas o presencia de condensaciones (Martínez y Artés, 2005).

Por este motivo, y al objeto de proporcionar valores de permeabilidad útiles y reales para el producto a envasar en atmósfera modificada, las empresas de películas plásticas deberían realizar ensayos técnicos para la determinación de permeabilidades a temperaturas más bajas incluyendo en éste los diferentes aditivos/barnices, microperforaciones, humedad relativa, etc. De esta forma se garantizaría que los datos proporcionados corresponden a las condiciones reales de conservación del producto IV Gama.

1.4. INNOVACIONES Y TENDENCIAS EN PRODUCTOS IV GAMA: ENVASADO ACTIVO COMO ALTERNATIVA A LA CONSERVACIÓN EN ATMÓSFERA MODIFICADA

1.4.1. Sistemas activos de envasado

Aunque el envasado en atmósfera modificada retrasa el deterioro de las frutas y hortalizas mínimamente procesadas, no siempre es suficiente para mantener su calidad durante todo el período de comercialización deseable. Por este motivo se está

promoviendo el uso de tecnologías de envasado activo, caracterizadas por establecer una interacción entre el envase/producto/medioambiente.

Distintas técnicas de envasado activo se han utilizado desde los años ochenta en productos muy variados tales como embutidos y quesos en países como Japón y Australia (Giambanco, 2009). Dicho sistema consiste en un conjunto de técnicas capaces de cambiar el estado del alimento envasado para extender su vida útil, mejorar sus propiedades organolépticas y de seguridad alimentaria, al mismo tiempo que mantiene la calidad del alimento (Rojas, 2006). Por tanto, un envasado se considera activo cuando, además de suponer una barrera entre el alimento y el exterior, ayuda de alguna otra forma a conservar el producto.

A continuación se resumen las principales funciones de los envases activos:

- Interactúan con el producto.
- Responden a cambios atmosféricos internos o de los propios productos.
- Modifican las condiciones de conservación de los productos con el fin de aumentar su duración y conservación.

En los siguientes apartados se describirán todos los sistemas para incorporar los elementos activos en productos de IV Gama **(Figura I.4.1)**, analizándose desde el que forma parte del propio producto, hasta el que es colocado en el exterior del envase (etiquetado inteligente).

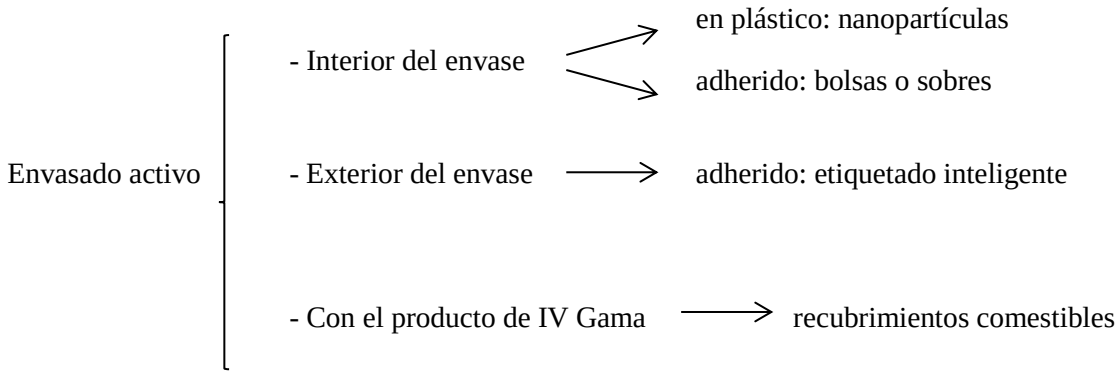


Figura I.4.1. Esquema sistemas activos que existen para productos IV Gama.
Fuente: Elaboración propia.

1.4.2. Componente activo localizado en el interior del envase.

Si el componente activo se localiza en el interior del envase, éste pasa a ejercer una función dinámica, ya que existe una continua interacción entre producto/envase/molécula activa al objeto de mejorar las condiciones internas de envasado.

1.4.2.1. Formando parte del material de envasado: nanopartículas

El sistema activo que ubica la molécula activa en el interior del material de envasado recibe el nombre de nanotecnología. De esta forma el envasado activo permite

que la técnica sea más atractiva para el consumidor, ya que no encuentra ningún elemento extraño en el interior del envase.

El empleo de sistemas nanotecnológicos tiene como objetivo la creación de películas plásticas con espesores mínimos, perfectamente adaptados a las necesidades del alimento. Las moléculas activas son introducidas en las películas plásticas mediante microencapsulación generando estructuras laminares para la fabricación de nanorecubrimientos constituidos por mono o multicapas mediante extrusión o compresión (Hernández-Izquierdo y Krochta, 2008; Rhim y Kim, 2014). En el momento de fabricación de las películas plásticas es importante tener en cuenta la heterogeneidad estructural de las moléculas activas (hidrófilas/hidrófobas, presentar o no carga superficial, ser polar/apolar, etc.), al objeto de establecer una u otra disposición en la formación de estructuras multicapas.

Cuando la molécula activa es encapsulada en la película plástica se forma un complejo molecular formado por polímero plástico-molécula activa provocando cambios en las propiedades físico-químicas del envase en el que está contenido. Así, debido a la migración y sorción controlada de las moléculas activas, en lugar de ceder al alimento sustancias indeseables se ceden sustancias con efecto beneficioso, previamente incorporadas al mismo, o bien se elimina por porción componentes indeseables del alimento. De esta manera, se producen numerosas modificaciones, tales como protección contra la luz y el oxígeno, modificación de su reactividad química, reducción de su volatilidad y aumento en su solubilidad acuosa. Además, se consigue facilitar el manejo de sustancias altamente hidrofóbicas, transformar compuestos líquidos o gaseosos a sólidos, proteger contra la degradación microbiana, modificar olores, sabores y colores, etc. (Madene et al., 2006).

De esta manera, se introducen sistemas que presenten distintos grados de impermeabilidad a humedad y gases; capaces de contener inhibidores, antimicrobianos, sustancias antioxidantes e inhibidores de la maduración, etc., permitiendo una protección efectiva e imperceptible (Morata, 2009; Ayala-Zavala et al., 2014; Muriel-Galet et al., 2015) **(Tabla I.4.1).**

Tabla I.4.1. Sistemas activos introducidos en el film plástico de envasado.

Aplicación	Descripción	Referencia
Antimicrobianos	Películas plásticas construidas con contenidos microbiocidas o bacteriostáticos de aceites esenciales, lisozima, sistema lactoperoxidasa, lactoferrina	Suppakul et al., 2003 ; Cagri et al., 2004; Valencia-Chamorro et al., 2011; Morelli et al., 2015
Antioxidantes	Incorporación de antioxidantes o captadores de oxígeno. Sistemas enzimáticos (glucosa oxidasa-glucosa, alcohol oxidasa-etanol)	Cagri et al, 2004; Ozdemir y Floros, 2004; Torres et al., 2015
Absorbedores de CO_2	Sistemas que reducen el contenido en CO_2	Aday et al., 2011
Inhibidores de encimas de maduración	Absorbentes de etileno como carbón activo, gel de sílice con $KMnO_4$, Kieseguhr, bentonitas, zeolitas, ozono, etc.	Ozdemir y Floros, 2004
Precursores/inhibidores de aromas, pigmentos, vitaminas	Adición de sustancias que se comportan en las películas plásticas como precursores de aromas o que inhiben la liberación de aromas del alimento. Adición de pigmentos y vitaminas.	Cagri et al, 2004; Ozdemir y Floros, 2004; Pastorelli et al., 2006

Fuente: Adaptado de Sacharow, 1976; Anon, 1979; Anon, 1982; Brydson, 1982; Ballantyne, 1986; Zagory y

Una vez localizados en ellos, migran al producto de IV Gama inmovilizando la sustancia indeseable en el material de envasado. Estas moléculas activas se caracterizan por poseer naturaleza variada ya que engloban ácidos orgánicos débiles (acético, benzoico, sórbico, cítrico, propiónico) o sus sales, enzimas (lisozima, glucosa oxidasa), bacteriocinas (nisina, pediocina), fungicidas sintéticos (imazalil), metales (plata, cobre, zirconio) o extractos naturales de plantas (romero, tomillo, orégano) (Han, 2003; Cha y Chinnan, 2004).

1.4.2.2. Adherido al material de envasado: bolsas o sobres

Además de la técnica anteriormente descrita para el establecimiento del envasado activo en productos mínimamente procesados, existen otras que consisten en la introducción en el interior del envase de pequeñas bolsas o sobres fabricados mediante materiales permeables en cuyo interior se sitúan las moléculas activas y las cuales son adheridas a la superficie interna del envase. El principal inconveniente que éstas presentan es el rechazo del producto por parte del consumidor, ya que existe riesgo de ingestión accidental del contenido en caso de producirse la rotura de la bolsa o sobre.

Los sistemas activos constituidos por bolsas o sobres, pueden ser clasificados en absorbedores (eliminando sustancias no deseadas como el oxígeno, exceso de agua, etileno, dióxido de carbono, olores y sabores indeseados, etc) y emisores **(TablaI.4.2)**, y que tendrán como finalidad extender la vida útil del producto (Gavara et al., 2009).

Tabla I.4.2. Sistemas activos adheridos al material de envasado.

Aplicación	Descripción	Beneficios en IV Gama	Referencia
Absorbedores			
O_2	Disminución (no su eliminación) de O_2 de forma controlada no llegando a condiciones anaeróbicas. Se basa en la oxidación de compuestos muy diversos: polvo de hierro, ácido ascórbico, dienos fotosensibles, enzimas (glucosa oxidasa y etanol oxidasa), ácidos grasos insaturados (oléico, linoléico y linolénico), H_2-paladio, alcaliglucosa, ditionita, estractos de arroz, levadura inmobilizados sobre sustrato sólido, etc., que son contenidos en materiales (bolsas) permeables al oxígeno e introducidos en el interior del envase	Eliminación decoloración, mohos, microorganismos aerobios, preservación sabor y características propias del producto, preservar nutrientes sensibles al O_2, extensión vida útil	Catalá et al., 2007; Catalá et al., 2008
CO_2	La absorción de parte del CO_2 dentro del envase de los productos de IV Gama. Al objeto de reducir los niveles de dicho gas se utilizan absorbedores cuyo agente activo es el hidróxido cálcico o el carbón activo.	Reducción de cambios de color, mejorar la textura del producto procesado, retrasar el desarrollo de bacterias, mohos y levaduras.	Suppakul et al., 2003; Catalá et al., 2008
H_2O / Humedad	La absorción de agua en estado líquido o gaseoso en el interior del envase. Los sistemas activos absorbedores de agua pueden ser sales de poliacrilato, amidas modificadas, copolímeros de almidón, etc., y están recubiertos de materiales microporosos. Los sistemas reguladores de humedad son sustancias humectantes, tales como como el propilenglicol.	Mejora las características físico-química y microbiológica.	García et al., 2006
Etileno	Corrige condiciones de etileno en el envase (oxidación del etileno a etilenglicol y éste a CO_2 y agua). Se realiza con $KMnO_4$ inmovilizado sobre un sustrato mineral inerte (gel de sílice, alúmina, carbón activo, vermiculita, zeolita, perlita, etc.) o con catalizadores metálicos, como el paladio, inmovilizado sobre carbón activo.	Ralentiza el reblandecimiento, disminuye la degradación de clorofila, aumentando la vida útil	Catalá et al., 2007; Catalá et al., 2008
Emisores			
Antimi-crobianos	Controla la posible contaminación del producto IV Gama por esporas y microorganismos que permanecen en estado latente hasta que las condiciones atmosféricas del medio (humedad, O_2, temperatura) sean las favorables para su desarrollo. Para ello se emplean sustancias volátiles antimicrobianas (hexanal, 1-hexenol, benzoato de metilo y 2-nonanona), metabisulfito, ClO_2, etanol que incorporadas al material de envase permiten controlar el crecimiento de hongos, mohos y bacterias	Disminuyen la degradación y deterioro causado por la oxidación además de controlar el crecimiento microbiano	Catalá et al., 2007; Catalá et al., 2008

1.4.3. Componente activo localizado en el exterior del envase: etiquetado inteligente

Si el componente activo se localiza en el exterior del envase, el sistema activo de envasado se denomina etiquetado inteligente, cuya función es el control de las condiciones de conservación de los alimentos e informar al consumidor sobre las mismas. Los envases inteligentes más habituales son los dispositivos indicadores de temperatura, de crecimiento microbiano y de gases.

De esta forma, un envase inteligente aporta al consumidor final importante información adicional a la inicialmente contenida en el envase, al objeto de indicar las condiciones de almacenamiento, maduración, estabilidad en la cadena de frío, y otros datos registrados durante las etapas posteriores al envasado.

El etiquetado inteligente está compuesto por etiquetas adheridas en los envases que mediante una indicación de manera clara, informa de variaciones en cuanto a condiciones óptimas de conservación del producto.

Como principales funciones de los envases inteligentes destacan:

- Mantienen, controlan e informan de las condiciones en las cuales los productos han sido envasados y conservados.
- Reaccionan a las condiciones del entorno a las que son sometidos los envases.
- Proporcionan información sobre historia de calidad de los productos contenidos en las etapas posteriores al envasado.
- Facilitan el uso y/o consumo de los productos contenidos, siendo la Industria Agroalimentaria la gran beneficiada.

En relación a los alimentos mínimamente procesados, como por ejemplo los listos para cocción, los envases inteligentes aportan novedosas opciones, ya que facilitan la forma de preparación y consumo de alimentos, sin necesidad de vaciar el contenido a otros medios para su cocción o preparación.

A continuación **(Tabla I.4.3)** se analizan distintos dispositivos empleados para este fin, tales como indicadores tiempo-temperatura, indicadores de madurez, válvula reguladora de gases, envasados caloríferos, etc.

Tabla I.4.3. Sistemas activos empleados como etiquetas inteligentes.

Aplicación	Descripción
Indicadores tiempo-temperatura	Consiste en la incorporación de etiquetas compuestas por productos químicos con un punto de fusión característico, junto al que se incorpora un compuesto colorante, los cuales permanecen separados por una guía por donde se difundirán los productos químicos una vez alcanzado el punto del fusión. De esta manera se determina un cambio de color cuando se alcance una determinada temperatura de fusión.
Indicadores de frescura	Indican mediante el etiquetado del deterioro o la falta de frescura del producto. De esta forma, se detectan metabolitos volátiles producidos durante la alteración de alimentos, como por ejemplo dióxido de carbono, aminas (en el caso del pescado), amoniaco y sulfuro de hidrógeno
Indicadores de caducidad	Indica la fecha óptima de consumo o el tiempo máximo de caducidad del producto alimentario, desde que ha sido abierto, siempre y cuando sea conservado en el frigorífico.
Indicadores de fuga	Aportan información sobre los niveles de oxígeno/dióxido de carbono en el interior del envase. Si se combinan estos sistemas con absorbedores de oxígeno, indican la eficacia del sistema activo interno, mediante una modificación de su color como resultado de la reacción química o enzimática. El colorante más utilizado para los indicadores de fuga es el azul de metileno, cuyo cambio de color se basa en una reacción de oxidación-reducción.
Indicadores de llenado	Consiste en comunicar al consumidor de datos relativos al llenado, como la cantidad exacta que contiene el envase. La información es transmitida al consumidor mediante sistemas de sondas y microchips en el momento en que se produce la apertura del envase.
Envases para la cocción de alimentos	Es una película plástica resistente a la cocción del alimento en horno microondas. En un orificio del plástico posee un componente céreo que, por la acción del calor, se funde y permite que la presión interior del envase se libere sin riesgo de explosión. Así, la humedad de los alimentos se conserva tras el proceso de cocción. Además incorporan válvulas en el interior de los envases de hortalizas mínimamente procesadas al objeto de alcanzar una temperatura y presión de vapor justas para la adecuada cocción de las hortalizas en un tiempo muy corto (5 minutos).
Envases refrigerantes	Formados por una pequeña bolsa de vinilo llena de agua, de manera que al ser abierto el envase, el líquido contenido en la bolsa refrigerante es comprimido y se evapora. Por consiguiente, el vapor substrae calor al producto, de modo que éste se enfría. El vapor que se precipita en la bolsa es captado mediante una materia secante a base de arcilla.
Etiquetas inteligentes RFID	Permiten la comunicación automática de la información ya que están provistos de un minúsculo microprocesador y una pequeña antena que recibe señal vía radiofrecuencia desde un emisor exterior y responde mediante una señal de identificación. Son capaces de almacenar y transmitir datos en tiempo real, para proporcionar una identificación y trazabilidad automática del producto envasado, legible mediante sistemas digitales. Así, se permite un control seguro en la distribución de productos, en el control de inventarios, en los registros de trazabilidad de productos, etc.

Fuente: Lee y Rahman, 2014.

1.4.4. Formando parte del producto mínimamente procesado: recubrimientos comestibles

Hasta la actualidad esta técnica ha sido aplicada mayoritariamente en frutas frescas (uvas, fresas, etc.) al objeto de aumentar la vida útil de éstas. Si bien, y debido a la gran demanda existente en el sector de frutas y hortalizas mínimamente procesadas, es cada vez más frecuente el empleo en éstas para aumentar su vida útil.

Por otro lado, es interesante el empleo de dicho sistema ya que al formar parte de alimentos, se evita la utilización de materiales de envasado no renovables, lo que constituye una ventaja para el medio ambiente.

Los recubrimientos y películas comestibles constituyen una estrategia potencial para reducir los efectos perjudiciales que provocan el procesado mínimo en los tejidos vegetales. Se trata de películas biodegradables que se adhieren a la superficie del alimento creando una atmósfera en torno a él pobre en oxígeno. De esta forma, su

aplicación favorece el control de procesos respiratorios típicos de los tejidos vivos, controla los procesos de deshidratación, permite la introducción de agentes antioxidantes, la incorporación de compuestos antimicrobianos y más recientemente, la incorporación de otras sustancias que podrían mejorar las características del producto final, tales como nutrientes, saborizantes e incluso microorganismos beneficiosos (Rojas, 2006).

La principal diferencia entre ambos sistemas activos adheridos al producto citados anteriormente, es que el recubrimiento comestible (RC) se define como una capa delgada de material comestible formado como revestimiento sobre el alimento, mientras que una película comestible (PC) es una capa preformada y delgada constituida con material comestible, la cual una vez elaborada puede ser colocada sobre el alimento o entre los componentes del mismo (McHugh y Senesi, 2000). De esta manera, la principal diferencia respecto a la aplicación de los mismos es los RC son aplicados en forma líquida sobre el alimento forma líquida sobre el alimento, generalmente por inmersión del producto en una solución, y las PC son preformadas como láminas sólidas las cuales son posteriormente aplicadas para cubrir el alimento.

De esta forma, su uso sobre el tejido vegetal cortado produce una atmósfera modificada activa en la fruta que retrasa su deterioro y maduración, reduce la pérdida de agua, retarda los cambios de color, mejora la apariencia, disminuye la pérdida de aromas y puede servir para la introducción de sustancias tales como antioxidantes y estabilizantes de textura (Olivas y Barbosa-Cánovas, 2005).

Para la formación de un RC es necesaria una solución que pueda construir una matriz estructural con suficiente adhesión (Debeaufor et al., 1998). Para ello, existen dos grandes grupos de materiales usualmente empleados en la elaboración de RC para frutas frescas cortadas: polisacáridos y proteínas.

1.4.4.1. Polisacáridos en la matriz de los recubrimientos comestibles

Entre los polisacáridos más empleados como base para formar RC se encuentran: celulosa, almidón, pectina, alginatos, chitosan, maltodextrinas, metilcelulosa, carboximetilcelulosa y gelano (Pavlath et al., 1993; Wong et al., 1994; Krochta y Mulder-Johnston, 1997; Yang y Paulson, 2000; Díaz-Sobac et al., 2001; Le Tien et al., 2001; Turhan et al., 2001; Rojas-Graü et al., 2007; Pérez-Gago et al., 2010; Dhall, 2013) **(Tabla I.4.4).**

Tabla I.4.4. Principales polisacáridos empleados como recubrimientos comestibles.

	Tipo	Características	Referencia
Polisacáridos	Celulosa y derivados	Polisacárido comestible pero no digerible formado por residuos de glucosa unidos por enlace ß (1-4). Está compuesto por unidades de D-glucosa que son altamente permeables al vapor de agua. Posee formas no solubles, tales como metilcelulosa (MC), y formas aniónicas, como la carboximetilcelulosa (CMC) o con grupos metilo e hidroxipropilo como la hidroxipropilmetilcelulosa (HPMC). Su empleo en recubrimiento produce una disminución en las cantidades de CO_2 y etileno emitidas	Kester y Fennema, 1986; Baldwin, 1999; Weber et al., 2002; Villalobos-Carvajal et al., 2009
	Almidón	Polisacárido comestible formado por unidades de glucosa unidas por enlace α (1-4) con ramificaciones con enlace α (1-6). Proporcionan baja permeabilidad al oxígeno	Donhowe y Fennema, 1994 ; Weber et al., 2002; Rhim y Ng, 2007; Chiumarelli y Hubinger, 2014
	Pectinas	Heteropolisacárido que forma parte de las paredes celulares de las plantas superiores. Están compuestos por residuos de ácido Dgalacturónico unidos por un enlace α (1-4) (homogalacturonano). Los grupos carboxilo de C6 pueden estar metil-esterificados o permanecer libres. Su empleo en recubrimiento produce una reducción en las tasas de producción de CO2 y etileno de hasta un 90 %	Wong et al., 1994; Cagri et al., 2004; Ayala-Zavala et al., 2013
	Dextrinas	Las dextrinas son un grupo de glúcidos de bajo peso molecular producidas por la hidrólisis del almidón. Si se incluyen formando parte de los recubrimientos comestibles proporcionan al alimento una textura crujiente	Cagri et al., 2004; Gonçalves et al., 2010
	Chitosán	Polisacárido comestible pero no digerible, comercialmente se produce por deacetilación de la quitina que es el elemento estructural del exoesqueleto de los crustáceos (cangrejos, gambas, etc.). El grado de deacetilación oscila en el chitosan comercial entre el 60-100 %. Prolonga la vida útil y mejora la calidad de frutas cortadas ya que presentan una alta permeabilidad selectiva frente a los gases, una ligera resistencia al vapor de agua, además de poseer propiedades antifúngicas	Krochta y de Mulder-Johnston, 1997; Ocio et al., 2007; No et al., 2007; Brown et al., 2008; Pilon et al., 2014
	Alginato cálcico de algas marrones	Polisacárido comestible gelificable sintetizado por las algas inicialmente como un polímero de ácido murónico, que posteriormente modifican transformando unidades de manurónico en gulurónico mediante una epimerización enzimática. El producto final contiene zonas formadas por gulurónico, zonas formadas por manurónico y zonas con gulurónico y manurónico alternados. Actúa como barrera a la humedad	Datta et al., 2008; Costa et al., 2012; Azarakhsh et al., 2014a, 2014b.
	Puré de frutas	Sustancias pécticas y celulósicas constituyendo los polisacáridos primarios en los purés de frutas. Aportan características sensoriales y propiedades barrera, las cuales permiten mejorar y extender la vida útil del producto mínimamente procesado	McHugh y Senesi, 2000; Rojas-Graü et al., 2007
	Gelano	Polisacárido secretado por *Pseudomona elodia*. Es un hidrocoloide multifuncional que puede ser usado en productos alimenticios que demanden gelificación, texturización, estabilización, suspensión y formación de películas proporcionando buenas propiedades mecánicas y de barrera	Yang y Paulon, 2000; Azarakhsh et al., 2014

Tabla I.4.4. Principales polisacáridos empleados como recubrimientos comestibles (continuación).

	Tipo	Características	Referencia
Polisacáridos	Mucílagos	Polisacáridos heterogéneos formados por diferentes azúcares y en general ácidos urónicos. No muy estudiado en frutas cortadas. Investigaciones han demostrado que el gel (mucílago) proveniente de la planta de sábila (Aloe vera) puede prolongar la conservación de productos frescos como uvas (aumentando la vida útil de éstas hasta 35 días comparado con uvas sin recubrir). Otro mucílago empleado en RC es el procedente de cactus, capaz de absorber grandes cantidades de agua, disolverse y dispersarse por sí mismo y formar soluciones viscosas. Éste ha sido aplicado en fresas, manteniéndo su color y firmeza original	Del-Valle et al., 2005; Dominguez-López, 2005; Serrano et al., 2006; Zambrano - Zaragoza et al., 2014
	Agar de Gelidium corneum	Polisacárido comestible gelificable. Es una mezcla de agarosa y agaropectina. La agarosa es un polímero lineal de peso molecular 120.000 con una estructura basada en la unidad – (1-3)-ß-Dgalactopiranosa- (1-4)-3,6-anhidro- α -L-galactopiranosa. Proporcionan propiedades antifúngicas	Ku et al., 2008

1.4.4.2. Proteínas en la matriz de los recubrimientos comestibles

Por otro lado, en cuanto a las proteínas más empleadas destacan: proteína de suero lácteo, proteína aislada, gluten de trigo, colágeno, zeína de maíz, soja y caseína **(Tabla I.4.5)** (Avena-Bustillos y Krochta, 1993; Gontard et al., 1993; Gagri et al., 2001; Sabato et al., 2001; Pérez-Gago et al., 2003).

Tabla I.4.5. Principales proteínas empleadas en recubrimientos comestibles.

	Tipo	Características	Referencia
Proteínas	Caseína	Proteína de la lecha soluble en agua que no confiere ningún tipo de color u olor diferente del alimento donde se aplica. No resiste a la difusión de agua, por lo que la incorporación de lípidos en recubrimientos de caseína provocan una buena protección para frutas y hortalizas contra la pérdida de agua y el pardeamiento oxidativo	Baldwin et al, 1995; Ponce et al., 2008
	Gelatina	Proteína animal comestible con alto contenido en glicina (1 de cada 3 residuos), prolina y 4-hidroxiprolina. Tiene características muy similares a la caseína	Ku y Song, 2007
	Zeína de maíz	Proteína procedente del maíz rica en prolamina que se puede utilizar como polímero termoextruible. Es insoluble en agua, pero soluble en soluciones acuosas de alcohol, glicerol y esteres de glicerol. Posee buenas propiedades de adhesividad y barrera al oxígeno, aunque por su elevada hidrofilidad y fragilidad requiere la adición de agentes plastificantes como glicerol. Ha sido clasificada GRAS* por la Food and Drug Administration (FDA)	Martin-Polo et al., 1992; Janes et al., 2002; Quintero- Salazar et al., 2005; Ku y Song, 2007
	Proteínas de soja	Proteína de semilla leguminosa formada por globulinas (legumininas 11S y vicilinas 7S), glicinas, ß-conglicininas y proteínas tipo gluten. Entre sus características destacan su fácil adherencia a superficies hidrofílicas y la reducción del crecimiento microbiano	Eswaranandam et al., 2004; Theivendran et al., 2006

Tabla I.4.5. Principales proteínas empleadas en recubrimientos comestibles (continuación).

Tipo	Características	Referencia
Proteínas de suero lácteo	Proteínas presentes en la leche y son consideradas de muy alta calidad. Contienen cinco grupos importantes: ß-lactoglobulinas, α - lactoalbuminas, inmunoglobulinas, albúmina de serum bovino y proteasa-peptonas. Poseen alta permeabilidad a los gases, reducen el pardeamiento enzimático y pérdidas de textura	McHugh y Krochta, 1994 Sonti et al., 2003; Robitaille et al., 2014
Mezcla de cúrcuma y caseína	Mezcla de cúrcuma y caseína con alcohol polivinílico y propilenglicol como agentes plastificantes y con Tween-80 como emulsionante. Proporcionan propiedades antifúngicas	Jagannatha et al., 2006; Lacroix y Vu, 2013

1.4.4.3. Lípidos en recubrimientos comestibles

Debido a que los productos procesados en IV Gama continúan respirando, se debe prestar especial atención a las propiedades barrera a los gases impuestas por cada recubrimiento. Por este motivo, generalmente se combinan dos o más proteínas/polisacáridos con el fin de mejorar el intercambio de gases, la adherencia y las propiedades de permeabilidad a la humedad (Baldwin et al., 1995).

Además de polisicaráridos y proteínas anteriormente descritas, es usual la adición de lípidos (ceras, acilgliceroles y ácidos grasos) al objeto de aumentar la barrera al vapor de agua (Cagri et al., 2004). Su empleo se realiza con el objeto de mejorar la flexibilidad y la funcionalidad de los recubrimientos. Entre los agentes plastificantes utilizados frecuentemente se encuentran: glicerol, polietilenglicol, sorbitol, aceites, ácidos grasos, ceras, etc., siendo el glicerol uno de los más utilizados (Rojas-Graü et al., 2007; Raybaudi-Massilia et al., 2010).

1.4.4.4. Aditivos en recubrimientos comestibles: antimicrobianos y antioxidantes

A pesar de la gran diversidad de moléculas activas a emplear según las condiciones internas del envase, la principal función de éstas es la disminución de microorganismos patógenos y alterantes de los productos mínimamente procesados.

Para este fin, existen un amplio número de sustancias no volátiles de acción antimicrobiana que se incorporan a los materiales poliméricos formando parte estructural de los mismos.

Una vez localizados en ellos, migran al producto de IV Gama inmovilizando la sustancia indeseable en el material de envasado. Estas moléculas activas se caracterizan por poseer naturaleza variada ya que engloban ácidos orgánicos débiles (acético, benzoico, sórbico, cítrico, propiónico) o sus sales, enzimas (lisozima, glucosa oxidasa), bacteriocinas (nisina, pediocina), fungicidas sintéticos (imazalil), metales (plata, cobre, zirconio) o extractos naturales de plantas (romero, tomillo, orégano) (Han, 2003; Cha y Chinnan, 2004; Severino et al., 2014).

Por su parte los diferentes compuestos antioxidantes pueden emplearse contra la oxidación, degradación y descoloración de productos IV Gama. Entre ellos destacan el pectonato, pectato, etanol, cisteína, derivados del maíz que incluyan BHA, BHT y ácido cítrico (Han et al., 2014b).

1.4.5. Aplicaciones de recubrimientos comestibles en productos IV Gama

A continuación se resumen los principales recubrimientos comestibles empleados en los productos IV Gama **(Tabla I.4.6)**, no existiendo ningún estudio sobre calabacín. Por ese motivo se ha estudiado dicho aspecto en un capítulo de Tesis.

Tabla I.4.6. Aplicación de recubrimientos comestibles en frutas y hortalizas de IV Gama.

Producto IV Gama	Recubrimiento	Aditivos y plastificantes	Referencia
Aguacate	Cera de Candelilla	Aloe vera, ácido elágico, ácido gálico	Saucedo-Pompa et al., 2007
Apio	CC, AMG	----	Shon y Haque, 2007
Berenjena	SPI, BW	AA, Cys, Gly	Ghidelli et al., 2010a; Ghidelli et al., 2014
Castaña de agua china	Chitosan	----	Pen y Jian, 2003
Caqui	WPI, BW	Ascorbato de sodio, Gly	Pérez-Gago et al., 2005b
Cebolla	SWP, SPI, CC	Sorbitol	Shon y Haque, 2007
Champiñón	Chitosan	----	Eissa, 2007
Lechuga	Alginato	$CaCl_2$	Tay et al., 2004
Litchi	Chitosan	----	Dong et al., 2003
Fresa	Chitosan	----	Campaniello et al., 2008
Mango	Chitosan	----	Chien et al., 2007
	CMC, chitosan, dextrina, ácido esteárico	AA, ácido cítrico, lactato de calcio	Ducamp-Collin et al., 2009; Plotto et al., 2010
	CMC, maltodextrina	Ascorbato de calcio, N-acetilcisteina	Plotto et al., 2004
Manzana	Alginato; pure de manzana	N-acetilcisteina, $CaCl_2$, orégano, aceite de limón, Gly	Rojas-Graü et al., 2007
	Alginato, gelano, aceite de girasol	N-acetilcisteina, $CaCl_2$	Rojas-Graü et al., 2008
	Alginato	N-acetilcisteina, glutationa, cinamon, clavo, aceite de limón, eugeniol, lactato clacico, ácido málico, Gly	Raybaudi-Massilia et al., 2008 b
	Puré de manzana, BW, aceite vegetal	AA, ácido cítrico, Gly	McHugh and Senesi, 2000
	Alginato, AMG, ácido linoleico	$CaCl_2$	Olivas et al., 2007
	Carragenano, CMC WPC	$CaCl_2$, Gly, PEG	Lee et al., 2003
	CMC, CC, WPI	$CaCl_2$, Gly	Le Tien et al., 2001
	Nature Seal, CMC, SPC	AA, PS, SB, aceite de soja, $CaCl_2$, Gly, PEG	Baldwin et al., 1996
	SWP, SPI, CC	Sorbitol	Shon and Haque, 2007

BW= cera de abeja; AMG= monoglicérido acetilado; CMC= carboximetilcelulosa; WPC= proteína de suero lácteo concentrada; CC= caseinato de calcio; SPC= proteína de soja concentrada; SWO= suero láctea en polvo; WPI= aislado de proteína de suero lácteo; HPMC= hydroxipropil-metilcelulosa; CW= cera de carnauba; MC= metilcelulosa; SPI= proteína de soja aislada; $CaCl_2$= cloruro de calcio; Gly= glicerol; AA= ácido ascórbico; PEG= polietilenglicol; Cys= cisteína; PS= sorbato de potasio; SB= benzoato de sodio.

Tabla I.4.6. Aplicación de recubrimientos comestibles en frutas y hortalizas de IV Gama (continuación).

Producto	Recubrimiento	Aditivos y plastificantes	Referencia
Manzana	WPI, BW	Gly	Pérez-Gago et al., 2003
	WPI, WPC, HPMC, BW, CW	Gly	Pérez-Gago et al., 2005a
	WPC, BW	AA, Cys, Gly	Pérez-Gago et al., 2006
	Cera de Candelilla	Aloe vera, ácido elágico, ácido gálico	Saucedo-Pompa et al., 2007
Melón	Alginato, gelano, pectina, aceite de girasol	$CaCl_2$, Gly	Oms-Oliu et al., 2008a
	Chitosan	$CaCl_2$, Gly	Chong et al., 2015
	CMC, maltodextrina	Canela, aceite de palma, aceite de limón, eugenol, geraniol, citral, ácido málico, lactato cálcico, Gly	Raybaudi-Massilia et al., 2008
	SPI	ácido málico, ácido láctico, Gly	Eswaranandam et al., 2006
Patata	Nature Seal, CMC, SPC	AA, PS, SB, aceite de soja, $CaCl_2$, Gly, PEG	Baldwin et al., 1996
	CMC, CC, WP	$CaCl_2$, Gly	Le Tien et al., 2001
	SWP, SPI, CC	Sorbitol	Shon y Haque, 2007
Papaya	Alginato, gelano	AA, $CaCl_2$, Gly	Tapia et al., 2007
Pera	Alginato, gelano, pectina	N-acetilcisteina, glutationa, $CaCl_2$, Gly	Oms-Oliu et al., 2008b
	MC, ácido esteárico	AA, PS, $CaCl_2$, Gly, PEG	Olivas et al., 2003
Plátano	Carragenano	AA, Cys, $CaCl_2$, Gly, PEG	Bico et al., 2009
	Cera de Candelilla	Aloe vera, ácido elágico, ácido gálico	Saucedo-Pompa et al., 2007
Pomelo	Microemulsión de cera	----	Baker y Hagenmaier, 1997
	SPI	Ácido cítrico, $CaCl_2$, Gly	Ghidelli et al., 2010b
Zanahoria	Alginato	Ácido cítrico, $CaCl_2$	Amanatidou et al., 2000
	Almidón		Lai et al., 2013
	Chitosan, almidón de ñame	Gly	Durango et al., 2006
	Chitosan, MC, ácido oleico	----	Simoes et al., 2009
	Goma de xantana	Lactato de calcio y gluconato	Vargas et al., 2009
	Celulosa	----	Mei et al., 2002
	Pectina, CMC, CC, WPI	Cinamaldehído, Gly	Peiyin y Barth, 1998
	HPMC, ester de sacarosa	----	Caillet et al., 2006
	CC, WPI	Gly	Villalobos-Carvajal et al., 2009
	SWP, SPI, CC	Sorbitol	Lafortune et al., 2005

BW= cera de abeja; AMG= monoglicérido acetilado; CMC= carboximetilcelulosa; WPC= proteína de suero lácteo concentrada; CC= caseinato de calcio; SPC= proteína de soja concentrada; SWO= suero láctea en polvo; WPI= aislado de proteína de suero lácteo; HPMC= hydroxipropil-metilcelulosa; CW= cera de carnauba; MC= metilcelulosa; SPI= proteína de soja aislada; $CaCl_2$= cloruro de calcio; Gly= glicerol; AA= ácido ascórbico; PEG= polietilenglicol; Cys= cisteína; PS= sorbato de potasio; SB= benzoato de sodio.

1.5. LA ESPECTROSCOPÍA EN EL INFRARROJO CERCANO (NIRS) PARA LA PREDICCIÓN DE PARÁMETROS DE CALIDAD EN FRUTAS Y HORTALIZAS

1.5.1. Principios de la técnica

La tecnología NIRS es una técnica espectroscópica no destructiva considerada como una alternativa que puede sustituir a los métodos analíticos tradicionales. Se caracteriza por ser rápida, no contaminante, de gran precisión y exactitud, siempre que se sigan los procedimientos adecuados para generar los modelos de predicción requeridos (Marten y Naes, 1989; Garrido et al., 1993).

El espectro electromagnético de la región NIR (*Near-Infrared*), se encuentra comprendido tras la región visible (ubicado éste desde los 380 a los 700 nm), es decir desde los 700 (25000 cm^{-1}) hasta los 2500 nm, correspondiente a la región del infrarrojo medio (MIR) (4000 cm^{-1}) (Osborne et al., 1993; Murray, 1999; McClure, 2003).

La espectroscopía NIR, al ser una técnica espectroscópica vibracional molecular, consiste en la absorción producida cuando la radiación electromagnética proveniente del infrarrojo cercano vibra a la misma frecuencia específica que los enlaces moleculares presentes en el producto analizado. Dichos enlaces moleculares son fundamentalmente del tipo X-H, donde X corresponde a átomos de carbono, nitrógeno u oxígeno. Estas absorciones son causadas principalmente por vibraciones y movimientos de tensión o alargamiento (*stretching*) y de flexión o deformación (*bending*) de los mismos (Shenk y Westerhaus, 1995a, 1995b; Lachenal, 2000; Miller, 2001). Debido a que el comportamiento de las moléculas reales se acerca más al de un oscilador anarmónico que al de un oscilador armónico perfecto, las absorciones NIR tienen lugar fundamentalmente como sobretonos y combinaciones de las vibraciones fundamentales existentes en la región del infrarrojo medio (Miller, 2001; Benson, 2003), aunque también se pueden observar absorciones electrónicas debidas al movimiento de electrones entre diferentes niveles energéticos.

En la región NIR, las bandas localizadas entre 700 – 1900 nm se corresponden con primeros, segundos y terceros sobretonos de absorciones fundamentales, mientras que las bandas localizadas entre 1900 y 2500 nm se corresponden con bandas de combinación de uno o más sobretonos **(Figura I.5.1)** (Shenk et al., 1992; Ciurczak, 2001).

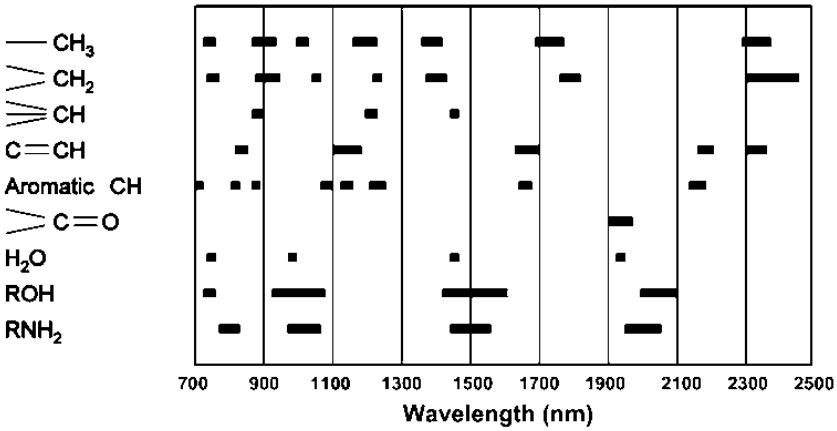


Figura I.5.1. Bandas con grupos funcionales en el espectro NIR.

Fuente: Spectroscopy solutions, Ltd.

Desde finales de los años sesenta, la instrumentación NIRS ha ido evolucionando incorporando como avances más significativos los relacionados con la mejora relación señal/ruido, la precisión en la longitud de onda, la disponibilidad de diferentes sistemas de interacción de la radiación con la muestra y la existencia de una gran variedad de accesorios para su utilización 'off-line' (de forma manual en el laboratorio), 'at-line' (de forma manual en el proceso analítico) o 'on line' (de forma automática en el proceso analítico) (Garrido et al., 2000, Van Kempen, 2001).

Por otro lado, según sea el modo de ubicación de los detectores con respecto a la muestra, el equipo NIR puede trabajar en: transmitancia, reflectancia, transmitancia o traansflectancia transmitancia (Shenk y Westerhaus, 1995a; Bergera et al., 2006) **(Figura I.5.2).**

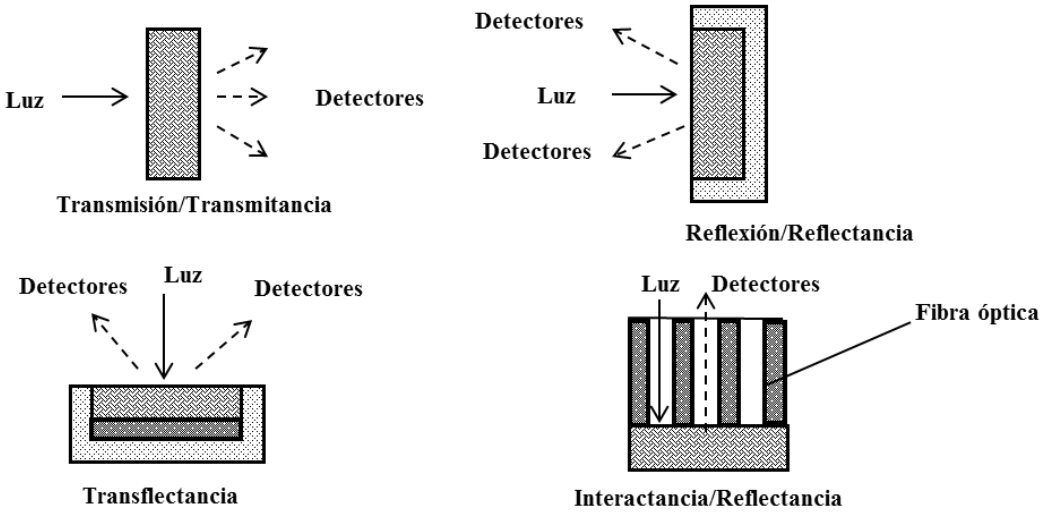


Figura I.5.2. Modo de análisis NIR según la posición de los detectores.

Fuente: Adaptado de Kawano, 2004.

Transmitancia: cuando un haz de luz monocromática (de una sola longitud de onda) incide sobre un cuerpo, parte de ese haz será absorbido y otra parte atravesará el medio. La transmitancia es la parte del total que no es absorbida por el cuerpo en el que incide. Se suele utilizar tanto para muestras homogéneas como para líquidos. Se conoce el paso óptico. Este sistema es ampliamente utilizado para muestras líquidas.

Reflectancia: es el efecto producido cuando un haz de luz incide sobre la superficie de un cuerpo, y éste lo devuelve al medio en mayor o menor proporción en función del tipo de material sobre el que incida la luz. El paso óptico es indeterminado, por no saber la profundidad a la que llegará la radiación NIRS a la muestra. En este caso la muestra puede ser opaca, pulvarizada, o de grosos superior a 1 cm. Espectral o difusa (la más utilizada).

Transflectancia: efecto combinado de la reflectancia y la transmitancia. Se conoce también como doble transmisión. En este caso parte de la luz incidente es reflejada en la muestra y otra parte la atraviesa, para ser reflejada por un material colocado en la cara opuesta de la muestra, siendo recogida por el detector.

Interactancia: una sonda de fibra óptica que entra en contacto directo con la superficie de la muestra para iluminarla y detectar la radiación reflejada por la misma (García Olmo, 2002). Con este tipo de análisis, se elimina el proceso de llenado de cápsulas o cubetas de medición, recogiendo el espectro directamente sobre la muestra y transmitiéndolo al instrumento mediante las fibras ópticas (Kawano, 2004).

1.5.2. Análisis quimiométrico

La quimiometría consiste en relacionar la información espectral proveniente de las muestras de las muestras a analizar con los correspondientes valores del parámetro a estudiar, los cuales han sido obtenidos previamente a partir de un método de referencia. Una vez desarrollado el modelo, éste permite predecir el contenido de otras muestras de características similares a las incluidas en el grupo de entrenamiento o calibración.

1.5.2.1. Análisis de Componentes Principales (ACP)

Es una técnica multivariante en la cual un número de variables más o menos correlacionadas son transformadas a otro número menor de variables no correlacionadas. Esta colinealidad significa que la matriz de datos X tiene algún tipo de variabilidad dominante que lleva la información principal; siendo el objetivo del ACP la eliminación de información redundante y la variabilidad debida al ruido (Coello y Maspoch, 2007).

El análisis de componentes principales busca las direcciones que expliquen la máxima variabilidad de las muestras, definiendo unos nuevos ejes que describirán el volumen en que se encuentran las mezclas, siendo los nuevos ejes los componentes principales o loadings.

Un espectro, medido a K longitudes de onda, constituye un conjunto de K variables que puede ser descrito matemáticamente como un vector. Se puede constituir un espacio de K dimensiones de forma que cada dimensión corresponda a una de las longitudes de onda, y se puede representar el mismo objeto como un punto en este nuevo espacio. Si tenemos M muestras, se pueden representar como otros tantos puntos en el espacio de K dimensiones. Si no tienen nada en común los unos con los otros, los M puntos estarán dispersos en el espacio, pero si los M espectros están relacionados los M puntos aparecerán agrupados. Por tanto, el ACP relacionará las direcciones en que están agrupados los M puntos de los objetos en el espacio de K variables, y reducir el sistema inicial K dimensional a uno A dimensional, con A < K, manteniendo la información relevante del sistema (Coello y Maspoch, 2007). La finalidad es desarrollar un modelo bilineal donde los valores de absorbancia NIR sean las variables originales, que tras combinarse formen otras componentes principales (Jackson, 1991).

El paso posterior al ACP es el cálculo de distancias entre muestras (espectros) en un espacio n-dimensional, generalmente a través de la distancia de Mahalanobis (Shenk y Westerhaus, 1995a, 1996). Además los procedimientos descritos detectan aquellas muestras con comportamiento diferente o anómalas (outliers), siendo muy importante su detección, interpretación y posible eliminación, ya que puede tratarse de una anomalía proveniente de la información espectroscópica o físico-química (Williams y Norris, 2001).

Finalmente, se define el colectivo de calibración, ya sea para la construcción de ecuaciones de calibración (análisis cuantitativo, llevado a cabo en la presente Tesis) como para modelos de clasificación (análisis cualitativo).

1.5.2.2. Análisis cuantitativo

Consiste en el empleo de la información espectral (espectros NIRS) para la determinación de uno o varios componentes en la muestras del producto analizado, empleándose en la mayoría de los casos calibraciones basadas en los métodos lineales de ajuste (asumiendo que la relación entre la señal espectral y la concentración de la ley de Lambert-Beer es lineal), existiendo también algoritmos no lineales.

Para el desarrollo de una calibración existen diferentes métodos de regresión lineal (Martens y Naes, 1989; Burns y Ciurczak, 1992, 2001), siendo los más utilizados en el análisis cuantitativo la Regresión Lineal Múltiple (RLM ó MLR), la Regresión por Componentes Principales (RCP ó PCR), la Regresión mediante Mínimos Cuadrados Parciales (RMCP ó PLS) y la Regresión mediante Mínimos Cuadrados Parciales Modificada (RMCPM ó MPLS), siendo éstas dos últimas las más empleadas en aplicaciones agroalimentarias (Shenk y Westerhaus, 1995b, Pérez-Marín et al., 2007).

Existen dos metodologías básicas para la construcción del modelo. Si se dispone de un número suficiente grande de muestras de composición conocida, éstas se dividen en dos conjuntos, uno que constituye propiamente dicho el conjunto de calibración y otro utilizado para comprobar la capacidad predictiva de los distintos modelos desarrollados (Test Set). Sin embargo, cuando el número de muestras disponible es relativamente pequeño, la metodología a seguir es la denominada validación cruzada (Cross validation), la cual utiliza muestras del bloque de calibración para comprobar el modelo. Mediante este método el conjunto de muestras de calibrado se divide en varios bloques o segmentos. De esta manera se evitan los sobreajustes, atendiendo al valor del error estándar de validación (SECV), que de forma aproximada se puede decir que es equivalente al error estándar de predicción (SEP) de 10 muestras elegidas al azar (Vandeginste et al., 1998; WinISI, 2000; Brereton, 2003).

El proceso de elección de número de factores o variables latentes comienza por establecer el número mínimo de SECV y a partir de éste se calcula el valor de SECV aceptable (5 % superior al mínimo). Se identificará el SECV aceptable, eligiéndose ese número de factores o variables latentes para desarrollar el modelo.

Durante este proceso también son detectadas muestras con altos residuales, diferencias entre el valor de referencia y el predicho. Se utiliza el criterio T, de forma que aquellas muestras cuyo residual dividido por el SECV del proceso supere el valor de 2,5 serán eliminadas del conjunto de calibración.

$$T = \frac{residual}{SECV}$$

Este procedimiento se repite dos veces para obtener finalmente el modelo (WinISI, 2000).

A continuación se describen los principales parámetros obtenidos en la ecuación de calibración:

RSQ: coeficiente de correlación múltiple: se utiliza para medir el grado con el que el calibrado ajusta sus datos

$$RSQ = 1 - \left(\frac{\sum_{i=1}^{N}(y_{cal\,i} - \hat{y}_{teo\,i})^2}{\sum_{i=1}^{N}(\hat{y}_{teo\,i} - \bar{y})^2}\right)$$

SEC: error estándar de calibración

$$SEC = 1 - \sqrt{\left(\frac{\sum_{i=1}^{N}(y_{cal\,i} - \hat{y}_{teo\,i})^2}{N-p-1}\right)}$$

BIAS: se define como la media de los residuales, siendo éstos la diferencia entre el valor del laboratorio ($y_{cal\,i}$) y el valor predicho ($\hat{y}_{teo\,i}$),

$$\text{residual, } f_{ij} = (y_{cal\,i} - \hat{y}_{teo\,i})$$

$$BIAS = \frac{1}{n}\sum_{i=1}^{N}(y_{cal\,i} - \hat{y}_{teo\,i})$$

SECV: error estándar de validación cruzada

$$\text{SECV} = \sqrt{\left(\frac{\sum_{i=1}^{N}(y_{cal\,i} - \hat{y}_{teo\,i} - BIAS)^2}{N-1}\right)}$$

Rango de aplicabilidad: valores máximo y mínimo del parámetro de referencia para los cuales se puede utilizar la ecuación

En las ecuaciones $y_{cal\ i}$ e $\hat{y}_{teo\ i}$, son respectivamente los datos calculados y teóricos para cada una de las i-muestras, $\bar{y}$ es el valor medio de los datos calculados, *N* es el número de muestras utilizadas en el calibrado, y *p* es el número de factores de la regresión.

Una vez obtenido el modelo se procede a la validación interna mediante la predicción de los valores del parámetro de referencia en las mismas muestras que finalmente forman parte del modelo. Algunos de los parámetros que se utilizan para estimar la bondad del modelo son:

RSQ: obtenido al realizar una regresión entre los valores de referncia y los prechichos mediante espectroscopía NIR.

SEP: error estándar de predicción

$$\text{SEP} = \sqrt{\left(\frac{\sum_{i=1}^{N}(y_{cal\,i} - \hat{y}_{teo\,i})^2}{N}\right)}$$

SEP (C): error corregido por el BIAS

$$\text{SEP (C)} = \sqrt{\left(\frac{\sum_{i=1}^{N}(y_{cal\,i} - \hat{y}_{teo\,i} - BIAS)^2}{N-1}\right)}$$

En estas ecuaciones $y_{cal\ i}$ e $\hat{y}_{teo\ i}$ son respectivamente, los datos calculados y teóricos para cada una de las i-muestras y N es el número de muestras utilizadas para realizar la validación interna.

RPD: capacidad de predicción del método

$$\text{RPD} = \frac{SD_{ref}}{SEP}$$

Se trata de la relación entre la desviación estándar de los datos obtenidos mediante el análisis de referencia y el error estándar de predicción de los mismos. Este valor idealmente debe ser superior a 2,5, si bien en los casos en los que SD_{ref} es menor que la unidad puede no llegarse a ese valor ideal (Williams y Sobering, 1993).

Además, para comprobar la robustez del modelo de calibración se realiza una validación externa, aplicando las ecuaciones a un conjunto de muestras que no pertenecen al colectivo de calibración. Algunos de los parámetros que se evalúan son:

Media de los residuales

Porcentaje de error respecto al valor de referencia

RMSE: error cuadrático medio

$$\text{RMSE} = \sqrt{\left(\frac{\sum_{i=1}^{N}(y_{cal\,i} - \hat{y}_{teo\,i})^2}{N}\right)}$$

En esta ecuación $y_{cal\ i}$ e $\hat{y}_{teo\ i}$ son respectivamente, los datos calculados y teóricos para cada una de las i-muestras y N es el número de muestras utilizadas para realizar la validación externa. La fórmula es la misma que se emplea en validación interna (para el caso del SEP), pero aplicada a muestras que no pertenecen al colectivo de calibración.

RER: error típico de predicción

$$\text{RER} = \frac{Rango\ (máx - \min)_{ref}}{SEP}$$

1.5.3. Aplicación del NIRS en el control de calidad de productos agroalimentarios

La tecnología NIRS ofrece una amplio potencial en la cuantificación de parámetros tanto físico-químicos (color, firmeza, sólidos solubles, acidez, pH, materia seca) como nutricionales (capacidad antioxidante, carotenoides totales, minerales, vitamina C, polifenoles, etc.) en un amplio rango de productos agroalimentarios. A continuación se resumen estudios recientes de la aplicación de dicha tecnología tanto en frutas **(Tabla I.5.1)** como en hortalizas, cereales ó plantas **(Tabla I.5.2).**

Tabla I.5.1. Aplicación de la tecnología NIRS en el control de calidad de frutas.

	Producto	Compuesto / Parámetro	Instrumento	Modo de análisis	Rango (nm)	Estadísticos		Referencia
						RPD	R^2_{cal}	
Frutas	Arándanos	Sólidos solubles	FT-NIR	Reflectancia	833 - 2780	2,52	0,96	Sinelli et al., 2008
		Polifenoles totales				2,05	0,87	
		Ácido ascórbico				2,06	0,91	
	Manzana	Materia seca	Red de Diodos	Reflectancia	1000 - 2500	2,7	0,86	Travers et al., 2014
		Sólidos solubles				2,6	0,85	
	Manzana	Vitamina C	Monocromador	Reflectancia	400 - 2500	2,0	0,8	Pissard et al., 2012
		Polifenoles				5,1	0,94	
		Sólidos solubles				4,3	0,94	
	Melón	Chroma (C*)	Red de diodos	Reflectancia	400 -1700	5,22	0,96	Sánchez et al., 2014
		Hue (hº)				2,33	0,82	
	Melón	Sólidos solubles	Red de diodos	Reflectancia	400 - 1700	2,05	0,76	Flores et al., 2008
	Naranja	Sólidos solubles	Monocromador	Reflectancia	1000 - 2499	6,12	0,98	Liu et al., 2015
		Acidez				3,16	0,92	
		Vitamina C				1,84	0,90	
		Índice de madurez				3,49	0,77	
	Pera	Sólidos solubles	Monocromador	Reflectancia	400 - 1800	2,26	0,91	Li et al., 2013
		pH				2,11	0,87	
		Firmeza				2,18	0,87	
	Plátano	Carotenoides totales	Monocromador	Reflectancia	367- 2388	2,55	0,84	Davey et al., 2009
	Sandía	Sólidos solubles	Red de diodos	Reflectancia	400 - 1700	1,69	0,65	Flores et al., 2008

Tabla I.5.2. Aplicación de la tecnología NIRS en el control de calidad de hortalizas, cereales y plantas.

	Producto	Compuesto / Parámetro	Instrumento	Modo de análisis	Rango (nm)	Estadísticos		Referencia
						RPD	R^2_{cal}	
Hortalizas / Cereales / Planta	Arroz	Capacidad antioxidante (ABTS)	FT-NIR	Reflectancia	1000 - 2500	6,21	0,82	Wu et al., 2015
		Capacidad antioxidante (DPPH)				4,07	0,93	
	Bambú	Capacidad antioxidante (ABTS)	FT-NIR	Reflectancia	800 - 2500	3,50	0,96	Wu et al., 2012
		Capacidad antioxidante (DPPH)				1,97	0,86	
		Capacidad antioxidante (FRAP)				2,34	0,91	
	Calabacín	Carotenoides totales (exocarp)	Monocromador	Reflectancia	400 - 2500	4,55	0,90	Martínez-Valdivieso et al.,
		Carotenoides totales (mesocarp)				4,31	0,95	2014a
	Calabacín	Minerales (exocarp)	Monocromador	Reflectancia	400 - 2500	2,37	0,84	Martínez-Valdivieso et al.,
		Minerales (mesocarp)				2,00	0,70	2014b
	Calabaza	Arabinosa	FT-NIR	Reflectancia	650 - 2500	-	0,96	Kurz et al., 2010
		Galactosa				-	0,96	
		Glucosa				-	0,98	
		Xilosa				-	0,97	
	Té	Capacidad antioxidante (DPPH)	Monocromador	Reflectancia	1000 – 2500	4	0,89	Chin et al., 2012
	Tomate	Sólidos solubles	Monocromador	Reflectancia	325 - 1075	0,54	0,77	Szuvandzsiev et al., 2014
		Polifenoles totales				1,97	0,75	
		Licopeno				0,15	0,72	
	Pepino	Firmeza	Monocromador	Interactancia	550 - 1390	1,1	0,52	Kavdir et al., 2007
		Chroma (C*)				2,23	0,83	
		Hue (hº)				1,66	0,76	
		Materia seca				1,52	0,65	
	Quinoa	Polifenoles totales	Monocromador	Reflectancia	1100 - 2000	4,33	0,94	Moncada et al., 2013
		Capacidad antioxidante (DPPH)				4,54	0,95	

Capítulo II. Justificación del tema

Chapter II. Justification of the topic

II. JUSTIFICACIÓN DEL TEMA

En la actualidad existe una gran demanda por parte del consumidor de alimentos frescos, naturales, con un contenido prácticamente nulo de aditivos o incluso libre de ellos, manteniendo sus propiedades nutricionales y sensoriales.

Durante las últimas décadas se ha producido en los países industrializados un cambio el estilo de vida, siendo un constante objetivo por parte de las industrias agroalimentarias el desarrollo de nuevas técnicas de conservación que garantizasen, con una mínima preparación por parte del consumidor, la máxima calidad del producto. Esta situación, junto a la enorme competencia de otras zonas hortofrutícolas, con menores costes de producción y a las grandes variaciones de precios de dichos productos a lo largo del año, ha impulsado evolución del sector alimentario hacia nuevos sistemas y formatos de comercialización en aras de aumentar el valor añadido de los productos elaborados.

Las frutas y hortalizas mínimamente procesadas o de IV Gama son productos naturales y sanos, de alta calidad nutricional, sensorial y microbiológica y que requieren de poco tiempo de preparación. El procesado mínimo de las frutas y hortalizas puede incluir operaciones como selección, lavado, pelado, cortado, desinfección, enjuagado, secado, envasado y conservación para obtener un producto fresco, sano, saludable y listo para ser consumido. Por tanto, el interés en la investigación y desarrollo de estas tecnologías de procesado es una respuesta a las demandas expresadas por la sociedad actual, que valora el consumo de productos similares a los frescos, alejados de los productos altamente procesados cuyas calidades nutricionales y sensoriales se consideran inferiores.

El crecimiento de este sector en España ha sido más que evidente incluso en época de crisis económica, creciendo el volumen de ventas a raíz del 123% desde el 2004 (FEHRCAREM, 2015). Así, en el año 2014 el volumen comercializado de frutas y hortalizas de IV Gama en España ascendió a 81,5 millones de kilos (con un incremento del 4,9% frente al año anterior), de los cuales 79 millones de kilos corresponden a hortalizas IV Gama y 2,5 millones de kilos a frutas de IV Gama (MAGRAMA, 2015).

Sin embargo, aunque las frutas y hortalizas mínimamente procesadas adquieren un rápido protagonismo en los puntos de venta por la comodidad de uso que conllevan y

por su apariencia de frescura y calidad, se tratan de productos más perecederos que el material original del que provienen (Cantwell, 1992; Shlimme, 1994; Watada et al., 1996).

Por este motivo, el desafío de las industrias procesadoras de productos de IV Gama es considerablemente alto, ya que para poder mantener la calidad de los productos e incrementar su vida útil se han puesto a punto nuevos sistemas de procesado, la mayoría basados en la combinación de refrigeración y atmósferas modificadas (AM), así como el desarrollo de recubrimientos comestibles o materiales de envasado.

Después del tomate y el pimiento, el calabacín es la tercera hortaliza más importante en volumen de producción en Almería (principal zona exportadora de hortalizas hacia Europa) alcanzando una superficie producción de 5 789 has que dan lugar a unas 354 156 Tn (Consejería de Agricultura, 2015). Este enorme volumen de producción es posible gracias a que Almería posee el 98 % de su superficie con el sistema de producción bajo invernadero, permitiendo este sistema de producción intensivo obtener hasta tres ciclos consecutivos de cultivo dentro de una misma campaña.

En relación a esta hortaliza, pocos son los trabajos realizados con calabacín en formato mínimamente procesado (Izumi y Watada, 1995; Izumi et al., 1996; Reyes et al., 2007), siendo inexistente en la literatura científica sobre la selección de genotipos/variedades, estados de madurez, momentos de recolección adecuados o sistemas que evalúen el efecto del envasado en atmósfera modificada que incluyan el empleo de recubrimientos comestibles a lo largo de la conservación del producto.

Por otro lado, la preocupación creciente por la calidad de los productos de consumo en fresco ha permitido que evolucionen las exigencias de los mercados y los consumidores hacia una industria alimentaria con una trazabilidad marcada. Entre los sistemas de control de calidad emergentes se encuentra la espectroscopía por reflectancia en el infrarrojo cercano (NIRS), definida como una técnica espectroscópica vibracional molecular no destructiva caracterizada por ser rápida, no contaminante, de gran precisión y exactitud, y considerada como una alternativa que puede sustituir y complementar a determinados métodos analíticos tradicionales (Marten y Naes, 1989; Garrido et al., 1993).

Además, debido a que es una tecnología relativamente nueva en España (década de los sesenta) (Garrido et al., 2000, Van Kempen, 2001) se encuentra en continuo

crecimiento, siendo recientes y en reducida cantidad los trabajos que reporten análisis de compuestos nutricionales con esta técnica, y hasta el presente ningún trabajo previo correspondiente a calabacín.

Por tanto, en este Trabajo de Investigación de Tesis Doctoral se plantea la puesta en valor de una de las hortalizas con más potencial en la actualidad, y compleja desde el punto de vista de su transformación y conservación en IV Gama. Para lo cual se estudiarán y evaluarán diversos morfotipos y variedades de calabacín por su potencial como producto de IV Gama, así como la aplicación de la tecnología NIRS y de diversas atmósferas modificadas destinadas a preservar la calidad físico-química, nutricional y microbiológica del producto transformado al objeto de aumentar la vida útil del mismo, abriendo así la puerta a un producto competitivo con mayores posibilidades de mercado.

II. JUSTIFICATION OF THE TOPIC

Consumers currently demand fresh and natural commodities practically without additives or even free of them, as well as maintaining their nutritional and sensory properties.

In the past decades, the industrialized countries have experienced an evolution in the consumer's lifestyle. The development of innovative food conservation techniques has been one of the main goals for the agrifood industry, in order to ensure the quality of the product throughout the shelf-life. This situation, with the rise in competitive markets from other horticultural areas with lower input production costs and the price market fluctuations during the year, has contributed to the evolution of the agrifood industry to new marketing systems and formats to increase the added value of these products.

Minimally processed fruit and vegetables are natural and healthy products with a high nutritional, sensorial and microbial quality in which a short preparation time is required. The minimal processing includes several operations such as selection, washing, peeling, cutting, disinfection, rinsing, drying, packaging and storage resulting in healthy beneficial fresh product ready to eat. Therefore, the interest in new processing technologies is the response to consumer's demands for fresh products with natural appearance, away from those highly processed products with important losses in the nutritional and sensory quality.

In Spain, the growth trend in this sector has been more than evident even during the economic crisis, increasing the ready-to-eat volume sale around 123% since 2004 (FEHRCAREM, 2015). Thus, in 2014 the minimally processed products sales in volume was up to 81.5 million kilos (increasing in 4.9% from the previous year), corresponding 79 million kilos to vegetables and 2,5 million kilos to fruit (MAGRAMA, 2015).

Although minimally processed products have experienced a record growth in sale points due to their preparation for consumption showing also a freshness in appearance and quality, they are more perishable products than the original one (Cantwell, 1992; Shlimme, 1994; Watada et al., 1996).

That is why, the challenge of the agrifood industry in maintaining quality and extending the product's shelf-life is considerably high due to the fact that new packaging systems are required, most of them consisting in the combination of modified atmosphere packaging (MAP) with refrigeration, as well as edible coatings or food packaging materials.

After tomato and pepper, summer squash is the third most produced vegetable in Almería (the main vegetable production area in Europe) with 354 156 Tm in the area of 5 789 Has (Consejería de Agricultura, 2015). This high volume is possible because in Almería 98 % of the area is under greenhouses, allowing this intensive production system to obtain up to three consecutive crop cycles within the same campaign.

In this regard, little information is available concerning summer squash fresh cut (Izumi and Watada, 1995; Izumi et al, 1996; Reyes et al, 2007; Lucera et al., 2010), being limited in the scientific literature studies about genotypes/varieties and maturity stages selection, optimal harvest date or systems to evaluate the effect of modified atmosphere packaging including edible coatings for preserving the quality of the product.

On the other hand, the growing concern of fresh product quality contributed to the development of a new food industry with a well-run traceability system. Among the emerging quality control technology systems are the Near Infrared Reflectance Spectroscopy (NIRS), defined as a spectroscopic molecular vibrational non-destructive technique characterized by rapid, non-polluting, high precision and accuracy, and being considered as a promising alternative for replacing or using in combination with the traditional analytical methods (Marten and Naes, 1989; Garrido et al., 1993).

In addition, due to the fact that NIRS is a relatively new technology in Spain (created during the 60's) (Garrido et al., 2000, Van Kempen, 2001) it is in continuous growth, being recent and in little number the studies that use this technique for nutritional quality control, non-existent until now in summer squash.

Therefore, this Doctoral Thesis is focused on the study of summer squash, a vegetable with a high potential, and difficult from the processing point of view. Therefore, we study and evaluate the suitability of several summer squash cultivars to be processed as a fresh cut product, the application of NIRS for predicting its quality attributes and we point out the advantages of different MAP conditions to preserve the physical, chemical, nutritional and microbiological quality as well as for extending the shelf-life, thus offering the opportunity to new competitive markets access.

Capítulo III. Objetivos

Chapter III. Objectives

III. OBJETIVOS

3.1. OBJETIVO GENERAL

Así, el principal objetivo de esta Tesis Doctoral ha sido aumentar la competitividad y las posibilidades de mercado de calabacín fresco, mediante el estudio del potencial de este producto para ser transformado a un producto de IV Gama.

3.2. OBJETIVOS ESPECÍFICOS

3.2.1. Análisis de la variabilidad genotípica de calabacín (*Cucurbita pepo* spp.) y selección de aquellos morfotipo/s que presenten un mayor potencial desde el punto de vista nutricional.

3.2.2. Influencia de factores precosecha (variedad, estado de madurez y momento de recolección) en la calidad del calabacín en fresco.

3.2.3. Influencia de factores precosecha (variedad y estado de madurez) y poscosecha (formato de corte, temperatura de conservación, atmósferas de conservación y tiempo de conservación) en la calidad del producto IV Gama.

3.2.4. Aplicación de sistemas activos (recubrimientos comestibles) que mejoren la calidad del calabacín procesado en IV Gama tanto en crudo como tras su cocinado.

3.2.5. Potencial de la tecnología NIRS para la predicción de compuestos nutricionales en calabacín.

III. OBJECTIVES

3.1. GENERAL OBJECTIVE

The main objective of this Doctoral Thesis is to increase the competitiveness and the opportunities of fresh summer squash in the market, by studying the potential of this product to be minimally processed.

3.2. SPECIFIC OBJECTIVES

3.2.1. Analyze the genotypic diversity of summer squash (*Cucurbita pepo* spp.) to select those with the best properties since the nutritional point of view.

3.2.2. Influence of preharvest factors (variety, maturity stage and harvest date) on the quality of fresh summer squash.

3.2.3. Influence of both preharvest (variety and maturity stage) and postharvest factors (cutting type, storage temperature, atmosphere conditions and storage time) on the quality of the fresh cut summer squash.

3.2.4. Use of active systems (edible coatings) to preserve the quality of summer squash after processing and cooking.

3.2.5. Potential of NIRS for predicting nutritional compounds in summer squash fruits.

Capítulo IV. Resultados y discusión

Chapter IV. Results and discussion

4.1

'Genotypic diversity of natural pigments and phytochemical compounds from exocarp and mesocarp of 27 Cucurbita pepo genotypes'

María Teresa Blanco-Díaz[a], Rafael Font[a], Damián Martínez-Valdivieso[b], Mercedes Del Río-Celestino[b]

[a] Department of Postharvest Technology and Food Industry.
[b] Department of Plant Breeding and Crop Biotechnology - IFAPA Centro La Mojonera - Camino San Nicolás, 1, 04745 La Mojonera, Almería, Spain

Submitted - Under review

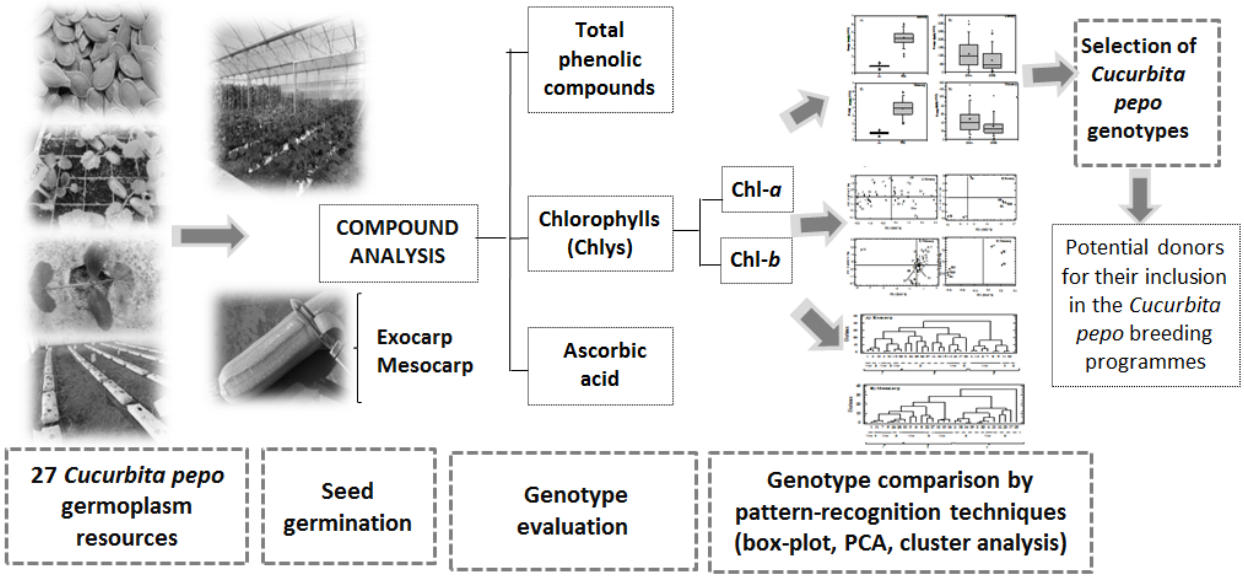
Abstract

The genotypic variability in crop species is crucial for crop improvement and consequently enables the development of more new valuable agricultural products. This study aimed to evaluate the ascorbic acid (AA), chlorophyll *a* (Chl-A), chlorophyll *b* (Chl-B), total phenolic compounds (TPC) as well as dry matter (DM) in different tissues (exocarp and mesocarp) of *Cucurbita pepo* fruits belonging to a 27 morphologically diverse germplasm accessions (14 traditional genotypes and 13 commercial hybrids from different countries). DM content ranged from 4.2 to 11.76%, while natural pigments were between 2602.2 and 5.85 µg/g DW (corresponding to Chl-A and Chl-B, respectively). Other antioxidant compounds registered higher values, 1.29 to 0.47 mg/g DW for AA and 5.49 to 1.98 mg/g DW for TPC. Additionally, pattern-recognition techniques including distribution, principal component analysis (PCA) and dendogram cluster analysis were used to compare accessions. PCA revealed that the first two components represented 76% and 84% of the total variability in exocarp and mesocarp tissues, respectively; while clusters regrouped more interesting genotypes with elevated values in chlorophylls, AA and TPC. Exocarp samples exhibited significantly higher content in all compounds studied, suggesting the fruit peel consumption from a functional point of view. This work also highlights the potential contribution of 'zucchini' morphotype to dietary intake requirements, being moderate in 'vegetable marrow' and 'pumpkin'. Genotypes rich in AA, Chl-A, Chl-B and TPC like those namely Ac-2, Ac-8, Ac-23 and Ac-27 could be exploited as potential donors for novel variety development in future breeding programmes.

Keywords: *Cucurbita pepo;* exocarp and mesocarp; ascorbic acid; chlorophylls; total phenolic compounds; recommended dietary allowance.

Abbreviations: AA: ascorbic acid; ANOVA: analysis of variance; Chl-A: chlorophyll *a*; Chl-B: chlorophyll *b*; CV: coefficient variation; DM: dry matter; DW: dry weight; FW: fresh weight; LSD: least significant differences; P: 'pumpkin'; PCA: principal component analysis, PC: principal component; RDA: recommended dietary allowance; SD: standard deviation; TPC: total phenolic compounds; VM: 'vegetable marrow'; Z: 'zucchini'.

Graphical abstract



1. Introduction

In recent decades, agriculture globalization and modernization in many developing countries has encouraged a simplification in the nutritional intake by consumers resulting in nutritional deficiencies promoting diets low in diversification but high in energy.

Cucurbitaceae is a large family including diverse groups which are originated from the tropics of the American continent, being introduced in Europe (about 500 years ago) (Whitaker, 1947). The *Cucurbita* genus comprises several species, five domesticated (*Cucurbita argyrosperma, Cucurbita ficifolia, Cucurbita maxima, Cucurbita mostacha* and *Cucurbita pepo*) and ten wild species (Robinson and Decker-Walters, 1997), with the *C. pepo* subspecie *pepo* the highest variability (Nesom, 2011). Additionally, four morphotypes widely grown around the world (pumpkin, vegetable marrow, cocozelle and zucchini) are included in the supspecie *pepo* (Ferriol et al., 2003; Paris et al., 2003).

Cucurbita pepo is mainly commercialized in countries around the Mediterranean Sea, such as Spain, Italy, France, Turkey and Greece. In particular, Almería (Southern Spain) is the main area of vegetable production in Europe, summer squash being one of the most important vegetable in production volume.

On the other hand, *Cucurbita pepo* is one of the seasonal vegetables that takes part of healthy nutrition due to it is low in calories, high in nutritional contains and medical value (Shokrzadeh et al., 2010) attributing these beneficial properties to its antioxidant/anti-radical, anticarcinogenic, anti-inflammatory, antiviral, and antimicrobial activities (Oloyede et al., 2012). In this respect, *Cucurbita pepo* is an excellent source of both natural pigments and antioxidant constituents such as chlorophyll *a* (Chl-A), chlorophyll *b* (Chl-B), total phenolic compounds (TPC) and ascorbic acid (AA) that play an important role in visual appearance and the nutritional value in the human diet (Tadmor et al., 2005; Blanco-Díaz et al., 2014). Furthermore, several epidemiological studies have indicated that high intake of these phytochemicals are associated with a reduced risk of a number of chronic diseases, such as atherosclerosis and cancer (Gundgaard et al., 2003; Gosslau and Chen, 2004).

In recent years, the evaluation of nutritional compounds in fruits belonging to *Cucurbitaceae* family has motivated works as those about dry matter, soluble sugars, and vitamin C on cucumber (Huang et al., 2009); carotenoids content on melon (Condurso et al., 2012); ascorbic acid and antioxidant activity on gourd (Karmakar et al., 2013). In this context, different nutritional properties have been recently attributed to edible parts of *Cucurbita pepo*, such fatty acids, carotenoids and vitamins on seeds (Procida, 2013); ash, crude protein, fiber, fat and protein free extract on edible flowers (Sotelo et al., 2007), or mineral composition on tissues of fruits (Villora et al., 2000; Martínez-Valdivieso et al., 2014).

However, as far as we know limited scientific literature referring to natural pigments and antioxidant compounds in *Cucurbita pepo* fruit is available, in spite of being the development of new varieties with high nutritional compounds one of the main goals for both consumers and plant breeding programmes. For this purpose, in the present study we have characterized and investigated the ascorbic acid, chlorophylls and total phenolic compounds as well as dry matter of a wide *Cucurbita pepo* fruits collection, distinguishing between exocarp and mesocarp tissues, corresponding to twenty seven traditional and marketed cultivars presenting different external coloration. Therefore, this study would provide a way of allowing breeders to select those superior genotypes for showing a high natural pigments and phytochemical compounds.

The objectives of the present work were to i) identify the variation in natural pigments and phytochemical composition of exocarp and mesocarp summer squash tissues ii) detect similarities among *Cucurbita pepo* genotypes and point out morphotype selection advantages, and iii) study the potential contribution of the different *Cucurbita pepo* morphotypes to required daily intake.

2. Material and methods

2.1. Plant material

A total of 27 genotypes of a *Cucurbita pepo* spp *pepo* germplasm world collection (from the European Central Cucurbits germoplasm database collection developed at COMAV and other countries) currently kept at the 'IFAPA Centre La Mojonera' were evaluated in this work **(Table IV.1.1).**

Table IV.1. 1 Botanical classification, morphotype, geographic origin and source of germplasm of 27 *Cucurbita pepo* genotypes included in this study.

Accession	Subspecies	Morphotype	Country of origin (Estate/Region)	Source of germoplasm
Ac-1	spp. pepo	vegetable marrow	Spain (Aragón)	COMAV
Ac-2	spp. pepo	zucchini	Spain (Aragón)	COMAV
Ac-3	spp. pepo	zucchini	Spain (Andalucía)	COMAV
Ac-4	spp. pepo	vegetable marrow	Spain (Andalucía)	COMAV
Ac-5	spp. pepo	vegetable marrow	Spain (Andalucía)	COMAV
Ac-6	spp. pepo	vegetable marrow	Spain (Asturias)	COMAV
Ac-7	spp. pepo	vegetable marrow	Spain (Canarias)	COMAV
Ac-8	spp. pepo	vegetable marrow	Spain (Cataluña)	COMAV
Ac-9	spp. pepo	vegetable marrow	Spain (Cataluña)	COMAV
Ac-10	spp. pepo	vegetable marrow	Spain (Cataluña)	COMAV
Ac-11	spp. pepo	pumpkin	Spain (Cataluña)	COMAV
Ac-12	spp. pepo	zucchini	Spain (Andalucía)	Commercial hybrid
Ac-13	spp. pepo	vegetable marrow	Spain (Unknown)	COMAV
Ac-14	spp. pepo	zucchini	Spain (Andalucía)	Commercial hybrid
Ac-15	spp. pepo	vegetable marrow	USA (Arizona)	USDA
Ac-16	spp. pepo	vegetable marrow	Israel (Unknown)	Israel
Ac-17	spp. pepo	zucchini	Spain (Andalucía)	Commercial hybrid
Ac-18	spp. pepo	zucchini	Spain (Andalucía)	Commercial hybrid
Ac-19	spp. pepo	zucchini	Spain (Andalucía)	Commercial hybrid
Ac-20	spp. pepo	zucchini	Spain (Andalucía)	Commercial hybrid
Ac-21	spp. pepo	zucchini	Spain (Andalucía)	Commercial hybrid
Ac-22	spp. pepo	zucchini	Spain (Andalucía)	Commercial hybrid
Ac-23	spp. pepo	zucchini	Spain (Andalucía)	Commercial hybrid
Ac-24	spp. pepo	zucchini	Spain (Andalucía)	Commercial hybrid
Ac-25	spp. pepo	zucchini	Spain (Andalucía)	Commercial hybrid
Ac-26	spp. pepo	zucchini	Spain (Andalucía)	Commercial hybrid
Ac-27	spp. pepo	zucchini	Spain (Andalucía)	Commercial hybrid

Summer squash samples used in this work were representative of traditional and commercial cultivars and were classified as follow: 14 traditional genotypes from different regions of Spain (4 belonging to 'Zucchini' morphotype, 9 to 'Vegetable marrow' morphotype, and 1 to 'Pumpkin' morphotype) which are cultivated in small orchards used for self-consumption; 1 genotype from Israel ('Vegetable marrow' morphotype) and 1 genotype from the United States ('Vegetable marrow' morphotype); 13 commercial hybrids belonging to the 'Zucchini' morphotype representatives of the main commercial varieties currently offered in the market.

Seeds of cited genotypes were germinated on wet filter paper in Petri dishes at room temperature for 2-4 days in dark conditions and they were transplanted into rock-wool cubes (Grodan BV, 6040KD Roermond, NL) in a greenhouse. When plants developed 3-4 leaves they were transferred to 1 m large rock-wool slabs at 2 plants/slab of density. Plants were grown in a greenhouse in the IFAPA Centre - La Mojonera, Almería, Spain

(36º 46' N, 2º 48' O) during 2011 following standard local cultural practices for both plant nutrition and insect pest and disease control.

Fruits (6-10) of each genotype were harvested at an immature stage because they are marketed this way (14 to 20 cm). The vegetables used in this work showed a good visual appearance without surface defects, matching the generally accepted commercial standards for this product.

Each fruit employed in this study was manually peeling to separate exocarp from mesocarp and these were immediately frozen in individual plastic bags at -80 ºC until analysis in dark conditions.

2.2. Genotypic variability in Cucurbita pepo

2.2.1. Dry matter

Freeze-drying was used for the determination of dry matter. Sample lyophilization was performed using freeze drier equipment (Telstar LyoQuest, Terrasa, Spain) at -55ºC under vacuum (133 x 10^{-3} mBar) for 96 h per sample. Then, the samples were ground and frozen at -80ºC for further analysis.

2.2.2. Ascorbic acid

The reference values for AA were obtained using an automatic titration (Metrohm, 862 Compact Titrosampler, Metrohm, US) by the iodine titration method (Blanco-Díaz et al., 2014).

Thus, 250 mg of freeze-dried sample was mixed with distilled deioniser water until final weight of 50 g and treated with 2 mL glyoxal solution (40%) (Sigma-Aldrich, Munich, Germany), stirred briefly and allowed to stand for 5 min. After the addition of 5 mL sulfuric acid (25%) (Merck, Darmstadt, Germany), it was titrated with iodine (0.01 mol/L) (Fisher Scientific, Loughborough, UK) up to the endpoint (EP1). The linearity of the method was determined using AA as an external standard (> 99% pure) (Sigma-Aldrich, Munich, Germany). Triplicate titrations were made for each standard solution. The regression equation and the regression coefficient (r^2=0.9997) (Sigma-Aldrich, Munich, Germany) values were obtained. Finally, the ascorbic acid content was expressed as mg/g dry weight (DW). All measurements were made in triplicates.

2.2.3. Chlorophyll *a* and *b*

The extraction and analysis of the chlorophylls pigments were carried out simultaneously, in order to avoid pigment degradation. Chlorophyll pigments were determined using a UV–Visible spectrometer (Thermo Fisher Scientific, Madison, Wisconsin, USA) and both chlorophylls, *a* and *b*, were determined according to the equations reported by Lichtenthaler and Wellburn (1983) using the method described by Blanco-Díaz et al. (2014).

Chlorophyll *a* =11.75 A_{662} - 2.35 A_{645}

Chlorophyll *b* =18.61 A_{645} - 3.96 $_{A662}$, where A is absorbance

Eight mL of acetone (Sigma-Aldrich, Munich, Germany) containing butylated hydroxytoluene (BHT) (1 mg/ml) (Sigma-Aldrich, Munich, Germany) were added to 150 mg of freeze-dried sample and were shaken by vigorous vortexing followed by ultrasonic bath during 30 min and finally centrifugated at 4000 rpm (Megafuge 1.0R, Heraeus Instruments, DJB Labcare, UK) for 10 min. This procedure was repeated until the sample became colorless. Then, an aliquot was taken from the supernatant for measurement of optical density at 662 and 645 nm in the spectrophotometer using quartz cuvettes with a cell path length of 1.0 cm. Finally, chlorophylls pigments *a* and *b* were expressed as μg/ g DW. All measurements were made in triplicates.

2.2.4. Total phenolic compounds

The concentrations of TPC in the *Cucurbita pepo* samples were obtained by the Folin-Ciocalteu reagent method using gallic acid as external standard (Blanco-Díaz et al., 2014).

To do this, 150 mg of freeze-dried sample was mixed with 5 ml of methanol (80%) (Panreac, Madrid, Spain) and was shaken by vigorous vortexing followed by centrifugation during 10 min at 4000 rpm. Then, 1 ml of the supernatant was mixed with 9 ml of distilled water in a falcon flask. An aliquot (1 ml) of extracts or standard solution of gallic acid was added to 25 ml volumetric flask, containing 9 ml of distilled deionised water. Then, 1 ml of Folin–Ciocalteu's phenol reagent (Sigma-Aldrich, Munich, Germany) was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution (Panreac, Madrid, Spain) was added to the mixture. The solution was diluted to volume (25 ml) with distilled deionised water. The solution was incubated at room temperature in the dark for 90 min, and the absorbance was read at 750 nm against a blank solution using quartz cuvettes with a cell path length of 1.0 cm. The regression equation and the regression coefficient (r^2=0.9984) values were obtained for the gallic acid (Sigma-Aldrich, Munich, Germany). Finally, results were reported in gallic acid equivalents (mg/g DW). All measurements were made in triplicates.

2.3. Statistical Analysis

To assess the variability of nutritional composition between genotypes, analysis of variance (ANOVA) with type III sums of squares was performed using the GLM (General Linear Model) procedure of the LSD (Least Significant Differences test) was used to compare means, and significance was accepted at $P \leq 0.05$ level. Dry matter, ascorbic acid, chlorophyll *a* and *b* and total phenolic compounds in both exocarp and mesocarp of the 27 *Cucurbita pepo* genotypes were subject to principal component analysis (PCA) and cluster dendogram analysis on the basis of distances computed. Statistical analysis was performed using SAS for Windows and Statgraphics plus 5.0 (Statistical Graphics Corp., Rockville, MD, USA).

3. Results and discussion

3.1. Dry matter

The descriptive analysis (mean, range, standard deviation and coefficient of variation) as well as statistical ANOVA of DM are summarized for exocarp **(Table IV. 1. 2)** and mesocarp **(Table IV. 1. 3)** *Cucurbita pepo* tissues.

Table IV.1. 2. Descriptive analysis (mean, minimum, maximum, standard deviation and coefficient variation) of dry matter, ascorbic acid (AA), chlorophyll a (Chl-A), chlorophyll b (Chl-B), and total phenolic compounds (TPC) in exocarp tissue from 27 *Cucurbita pepo* accessions.

	Exocarp				
Accession	**Dry matter (%)**	**AA (mg/g DW)**	**Chl-A (µg/g DW)**	**Chl-B (µg/g DW)**	**TPC (mg/g DW)**
Ac-1	6.92 d-g	0.88 c-f	1771.0 b-d	922.2 d-f	4.19 g
Ac-2	6.09 d-g	0.78 e-g	2128.6 a-c	1701.9 a-c	4.52 a-e
Ac-3	6.66 d-g	0.74 f-g	1333.3 d-h	799.3 e-h	4.69 a-c
Ac-4	6.91 d-g	0.87 c-f	364.0 i-k	264.6 h-j	4.03 b-g
Ac-5	6.11 d-g	0.86 d-f	2186.5 a-b	838.7 e-g	3.98 b-g
Ac-6	6.97 d-g	0.80 e-g	1052.0 e-i	328.8 g-j	4.02 b-g
Ac-7	7.90 b-f	0.98 a-e	570.8 i-k	151.4 j	5.29 a
Ac-8	5.64 e-g	0.75 e-g	1064.2 e-i	256.9 h-j	4.84 a-b
Ac-9	5.13 d-e	0.75 f-g	804.9 g-j	325.2 g-j	4.55 a-e
Ac-10	6.16 d-g	0.78 e-g	1536.2 b-f	670.2 e-j	3.31 g
Ac-11	5.45 d-e	0.86 d-f	365.0 i-k	247.2 i-j	4.98 a-b
Ac-12	7.47 c-f	0.68 f-h	1348.2 d-h	1429.2 b-d	4.08 b-g
Ac-13	5.95 d-g	0.78 e-g	38.1 k	311.8 g-j	4.47 a-e
Ac-14	8.22 b-e	0.42 i	1771.0 b-d	1200.8 c-e	3.34 g
Ac-15	10.14 a-c	0.81 d-g	426.1 i-k	431.7 f-j	3.26 g
Ac-16	8.27 b-e	0.80 e-g	1430.7 c-g	782.1 e-i	3.59 d-g
Ac-17	8.66 b-d	0.77 e-g	948 f-j	576.2 h-j	3.19 g
Ac-18	11.76 a	0.46 h-i	1857.1 b-d	1206.2 c-e	3.37 f-g
Ac-19	11.59 a	0.50 h-i	1357.7 d-h	732.2 e-i	3.99 b-g
Ac-20	7.76 b-f	1.03 a-d	2590.7 a	1872.6 a-b	3.22 g
Ac-21	6.48 d-g	0.77 e-g	2115.6 a-c	960.4 d-f	3.53 d-g
Ac-22	4.20 e	0.69 e-g	1743.2 b-e	798.2 e-h	3.64 c-g
Ac-23	10.63 a-b	1.11 a-b	246.8 j-k	776.2 e-i	4.43 a-f
Ac-24	6.54 d-g	0.63 g-i	1636.8 b-f	2168.1 a	3.51 e-g
Ac-25	5.54 d-e	0.98 a-e	702.5 h-k	545.6 f-j	4.57 a-d
Ac-26	6.38 d-g	1.09 a-c	1868.8 b-d	1856.0 a-b	4.21 b-g
Ac-27	5.78 d-g	1.21 a	2602.2 a	1610.1 b-c	4.88 a-b
Range	4.2-11.76	0.42-1.21	38.1-2602.2	151.4-2168.1	3.19-5.29
Mean	7.23	0.81	1328.14	880.14	4.06
SD	1.92	0.18	721.52	570.55	0.61
CV (%)	26.61	23.06	54.32	64.82	15.09

SD= standard deviation

CV= coefficient variation

Means followed by different letters are statistically different according LSD test at $p \leq 0.05$

Table IV.1. 3. Descriptive analysis (mean, minimum, maximum, standard deviation and coefficient variation) of dry matter, ascorbic acid (AA), chlorophyll *a* (Chl-A), chlorophyll *b* (Chl-B), and total phenolic compounds (TPC) in mesocarp tissue from 27 *Cucurbita pepo* accessions.

	Mesocarp				
Accession	**Dry matter (%)**	**AA (mg/g DW)**	**Chl-A (µg/g DW)**	**Chl-B (µg/g DW)**	**TPC (mg/g DW)**
Ac-1	5.16 e-h	0.82 c-g	42.44 c-f	12.27 e-f	3.43 c-g
Ac-2	6.72 b-c	0.92 b-e	78.05 b-c	25.67 e-f	3.94 a-f
Ac-3	5.90 b-f	0.95 b-d	66.05 b-e	34.42 d-f	3.82 b-f
Ac-4	6.07 b-e	0.99 a-c	35.37 c-f	26.58 e-f	3.51 c-g
Ac-5	4.50 h	0.64 e-h	51.00 b-f	14.90 e-f	2.99 d-g
Ac-6	4.79 f-h	0.66 d-h	94.98 b	66.26 b-c	3.73 b-f
Ac-7	5.38 d-h	0.67 d-h	48.16 b-f	31.58 d-f	3.86 a-f
Ac-8	5.42 d-h	0.86 b-e	12.52 f	90.76 b	5.00 a-c
Ac-9	4.90 e-h	0.93 b-e	30.18 c-f	20.56 e-f	4.07 a-e
Ac-10	5.66 c-h	0.82 c-g	37.01 c-f	27.11 e-f	4.18 a-d
Ac-11	5.50 c-h	0.76 c-h	48.45 b-f	26.33 e-f	3.47 c-g
Ac-12	6.04 b-e	1.12 a-b	50.40 b-f	26.45 e-f	5.26 a-b
Ac-13	5.10 e-h	0.54 g-h	67.98 b-d	31.23 d-f	2.36 f-g
Ac-14	6.43 b-d	1.01 a-c	24.31 d-f	29.10 e-f	4.21 a-d
Ac-15	5.75 c-g	0.47 h	74.52 b-c	18.62 e-f	1.98 g
Ac-16	5.42 d-h	0.55 g-h	21.84 d-f	11.26 e-f	2.05 g
Ac-17	8.32 a	1.02 a-c	45.85 c-f	28.21 e-f	5.49 a
Ac-18	7.01 b	0.93 b-e	58.38 b-f	25.49 e-f	4.68 a-c
Ac-19	6.58 b-d	0.93 b-e	20.85 d-f	37.45 c-e	4.51 a-d
Ac-20	6.08 b-e	0.92 b-e	54.86 b-f	28.32 e-f	3.62 b-g
Ac-21	5.76 c-g	1.02 a-c	18.94 e-f	5.85 f	4.26 a-d
Ac-22	4.63 g-h	1.14 a-b	57.52 b-f	61.20 b-d	5.05 a-c
Ac-23	6.98 b-e	1.29 a	15.94 f	34.61 d-f	4.25 a-d
Ac-24	5.09 e-h	0.85 b-f	42.83 c-f	26.66 e-f	4.32 a-d
Ac-25	4.54 g-h	0.92 b-e	470.35 a	312.02 a	2.52 e-g
Ac-26	5.03 e-h	0.89 b-e	64.62 b-e	30.93 d-f	3.83 b-f
Ac-27	4.43 h	0.93 b-e	40.38 c-f	67.87 b-c	4.42 a-d
Range	4.43-8.32	0.47-1.29	12.52-470.35	5.85-312.02	1.98-5.49
Mean	5.67	0.87	61.99	42.65	3.88
SD	0.91	0.19	84.12	57.01	0.91
CV (%)	16.12	21.83	135.70	133.66	23.52

SD= standard deviation
CV= coefficient variation
Means followed by different letters are statistically different according LSD test at $p \leq 0.05$.

Dry matter (DM) values in summer squash exocarp (mean of 7.23%) **(Table IV. 1. 2)** were higher than in mesocarp (mean of 5.67%), increasing exocarp DM values by ~30%. Also a wide range in DM content was found in the exocarp tissue (4.2 to 11.76%) (CV= 26.61%) (Table 2), showing higher values in the exocarp genotypes from Ac-18 (11.76%) > Ac-19 (11.59%) > Ac-23 (10.63%) > Ac-15 (10.14%), and lower content in the exocarp genotypes from Ac-22 (4.20%) < Ac-9 (5.13%) < Ac-11 (5.45%) < Ac-25 (5.54%). On the contrary, less range in DM than those described

previously was observed in the mesocarp of the samples (4.43 to 8.32%) (CV= 16.12%) **(Table IV.I. 3)**, displaying the greater values in the mesocarp genotypes from Ac-17 (8.32%) > Ac-18 (7.01%) > Ac-2 (6.72%) > Ac-19 (6.58%), while the lower DM content was found in the mesocarp genotypes from Ac-27 (4.43%) < Ac-5 (4.50%) < Ac-25 (4.54%) < Ac-22 (4.63%). In addition, considering the whole *Cucurbita pepo* fruit (both exocarp and mesocarp tissues), the greatest variability was found in 'zucchini' due to this morphotype registering the highest (Ac-18 and Ac-19) and the lowest (Ac-22 and Ac-25) summer squash DM contents.

These values are in agreement with fruits from 'zucchini' morphotype in which DM studied were close to 4.8% (Mattila and Hellström, 2007), while winter squash fruits displayed higher DM content ~10% (Conti et al., 2015). On the other hand, similar trends (referring to $DM_{exocarp} > DM_{mesocarp}$) in *Cucurbita pepo* fruits described in this work were achieved in squash fruits (*Cucurbita maxima*) by other authors (Jacobo-Valenzuela et al., 2011).

In accordance with the DM limits established by Corrigan et al. (2006) in other *Cucurbita* samples, DM values below 11.3% are considered as low, values between 11.3-19.6% can be recognized as medium, and those between 21.1-33.2% are described as high content. In this study, after averaging DM of both tissues, *Cucurbita pepo* morphotypes were classified as follows, zucchini (6.77%) > vegetable marrow (6.12%) > pumpkin (5.47%), all of them belonging to the low DM content group.

The differences in DM among accessions may be due to the genetic and agro-ecological factors, DM being an important parameter for presenting positive correlations with sensory and textural attributes in *Cucurbita* fruits (Corrigan et al., 2001). Therefore, it seems reasonable to believe that cultivated or commercial hybrid *Cucurbita pepo* accessions with higher DM (~7%) will be more appreciated than traditional genotypes (DM ~6%) by consumers.

3.2. Ascorbic acid

Differences in AA content in the investigated traditional and commercial cultivars from *Cucurbita pepo* germoplasm tissues are shown for exocarp **(Table IV.1.2 and Fig. IV. 1.1A)** and mesocarp tissues **(Table IV.1.3 and Fig. IV.1.1C).**

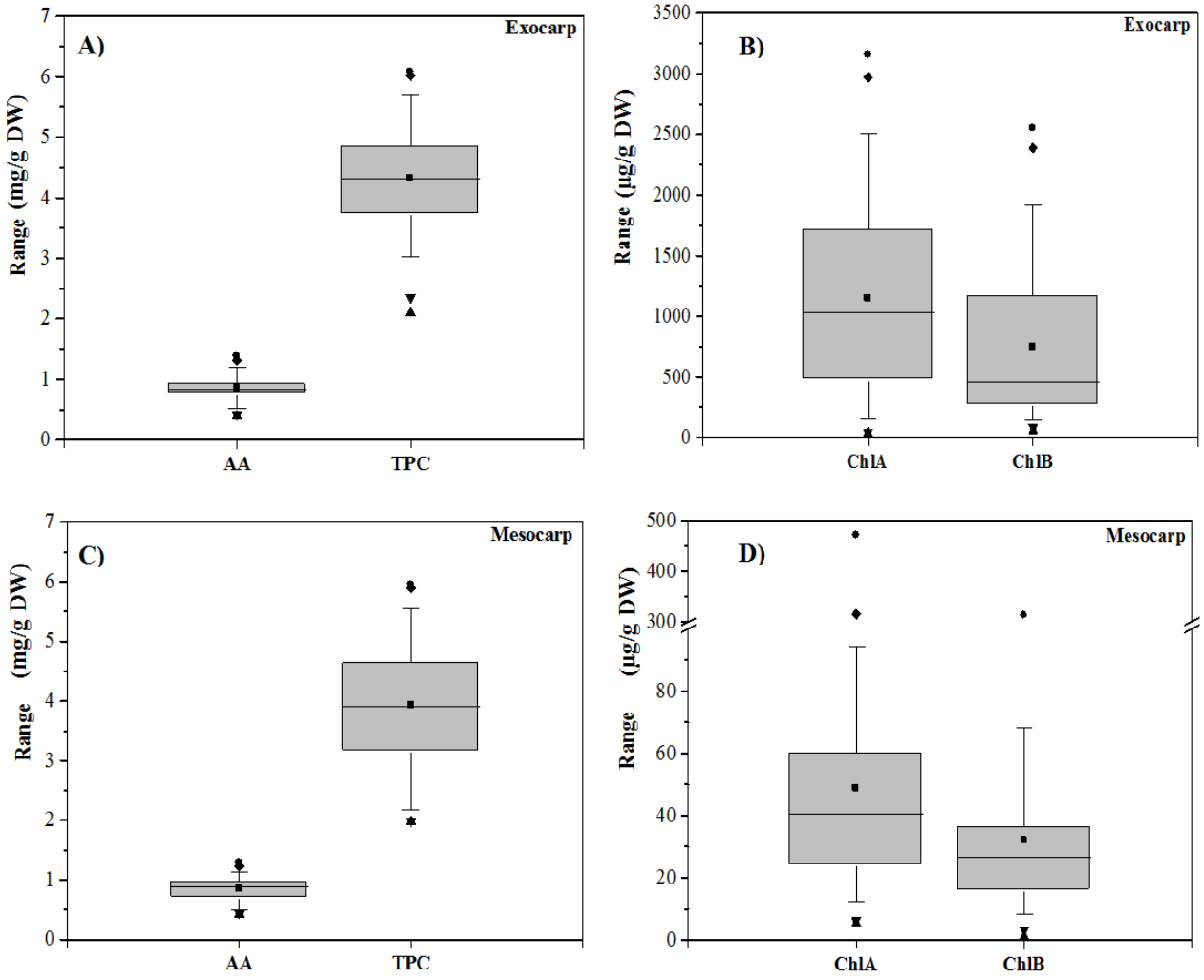


Figure IV.1. 1. Box and whisker plots of 27 *Cucurbita pepo* accessions in exocarp tissues for (A) ascorbic acid and total phenolic compounds and (B) chlorophyll *a* and chlorophyll *b*; and in mesocarp tissues for (C) ascorbic acid and total phenolic compounds and (D) chlorophyll *a* and chlorophyll *b*. The line in the box represents the median while the top and bottom of the box represent the 75th percentile and 25th percentile respectively. The ends of the whiskers represent the minimum and maximum of the data.

(0.81±0.18 vs. 0.80±0.19 mg/g DW, respectively). However, AA values in exocarp registered higher variability than those from mesocarp samples ($CV_{exocarp}$= 23.06% vs. $CV_{mesocarp}$= 21.83%, respectively). As expected, approximate range in AA content was obtained in both exocarp and mesocarp tissues (0.42 to 1.21 and 0.47 to 1.29 mg/g DW, respectively).

Evaluating the AA (mg/g DW) content in the *Cucuribita pepo* exocarp genotypes, higher values were found in Ac-27 (1.21) > Ac-23 (1.11) > Ac-26 (1.09) > Ac-20 (1.03), whereas lower values were registered in Ac-14 (0.42) < Ac-18 (0.46) < Ac-19 (0.50) < Ac-24 (0.63).

Among the mesocarp tissues tested, AA (mg/g DW) values in Ac-23 (1.29) > Ac-22 (1.14) > Ac-12 (1.12) > Ac-21 (1.02) were significantly higher than those found in

'vegetable marrow' morphotypes from Ac-15 (0.47) < Ac-13 (0.54) < Ac-16 (0.55) < Ac-5 (0.64).

Recent studies reported by Aquino-Bolaños et al. (2013) and Karmakar et al. (2013) demonstrated that the higher amounts of AA in edible parts of zucchini fruits were in flowers (~16 mg/100 FW), while ridge gourd fruits (*Luffa acutangula*) (also belonging *Cucurbitaceae* family) had similar AA contents as values found in this study. With respect to other by-products from the *Cucurbita pepo* family such as pumpkin seed oil or pumpkin flesh oil they were characterized for having low vitamin C content, ~0.08 and 0.03 mg/100g FW, respectively (Juknevičiene et al., 2013).

It is well known that AA acts as a cellular antioxidant, but also facilitates intestinal absorption of iron and maintenance of plasma iron in reduced form (Smirnoff, 2000). Hence, both exocarp and mesocarp tissues from Ac-23 could be recommended for providing higher content in ascorbic acid in *Cucurbita pepo* plant breeding programmes, while mesocarp selection from 'vegetable marrow' may not be appropriate in the development of new varieties for having low ascorbic acid content.

3.3. Chlorophyll a and b

Summer squash samples from the *Cucurbita pepo* germoplasm differed in terms of AA, tissue selection having a strong influence in chlorophyll content, the exocarp **(Table IV. 1. 2 and Fig. IV.1.1B)** showing higher natural pigments than the mesocarp **(Table IV.1. 3 and Fig. IV.1.1D).** In fact, exocarp exhibited in chlorophyll *a* and chlorophyll *b* contents around 21-fold higher compared to mesocarp (61.99 vs. 1328.14 µg/g DW; 42.65 vs. 880.14 µg/g DW, for exocarp and mesocarp, respectively). In spite of the fact that *Cucurbita pepo* mesocarps showed higher variability between samples in chlorophyll values (CV= 135.70% and CV= 133.66% for Chl-A and Chl-B), the exocarp tissues registered higher chlorophyll range ($Chl\text{-}A_{range}$= 38.1-2602 µg/g DW and $Chl\text{-}B_{range}$= 151.4-2168.1 µg/g DW).

Analyzing the Chl-A (µg/g DW) content several exocarps could be classified as rich in this natural pigment Ac-27 (2602.2) > Ac-20 (2590.7) > Ac-5 (2186.5) > Ac-2 (2128.6) whereas others displayed lower amounts of this pigment Ac-13 (38.1) < Ac-23 (246.8) < Ac-4 (364.0) < Ac-11 (365.0).

In mesocarp Chl-A values were also distributed in two groups. On one hand those genotypes with an abundant Chl-A (µg/g DW) content [Ac-25 (470.35) > Ac-6 (94.98) > Ac-2 (78.05) > Ac-15 (74.52)], and on the other hand those genotypes with a poor content in this compound [Ac-8 (12.52) < Ac-23 (15.94) < Ac-21 (18.94) < Ac-19 (20.85)].

Analyzing the Chl-B (µg/g DW) content several exocarps were shown to be a good source of this pigment Ac-24 (2168.1) > Ac-20 (1872.6) > Ac-26 (1856) > Ac-2 (1701.9) > Ac-27 (1610.1), whereas other *Cucurbita pepo* genotypes displayed lower amounts of this pigment Ac-7 (151.4) < Ac-11 (247.2) < Ac-8 (256.9) < Ac-4 (264.6).

Although mesocarp presented small quantities of Chl-B, two groups were also observed. Among the mesocarp tissues tested, Chl-B (µg/g DW) content in Ac-25 (312.02) > Ac-8 (90.76) > Ac-27 (67.87) > Ac-6 (66.26) were statistically higher than those found in 'vegetable marrow' morphotypes from Ac-21 (5.85) < Ac-16 (11.26) < Ac-1 (12.27) < Ac-5 (14.90).

In higher plants, such as zucchini, chlorophyll *a* to *b* is present in approximately 3 to 1 ratio, however, the ratio varies with growth conditions and environmental factors. In agreement with other ratio limits established by Lichtenthaler et al. (1982) plant species exposed to sun (high-light plants) tend to have higher ratios (~3.2 to 4.0) than shade (low-light) plants (~2.6 to 3.2). Thus, considering the whole fruits (exocarp+mesocarp) and calculating the chlorophyll *a/b* ratio for each morphotype **(Table IV.1.4),** 'vegetable marrow' reached the highest values (*a/b* ratio of 2.11) suggesting that fruits from this morphotype are able to receive higher amounts of sun light, especially those fruits from regions characterized by having more hours with sun light such as 'The Canary Islands' (the highest *a/b* ratio in Ac-7= 3.38).

Other *Cucurbita pepo* morphotypes studied presented similar ratios, 1.40 and 1.51 for 'zucchini' and 'pumpkin', respectively. In general, ratios in 'zucchini' or 'pumpkin' found in this work were similar to those found in tomatoes (0.17-1.78) (Lenucci et al., 2012) or green leaf lettuce (0.80-1.43) (Caldwell and Britz, 2006), while ratios from 'vegetable marrow' were close to red leaf lettuce (1.92-2.93) (Caldwell and Britz, 2006), green pepper (~2.05) or spinach (~2.70) (Sánchez et al., 2014); registering B*rassica oleracea* higher values (~4.5) (Lefsrud et al., 2007).

Referring to structure, chlorophyll *b* is similar to chlorophyll *a*, except for having an aldehyde group (-CHO) in place of the methyl group at C-7, this small structural difference between both molecules generates significant differences in absorption spectra as well as in the antioxidant properties (Lanfer-Márquez and Sinnecker, 2008). Previous reports have highlighted the antimutagenic behaviour of chlorophyll *a*, positioning the antioxidant activity of chlorophyll *a* around 3-fold higher compared to chlorophyll *b* (Ferruzzi et al., 2002). Among chlorophyll health properties are wound healing, the reduction of inflammation, oxygenation of the blood, neutralization of free radicals, encouraging the growth of beneficial intestinal flora, supporting the immune system as well as correcting the effects of anemia in blood and having beneficial effects in odor suppression (Humphrey, 2004). Hence, Ac-2 and Ac- 27 (both exocarp and mesocarp tissues) could be considered as promising genotypes in parental recombination for higher contribution in Chl-A and Chl-B, respectively.

Table IV.1. 4. Total chlorophyll *a*, total chlorophyll *b*, chlorophyll *a/b* ratio and total chlorophylls content in 27 the *Cucurbita pepo* accessions and morphotypes studied.

Accession	Total chlorophyll *a* (exocarp+mesocarp)	Total chlorophyll *b* (exocarp+mesocarp)	Ratio *a/b*	Total chlorophylls *(a+b)*
Ac-1	1813.44	934.47	1.94	2747.91
Ac-2	2206.65	1727.57	1.27	3934.22
Ac-3	1399.35	833.72	1.67	2233.07
Ac-4	399.37	291.18	1.37	690.55
Ac-5	2237.5	853.6	2.62	3091.1
Ac-6	1146.98	395.06	2.90	1542.04
Ac-7	618.96	182.98	3.38	801.94
Ac-8	1076.72	347.66	3.09	1424.38
Ac-9	835.08	345.76	2.41	1180.84
Ac-10	1573.21	697.31	2.25	2270.52
Ac-11	413.45	273.53	1.51	686.98
Ac-12	1398.6	1455.65	0.96	2854.25
Ac-13	106.08	343.03	0.30	449.11
Ac-14	1795.31	1229.9	1.45	3025.21
Ac-15	500.62	450.32	1.11	950.94
Ac-16	1452.54	793.36	1.83	2245.9
Ac-17	993.85	604.41	1.64	1598.26
Ac-18	1915.48	1231.69	1.55	3147.17
Ac-19	1378.55	769.65	1.79	2148.2
Ac-20	2645.56	1900.92	1.39	4546.48
Ac-21	2134.54	966.25	2.20	3100.79
Ac-22	1800.72	859.4	2.09	2660.12
Ac-23	262.74	810.81	0.32	1073.55
Ac-24	1679.63	2194.76	0.76	3874.39
Ac-25	1172.85	857.62	1.36	2030.47
Ac-26	1933.42	1886.93	1.02	3820.35
Ac-27	2642.58	1677.97	1.57	4320.55

	Chlorophyll *a/b* ratio		
Morphotype	***Vegetable marrow***	***Zucchini***	***Pumpkin***
Range	0.30-3.38	0.32-2.20	-
Mean	2.11	1.40	1.51
SD	0.92	0.49	-
CV (%)	43.61	65.01	-

SD= standard deviation

CV= coefficient variation

3.4. Total phenolic compound

The concentrations of TPC in *Cucurbita pepo* were summarized for exocarp **(Table IV.1.2 and Fig. IV.1.1A)** and mesocarp **(Table IV.1.3 and Fig. IV.1.1C).** Approximate values in mean and SD for TPC were found in both exocarp and mesocarp tissues (4.06±0.61 vs. 3.88±0.91 mg/g DW, respectively), while mesocarp presented a slightly higher TPC range ($range_{mesocarp}$= 1.98-5.49 vs. $range_{exocarp}$= 3.19-5.29 mg/g DW). These differences between tissues promoted a higher coefficient of variation in mesocarp ($CV_{mesocarp}$= 23.52% vs. $CV_{exocarp}$= 15.09%).

According to TPC in the *Cucuribita pepo* exocarp genotypes, the higher values (mg/g DW) were found in Ac-7 (5.29) > Ac-11 (4.98) > Ac-27 (4.88) > Ac-8 (4.84), whereas the lower values were registered in Ac-17 (3.19) < Ac-20 (3.22) < Ac-15 (3.26) < Ac-10 (3.31).

Throughout the mesocarp tissues tested, TPC (mg/g DW) in Ac-17 (5.49) > Ac-12 (5.26) > Ac-22 (5.05) > Ac-8 (5) were significantly greater than those found in 'vegetable marrow' morphotypes from Ac-15 (1.98) < Ac-16 (2.05) < Ac-13 (2.36) < Ac-25 (2.52). In our study, as reported in other vegetables like cucumber, potato, tomato or eggplant (Ji et al., 2011; Carrillo-López and Yahia, 2013), the exocarp tissue reached the highest values in TPC.

In addition, results indicated in this work were higher than TPC found in *Cucurbita pepo* seeds (0.009 mg/100g FW) (Amutha et al., 2014), in other zucchini fruits (0.36 mg/100g FW) (Mattila and Hellström, 2007) or even in other species belonging to the *Cucurbita pepo* family like *Lagenaria siceraria* and *Luffa cylindrical* (0.15 mg/100g FW) (Irshad et al., 2014).

With reference to other vegetables, lower TPC values than those above described were found in radish (0.10 mg/100g FW), red or white cabbage (1.2 and 1.8 mg/100g FW, respectively), whereas our values were close to other vegetables (broccoli TPC = 12 mg/100g FW and carrot TPC = ~20 mg/100g FW) (Mattila and Hellström, 2007).

Phenolics are characterized by having at least one aromatic ring with one or more hydroxyl groups attached and more than 8000 polyphenolic compounds have been identified in various plant species (Strack, 1997). Referring to the health benefit, it is well established that polyphenol-rich diets provide significant protection against the development and progression of many chronic pathological conditions including cancer, diabetes, cardio-vascular problems and aging. Besides, in vegetables polyphenols contribute to the color, flavor, odor and oxidative stability (Pandey and Rizvi, 2009), increasing their quality. Therefore, both exocarp and mesocarp tissues from Ac-8 could be selected for improving phenolics compounds in the development of new *Cucurbita pepo* cultivars.

3.5. PCA and Dendogram analysis

To study which parameters contributed most to the total data variation, PCA was applied to the mean of each constituent in both exocarp and mesocarp *Cucurbita pepo* tissues, including the grouping of morphotypes studied ('vegetable marrow', 'zucchini' and 'pumpkin').

Regarding exocarp **(Fig. IV.1.2A)** a total of 75.89% of difference was explained by relation between PC1 and PC2, also obtaining four more components (representing 12.76; 7.51; 3.65 and 0.16 % of variance). Most exocarp samples from 'vegetable marrow' morphotypes are located on the left half of the plot, while the 'zucchini' samples are situated on right side of **Fig. IV.1.2A.**

Interestingly, 'pumpkin' is located more closely to 'vegetable marrow' than 'zucchini' samples, indicating that its exocarp composition is quite similar to 'vegetable marrow'. According to the distribution of the evaluated parameters in exocarp **(Fig. IV.1.2B),** the total chlorophylls (as well as both forms Chl-A and Chl-B) were responsible for the separation in PC1 while DM was mainly linked to the PC2 separation. Positive values for PC1 indicated genotypes with higher chlorophylls (Ac-20, Ac-24 and Ac-27). The highest PC2 values corresponded to genotypes with high DM (Ac-18, Ac-19 and Ac-15), while the group of genotypes with negative values in PC2 denoted high AA and TPC (Ac-27, Ac-26 and Ac-25).

Concerning mesocarp **(Fig. IV.1.2C),** PC1 was responsible for 53.43% while PC2 accounts for 30.71% of the variability observed in this tissue. Moreover, another three components were also obtained (with 11.20; 3.77 and 0.87 % of variance). All mesocarp samples from 'vegetable marrow' are located in the upper-right-hand quadrant of **Fig. IV.1.2C**, with the exception of Ac-8 and Ac-4, whereas the 'zucchini' morphotypes are in the lower part of the Figure. Also mesocarp from 'pumpkin' was more comparable with 'vegetable marrow' samples. Negative values for PC1 **(Fig. IV.1.2D)** indicated genotypes with higher chlorophylls (Ac-17 and Ac-23), whereas the highest values in PC2 **(Fig. IV.1.2D)** corresponding to genotypes with high DM, AA and TPC (Ac-17, Ac-25 and Ac-23).

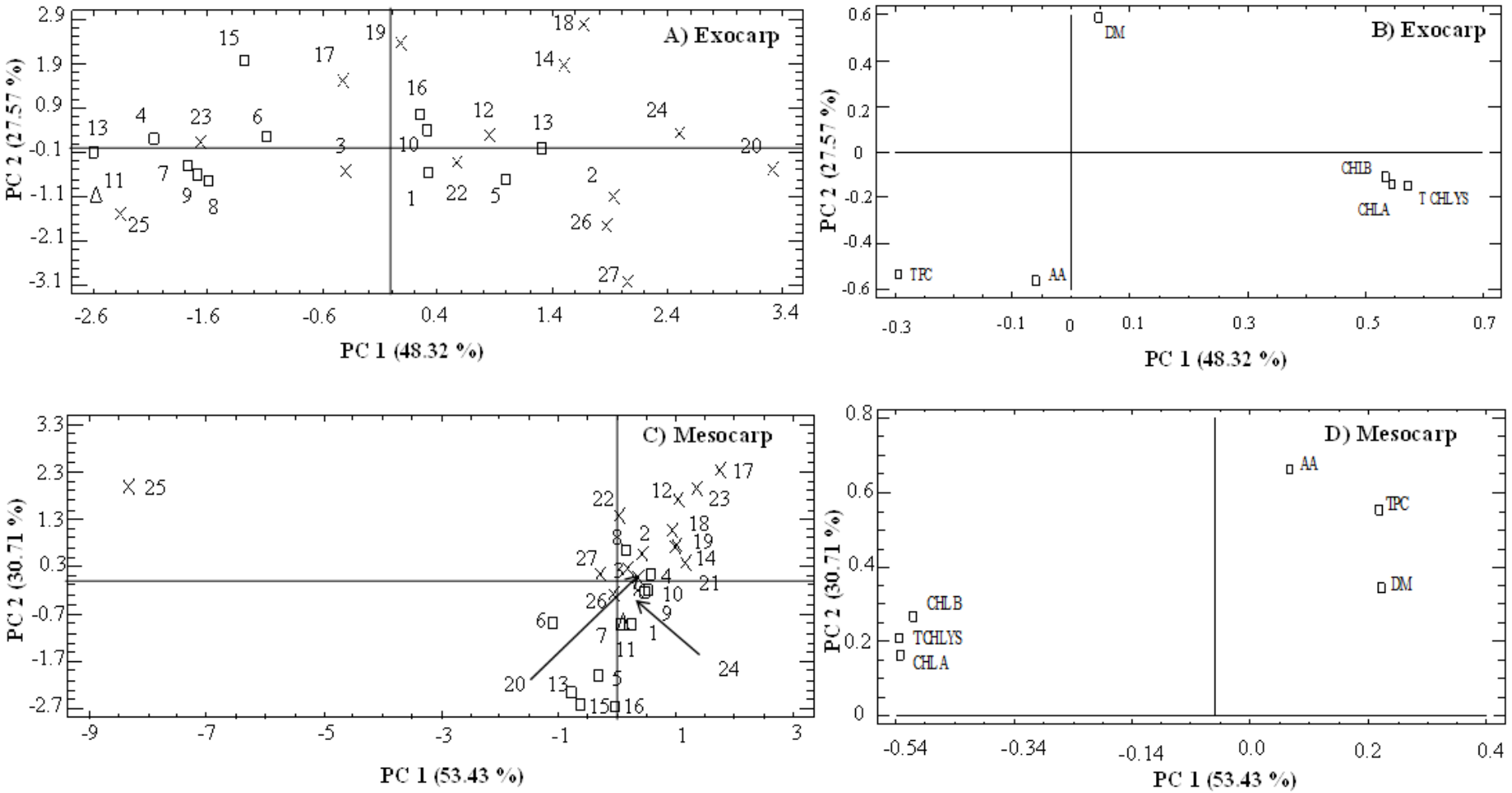


Figure IV.1. 2. Principal component analysis (PCA) plots of 27 *Cucurbita pepo* accessions. (A) PCA scores plot for exocarp tissues, (B) loading plots of exocarp tissues for different variables on PC1 and PC2, (C) PCA scores plot for mesocarp tissues, (D) loading plots of mesocarp tissues for different variables on PC1 and PC2.

A dendogram analysis was carried out using Ward's method of agglomeration and Euclidean distances to measure the similarity between exocarp **(Fig. IV.1.3A)** and mesocarp **(Fig. IV.1.3B)** samples, considering as variables all the analyzed chemical parameters. As can be seen, no well defined class structure was apparent in both *Cucurbita pepo* tissues. Rather, samples clustered in three different regions in exocarp **(Fig. IV.1.3A)**: center (Cluster 2 comprising 13 samples) showing all 'zucchini' samples (84.6%), except two from 'vegetable marrow' (15.4%); right (Cluster 3 containing 8 samples) presenting all 'vegetable marrow' samples (75%) which were clearly distinguished from the other 'pumpkin' (12.5%) and 'zucchini' (12.5%) samples; and left (Cluster 1 including 6 samples) constituted by 'zucchini' (50%) and 'vegetable marrow' samples (50%) (distributed between 'zucchini' samples).

Moreover, samples from mesocarp **(Fig. IV.1.3B)** were also clustered in three different regions: right (Cluster 3 comprising 12 samples) displaying all 'zucchini' samples (91.7%), except one from 'vegetable marrow' (8.3%); center (Cluster 2 containing 9 samples) showing all 'vegetable marrow' samples (77.3%), except two from 'zucchini' (22.2%); right (Cluster 1 including 6 samples) constituted by 'vegetable marrow' (50%) together with 'zucchini' (33.3%) and 'pumpkin' (16.7%). For both exocarp and mesocarp tissues, cluster 2 and 3 regrouped more interesting *Cucurbita pepo* genotypes characterized by a higher content in chlorophylls and elevated values in AA and TPC, respectively.

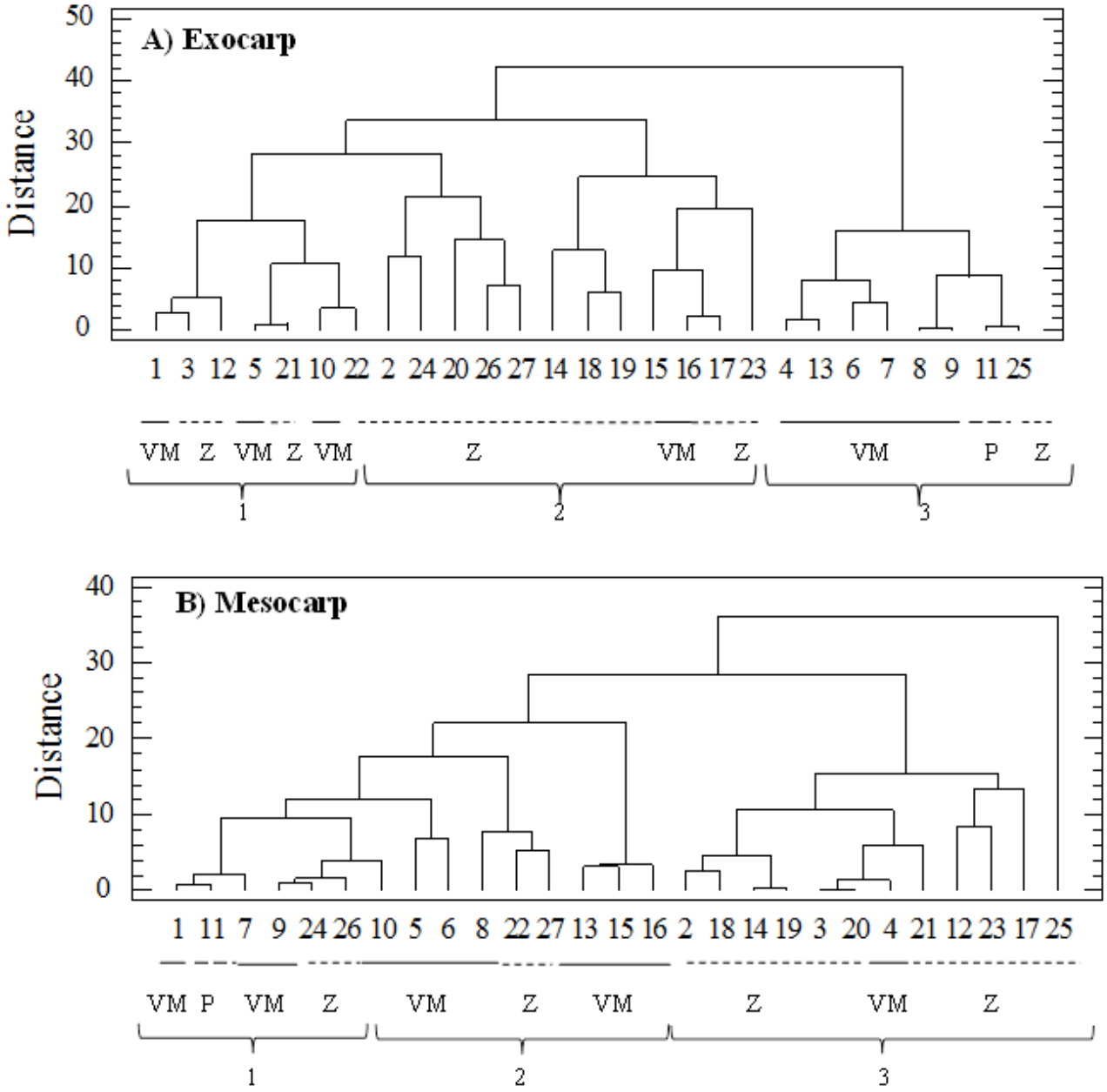


Figure IV.1. 3. Dendrogram of hierarchical cluster analysis of *Cucurbita pepo* (A) exocarp and (B) mesocarp.

3.6. Potential contribution to dietary requirements

Estimates of the required daily intake of vitamin C, chlorophylls and total phenolics for an adult person (both men and women) are shown in **Table IV.1.5.** Among them total phenolics (449-1377 mg/day) as well as vitamin C (60-80 mg/day) are the most important required constituents in human health, while a rich source of chlorophylls is not necessary (0.69-1.56 mg/day). Concerning the required intake, increasing fruit and vegetable consumption to between 400 to 800 g/day is a well-known public health strategy. For this reason the Joint FAO/WHO Expert Consultation on diet, nutrition and the prevention of chronic diseases, recommends a daily intake of fruits and vegetables higher than 400 g per person, suggesting serving each color selection included in the seven color classes (red, yellow-green, red-purple, orange, orange-yellow, green, white-green) (Yahia, 2009).

In this study vitamin C values from 'pumpkin' grown in Spain (around 8.9 mg/200 g) were slightly higher than when this morphotype is grown in Africa (~4 mg/200 g) (Van Jaarsveld et al., 2014). In more detail, 200 g summer squash from 'zucchini' morphotype can supply the highest % of the adult RDA in vitamin C (17-18%), while moderate % of RDA is provided by 'vegetable marrow' and 'pumpkin' (between 13 and 14% of RDA) (Table 5). However, these values are lower than those observed in dark-green leafy vegetables (in which 100 g contributes more than 50% of the RDA) (Van Jaarsveld et al., 2014) or tomato (contributing 100 g around 20 % of RDA) (Yahia, 2009).

In relation to total chlorophylls, the estimated average amount of these compound nutrients in *Cucurbita pepo* morphotypes studied were as follows 'zucchini' (2.95 mg/200 g) > 'vegetable marrow' (1.58 mg/200 g) > 'pumpkin' (0.68 mg/200 g). Summer squash fruits (included in the green vegetable group), with a chlorophyll potent inhibition up to 77% are highly implicated in the dietary management of human cancer risk as well as cancer intervention (Breinholt et al., 1955). Nonetheless, in spite of their chemopreventive properties, limited information is available about the potential contribution of vegetables on chlorophyll dietary requirements (% of RDA). In this study, 'zucchini' could be described as an excellent source of chlorophylls, containing at least 263% of RDA, showing a potential contribution around 2-fold and 4-fold higher than 'pumpkin' and 'vegetable marrow' morphotypes (~145% and ~60% of the RDA, respectively) **(Table IV.1.5).** For this reason, the ingestion of whole *Cucurbita pepo* fruits including exocarp ensures the daily requirements of these pigments.

On the other hand, the medicinal property of *Cucurbita pepo* may be attributed to the presence of phenolic compounds which counteract the free radicals. Values reported in other *Cucurbita pepo* fruits from India were 2-fold higher (~50 mg/100 g FW) than Spanish fruits described in this study. Potential contribution of these compounds towards the dietary requirements is fairly low **(Table IV.1.5)** positioning between 5 % (for 'vegetable marrow' and 'pumpkin') and 7% (for 'zucchini'). However, in spite of its low

allowance, recent studies (Amutha et al., 2014; Boaduo et al., 2014) revealed the use of *Cucurbita pepo* for obesity and type II diabetes mellitus prevention.

Therefore, taking into account the potential contribution described in this work, summer squash as a green vegetable, could be considered as part of the 5 servings of fruits and vegetables daily recommended for reducing up to 28% the risk of cardiovascular disease (Hung et al., 2004).

Table IV.1. 5. Nutrients supplied by 200 g of fresh *Cucurbita pepo* fruit from different morphotypes. Recommended dietary allowance (RDA) for men and women and potential contribution (%RDA) to nutrient requirements of this life stage.

Element	RDA (mg/day)				Unit	*Cucurbita pepo* morphotypes				
	Men		Women			*Vegetable marrow*		*Zucchini*		*Pumpkin*
	Range (min-max)	Mean	Range (min-max)	Mean		Range (min-max)	Average value	Range (min-max)	Average value	Average value
Ascorbic acid	60-80[a]	70	60-70[a]	65	mg/200 g FW	7.38-12.06	9.60	8.18-20.86	11.78	8.9
					% RDA men		14		17	13
					% RDA women		15		18	14
Total chlorophylls	0.73-1.45[b]	1.09	0.69-1.56[b]	1.12	mg/200 g FW	0.44-3.08	1.58	1.06-4.54	2.95	0.68
					% RDA men		145		270	62
					% RDA women		141		263	60
Total phenolic compounds	461-1377[c]	919	449-1185[c]	817	mg/200 g FW	37.82-62.54	46.17	36.8-76.78	55.47	46.24
					% RDA men		5		6	5
					% RDA women		6		7	6

[a] Referred to vitamin C (Cuervo et al. 2010).
[b] Balder et al. 2006.
[c] Ovaskainen et al. 2008

4. Conclusions

The results obtained in this work confirmed that exocarp and mesocarp tissues of *Cucurbita pepo* have a great potential to be exploited due to a large variability in dry matter as well as natural pigments and nutritional compound profile, genotype selection being a key factor for increasing the desirable attributes.

Eight fruit tissues were identified for registering higher contents, four of them on chlorophylls such as chlorophyll *a* (Ac-7 and Ac-25, exocarp and mesocarp, respectively) and chlorophyll *b* (Ac-24 and Ac-25, exocarp and mesocarp, respectively), two on ascorbic acid (Ac-27 and Ac-23, exocarp and mesocarp, respectively) and, another two on total phenolic compounds (Ac-7 and Ac-17, exocarp and mesocarp, respectively). In relation to whole fruit, four accessions presented high levels in both tissues referring to chlorophylls (Ac-2 and Ac-27), ascorbic acid (Ac-23) and total phenolic compounds (Ac-8), while desirable content in dry matter corresponded to the commercial hybrid accessions.

Additionally, the 'zucchini' morphotype was superior to 'vegetable marrow' and 'pumpkin' in all constituents analyzed. In adults, 200 g of this morphotype have an excellent contribution to the recommended dietary allowance for chlorophylls (270% of RDA), being considerable for vitamin C and total phenolic compounds (18 and 7 % of RDA, respectively).

In the near future, these accessions may be used directly for human consumption or developing novel and more nutritious cultivars achieving one of the main goals for consumers and plant breeding programmes.

Acknowledgements

This work was supported by the project RTA2009-00036-00-00 and RTA2009-00039-00-00 (INIA) and FEDER funds. Authors thank to the germoplasm seed sources for providing the material used in this study and to Nicolas Davies for his help in the grammatical revision of the manuscript. M.T. Blanco-Díaz acknowledges the financial support from the Spanish Ministry of Economy and Competitiveness as a contracted researcher of the Program 'Researcher Training' (Formación de Personal Investigador, FPI-INIA).

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4.2

'Effect of fruit ripening stage and harvest date on physical properties, proximate composition and health promoting compounds on six coloured zucchini varieties'

María Teresa Blanco-Díaz[a], Mercedes Del Río-Celestino[b], Rafael Font[a]

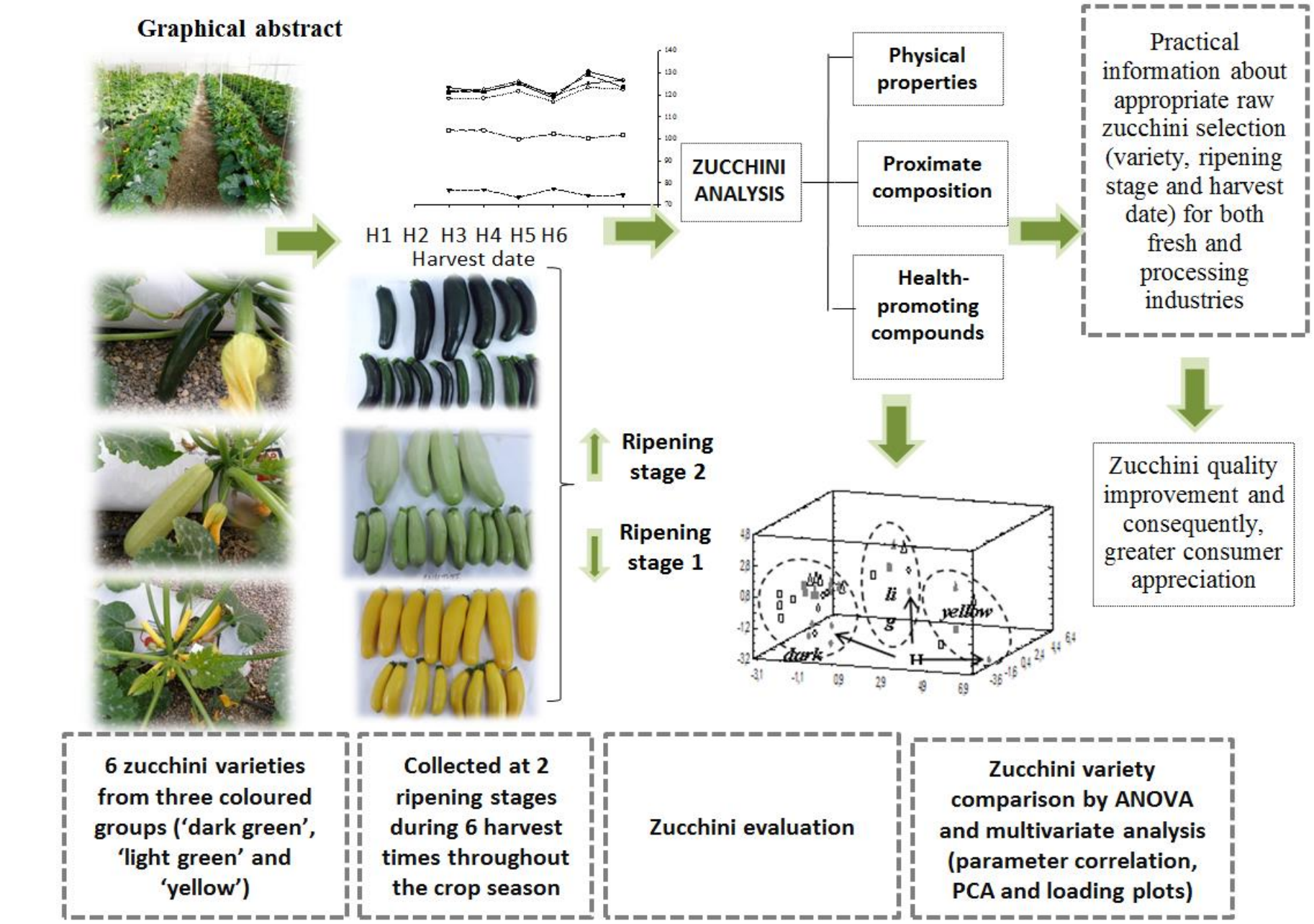
[a] Department of Postharvest Technology and Food Industry - IFAPA Centro La Mojonera - Camino San Nicolás, 1, 04745 La Mojonera, Almería, Spain
[b] Department of Plant Breeding and Crop Biotechnology - IFAPA Centro La Mojonera - Camino San Nicolás, 1, 04745 La Mojonera, Almería, Spain

In preparation

Abstract

Pre-harvest handling is a key factor on physicochemical and nutritional properties contributing to better postharvest quality of the product. This is the first report evaluating quality attributes of 6 varieties belonging to three coloured zucchini groups (dark green, light green and yellow) at 2 ripening stages (Rs 1 and Rs 2) and at 6 harvested dates (H1 to H6) during the production cycle. Parameters analyzed included physical properties (morphology, colour and texture), proximate composition (dry matter, pH, titrable acidity, soluble solids and chlorophylls) as well as health-promoting compounds (ascorbic acid, vitamin C, total carbohydrates, carotenoids, phenolic compounds and antioxidant activity). Light green zucchini ('Amalthée') showed the highest size and texture parameters, while dark green zucchini ('Cronos', 'Cassiope', 'Natura' and 'Sinatra') presented the greatest values in both chlorophylls *a* and *b* (~13 mg/100g FW) as well as total carbohydrates (~59 mg/g FW). Interestingly, yellowed zucchini ('Parador') could be recognized as an excellent source in soluble solids (4.48 ºBrix) together with ascorbic acid (23.56 mg/100g FW), carotenoids (3.55 mg/100g FW) and phenolics (25.59 mg/100g FW). Clear differences in colour, texture, pH, chlorophylls and nutritive compounds were detected between ripening stages, increasing antioxidant properties at Rs 1 ($P < 0.001$); while harvest date mainly affected the zucchini´s physical properties ($P < 0.0001$). Additionally, strong correlations between parameters and three well-defined groups were identified in principal component analysis. In general, harvest dates from H2 to H5 (middle April to end of May) could lead to zucchini quality improvement and consequently, a greater consumer appreciation for both fresh and minimally processed zucchini, thus producing higher market prices.

Keywords: dark green zucchini; light green zucchini; yellow zucchini; ripening stage; harvest date; physical properties; proximate composition; health-promoting compounds.


Graphical abstract
H1 H2 H3 H4 H5 H6
Harvest date
ZUCCHINI ANALYSIS
Physical properties
Proximate composition
Health-promoting compounds
Practical information about appropriate raw zucchini selection (variety, ripening stage and harvest date) for both fresh and processing industries
Zucchini quality improvement and consequently, greater consumer appreciation
Ripening stage 2
Ripening stage 1
dark
li
yellow
H
6 zucchini varieties from three coloured groups ('dark green', 'light green' and 'yellow')
Collected at 2 ripening stages during 6 harvest times throughout the crop season
Zucchini evaluation
Zucchini variety comparison by ANOVA and multivariate analysis (parameter correlation, PCA and loading plots)

1. Introduction

Zucchini is a vegetable belonging to the *Cucurbitaceae* family originating from America and Mexico, although it is widely grown and commercialized around the world. Particularly, in the main vegetable production area in Europe as is Almería (Southern Spain), where zucchini is the principal *Cucurbitaceae* morphotype produced, reaching more than 350000 tonnes during the 2012/2013 season (CAPMA, 2013).

As with other vegetables, the beneficial health effects of zucchini are attributed to their natural antioxidants such as vitamin C, polyphenols and carotenoids, contributing positively to the daily nutritional intake and also protecting against certain chronic diseases, including obesity, cardiovascular disease, accelerated ageing and some types of cancer (FAO, 2004).

Zucchini fruit also contains considerable amounts of carbohydrates and chlorophylls (Blanco-Díaz et al., 2014). Referring to carbohydrates, recent studies highlight the important health benefits of this fruit, formerly considered solely as an energy source. Studies described the potential of carbohydrates as a major substrate for fermentation in the colon, extent of digestion and fermentation in the gut and enhance physical performance through glycogen loading (FAO, 1998). The benefits of chlorophylls have been described as exhibiting a strong anticarcinogenic activity (Donaldson, 2004; Li et al., 2007) as well as other effects such as a wound healing accelerant and controlling urinary and fecal odors in patients (Young et al., 1980).

Nevertheless, unfortunately the supplementation of phytochemicals for consumers has not generally resulted in a significantly decreased incidence of cardiovascular events, being the 'benefit of the whole vegetable greater than the sum of its parts' (Higdon and Drake, 2007). This provoked consumers to demand detailed information about the nutritional value and ingredient composition of vegetables, requesting daily health recommendations. Because of that, several public health campaigns for increasing the vegetable consumption were promoted by governments around the world such as the '5 a Day' program (minimum of 5 daily servings or at least 400 g) and more recently the 'Fruits & Veggies Matter More' campaign.

On the other hand, the vegetables' postharvest quality (physicochemical and nutritional properties) depend on several pre-harvest factors, among them are the botanical variety (cultivar), ripening stage as well as the harvest date (Sams, 1999; Gil et al., 2012; Valero and Serrano, 2013). In horticultural crops, data from literature about the correlation between cited factors with physicochemical and nutritional compounds is not always linear and shows different trends during the harvest date. For example, cultivar selection played a key role in several nutritional compounds such as sugars and phenols on soybean and cabbage (Shong et al., 2013; Park et al., 2014). In relation to ripening stage, dry matter, total soluble solids, ascorbic acid, phenols and carotenoids increased in tomatoes with ripening (Opara et al., 2012; García-Valverde et al., 2013);

while a decrease in total phenols was observed during ripening in carob (Benchikh et al., 2014). Harvest date also produced significant changes in artichokes, the best quality results in colour, total phenols, antioxidant activity occurring when the product was harvested under low temperatures (Ricci et al., 2013). However, total phenol compounds in peppers increased towards the end of the season (Marín et al., 2008).

Thus, an effective zucchini genotype management, optimal harvest date identification, adequate physicochemical and nutritional characterization have repercussions in efficient postharvest handling equipment, zucchini manufacturing and optimization of bioprocesses resulting also in greater final quality of the product and higher prices at the market. To our knowledge this is the first report on the effect of different ripening stages during the full zucchini season on physicochemical and nutritional properties, in spite of the determination of these parameters being a fundamental role for both international seed companies and the agro-food chain.

Therefore, the objective of this study was to provide information for the zucchini industry to guide their appropriate raw material selection towards an increased product quality. For this purpose, we evaluated physical, proximate composition and health-promoting compounds on six dark green, light green and yellow coloured zucchini varieties at two ripening stages and harvested at six different dates during the full production cycle of this vegetable.

2. Material and methods

2.1. Plant material

A total of six zucchini squash cultivars (*Cucurbita pepo* spp. *pepo*) from different international seed companies were studied belonging to the three different zucchini coloured groups usually commercialized around the world, four to dark green: 'Cronos' (Syngenta Crop Protection, Co. Ltd), 'Cassiope' (Gautier Semences, Co. Ltd), 'Natura' (Enza Zaden, Co. Ltd) and 'Sinatra' (Clause, Co. Ltd); one to light green: 'Amalthée' (Gautier Semences, Co. Ltd); and one to yellow colour: 'Parador' (Gautier Semences, Co. Ltd) were germinated (January 28th), transplanted and grown in greenhouses of the Centre IFAPA La Mojonera (Almería, Spain) (36º 46' N, 2º 48' O). Each zucchini cultivar was collected every two/three weeks according to two ripening stages widely used when this vegetable is exported, ripening stage 1 (Rs 1) (12-18 cm) and ripening stage 2 (Rs 2) (18-25 cm). Ten zucchini fruits per cultivar and ripening stage were harvested at different dates during the 2012-2013 spring season: H1 (April 8th), H2 (April 15th), H3 (May 8th), H4 (May 13th), H5 (May 29th) and H6 (June 24th).

2.2. Physical analyses

2.2.1. Weight, diameter and length

Each fruit mass (g) was measured individually using a sensitive balance (±0.05, Precisa Instruments Ltd., Switzerland), while diameter (mm) and length (cm) were measure with a digital caliper and flexible tape, respectively.

2.2.2. Colour

Colour was measured using a CM-700d spectrophotometer equipped with D65 illuminant source (Minolta, Ramsey, NJ, USA) taking four measurements on the opposite sides fruit, among the middle of the diameter region of the zucchini. The instrument was previously calibrated on a white tile at an observation angle of 0. The values were expressed using the CIE L*a*b* system, chroma (C*) and hue angle (hº) were also determined.

2.2.3. Texture

Firmness was determined following the method previously reported on zucchini by Brew et al., 2006. The use of a texture analyzer (TA-XT-Plus, Stable Micro System, Surrey, UK) calibrated with a 5-kg weight and equipped with a 4-mm diameter probe was used to assess the firmness on the middle region of the zucchini fruit. The insert distance was 5 mm, with a stroke speed of 50 mm min^{-1}. Firmness was expressed in Newtons (N).

2.3. Proximate composition

2.3.1. Dry matter

Freeze-drying was used for the determination of dry matter. Sample lyophilization was performed using freeze drier equipment (Telstar LyoQuest, Terrasa, Spain) at -55ºC under vacuum (133 x 10^{-3} mBar) for 96 h per sample. Then, the samples were ground and frozen at -80ºC for further analysis. Results were expressed in percent of dry matter (%).

2.3.2. pH, titrable acidity and total soluble solids

At each harvest date zucchini cultivars at different ripening stages were homogenized using a commercial blender (Moulinex, Barcelona, Spain). Then, the pH of the juice was measured using a pH-meter (GLP 21^{+}, Crison, Barcelona, Spain), while for titrable acidity (TA) 10 ml of juice was titrated with 0.1 N NaOH to an endpoint of pH 8.1 (AOAC, 1984) and expressed as g of malic acid per 100 ml of juice (% malic acid), the most predominant acid in this vegetable (Wang and Buta, 1999). For total soluble solids (TSS) of the juice was measured with a hand refractometer (SMART-1, Atago, Japon) and expressed as ºBrix at 20 ºC.

2.3.3. Chlorophyll *a*, chlorophyll *b* and total chlorophylls

The extraction and analysis of the chlorophylls pigments were carried out simultaneously, in order to avoid pigment degradation. Chlorophyll pigments were determined using a UV–Visible spectrometer (Thermo Fisher Scientific, Madison, Wisconsin, USA) and both chlorophylls, *a* and *b*, were determined using the method

described by Blanco-Díaz et al. (2014) and according to the equations reported by Lichtenthaler and Wellburn (1983):

Chlorophyll *a* =11.75 A_{662} - 2.35 $A_{645,}$

Chlorophyll *b* =18.61 A_{645} - 3.96 A_{662},

Total chlorophylls = chlorophyll *a* + chlorophyll *b*, where

A is absorbance

Both chlorophylls pigments *a* and *b* as well as total chlorophylls were expressed as mg/ 100g FW.

2.4. Health-promoting compounds

2.4.1. Ascorbic acid and vitamin C

The reference values for ascorbic acid (AA) were obtained using an automatic titration (Metrohm, 862 Compact Titrosampler, Metrohm, US) by the iodine titration method (Blanco-Díaz et al., 2014). Finally, the ascorbic acid content was expressed as mg/ 100 g FW.

The total vitamin C analysis of fresh-cut zucchini were carried out using a UV-spectrophotometry (Thermo Fisher Scientific, Madison, WI, USA) method described by Rahman-Khan et al. (2006), with minor modifications, consisting on adapting the ground freeze-dried sample (200 mg) and the volume of 85% H_2SO_4 (Merck, Darmstadt, Germany) added (10 mL) when the samples were cooled in the ice bath for 10 min. As a result, the absorbance of the coloured solution was read at 521 nm against a blank solution using quartz cuvettes with a cell path length of 1.0 cm. The regression equation and the regression coefficient (r^2=0.9964) values were obtained for the ascorbic acid. Vitamin C results were expressed as mg/ 100 g FW.

2.4.2. Total carbohydrates and carotenoids

Total carbohydrates were estimated by anthrone method (Hedge and Hofreiter, 1962). Freeze-dried zucchini samples (250 mg) with 50 ml distilled deionised water were sonicated on an ultrasonic bath for 20 min and centrifuged for 5 min at 4000 rpm. An aliquot (1ml) of extracts was added to 15 ml tube vials and then, 2 ml of 75% H_2SO_4 solution (Merck, Darmstadt, Germany) were added to the mixture and vortex for 20 s. Anthrone reagent was prepared diluting 1g of anthrone (Sigma-Aldrich, Madrid, Spain) in 20 mL of ethanol (Panreac, Madrid, Spain) and mixing with 480 mL of 75% H_2SO_4. Finally 4 mL of anthrone reagent were added and vortex for other 20 s. Samples tubes vials were placed in a water bath and incubated at 100 ºC for 15 min and finally cooled down at room temperature. The absorbance against prepared reagent blank was introduced in a pair of 10 mm quartz cells and taken at 620 nm using a UV-spectrophotometry (Thermo Fisher Scientific, Madison, WI, USA). The regression equation and the regression coefficient (r^2=0.9964) values were obtained for standard solution of D-glucose (Sigma-Aldrich, Madrid, Spain). Total carbohydrates content of zucchini samples was expressed as mg/ 100 g FW.

Total carotenoids were determined using the procedure described by Blanco-Díaz et al. (2014) and calculate according equations described by Lichtenthaler and Wellburn (1983):

Total carotenoids = $[(1000\ A_{470}) – (2.27\ Chl\text{-}B) – (81.4 Chl\text{-}A)]/227$ where A is absorbance

The aliquot from the supernatant was UV-spectrophotometry measured with the optical density was measured at 470 nm using quartz cuvettes with a cell path length of 1.0 cm. Finally, total carotenoids were expressed as mg/ 100 g FW.

2.4.3. Total phenolic compounds and antioxidant activity

Total phenolic compounds (TPC) in the zucchini fruits were obtained by the Folin-Ciocalteu reagent (Sigma-Aldrich, Munich, Germany) method using gallic acid (Sigma-Aldrich, Munich, Germany) as external standard (Blanco-Díaz et al., 2014). The absorbance was read at 750 nm against a blank solution using quartz cuvettes with a cell path length of 1.0 cm. Finally, results were reported in gallic acid equivalents (mg/ 100 g FW).

The antioxidant assay was performed following the procedure described by Brand-Williams et al. (1995) with some modifications. Four grams of fresh zucchini were homogenized in an ultraturrax (IKA Werke GmbH & Co., Staufen, Germany) for 1 min with 3 mL of methanol (80%) (Panreac, Madrid, Spain). The homogenate was filtered and then centrifuged at 5 ºC at 9000 rpm for 5 min. The pellet was discarded and the supernatant was frozen at -80 ºC until analysis in dark conditions. Reactions started by pipetting 50 µL of the sample into 0.95 mL of diphenylpicrylhydrazyl solution (DPPH) (Sigma-Aldrich, Madrid, Spain). The cuvettes were closed with a stopper and secured with parafilm to prevent evaporation during 24 h in darkness. The absorbance was read using a UV-spectrophotometry (Thermo Fisher Scientific, Madison, WI, USA) at 515 nm, after 24 h in darkness. The regression equation and the regression coefficient (r^2=0.9981) values were obtained for Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (Sigma-Aldrich, Barcelona, Spain). Results were reported in Trolox equivalents (mg/ 100 g FW).

2.5. Statistical analysis

Experiments were performed using a completely randomized design. Data from the study was analyzed statistically by three-way ANOVA (cultivar x ripening stage x harvest date) with type III sums of squares using the GLM (General Linear Model) procedure with least significant difference (LSD) at $P < 0.05$. Pearson correlation analysis was performed to corroborate relationships between parameters. Data from physical analyses, proximate composition and health-promoting compounds were also subject to principal component analysis (PCA) to study which parameters contributed most to the total data variation. Statistical analyses were carried out using SAS for

Windows and Statgraphics software version Plus 5.0 (Statistical Graphics Corp., Rockville, MD, USA).

3. Results and discussion

3.1. Physical properties

3.1.1. Morphology

Table IV.2.1 summarizes morphology data (weight, width and length) in the six cultivars studied at two ripening stages. Light green zucchini ('Amalthée') could be defined as a large-size zucchini group at both ripening stages, showing significant differences ($P < 0.0001$) from the rest of cultivars studied for weight (125.63 and 487.96 at Rs 1 and Rs 2, respectively) and for diameter ($P < 0.0001$) (36.80 and 60.23 mm at Rs 1 and Rs 2, respectively) **(Table IV.2.1)**, but not for length. In contrast, dark green zucchini ('Sinatra') is characterized by low diameter at both ripening stages (28.66 and 46.18 mm at Rs 1 and Rs 2, respectively), reaching this group ('Cronos', 'Cassiope' and 'Natura') the intermediate size. In accordance with *Cucurbita* weight groups established by Balkaya et al. (2010), all zucchini samples studied could be considered as light fruits (< 5 kg) while values in length are in the same range that in other zucchini cultivars (Biesiada et al., 2007).

Three way interaction (cultivar x ripening stage x harvest date) ($P < 0.05$) was found for both weight and diameter of the zucchini fruits, while zucchini length was affected only by ripening stage ($P < 0.0001$) **(Table IV.2.1)**.

Table IV.2. 1. Physical properties of zucchini cultivars at different ripening stages.

		Weight (g)	Diameter (mm)	Length (cm)	Colour parameters					Texture (N)
					L*	a*	b*	C*	h°	
Ripening stage 1										
Dark green	Cronos	89.15 b	29.87 bc	13.73 ab	32.43 b	-4.99 b	9.20 c	10.40 c	118.63 a	23.11 ab
	Cassiope	100.62 b	32.83 b	14.12 ab	34.49 b	-5.35 b	10.88 c	12.28 c	118.18 a	23.08 ab
	Natura	100.74 b	32.10 b	14.15 ab	34.19 b	-4.70 b	8.60 c	9.84 c	121.30 a	22.26 b
	Sinatra	91.93 b	28.66 c	13.64 b	33.73 b	-5.32 b	9.94 c	11.43 c	119.40 a	22.40 b
Light green	Amalthée	125.63 a	36.80 a	12.61 b	66.57 a	-7.29 b	31.93 b	32.84 b	102.88 b	24.43 a
Yellow	Parador	86.13 b	29.94 bc	15.79 a	63.11 a	12.88 a	54.97 a	57.32 a	85.62 c	22.14 b
Ripening stage 2										
Dark green	Cronos	385.65 b	51.19 bc	23.98 a	30.15 d	-4.71 bc	7.48 d	8.86 a	123.60 a	20.54 d
	Cassiope	388.72 b	51.32 b	22.50 ab	32.37 c	-5.12 c	9.34 c	10.67 c	120.03 b	23.05 bc
	Natura	353.04 b	49.05 bc	22.18 ab	29.89 d	-4.01 b	6.57 d	7.73 d	123.56 a	23.25 ab
	Sinatra	334.40 b	46.18 c	21.19 b	30.56 d	-4.01 b	6.45 d	7.65 d	123.45 a	21.51 cd
Light green	Amalthée	487.96 a	60.23 a	21.75 b	73.77 a	-6.60 d	30.71 b	31.43 b	102.04 c	23.19 bc
Yellow	Parador	301.22 b	46.03 c	21.14 b	71.53 b	17.83 a	66.06 a	68.51 a	74.93 d	24.95 a
Interactions										
	Cultivar (Cv)	****	****	ns	****	****	****	****	****	****
	Ripening stage (Rs)	****	****	****	ns	***	ns	ns	ns	ns
	Harvests dates (Hd)	****	****	ns	**	**	***	***	***	****
	Cv * Rs	***	***	ns	****	**	*	*	****	****
	Cv * Hd	*	***	ns	ns	ns	**	**	ns	****
	Rs * Hd	****	****	ns	ns	ns	*	ns	ns	*
	Cv * Rs * Hd	*	*	ns	*	ns	ns	ns	*	*

ns= not significant
* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$
**** $P \leq 0.0001$

During the zucchini season, differences in weight between cultivars were more noticeable at Rs 2 **(Fig. IV.2.1B)**, while at Rs 1 only showing significant differences between cultivars at the end of the season **(Fig. IV.2.1A)**. This can be attributed to zucchini is a non-climateric fruit characterized by the first exponentially fruit expansion phase, increasing fruit size in large as a result of cell expansion rather than an increase in the number of cells (Maynard and Hochmuth, 2007), offering the opportunity to be harvested during the rapid period of growth. After this point, during the second phase of development, the fruit´s growth rate slows, as can be observed zucchini fruits at Rs 2 **(Fig. IV.2.1B)**. Thus, a strong influence of the harvest date occurs on zucchini weight ($P < 0.0001$) **(Table IV.2.1)**, contributing the high temperature (usually during the summer season) and the water supply to reduce zucchini fruit size (Kader and Rolle, 2004) and as a consequence the weight (Maynard and Hochmuth, 2007).

In our study, with reference to fruit weight, early harvest dates, H2 to H4 (from April 15th to the middle of May) were adequate for all Rs 1, weight decreasing during later picking times **(Fig. IV.2.1A)**. However, all cultivars at Rs 2 from the middle of the zucchini season, H3 to H5 (from May 8th to May 29th) were accompanied with more weight variation **(Fig. IV.2.1B)**. This suggests that earlier and middle harvest times (H2 to H5) could lead to a zucchini fruit quality improvement due to fruits with a bigger size being more appreciated by consumers, therefore reaching higher market prices than the smaller sizes. Indeed, dimensions described in this work can be used to discriminate between cultivars, ripening size and also for designing fresh zucchini processing machines.

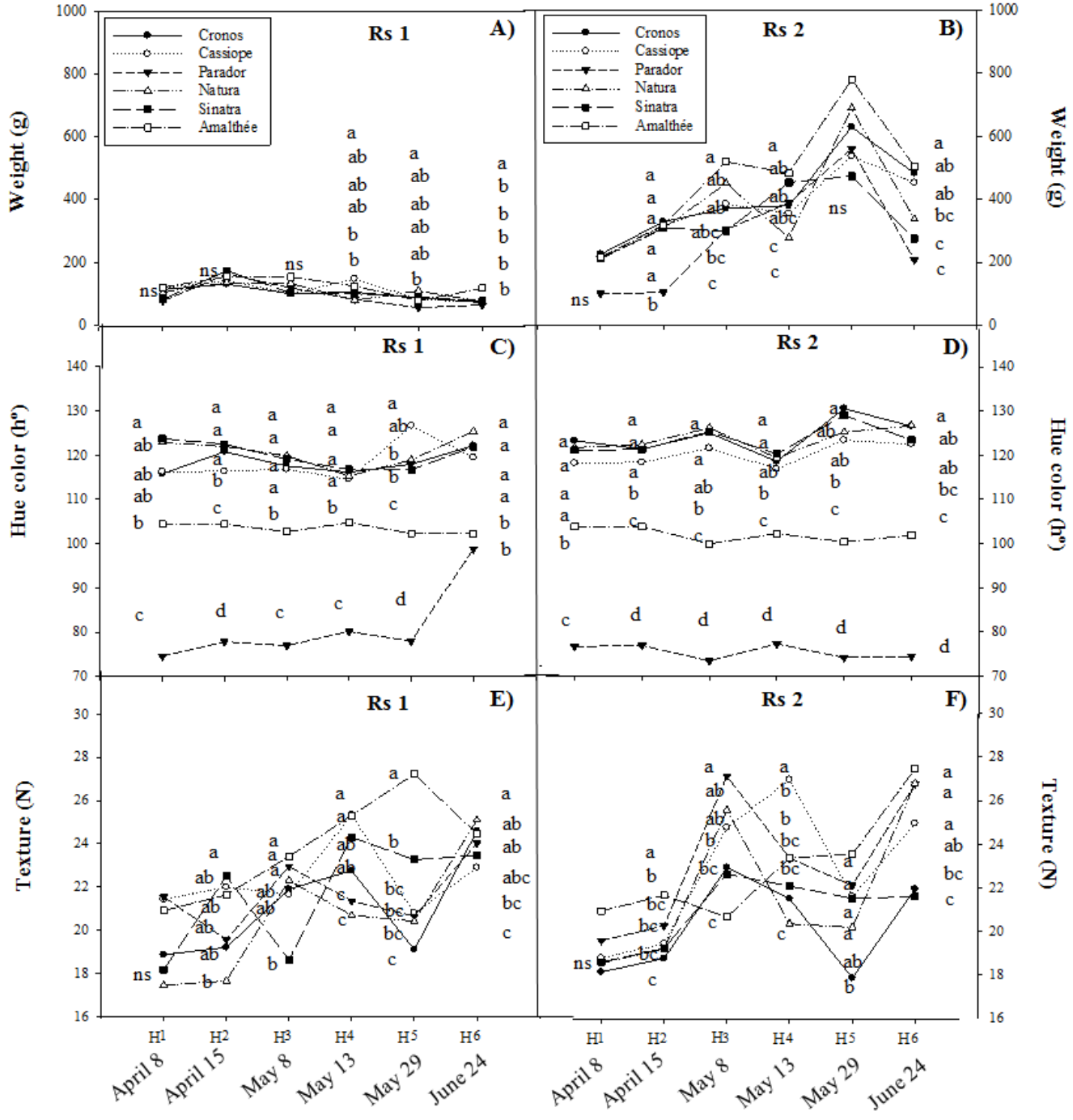


Figure IV.2. 1. Physical properties of zucchini cultivars at different ripening stages at six harvest times. Means followed by different letters are statistically different according to LSD test at $P \leq 0.05$.

3.1.2. Colour and texture

Differences in colour strongly depended on the zucchini colour group classification, dark green, light green or yellow ($P < 0.0001$) **(Table IV.2.1)**. For example, the dark green zucchini colour group ('Cronos', 'Cassiope', 'Natura' and 'Sinatra') at Rs 1 visually were perceived as less darker green values (L* takes higher values), changing gradually to darker green values when Rs 2 was reached, decreasing the L* values to between 6-13%. On the contrary, yellow ('Parador') and light green ('Amalthée') zucchini groups were darker during Rs1 (L* takes lower values) changing to lighter, increasing L* values by 13 and 11% for 'Parador' and 'Amalthée', respectively. All zucchini coloured groups at the early developmental stages (Rs 1), the green–red coordinate, a*, increased significantly when Rs 2 ($P < 0.001$), 'Parador' fruits registering the highest changes turning from yellow to dark orange during the fruit ripening. Concerning hue values (h^o value), three well cultivar-differentiated groups were also observed ($P < 0.0001$) **(Fig. IV.2.1C and IV.2.1D)**. Firstly dark green colour zucchini group with the highest values, ranging from Rs 1 (h^o value ~118.18) to Rs 2 (h^o value ~123.60 corresponding to more green colour); while light green and yellow zucchini reached intermediate (from 102.88 to 102.04, corresponding both ripening stages to light whitish yellow) and the lowest levels (from 85.62 to 74.93, meaning that with fruit growth changes in colour were from yellow–orange to orange–red tones) **(Table IV.2.2)**. In agreement with results described in this work, changes in colour across the entire surface were associated to the ripening stage in other *Cucurbitaceae* fruits (Paris and Nerson, 1986).

A three way interaction (cultivar x ripening stage x harvest date) ($P < 0.05$) was found for L* and h^o values, while for a*, b* and C* a two way interaction was observed (cultivar x harvest date) ($P < 0.01$) **(Table IV.2.1).** In general at both ripening stages, h^o values from the dark green zucchini progressively declined during the fruit season H1 to H4 **(Fig. IV.2.1C and IV.2.1D)**, showing at the end of the season from H4 to H6 (May 13th to June 24th) a slight increase. Even though a no evident tendency was registered in light green or yellow zucchinis, except in yellow zucchini at Rs 1 during the end of the season (H6). This fact could be promoted by tissue dehydration due to the high plant tissues transpiration and water evaporation expected during the summer season H5 to H6 (from May 29th to June 24th) contributing to changes in zucchini hue colour parameter (Imam et al., 2005).

As well as for cantaloupe (Beaulieu and Jeanne, 2007) or muskmelon (Parveen et al., 2012), zucchini at Rs 1 displayed slightly higher values in texture than the Rs 2 (around 11.1% for 'Cronos', 5.0% for 'Amalthée', 3.9% for 'Sinatra' and 0.1% for 'Natura'), considering all zucchini samples from this study as firm fruits (texture < 28.5 N) Balkaya et al. (2010). Cultivar had an important influence on texture ($P < 0.0001$), light green ('Amalthée') registering the highest values at Rs 1 (24.43 N), especially at H4 to

H5 (from May 8th to May 29th) as it is shown in **Fig. IV.2.1E**. Also at Rs 2, light green zucchini clearly presented the highest firmness values, during the first harvest dates of the season H1 to H2 (from April 8th to April 15th) and at the end of the season H5 to H6 (from May 29th to June 24th) **(Fig. IV.2.1F)**. Moreover, a three way interaction (cultivar x ripening stage x harvest date) ($P < 0.05$) was found for texture during the middle of the season H3 to H4 (from May 8th to May 13th), 'Parador' and 'Cassiope' being the most firmness cultivars. Tissue dehydration also affects to the turgor and cell wall composition (Sams, 1999), showing zucchini fruits firmer values at the end of the season (from H4 to H6) (Luna et al., 2012). Therefore, it is interesting to point out that for both Rs 1 and Rs 2 colour and texture quality improvement early harvest dates from H1 to H4 (April 8th to May 13th). Likewise, dark to a lighter green zucchini selection could be suggested for increasing consumer acceptability due to these fruits presenting higher regularity as well as the best results in maintaining initial colour during storage (Loy, 2004).

3.2. Proximate composition

3.2.1. Dry matter, pH, titrable acidity, total soluble solids

In spite of non-significant differences between cultivars in dry matter (DM) being found in zucchini at Rs 1, 'Cronos' and 'Amalthée' at Rs 2 showed significantly higher DM content (6.25 and 6.17%, respectively). Zucchini fruits such as *Cucurbita moschata* presented low DM levels (contributing these to the popular smooth and pasty texture usually observed in sauces, soups, cakes and puddings) (Daniel et al., 1995) and small differences between immature fruits, positioning DM values far from butternut or acorn squash both characterized by good eating quality when DM content is close to 18-20% (Loy, 2011). According to pH, despite no significant differences being found between zucchini cultivars, values were influenced by ripening stage ($P < 0.01$) **(Table IV.2.2)**. In fact, in general around 2-3% higher values in pH were found at Rs 1, whereas differences between cultivars ($P < 0.05$) were only shown for titrable acidity (TA) **(Table IV.2.2)**. Referring to total soluble solids (TSS), values were found ranging from 3.87 to 4.48 at Rs 1 and from 3.76 to 4.18 at Rs 2 **(Table IV.2.2)**. Zucchini cultivar ($P < 0.05$) significantly affected TSS, identifying different groups classified as follows $TSS_{yellow} > TSS_{light\ green} > TSS_{dark\ green}$, 'Parador' presenting the highest TSS values at both ripening stages (4.48 and 4.18 º Brix at Rs 1 and Rs 2, respectively) **(Table IV.2.2).** According to Balkaya et al. (2010) and in line with other immature fruits such as muskmelon and butternut squash (~5 ºBrix) (Roura et al., 2007), zucchini could be catalogued in the low TSS content group (TSS= 3-7%).

Throughout the zucchini season, no significant interaction was observed for DM, while a three way interaction (cultivar x ripening stage x harvest date) ($P < 0.0001$) was found for TA **(Table IV.2.2)**. Concerning pH it was mainly affected by harvest date ($P <$

0.0001), cultivar being the main factor affecting TSS ($P < 0.05$) **(Table IV.2.2)**. In common with brassicas (Biesiada et al., 2007) or pepper (Valero et al., 2014) higher TSS and TA were detected in zucchini fruits during the course of the harvest season **(Fig. IV.2.2A, IV.2.2B, IV.2.2C and IV.2.2D)**, this could be linked to the moderate water stress contributing to increase the contents of these compounds (Kader and Rolle, 2004). Therefore, in terms of both TSS and TA early and middle harvest dates, H1 to H4 (from April 8th to May 13th), are adequate to avoid water stress temperature-dependent on zucchini fruits. Regarding the potential uses of zucchini as raw material for processing, yellow zucchini ('Parador') at both ripening stages would be ideal for the production of baby food elaboration due to its high DM (associated with good squash taste) and sugar content (Harvey et al., 1997). On the contrary, in the production of pies or frozen food in which not a high level in TSS are required (Loy, 2011), both light and dark coloured green zucchini are suggested.

Table IV.2. 2. Proximate composition of zucchini cultivars at different ripening stages.

		Dry matter (%)	pH	Titrable acidity	TSS (ºBrix)	Chl-A (mg/100 g FW)	Chl-B (mg/100 g FW)	Total Chllys (mg/100 g FW)
Ripening stage 1								
Dark green	Cronos	6.17 a	6.40 b	0.06 b	3.99 bc	9.54 a	3.81 a	13.35 a
	Cassiope	5.90 a	6.40 b	0.06 b	3.87 c	8.23 a	3.39 a	11.62 a
	Natura	5.93 a	6.50 ab	0.06 b	4.03 bc	9.27 a	3.79 a	13.06 a
	Sinatra	6.00 a	6.39 b	0.07 b	4.00 bc	8.95 a	3.57 a	12.52 a
Light green	Amalthée	6.22 a	6.39 b	0.06 b	4.22 ab	2.22 b	0.84 b	3.07 b
Yellow	Parador	6.25 a	6.58 a	0.09 a	4.48 a	0.24 c	0.16 c	0.41 c
Ripening stage 2								
Dark green	Cronos	6.25 a	6.18 a	0.06 a	3.96 ab	6.52 b	2.58 b	9.10 b
	Cassiope	6.04 ab	6.42 a	0.06 a	3.94 ab	6.39 b	2.58 b	8.97 b
	Natura	5.70 ab	6.37 a	0.05 a	3.76 b	7.35 a	2.93 a	10.28 a
	Sinatra	5.57 b	6.38 a	0.06 a	4.01 ab	6.41 b	2.62 ab	9.07 b
Light green	Amalthée	6.17 a	6.43 a	0.05 a	3.93 ab	1.14 c	0.54 c	1.68 c
Yellow	Parador	5.91 ab	6.44 a	0.06 a	4.18 a	0.19 d	0.18 d	0.38 d
Interactions								
	Cultivar (Cv)	ns	ns	*	*	****	****	****
	Ripening stage (Rs)	ns	**	ns	ns	***	**	***
	Harvests dates (Hd)	ns	***	ns	ns	ns	ns	ns
	Cv * Rs	ns	ns	ns	ns	ns	ns	ns
	Cv * Hd	ns	ns	ns	ns	ns	ns	ns
	Rs * Hd	ns	ns	**	ns	ns	ns	ns
	Cv * Rs * Hd	ns	ns	****	ns	ns	ns	ns

ns= not significant
* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$
**** $P \leq 0.0001$

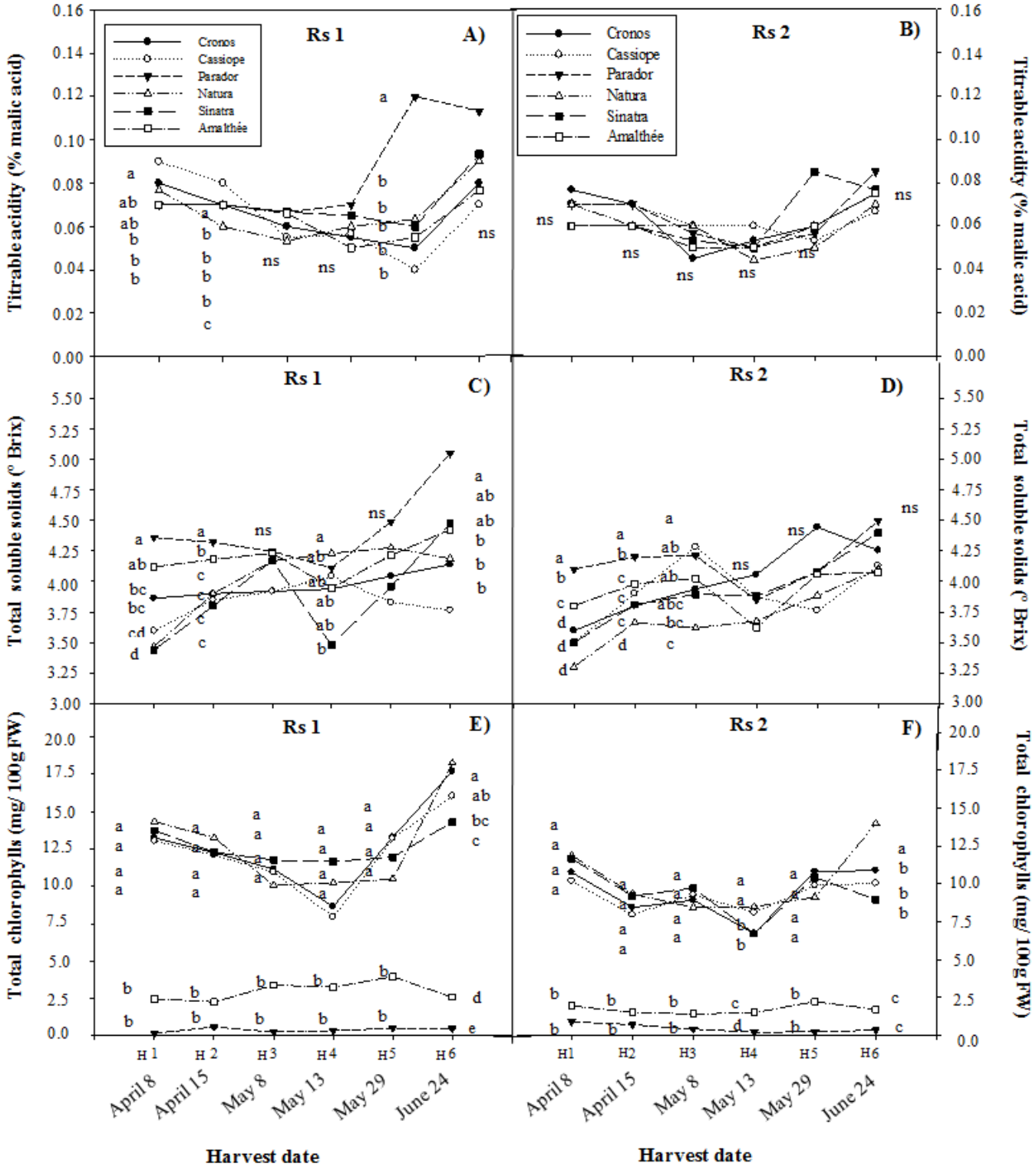


Figure IV.2. 2. Proximate composition of zucchini cultivars at different ripening stages at six harvest times. Means followed by different letters are statistically different according to LSD test at $P \leq 0.05$.

3.2.2. Chlorophyll *a*, chlorophyll *b* and total chlorophylls

Concentrations of chlorophyll *a*, chlorophyll *b* as well as the combination of both chlorophylls are shown in **Table IV.2.2**. Due to chlorophylls contribute to the green zucchini colour, natural pigments detected in the yellow zucchini were in much lower amounts, showing only dark and light green groups with the genetic capacity to synthesize chlorophylls. As expected, levels were the highest in dark green fruits (ranging from 8.97 to 13.35 mg/100 g FW), intermediate in light green fruits (between 1.68 and 3.07 mg/100 g FW), and low in yellow fruits (from 0.38 to 0.41 mg/100 g FW), predominating chlorophyll *a* as a major component in all samples. In fact, dark green zucchini fruits could be identified as a group with an excellent retention of sun light (chlorophyll *a/b* ratio ~2.5), while light green group and yellow groups could be recognized to receive moderate (chlorophyll *a/b* ratio ~2) and low (chlorophyll *a/b* ratio ~1.5) amounts of sun light, respectively (Hikosaka and Terashima, 1995).

Chlorophyll content was influenced by ripening stage ($P < 0.001$), Rs 1 increasing the amounts of these compounds by 7 to 45% (~25% for green, ~45% light green and ~7% yellow colour groups). These results are in agreement with previous studies confirming that chlorophylls were the natural pigments most abundant in immature cucumbers (Handley et al., 1983), peppers (Gómez-Ladrón de Guevara and Pardo-González, 1996) or tomatoes (Choi et al., 2010).

Significant interaction was shown by cultivar ($P < 0.0001$) in chlorophylls *a*, *b* and total content. Also ripening stage affected chlorophyll content, but with different intensities ($P < 0.01$) for chlorophyll *b* and ($P < 0.001$) for chlorophyll *a* and total chlorophylls.

The variation of colour stability in dark green zucchini was influenced by the temperature and illumination, registering significant increases in chlorophyll content at both ripening stages during late harvest dates, H4 to H6 **(Fig. IV.2.2E and IV.2.2F).**

Hence, to guarantee the highest chlorophyll content and consequently the greatest anticarcinogenic effect from green pigments, dark green at Rs 1 zucchini collected during early harvest, from H1 to H4 (April 8th to May 13th) would be suitable, thus avoiding the usually wide variation on surface colour that normally occurs at the end of the season.

3.3. Health-promoting compounds

3.3.1. Ascorbic acid and vitamin C

The average ascorbic acid and vitamin C content in the six zucchini varieties harvested at Rs 1 and Rs 2 are given in **Table IV.2.3**. Surprisingly, yellow fruits were highlighted by registering the highest ascorbic acid content at both ripening stages (23.56 mg/ 100 g FW for Rs 1 and 16.15 mg/ 100 g FW for Rs 2, respectively) while light green zucchini exhibited the highest values for vitamin C (30.39 mg/ 100 g FW for Rs 1 and 23.50 mg/ 100 g FW for Rs 2, respectively). On the other hand, similar values were observed in zucchini corresponding to dark green group (from 12.51 to 20.57 mg/100 g FW of

ascorbic acid and from 18.27 to 23.28 mg/ 100 g FW of Vitamin C) and light green group (from 16.60 to 19.56 of mg/ 100 g FW of ascorbic and from 23.50 to 30.39 mg/ 100 g FW of vitamin C). In general, values of ascorbic acid described in this work are in agreement with some *Cucurbita pepo* fruits including zucchini and pumpkin (~13 to ~20 mg/ 100 g FW) (Uusiku et al., 2010; Tarrago-Trani et al., 2012; Palmers et al., 2014), but lower than other fruits from the *Cucurbitaceae* family such as gourd (32 mg/100 g FW) (Kulkarni and Vijayanand, 2012). Moreover, results were consistent with prior studies, demonstrating that vitamin C is surface colour dependent, concluding that red and orange paprika contained 2 fold-higher ascorbic acid with respect to green ones (Chuah et al., 2008), while yellow tomatoes increased in 8% the vitamin C levels compared to the red conventional ones (Georgé et al., 2011). Interestingly, zucchini fruits at Rs 1 were identified as an excellent source of both ascorbic acid and vitamin C, decreasing those compounds with ripening stage ($P < 0.05$). Also as the fruit ripening, the content in vitamin C decreased in both 'Cherry' and conventional tomato (Raffo et al., 2002; Opara et al., 2012), bell pepper (Yahia et al., 2001; Fox et al., 2005) as well as in eggplant (Zaro et al., 2014). Concerning harvest date, no significant interaction was found for either ascorbic acid or vitamin C during the season **(Fig. IV.2.3A and IV.2.3B).**

Table IV.2. 3. Health-promoting compounds of zucchini cultivars at different ripening stages.

		Ascorbic acid (mg/100 g FW)	Vitamin C (mg/100 g FW)	Total carbohydrates (mg/g FW)	Total carotenoids (mg/100 g FW)	TPC (mg/100 g FW)	DPPH (mgTE/100 g FW)
Ripening stage 1							
Dark green	Cronos	18.21 ab	22.19 b	50.28 a	1.54 b	18.71 c	5.39 b
	Cassiope	16.92 b	23.28 b	47.04 bc	1.42 b	21.87 bc	7.14 a
	Natura	16.95 b	21.55 b	49.04 ab	1.57 b	20.76 bc	6.73 ab
	Sinatra	20.57 ab	22.19 b	49.41 ab	1.56 b	29.91 a	6.97 ab
Light green	Amalthée	19.56 ab	30.39 a	47.08 bc	0.55 c	29.34 a	6.82 ab
Yellow	Parador	23.56 a	24.65 b	42.26 c	3.55 a	25.59 ab	6.86 ab
Ripening stage 2							
Dark green	Cronos	12.51 c	18.27 b	51.19 ab	1.09 b	13.55 b	4.21 a
	Cassiope	15.12 bc	20.26 ab	55.39 ab	1.03 b	15.96 ab	4.02 a
	Natura	16.44 abc	18.58 b	59.06 a	1.19 b	13.78 b	4.07 a
	Sinatra	14.91 bc	20.73 ab	52.41 ab	1.09 b	15.63 ab	4.96 a
Light green	Amalthée	16.60 ab	23.50 a	50.50 b	0.41 c	15.17 ab	4.16 a
Yellow	Parador	17.46 a	18.82 ab	54.13 ab	3.32 a	17.30 a	4.72 a
Interactions							
	Cultivar (Cv)	*	ns	ns	****	*	ns
	Ripening stage (Rs)	*	*	*	**	***	***
	Harvests dates (Hd)	ns	ns	ns	ns	ns	ns
	Cv * Rs	ns	ns	ns	ns	**	ns
	Cv * Hd	ns	ns	ns	ns	ns	ns
	Rs * Hd	ns	ns	ns	ns	ns	ns
	Cv * Rs * Hd	ns	ns	*	ns	ns	ns

ns= not significant
* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$
**** $P \leq 0.0001$

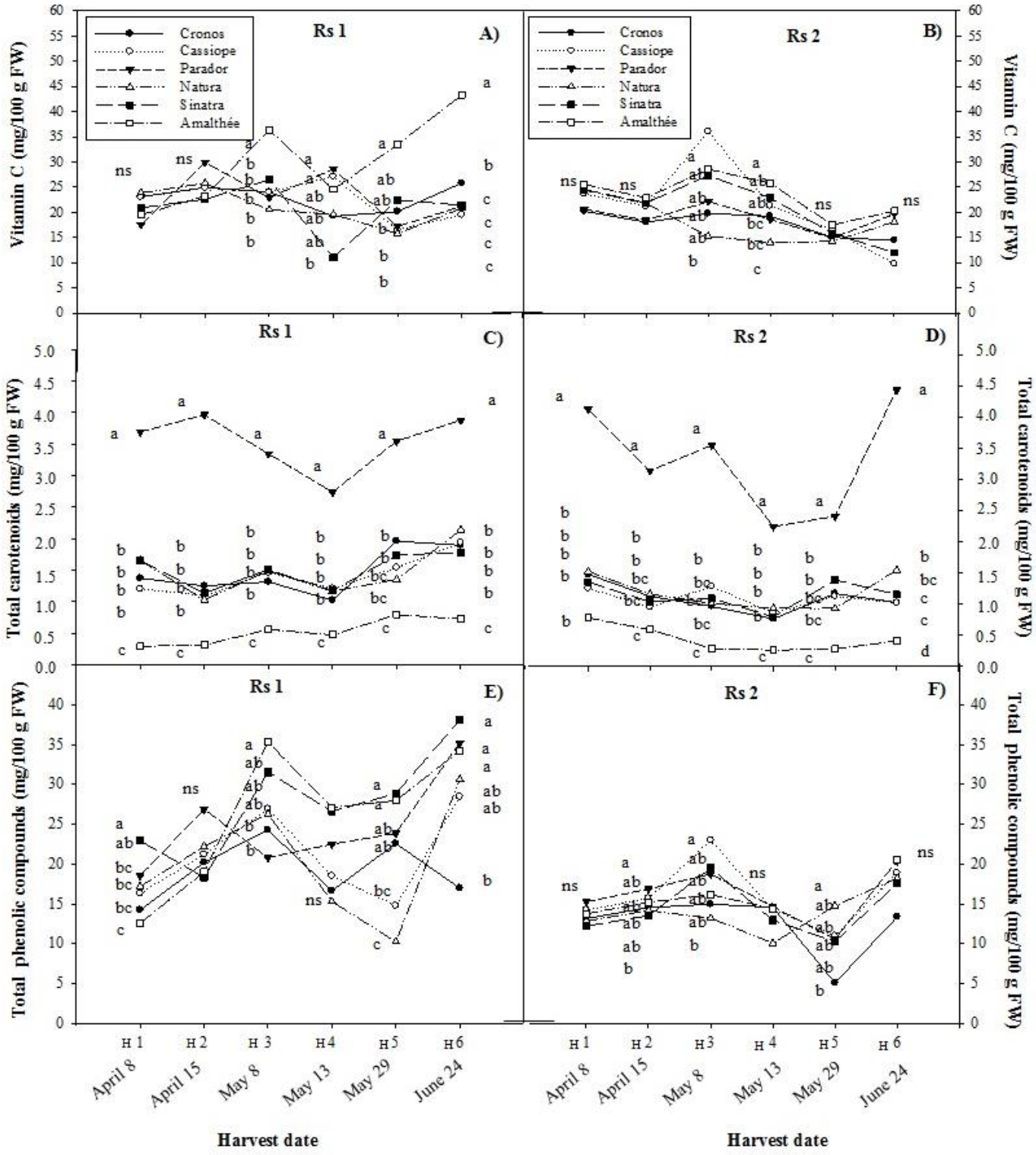


Figure IV.2. 3. Health-promoting compounds of zucchini cultivars at different ripening stages at six harvest times. Means followed by different letters are statistically different according to LSD test at $P \leq 0.05$.

Referring to the health benefit, it is well established that vitamin C is the most important vitamin in fruits and vegetables for human nutrition, contributing to reduce the incidence of diabetes and impaired glucose tolerance in a cohort study (Feskens et al., 1995). In this study, to ensure high content of those compounds ripening stage 1 selection of yellow zucchini (‘Parador’) for ascorbic acid or light green zucchini (‘Amalthée’) for vitamin C are suggested. However, due to vitamin C being highly sensitive to oxidation adequate preparation steps involved during industrial processing such as reducing the sodium hypochlorite in the washing water or washing the whole fruit as a previous step before slicing (Barrett et al., 2005) are important recommendations for initial vitamin C preservation.

3.3.2. Total carbohydrates and carotenoids

Total carbohydrates of dark green, light green and yellow zucchini corresponding to the mean values of the six harvest dates are shown in **Table IV.2.3**. Dark green zucchini (‘Cronos’, ‘Cassiope’, ‘Natura’ and ‘Sinatra’) registered the highest values in carbohydrates (ranging from 47.04 to 50.28 mg/g FW at Rs 1 and from 51.19 to 59.06 at Rs 2), whereas light green (‘Amalthée’) and yellow (’Parador’) zucchinis were classified as intermediate or low levels, depending on the ripening stage. Values in total carbohydrates studied in this work were slightly higher than *Cucurbita pepo* fruits described in literature (30 - 40 mg/g FW) (Uusiku et al., 2010; Palma et al., 2014). Sugars and starch are the major carbohydrate constituents in fresh squash (from 50 to 70%) (Phillips, 1946) with starch breakdown and substantial accumulation of glucose, fructose and sucrose occurring during ripening. In agreement with cucumber (Handley et al., 1983) or pumpkin (Oloyede et al., 2012) an increase in carbohydrate content was observed with ripening ($P < 0.05$) ranging from 1.80% (for ‘Cronos’) to 28.08% (for ‘Parador’). The harvest date affected total carbohydrates, only when interaction with cultivar and ripening stage were considered (cultivar x ripening stage x harvest date) ($P < 0.05$) **(Table IV.2.3)**. In fact, in this study as in other zucchini fruits (Rouphael and Colla, 2005) total carbohydrates in fruits harvested during the spring season were lower compared to fruits picked during the summer season.

When evaluating the carotenoid content in zucchini cultivars on both ripening stages during the season, significant results were found between cultivars ($P < 0.001$) **(Table IV.2.3)**. A wide range in carotenoid content was obtained, from 0.41 to 3.55 mg/ 100 g FW, corresponding to light green zucchini (‘Amalthée’) at Rs 2 and yellow zucchini (‘Parador’) at Rs 1, respectively. Taking into account other European countries, Spanish values reported in this study were between 13 and 18-fold higher than fruits from Luxembourg (0.083 mg/ 100 g FW) (Biehler et al., 2012). Contents in total carotenoids followed a similar pattern to that observed for ascorbic acid and vitamin C due to they were linked to surface colour, yellow zucchini displaying the highest values at both stages. In more detail, values varied from yellow zucchini (3.32 to 3.55 mg/ 100 g

FW) > dark green zucchini (1.03 to 1.57 mg/ 100 g FW) > light green zucchini (0.41 to 0.55 mg/ 100 g FW). A similar pattern was reported by Chuah et al. (2008), Marín et al. (2008) and Georgé et al. (2011), with carotenoid content increasing red pepper and orange paprika by 80-90% and 60% detected in the green variety. With respect to ripening development, as previously mentioned, values in Rs 1 were in higher concentrations than in Rs 2 ($P < 0.01$). The highest differences between ripening stages were observed in both dark and light green zucchini, 27.63% and 25%, respectively, while moderate was detected in yellow zucchini (6.47%). These results agree with Rodriguez-Amaya (2001) who described a notable reduction in carotenoid levels at development progresses in green fruits.

Through zucchini samples studied in this work, dark green zucchini displayed the highest carbohydrate levels. On the other hand, Rs 1 from yellow-orange and dark green zucchinis are recommended by contributing to the highest carotenoid intake **(Fig. IV.2.3 C and IV.2.3D)**, promoting lower lung cancer rates as well as playing an important role in decreasing the incidences of cataracts and preserving the retina (Ziegler et al., 1996; Rodriguez-Amaya, 2001).

3.3.3. Total phenolic compounds and antioxidant activity

Table IV.2.3 summarizes the data for total phenolic compounds (TPC) and antioxidant activity (DPPH) on the two ripening stages of the six cultivars studied. As described previously, vitamin C and carotenoid levels also reached a high TPC content within yellow zucchini (25.59 and 16.36 mg/100 g FW at Rs 1 and Rs 2, respectively), being in line with other yellow paprika or tomatoes (Chang and Liu, 2007; Georgé et al., 2011). Ripening stage was an important factor in phenolic concentration ($P < 0.001$), zucchinis at Rs 1 showing the highest values. In more detail, the differences between ripening stages were very high in 'Amalthée' (48.29%) and 'Sinatra' (47.74%), intermediate in 'Natura' (33.62%) and 'Parador' (32.39%), and moderate in 'Cronos' (27.57%) and 'Cassiope' (27.02%). The evident increase during the zucchini cycle in TPC that occurred throughout the Rs 1 compared to the Rs 2 **(Fig. IV.2.3E and Fig. IV.2.3F),** could be partially due to the size reduction of the fruits (Bai et al., 2009). Indeed, this agrees with recent studies (Sharma and Ramana Rao, 2013; Tundis et al., 2013) in pumpkin or peppers in which young fruits contained 25% higher TPC.

On the other hand, the antioxidant activity of fruits and vegetables is important for nutritional value assessing its measurement allows the evaluation of this nutritional variable. The scavenging effects on the six zucchini cultivars among the DPPH test were examined at concentrations ranging from 7.4 mg/ 100 g FW in 'Cassiope' to 5.30 mg/ 100 g FW in 'Cronos' **(Table IV.2.3)**. Intermediate values were obtained in the rest of the cultivar tested, with DPPH values ranging from 6.82 to 6.97 mg/ 100 g FW. Antioxidant activity levels in acorn squash (~1.5 µmol Trolox equiv/mL) (Boivina et al., 2009) were lower than values reported in this work. As expected, differences

between ripening stages were also found ($P < 0.001$), due to the radical scavenging activity being related to the phenol content. Ripening stage 1 registered the major DPPH values, positioning 'Cassiope' with the highest differences between ripening stages (~44%). In spite of not showing significant differences in DPPH in zucchini fruits at Rs 2, 'Sinatra' and 'Parador' could be recognized as remarkable antioxidant activity content cultivars, with values of 4.96 and 4.72 mg/ 100 g FW, respectively. In general, the nature of these compounds depends on the vegetable considered. For instance, in tomato and pepper total antioxidant capacity increases with maturity (Vicente et al., 2009), while in eggplant, as in zucchini, DPPH decreased with fruit development (Zaro et al., 2014).

In our study, from the antioxidant perspective, zucchins at ripening stage 1 are the most suitable size for increasing both phenolic and antioxidant activity, and especially 'Sinatra' for phenolics and 'Cassiope' for radical scavenging activity. At Rs 2, 'Parador' could be considered as a promising cultivar for containing a high content in both compounds.

3.4. Multivariate analysis

3.4.1. Correlations between parameters

Pearson correlation was used to investigate the interrelationships among parameters studied for each ripening stage **(Table IV.2.4)**. Referring to physical parameters, results revealed that the highest correlation in morphology of zucchini fruits was observed for weight. Thus, weight was highly correlated with diameter and length at both ripening stages ($r = \sim 0.8$, $P < 0.0001$). On one hand moderate correlations at both ripening stages between L* and a* ($r = \sim 0.60$, $P < 0.0001$), while strong for the rest of the colour-dependent parameters (particularly for b* and C*, with $r = 0.99$, $P < 0.0001$).

Both chlorophyll *a* and *b* as well as total chlorophylls showed at both ripening stages great correlations with colour parameters ($P < 0.0001$), confirming that chlorophylls are the main pigments responsible for the colour changes (especially for the lightness, L*, registering highest values $r > 0.88$, $P < 0.0001$), as well as between them ($r = 0.99$, $P < 0.0001$). Also carotenoid concentration played an important role at both ripening stages in values of the different CIELAB colour coordinates (L*, a*, b* C* and h^o), a* parameter showing the most suitable values ($r = \sim 0.90$, $P < 0.0001$) for distinguishing between carotenoid concentrations.

The importance of appropriate ripening stage selection also played an important role in health-promoting compounds. In fact, at Rs 2, length ($r = -0.49$, $P < 0.01$) and vitamin C ($r = 0.44$, $P < 0.01$) presented weak correlations with DPPH content.

Table IV.2. 4. Correlation coefficients of physico-chemical properties, proximate composition and nutritional compounds of zucchini fruits.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Weig.	*1*																	
	1																	
2. Diam.	*0.87d*	*1*																
	0.83d	**1**																
3. Long.	*0.78d*	*0.62d*	1															
	0.73d	**0.41b**	**1**															
4. L*	*ns*	*ns*	*ns*	*1*														
	ns	**ns**	**ns**	**1**														
5. a*	*ns*	*ns*	*ns*	*0.68d*	*1*													
	ns	**-0.28a**	**ns**	**0.55d**	**1**													
6. b*	*ns*	*ns*	*ns*	*0.95d*	*0.85d*	*1*												
	ns	**ns**	**ns**	**0.82d**	**0.90d**	**1**												
7. C*	*ns*	*ns*	*ns*	*0.94d*	*0.85d*	*0.99d*	*1*											
	ns	**ns**	**ns**	**0.81d**	**0.91d**	**0.99d**	**1**											
8. h°	*ns*	*ns*	*ns*	*-0.89d*	*-0.85d*	*-0.95d*	*-0.95d*	*1*										
	ns	**ns**	**ns**	**-0.87d**	**-0.86d**	**-0.99d**	**-0.98d**	**1**										
9. Text.	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*1*									
	ns	**ns**	**-0.30a**	**ns**	**ns**	**ns**	**ns**	**ns**	**1**									
10. D.m.	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*1*								
	ns	**0.38b**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**1**								
11. pH	*ns*	*ns*	*ns*	*0.27a*	*ns*	*0.33b*	*0.33b*	*-0.29a*	*ns*	*ns*	*1*							
	ns	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**1**							
12. TA	*-0.28a*	*-0.32b*	*ns*	*0.39b*	*0.54d*	*0.48c*	*0.48c*	*-0.46c*	*ns*	*ns*	*0.38b*	*1*						
	ns	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**1**						
13. TSS	*ns*	*ns*	*ns*	*0.42c*	*0.38b*	*0.41c*	*0.41c*	*-0.39b*	*ns*	*0.31b*	*ns*	*0.45c*	*1*					
	ns	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**1**					
14. Chl a	*ns*	*ns*	*ns*	*-0.90d*	*-0.61d*	*-0.88d*	*-0.88d*	*0.84d*	*ns*	*ns*	*-0.32b*	*-0.31a*	-0.37b	*1*				
	ns	ns	ns	**-0.93d**	**-0.64d**	**-0.87d**	**-0.87d**	**0.91d**	**ns**	**ns**	**ns**	**ns**	**ns**	**1**				
15. Chl b	*ns*	*ns*	*ns*	*-0.88d*	*-0.56d*	*-0.85d*	*-0.85d*	*0.82d*	*ns*	*ns*	*-0.33b*	*-0.27a*	*-0.36b*	*0.99d*	*1*			
	ns	**ns**	**ns**	**-0.91d**	**-0.65d**	**-0.87d**	**-0.86d**	**0.90d**	**ns**	**ns**	**ns**	**ns**	**ns**	**0.99d**	**1**			
16. Total chylls	*ns*	*ns*	*ns*	*-0.90d*	*-0.60d*	*-0.87d*	*-0.87d*	*0.84d*	*ns*	*ns*	*-0.32b*	*-0.30a*	*-0.37b*	*0.99d*	*0.99d*	*1*		
	ns	**ns**	**ns**	**-0.93d**	**-0.64d**	**-0.87d**	**-0.87d**	**0.91d**	**ns**	**ns**	**ns**	**ns**	**ns**	**0.99d**	**0.99d**	**1**		
17. T. carot.	*-0.35b*	*-0.37b*	*ns*	*0.47c*	*0.89d*	*0.68d*	*0.68d*	*-0.66d*	*ns*	*ns*	*ns*	*0.57d*	*0.39b*	*-0.33b*	*-0.29a*	*-0.32b*	*1*	
	-0.38b	**-0.41b**	**ns**	**0.28a**	**0.87d**	**0.70d**	**0.71d**	**-0.65d**	**ns**	**ns**	**ns**	**ns**	**0.32a**	**-0.36b**	**-0.37b**	**-0.36b**	**1**	
18. Asc. acid	*-0.28a*	*-0.26a*	*-0.33b*	*ns*	*ns*	*0.27a*	*0.27a*	*-0.26a*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*1*
	ns	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**1**

Ripening stage 1 is marked in italics and ripening stage 2 is marked in bold.

ns= not significant; a= * $P \leq 0.05$; b= ** $P \leq 0.01$; c= *** $P \leq 0.001$; d= **** $P \leq 0.0001$

Table IV.2. 4. Correlation coefficients of physico-chemical properties, proximate composition and nutritional compounds of zucchini fruits (continued).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
19.Vit C	*ns* **ns**	*ns* **ns**	*ns.* **-0.48c**	*0.32a* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **0.28a**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*-0.35b* **ns**	*-0.37b* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*1* **1**		
20.Tcarb	*ns* **ns**	*ns* **ns**	*ns* **ns**	*-0.36b* **ns**	*-0.44c* **ns**	*-0.44c* **ns**	*-0.44c* **ns**	*0.41c* **ns**	*ns* **ns**	*ns* **ns**	*-0.30a* **ns**	*-0.29a* **-0.37a**	*ns* **ns**	*0.37b* **ns**	*0.37b* **ns**	*0.37b* **ns**	*-0.35b* **ns**	*-0.36b* **ns**	*ns* **ns**	*1* **1**	
21. DPPH	*ns* **-0.33a**	*ns* **ns**	*ns* **-0.49c**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*-0.25a* **ns**	*ns* **0.44b**	*ns* **ns**	*1* **1**
22. TPC	*ns* **ns**	*ns* **ns**	*-0.34b* **-0.46b**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **0.46b**	*ns* **ns**	*-0.36b* **ns**	*ns* **0.33a**	*ns* **0.35a**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **ns**	*ns* **0.38b**	*ns* **-0.40b**	*ns* **ns**

Ripening stage 1 is marked in italics and ripening stage 2 is marked in bold.
ns= not significant; a= * $P \leq 0.05$; b= ** $P \leq 0.01$; c= *** $P \leq 0.001$; d= **** $P \leq 0.0001$

3.4.2. PCA and loading plot

PCA was applied to the mean of each constituent in both ripening stages, including the zucchini grouping colour (‘dark green’, ‘light green’, ‘yellow’) of each cultivar. Regarding ripening stage 1 **(Fig. IV.2.4A)** a total of 64.73% of difference was explained by relation between PC1, PC2 and PC3.

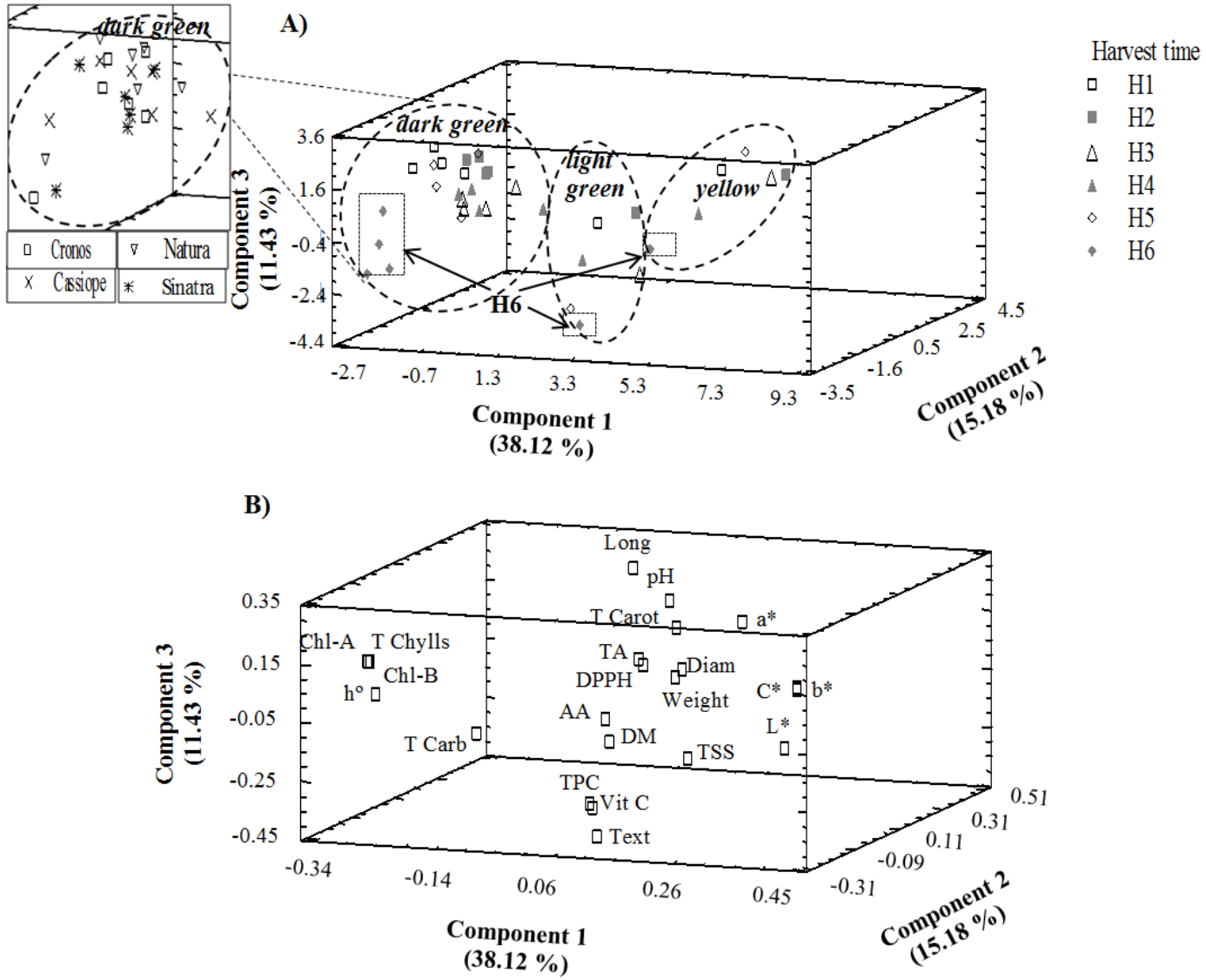


Figure IV.2. 4. Principal component analysis (PCA) plots of coloured zucchini fruits at ripening stage 1. (A) PCA scores plot for dark green (‘Cronos’, ‘Cassiope’, ‘Natura’ and ‘Sinatra’), light green (‘Amalthée’) and yellow (‘Parador’) at different harvest times (H1, H2, H3, H4, H5 and H6); (B) loading plots of zucchini fruits at ripening stage 1 for different variables on PC1, PC2 and PC3.

All dark green samples from ripening stage 1 are located on the left half of the plot, while light green and yellow zucchinis are situated in the center and on the right side of **Fig. IV.2.4A**, respectively. According to the distribution of the evaluated parameters in Rs 1 **(Fig. IV.2.4B)**, the colour parameters (L*, a*, b*, C* and h^o) as well as total chlorophylls (including forms Chl-A and Chl-B) and sugars (TSS and total carbohydrates) were responsible for the separation in PC1 while morphological parameters (weight, diameter, length) and antioxidant activity were mainly linked to the PC2 separation. Moreover, texture, proximate composition attributes (DM, pH, TA) as well as health-promoting compounds (T Carot, vit C, AA and TPC) contributed to divide in PC3. Positive values for PC1 indicated cultivars with higher values in colour parameters (L*, a*, b*, C*) and TSS (light green and yellow zucchini groups). The highest PC2 values corresponded to cultivars with high morphological properties (light green zucchini group) and antioxidant capacity (cv. 'Cassiope'), while light green cultivars with negative values in PC3 denoted high texture, DM, Vit C and TPC (cv. 'Amalthée').

Concerning ripening stage 2 **(Fig. IV.2.5A)**, PC1 was responsible for 37.14% while PC2 and PC3 accounts for 19.22 % and 11.28% of the variability observed in this size, respectively. In common with Rs 1, all dark green samples are located on the left hand of the plot, positioning light green and yellow zucchini in the center and on the right side of the **Fig. IV.2.5A**. Referring to the distribution of the evaluated parameters in Rs 2 **(Fig. IV.2.5B)**, the colour parameters (L*, a*, b*, C* and h^o) as well as total chlorophylls (and both forms Chl-A and Chl-B) were responsible for the separation in PC1 while morphological parameters (weight, diameter, length), proximate composition parameters (DM, pH and texture) and some nutritional constituents (T carb, DPPH and TPC) were mainly linked to the PC2 separation. Nevertheless, other attributes from approximate composition (TA and TSS) as well as health-promoting compounds (T Carot, Vit C, AA) contributed to PC3 division. Negative values for PC1 **(Fig. IV.2.5B)** indicated cultivars with higher pigments (h^o values, Chl-A, Chl-B and T Chylls) (dark green zucchini group), whereas the highest values in PC2 **(Fig. IV.2.5B)** corresponding to cultivars with high morphological traits (weight, diameter, length) (light green zucchini group) and proximate composition (Texture, pH, TA) (yellow zucchini group). Furthermore, negative values in PC3 denoted high TA, TSS, T Carot (yellow zucchini group) and AA (light green group).

Interestingly, for both ripening stages late zucchini harvest date samples (June 24th) are located in the bottom of each group, indicating the loss of the major parameters studied as a result of a decrease in the product´s quality.

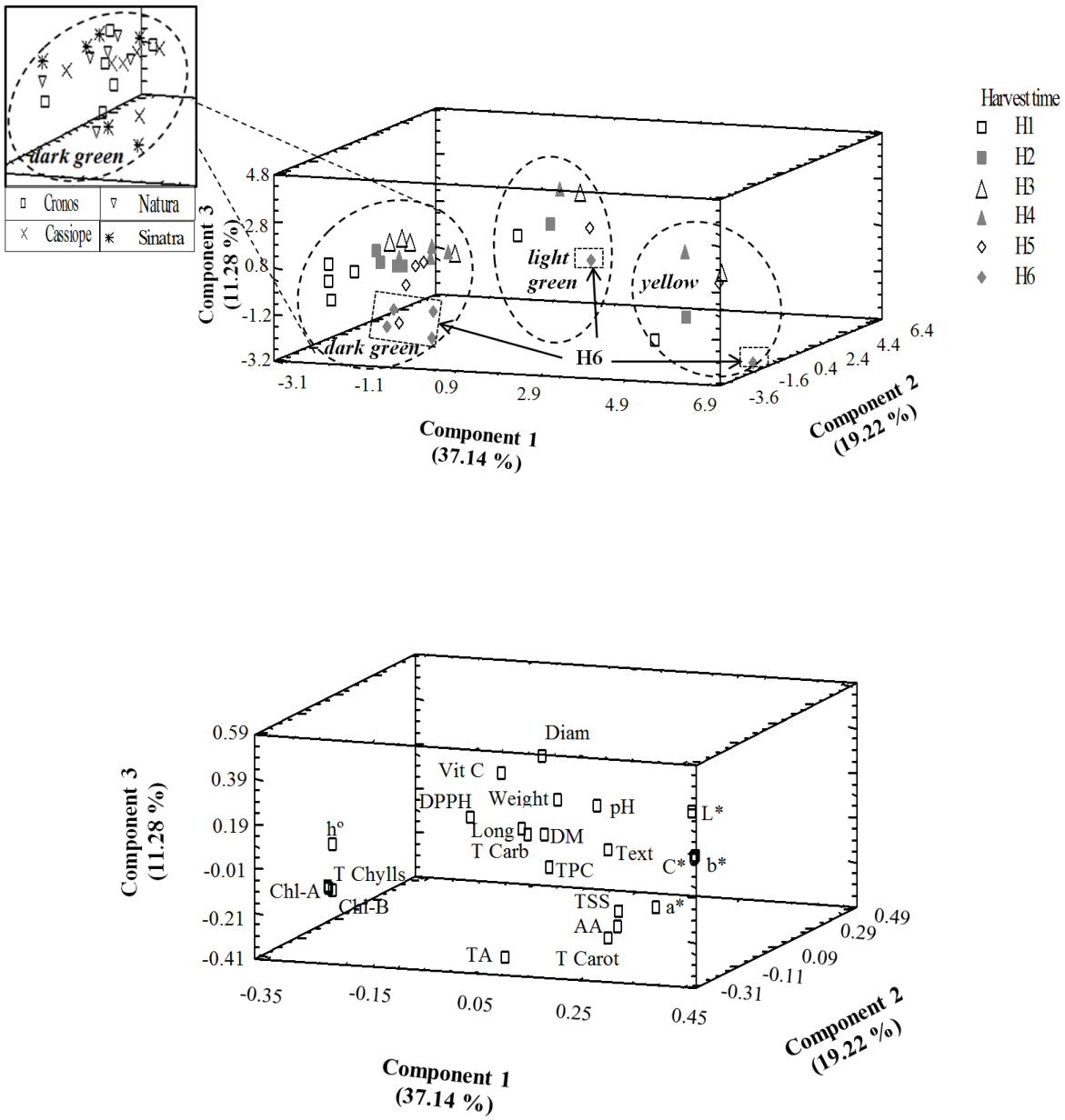


Figure IV.2. 5. Principal component analysis (PCA) plots of coloured zucchini fruits at ripening stage 2. (A) PCA scores plot for dark green ('Cronos', 'Cassiope', 'Natura' and 'Sinatra'), light green ('Amalthée') and yellow ('Parador') at different harvest times (H1, H2, H3, H4, H5 and H6); (B) loading plots at ripening stage 2 for different variables on PC1, PC2 and PC3.

4. Conclusions

This study demonstrated that zucchini has pre-harvest handling potential for providing higher physical quality and more nutritious fruits. Among strategies studied genotype was the main factor affecting major evaluated parameters, suggesting the selection of different zucchini coloured groups depending on results desired. In fact, an increase in the physical properties was observed in light green zucchini selection, whereas dark green and yellow zucchini were identified as a good source of chlorophylls and health-promoting compounds, respectively.

On the other hand, antioxidant compounds decreased with the ripening stage in all varieties tested. Zucchini genotype and ripening stage significantly affected physical, proximate composition and health-promoting compounds, while harvest date mainly affected physical properties. Results from the six harvest times during the production cycle of the vegetable suggested that early and middle harvest dates (from middle April to middle of May) are the best from a zucchini quality point of view, while late harvest dates (end May and June) are linked to tissue dehydration contributing to a decreased fruit size and promoting colour alterations. Our findings will provide useful information to consumers and processing industries for improving physical, proximate composition and health-promoting compounds in zucchini fruits by adequate management of pre-harvest factors.

Acknowledgements

This work was supported by the project PP.TRA2010.2012 (TRANSFORMA) and FEDER funds. Authors thank to the seed companies above mentioned for providing the material used in this study and to Nicholas Davies for grammatical revision of the manuscript. M.T. Blanco-Díaz acknowledges the financial support from the Spanish Ministry of Economy and Competitiveness as a contracted researcher of the Program 'Researcher Training' (Formación de Personal Investigador, FPI-INIA).

The results obtained in this paper reflect only the authors' view, and the community is not liable for any use that may be made of the information contained herein.

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4.3

'Quality of fresh-cut zucchini as affected by cultivar, maturity at processing and packaging'

María Teresa Blanco-Díaz, Antonio Pérez-Vicente, Rafael Font

Department of Postharvest Technology and Food Industry- IFAPA Centro La Mojonera

Camino San Nicolás, 1, 04745 La Mojonera, Almería, Spain

Submitted - Under review

Abstract

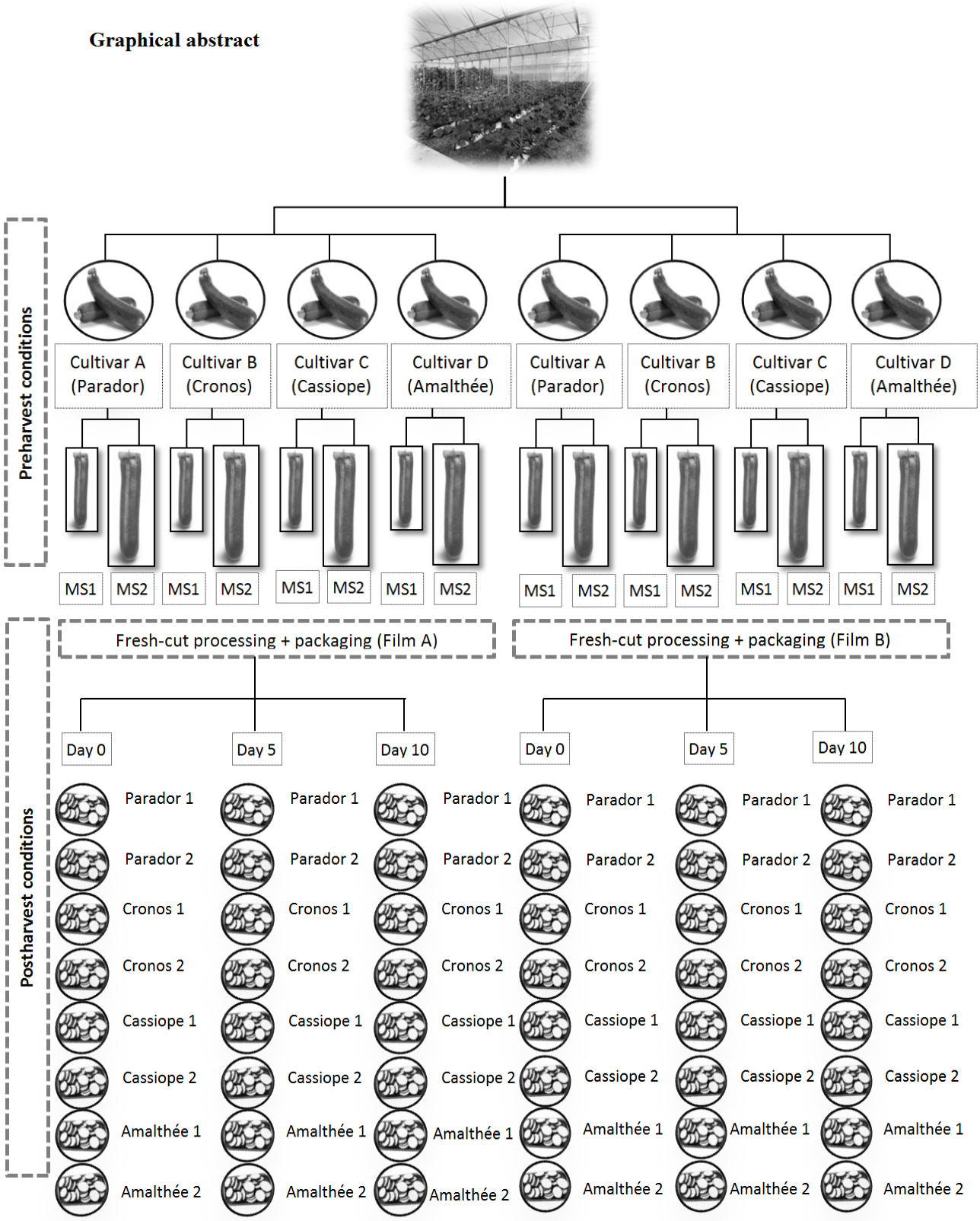
Appropriate plant material at processing and film packaging selection has a strong influence on product quality preservation. This is the first report studying the feasibility for minimal processing of four zucchini cultivars at two maturity stages, immature (MS1) and mature (MS2), stored at 6 ºC during 10 days under different packaging conditions. The treatments consisted in a 4x2x2x3 factorial combination of variety, maturity at processing, film packaging and storage time, including correlation studies between quality parameters. Negative effect on final quality after processing at MS1 was observed due to a higher respiration rate (between 15.91-20.12% than at MS2), CO_2 production and soluble solids consumption contributing to loss of firmness, slice discoloration and less overall quality. After 10 days under 25 µm film, cv. 'Cronos' showed the highest values in total chlorophylls (9.03 mg/100 g FW), while slices at MS1 suffered a noticeable oxidative stress increasing the total phenolic content (cv. 'Parador'>'Amalthée'>'Cronos'>'Cassiope'). At the end of storage, the highest vitamin C content was observed in trays sealed with 25 µm film (increasing cv. 'Cronos' at MS1 initial values by 228.09%). In conclusion, processing more mature zucchini and 25 µm film packaging were the most effective combination for preserving quality.

Keywords: food quality, minimal processing, packaging, physicochemical properties, nutritional quality.

Key findings:

- More maturity fresh-cut zucchini has better quality under MAP conditions.
- Passive MAP under high-permeability films improves fresh-cut zucchini quality.
- Fresh-cut zucchini cultivars should be selected to reduce color and firmness loss as well as preserving nutritional compounds.

Graphical abstract



MS1 or 1= maturity stage 1; MS2 or 2= maturity stage 2; Film A= 25 thickness µm; Film B= 40 thickness µm.

Figure IV.3. 1. Experimental procedure scheme.

1. Introduction

Fruit and vegetable cultivar selection has been proven to play a key role in the new fresh-cut products market, with the development of new varieties that preserve initial freshness after fresh-cut processing being one of the main goals for food industry. Although the quality of raw materials is one of the most important factors many other actions can influence the final fresh-cut quality, such as environmental factors, fruit maturity, postharvest handling, processing and the storage period.[1]

Cucurbita pepo L. is a large family with a high variability including 9 morphotypes (pumpkin, acorn, vegetable marrow, cocozelle, zucchini, straightneck, scallop, crockneck, ornamental gourd).[2] Zucchini is the best morphotype for size and form characteristics to be used in the food industry. In relation to the main area of vegetable production in Europe, which is Almería (Southern Spain), fresh zucchini is one of the most important exported vegetable. For this reason, different alternatives for increasing the added value of this vegetable should be considered by the food industry.

It is generally accepted that the physical damage that occurs during fresh-cut processing causes a respiration rate increase, biochemical and nutritional changes. This may result in a color, texture and sensory quality degradation, of the fresh-cut produce and development of browning during storage.[3] These changes are often cultivar-dependent and thus the incidence of decay during storage is particular among them.[4-6] For this reason, appropriate plant material selection before processing has a strong influence on zucchini metabolism and can reduce the product's shelf-life, nutritional and sensorial quality, as a consequence of the stress after cutting. In addition, sugar and acid content are crucial factors that influence taste and consumer demand, and material selection should consider both factors.[6]

On the other hand, fruit ripening is a sequence of biochemical events that affect to physiological maturity from inedible fruit to edible and tasty products. Major changes during ripening of fleshy fruit include loss of flesh firmness, degradation of chlorophyll, and formation of pigments, flavors, and aromas.[7]

Concerning fresh-cut products, more active metabolism and usually faster deterioration occur as a consequence of a higher respiration rate, thus contributing to a rapid loss of acids, sugars and other components that determine flavor quality and nutritional value.[8] Hence, assessing the appropriate maturity stage of commodities is of great interest in order to improve the quality and extend the shelf-life of fresh and minimally processed products. This fact motivated works such as those of Getinet *et al.*,[9] on tomato, Gil et al.,[10] on leafy vegetables, Pérez-López *et al.*,[11] on peaches, as well as Gorny et al.,[12] on fresh-cut pears and Martínez-Sánchez *et al.*,[13] on fresh-cut lettuce.

Low temperatures slow down fresh-cut metabolic processes, even though the use of a dynamic process of altering gaseous composition by modified atmosphere packaging (MAP) is useful to preserve quality of ready-to-cook fresh meal.[14] For this reason the

film selection to get the benefit of adequate modified atmosphere inside the package plays an important role in the shelf-life of the product.[15-17] Concerning fresh-cut zucchini, little is known about quality changes during storage under MAP conditions.[18] In this respect, the scientific reports including comparative studies of several zucchini cultivars at different processing maturity stages under MAP conditions, as well as their respiratory response, quality changes and nutritional compounds are required for both, agricultural producers and processing industries. This is the first report on the study of cultivars, maturity stage and film packaging on physicochemical, nutritional and sensory quality of fresh-cut zucchini during time of storage.

The objective of this study was to evaluate the effect of different packaging films on the postharvest quality and shelf-life of four zucchini cultivars widely commercialized by international seed companies, being those processed at two different maturity stages. Respiration rate, color, firmness, soluble solids, pH and total chlorophylls were considered as physicochemical parameters, while sensorial evaluation and nutritional compounds (total phenolic content and total vitamin C) were also evaluated. Correlation studies between the considered parameters were also conducted.

2. Material and methods

2.1. Plant material

Zucchini squash seeds (*Cucurbita pepo* spp. *pepo*) from different international seed companies cv. 'Parador' (Clause, Ltd), cv. 'Cronos' (Syngenta Crop Protection AG, Ltd), cv. 'Cassiope' (Gautier Semences, Ltd) and cv. 'Amalthée' (Gautier Semences, Ltd) were germinated, transplanted and grown in greenhouses of the Centre IFAPA La Mojonera (Almería, Spain) (36º 46' N, 2º 48' O). The fruits were collected on December according to two commercial zucchini sizes widely used when this vegetable is exported (less mature, zucchini harvest time at 'maturity stage 1' (MS1) (14-18 cm), and more mature or 'maturity stage 2' (MS2) (18-21 cm). Samples were stored in darkness at 6 ± 1 ºC and 90% relative humidity (RH) for 12 h before processing.

2.2. Washing, packaging and storage

Intact summer squash fruits were pre-washed in tap water for 1min to eliminate soil and insect residues and cut into slices (0.6 $\pm$ 0.01 cm thick) with a commercial equipment (SAMMIC CA-300, Guipúzcoa, Spain). Fresh-cut zucchini were washed for 2 min in 150 mg L^{-1} free chlorine solution (NaOCl) adjusted to pH 6.5 with citric acid. The excess surface water remaining on the zucchini slices was removed by centrifuging at 900 rpm for 4 min (SAMMIC ES-150, Guipúzcoa, Spain). Passive MAP conditions were developed after packaging zucchini slices (150 g) in trays with a gas transmission area of 154.54 cm^2 (1/4 GA, cod. G40T, Ilpra Systems, Mataró, Spain) and they were overwrapping by thermosealed on the top with two different bioriented polypropylene films (BOPP). The first one (25 µm thickness) was a high-permeability, with an oxygen

transmission rate (OTR) of 1300 (mL O_2 m^{-2} d^{-1}), while the other film (40 µm thickness) was a low-permeability, with an oxygen transmission rate (OTR) of 800 (mL O_2 m^{-2} d^{-1}) at 23 ºC (Derprosa, Jaén, Spain). Three replicates of each film packaging, cultivar, maturity stage and day of storage were packaged and stored under refrigeration conditions (6 ºC). Quality attributes were determined at initial moment (0 day), 5 and 10 days of storage **(Figure IV.3.1. graphical abstract).** For nutritional characterization about 80 g of fresh-cut samples of each package were freeze-dried (Telstar, Terrasa, Spain) until constant weight. Then, samples were ground with an industrial milled and stored at -80 ºC until chemical analyses in dark conditions.

2.3. Physicochemical analyses

2.3.1. Respiration rate and gas composition within the tray

Respiration rate (RR) (mL CO_2 kg^{-1} h^{-1}) was measured using a closed system. Whole zucchini fruits (3 fruits, about 450 g) and sliced zucchini (about 250 g) were placed in 3.65 L hermetic glass jars with a septum in the lid for sampling gas in the headspace at different times and stored at 6 ºC. Three replicates were analyzed for each whole or sliced zucchini cultivars and maturity stages. Gas sampling was monitored daily by means of a needle connected to an O_2/CO_2 gas analyser (CheckMate 3 PBI Dansensor, Ringsted, Denmark) with an accuracy of 0.5% using a septum in the lid for sampling gas in the headspace at different times.[19] This head space gas analyser is based on a highly stable zirconium electrochemical sensor to record the O_2 content and a mini-IR spectrophotometer detector to record the CO_2 content. The rates of carbon dioxide production (R_{CO2}) were determined by fitting experimentally obtained data on y_{CO2} with Eq. (1)

$$y_{CO2} = y^{i}_{CO2} - \frac{R_{CO2}\,W}{V_f}(t - t_i)\; x\; 100 \qquad (1)$$

where y^{i}_{CO2} and y_{CO2} are, respectively, CO_2 concentration (%) at the initial time t_i (h) (or time zero) and at time t (h). R_{CO2} is RR in mL kg^{-1} h^{-1} and W is the total weight of the product (kg). V_f is the free volume inside the glass jar (mL), which is the total volume of the glass jar minus the volume occupied by the sample.

Daily or immediately before opening the trays for the quality evaluations, the gas composition (O_2, CO_2) within the tray was measured through a septum by using a needle connected to the O_2/CO_2 gas analyzer. All measurements were made in triplicates.

2.3.2. Color

Color was measured using a CM-700d spectrophotometer equipped with D65 illuminant source (Minolta, Ramsey, NJ, USA) taking three measurements on the mesocarp region of the sliced zucchini. The instrument was previously calibrated on a white tile at an observation angle of 0. The values were expressed using the CIE *L*a*b** system, hue angle (hº) was also determined. At each storage time, the mean of five tissues pieces

(slices) were measured per tray for each cultivar (‘Parador’, ‘Cronos’, ‘Cassiope’ and ‘Amalthée’), processing maturity (MS 1 and MS2) and film packaging (25 µm and 40 µm). All measurements were made in triplicates.

2.3.3. Firmness

Firmness was determined following the method previously reported on zucchini by Brew *et al.,*[20] The use of a texture analyzer (TA-XT-Plus, Stable Micro System, Surrey, UK) calibrated with a 5-kg weight and equipped with a 4-mm diameter probe was used to assess the mesocarp firmness on sliced zucchini mesocarp region. The insert distance was 3 mm, with a stroke speed of 50 $mm \cdot min^{-1}$. At each storage time, the mean of five slices were measured per tray for each cultivar (‘Parador’, ‘Cronos’, ‘Cassiope’ and ‘Amalthée’) at different maturity stage (MS1 and MS2) and packaging films (25 µm and 40 µm), and the firmness was expressed in Newtons (N). All measurements were made in triplicates.

2.3.4. Total soluble solids (TSS) and pH

Three replicates of fresh-cut zucchini from each treatment were homogenized using a commercial blender (Moulinex, Barcelona, Spain). TSS of the juice was measured with a hand refractometer (SMART-1, Atago, Japón) and expressed as ºBrix at 20 ºC. Then, the pH of the juice was measured using a pH-meter (GLP 21+, Crison, Barcelona, Spain).

2.3.5. Total chlorophylls

The extraction and analysis of the chlorophylls pigments were carried out simultaneously, in order to avoid pigment degradation. Chlorophyll pigments were determined using a UV–Visible spectrometer (Thermo Fisher Scientific, Madison, Wisconsin, USA) and both chlorophylls, *a* and *b*, were determined according to the equations reported by Lichtenthaler and Wellburn[21] , using the method described by Blanco-Díaz *et al.,* [22] Total chlorophylls were calculated as chlorophyll *a* + chlorophyll *b*, and expressed as mg per 100 g fresh weigh (FW). All measurements were made in triplicates.

2.4. Sensory evaluation

After equilibrium at room temperature, a panel of five trained judges using a 9-point scale was used (9 = excellent, 7 = very good, 5 = good, limit of marketability, 3 = fair, limit of usability and 1 = poor, unusable)[23] in this study to quantitatively determined the ‘overall quality’ of zucchini samples. Panelists were asked to base their decision on the samples ‘overall quality’ taking into account their color, odor, and firmness. A score equal to 5 was used as the threshold for the produce acceptability. Three number codes were used to name samples in order to avoid the identification of the applied treatments.

2.5. Nutritional compounds

2.5.1. Total phenolic content

Total phenolic content (TPC) in the fresh-cut samples were obtained following by the Folin-Ciocalteu reagent method using gallic acid as external standard[24] with slightly modifications.[22] Results were expressed as mg per 100 g fresh weigh (FW). All measurements were made in triplicates.

2.5.2. Vitamin C

The total vitamin C analysis (ascorbic acid + dehydroascorbic acid) of fresh-cut zucchini were carried out using a UV-spectrophotometry (Thermo Fisher Scientific, Madison, WI, USA) method described by Rahman-Khan et *al.*,[25] with minors modifications consisting on adapting the ground freeze-dried sample (200 mg) and the volume of 85% H_2SO_4 added (10 mL) when the samples were cooled in the ice bath for 10 min. The absorbance of the coloured solution was read at 521 nm against a blank solution using quartz cuvettes with a cell path length of 1.0 cm. The regression equation and the regression coefficient (r^2=0.9964) values were obtained for the ascorbic acid. Results were expressed as mg per 100 g fresh weigh (FW). All measurements were made in triplicates.

2.6. Statistical analysis

Statistical analyses were carried out using the Statistix software version 9.0 (Analytical Software, Tallahassee, FL, USA). Experiments were performed using a completely randomized design. Data from the study were analyzed statistically by four-way ANOVA (cultivar x maturity at processing x film packaging x storage time) with least significant difference (LSD) at $P \leq 0.05$. Pearson correlation analysis was performed to corroborate relationships between parameters.

3. Results and discussion

3.1. Respiration rate and gas composition within the tray

RCO_2 of zucchini fruit were influenced by cultivar, maturity stage, damaged tissue after the cutting process and storage time, with the maturity stage being the primary factor that influenced the respiration rate **(Figure IV.3.2A to IV.3.2D)**.

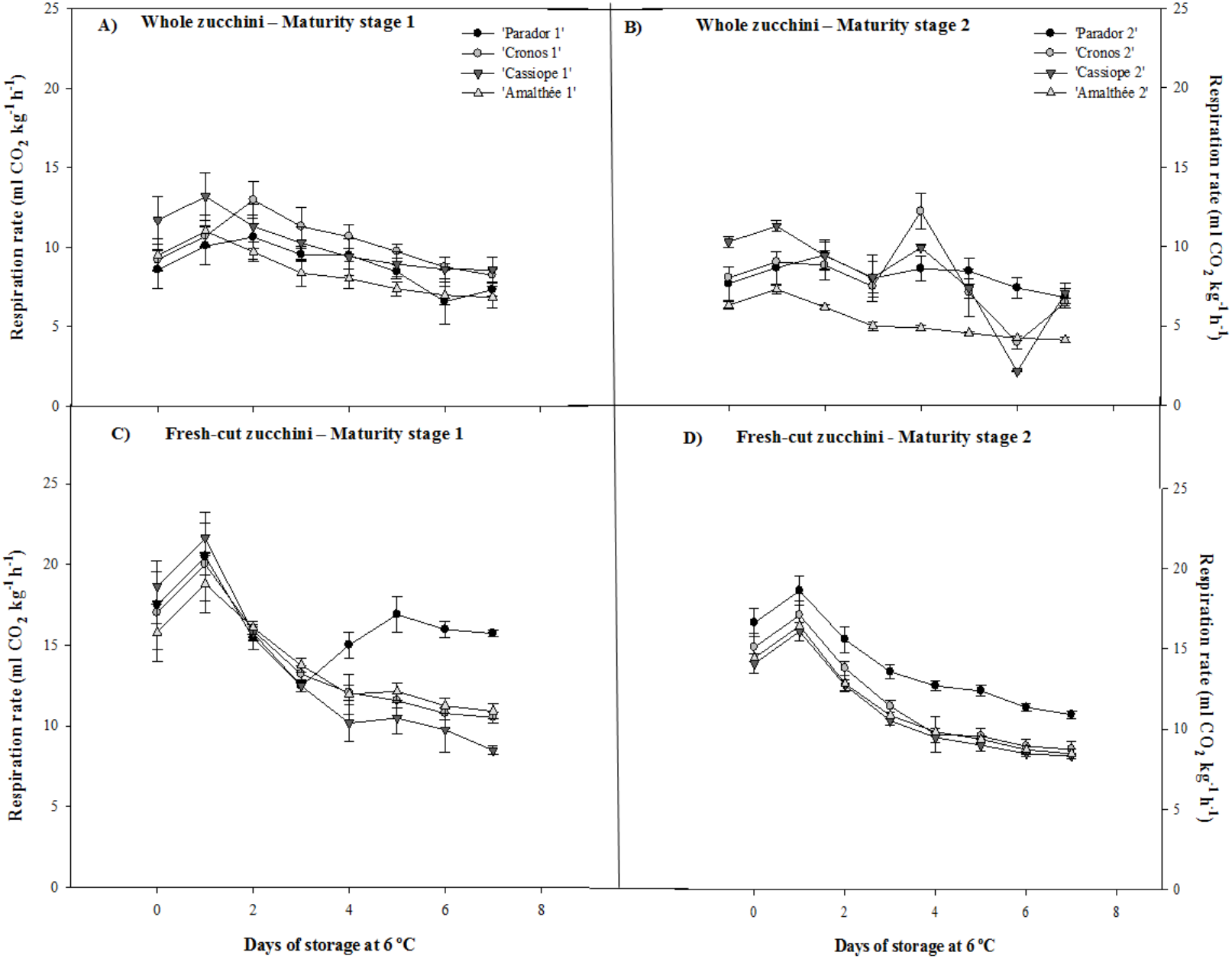


Figure IV.3. 2. Respiration rate of whole zucchini cultivars at 'maturity stage 1' (A) and at 'maturity stage 2' (B) during storage at 6 ºC; and in fresh-cut zucchini cultivars at 'maturity stage 1' (C) and at 'maturity stage 2' (D) during storage at 6 ºC. Vertical bars indicate the standard error of the means (n = 3).

For example, whole fruit exhibited more evident differences among cultivars at MS2 (respiration rate values fluctuated from 4.10 to 12.21 mL CO_2 kg^{-1} h^{-1}) **(Figure IV.3.2B)**, while for fresh-cut zucchini the effect of the cultivar selection was more obvious at MS1 (respiration rate values changed from 8.49 to 21.64 mL CO_2 kg^{-1} h^{-1}) **(Figure IV.3.2C)**. Considering both maturity stages during storage time, fresh-cut zucchini cv. 'Parador' showed the highest values on respiration rate, especially at MS1 (14.98 mL CO_2 kg^{-1} h^{-1}) **(Figure IV.3.2C)**. In this work, respiration rate differences among fresh-cut zucchini cultivars were higher than other cultivars 'Sofia' and 'Diamante' studied by Lucera *et al.,*[18] These results indicate the positive effect of MS2 to reduce the respiration rate during storage time, showing fresh-cut zucchini at MS1 a higher respiration rate (about 15.91-20.12%) than the same cultivar at MS2 (16.39% for cv. 'Parador'; 15.91% for cv. 'Cronos'; 16.28% for cv. 'Cassiope; and 20.12 % for cv. 'Amalthée'). These results agree with other studies concluding that less mature fruit like cucumber (belonging also to the *'Cucurbitaceae'* family)[26] or tomatillos[27] displayed a higher respiration rate than those more mature fruit.

Statistical analysis showed a four-way interaction (cultivar x maturity at processing x film packaging x storage time) for gas composition within the tray **(Table IV.3.1).** The highest respiration rate was found during the first days of storage, when higher O_2 ($r = 0.79$, $P < 0.001$) and lower CO_2 ($r = -0.78$, $P < 0.001$) levels were registered **(Table IV.3.1).**

Table IV.3. 1. Four-way ANOVA in physicochemical and nutritional quality parameters as affected by cultivar, maturity stage, film packaging and storage time.

	O_2	CO_2	Color			Firmness (N)	TSS (ºBrix)	pH	Total chlorophylls (mg/100 g FW)	Overall quality	Total phenolic compounds (mg/100 g FW)	Vitamin C content (mg/100 g FW)
			L*	b*	hº							
Cultivar (Cv)	****	****	****	ns	****	***	****	****	****	****	****	****
Maturity stage (Ms)	****	****	****	****	****	****	ns	****	****	ns	****	**
Film packaging (F)	****	****	****	****	****	***	*	****	**	*	****	****
Storage time (S)	****	****	****	ns	****	ns	****	****	ns	****	****	****
Cv x F	ns	****	*	ns	ns	****	ns	****	**	ns	****	ns
Cv x Ms	****	****	****	****	****	****	ns	****	****	ns	****	***
Cv x S	****	****	ns	ns	**	ns	ns	****	*	ns	****	****
Ms x S	****	****	ns	**	ns	ns	***	****	ns	***	****	ns
F x S	****	****	ns	ns	ns	ns	ns	****	ns	ns	***	ns
F x Ms	ns	****	****	*	**	ns	ns	****	**	ns	****	ns
Cv x Ms x F	****	****	****	ns	**	***	ns	****	**	ns	****	**
Cv x Ms x S	**	*	ns	ns	*	ns	***	****	ns	***	****	**
Cv x F x S	ns	**	ns	ns	*	ns	ns	ns	ns	ns	****	**
Ms x F x S	****	****	ns	ns	ns	ns	ns	ns	ns	ns	****	ns
Cv x Ms x F x S	**	*	ns	ns	*	ns	ns	ns	ns	ns	****	**

ns= not significant
* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$
**** $P \leq 0.0001$

After 10 days of storage the highest CO_2 levels were found in trays sealed with 40 µm polypropylene film in cv. 'Amalthée' at both maturity stages (23.74 kPa at MS1 **(Figure IV.3.3B)** and 20.46 kPa at MS2 **(Figure IV.3.3D)**; cv. 'Parador' was the fastest in CO_2 production in all treatments studied **(Figure IV.3.3A to IV.3.3D)**. Thus, on the basis of the gas composition inside the trays among the different zucchini cultivars studied, cv. 'Amalthée' and cv. 'Parador' could be considered as high CO_2 producers. Similar results have been previously described by Tudela *et al.,*[28] who after studying genetic influence on six fresh-cut lettuce cultivars, considered two of them as high CO_2 producers.

In relation to O_2 concentration inside the trays, higher levels were found when 25 µm overwrapping film was used **(Figure IV.3.3A and IV.3.3C).** In fact, very low O_2 concentrations (0.0-0.2 kPa) were detected inside the trays overwrapped with 40 µm film, while O_2 levels inside the trays overwrapped with 25 µm film (0.41-0.68 kPa) were close to those values recommended for fresh-cut zucchini storage.[29,30]

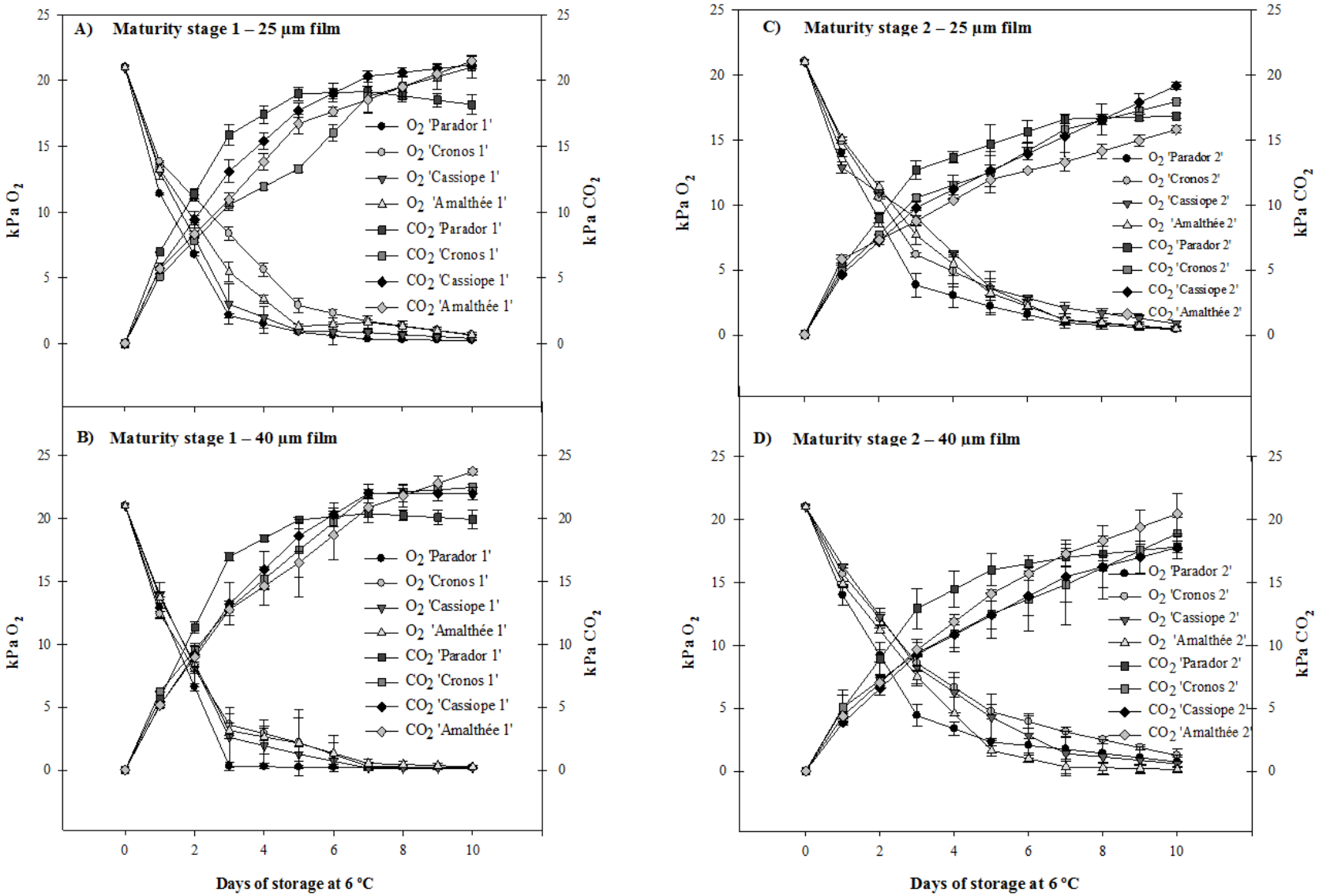


Figure IV.3. 3. Changes in headspace partial pressure of O_2 and CO_2 (kPa) of fresh-cut zucchini cultivars at 'maturity stage 1' in trays sealed with 25 µm film (A) and with 40 µm film (B) for 10 days at 6 ºC; and at 'maturity stage 2' in trays sealed with 25 µm film (C) and with 40 µm film (D) for 10 days at 6 ºC. Vertical bars indicate the standard error of the means (n = 3).

3.2. Color

There was a three-way interaction for L* values (cultivar x maturity stage x film packaging) **(Table IV.3.1)**. Initially (at day 0) higher values of L* were found in zucchini slices at MS1 than in the same cultivar at MS2, which is in agreement with other products also belonging to *'Cucurbitaceae'* family like melon cubes.[31] Correlation between L* values and O_2 levels ($r = 0.28$, $P < 0.001$) were higher than for CO_2 levels ($r = -0.25$, $P < 0.01$) **(Table IV.3.2)**, meaning that the O_2 levels inside the trays affected more to fresh-cut zucchini lightness loss **(Table IV.3.3 and Table IV.3.4)**. After 5 days of storage, a similar increment in L* values for all cultivars at MS2 were registered in samples stored in trays sealed with 40 µm film, decreasing those values throughout the storage period. The decrease in the L* values could be due to a combination of high CO_2 levels ($r = -0.25$, $P < 0.01$) with low O_2 levels ($r = 0.28$, $P < 0.001$), more obvious when 40 µm film was used **(Table IV.3.4)**. These results are in accordance with other authors who observed lightness losses after high CO_2 exposition level on sliced potatoes,[32] sliced apples,[33] or cubed papaya.[34]

Interactions found for b* were the lowest (two-way interaction, cultivar x maturity stage); while, on the contrary, the highest interactions were detected for h^o values (four-way interaction, cultivar x maturity stage x film packaging x storage time). In agreement with other authors[35,36] slices from delayed-harvest (MS2) presented lower h^o values (associated with yellow area) than less mature fruits (MS1) **(Table IV.3.3 and Table IV.3.4)**. Initially (at day 0) on zucchini slices at MS1, significant ($P < 0.05$) differences among cultivars in b* values were more evident (34.42; 27.77; 31.23; 25.13 for 'Parador', 'Cronos', 'Cassiope' and 'Amalthée, respectively) and also their variations during storage time, as a consequence of a rapid yellowing of these samples **(Table IV.3.3 and Table IV.3.4)**. In general, zucchini slices in trays overwrapped with 25 µm film experienced the lowest b* and the highest h^o values, indicating that higher O_2 and lower CO_2 levels inside the trays contributed to less discoloration in zucchini slices.

Table IV.3. 2. Pearson's correlation coefficients and significance for physicochemical properties and nutritional compounds of fresh-cut zucchini.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1.Respiration rate	1												
2. O_2	0.79 ****	1											
3. CO_2	-0.78 ****	-0.97 ****	1										
4. L* value	ns	0.28 ***	-0.25 **	1									
5. b* value	0.66 ****	0.37 ****	-0.36 ****	-0.28 ***	1								
6. h^o value	ns	0.21 **	-0.20 **	0.25 **	ns	1							
7. Firmness	ns	0.23 **	-0.24 **	0.19 **	ns	ns	1						
8. TSS	0.46 ****	0.47 ****	-0.50 ****	ns	0.26 ***	-0.19 *	ns	1					
9. pH	-0.68 ****	-0.84 ****	0.88 ****	ns	-0.31 ****	-0.17 *	ns	-0.61 ****	1				
10. Total chlorophylls	-0.24 **	ns	ns	ns	-0.20 **	0.23 **	0.30 ***	0.20 **	ns	1			
11. Overall quality	-0.60 ****	0.75 ****	-0.77 ****	0.40 ****	0.16 *	0.41 ****	0.20 **	0.36 ****	-0.74 ****	ns	1		
12. Total phenolic compounds	-0.25 ***	-0.55 ****	0.62 ****	-0.30 ***	ns	-0.30 ***	-0.24 **	-0.36 ****	0.65 ****	ns	-0.59 ****	1	
13. Vitamin C	-0.55 ****	-0.61 ****	0.65 ****	-0.23 **	-0.26 ***	-0.40 ****	ns	-0.43 ****	0.67 ****	ns	-0.65 ****	0.53 ****	1

ns= not significant; * = $\leq$ 0.05; ** = $\leq$ 0.01; *** = $\leq$ 0.001; **** = $\leq$ 0.0001.

Table IV.3. 3. Effect of cultivar and maturity stage on firmness, color parameters, TSS and pH of fresh-cut zucchini slices at 6 ºC under MAP conditions (25 µm film) during 10 days.

25 µm film		Maturity stage – 1			Mature stage – 2		
Parameter	**Cultivar**	**0**	**5**	**10**	**0**	**5**	**10**
Firmness (N)	**Parador**	10.03 ± 0.29A^{a}	6.98 ± 1.03B^{b}	7.16 ± 0.43C^{b}	10.18 ± 1.36A^{b}	12.58 ± 0.61A^{a}	11.83 ± 0.94A^{ab}
	Cronos	10.86 ± 1.08A^{a}	9.71 ± 0.97A^{a}	7.67 ± 0.52BC^{b}	9.73 ± 1.54A^{a}	8.46 ± 0.58C^{a}	8.64 ± 0.37B^{a}
	Cassiope	10.51 ± 0.82A^{a}	9.03 ± 0.40A^{a}	9.30 ± 1.13A^{a}	9.69 ± 0.45A^{a}	10.20 ± 0.59B^{a}	9.38 ± 0.88B^{a}
	Amalthée	10.71 ± 0.56A^{a}	9.62 ± 0.60A^{a}	8.57 ± 0.55AB^{b}	10.12 ± 0.95A^{a}	8.87 ± 0.91C^{a}	9.19 ± 0.54B^{a}
L* value							
	Parador	84.01 ± 0.46B^{a}	83.93 ± 0.05B^{ab}	83.13 ± 0.59C^{b}	83.54 ± 0.44B^{a}	82.80 ± 0.45C^{a}	81.62 ± 0.41C^{b}
	Cronos	85.63 ± 0.90A^{a}	82.85 ± 0.53C^{b}	82.04 ± 0.34BC^{b}	85.00 ± 0.16A^{a}	84.55 ± 0.11AB^{b}	84.08 ± 0.21AB^{c}
	Cassiope	84.13 ± 0.56B^{a}	84.19 ± 0.44AB^{a}	83.80 ± 0.94AB^{a}	85.19 ± 0.32A^{a}	84.05 ± 0.96B^{ab}	83.37 ± 0.15B^{b}
	Amalthée	86.36 ± 0.34A^{a}	85.04 ± 0.66A^{b}	84.60 ± 0.38A^{b}	85.26 ± 0.45A^{a}	85.07 ± 0.12A^{a}	84.43 ± 0.64A^{a}
b* value							
	Parador	34.42 ± 0.82A^{a}	29.66 ± 0.80A^{b}	27.36 ± 0.80A^{c}	26.15 ± 2.87A^{a}	23.37 ± 0.42A^{ab}	22.31 ± 0.47A^{b}
	Cronos	27.77 ± 0.68C^{a}	24.43 ± 0.57B^{b}	23.08 ± 0.45B^{c}	22.55 ± 2.27AB^{a}	20.54 ± 0.46C^{a}	20.07 ± 0.64B^{a}
	Cassiope	31.23 ± 1.04B^{a}	23.97 ± 0.93BC^{b}	22.48 ± 0.80B^{b}	22.53 ± 1.28AB^{a}	21.79 ± 0.63B^{a}	21.61 ± 0.41A^{a}
	Amalthée	25.13 ± 0.51D^{a}	22.66 ± 0.90C^{b}	20.84 ± 0.63C^{c}	19.54 ± 0.54B^{a}	19.12 ± 0.69D^{a}	19.00 ± 0.20C^{a}
h^{o} value							
	Parador	93.64 ± 0.79B^{a}	93.70 ± 0.09B^{a}	91.14 ± 0.83B^{b}	93.35 ± 0.89B^{a}	92.46 ± 0.91C^{ab}	91.78 ± 0.31D^{b}
	Cronos	95.45 ± 0.93A^{a}	96.03 ± 0.52A^{a}	95.99 ± 0.60A^{a}	93.76 ± 0.28B^{a}	93.57 ± 1.20C^{a}	93.19 ± 0.18C^{a}
	Cassiope	96.79 ± 0.17A^{a}	96.87 ± 0.78A^{a}	96.43 ± 0.13A^{a}	96.50 ± 1.85A^{ab}	97.58 ± 0.57A^{a}	94.33 ± 0.79B^{b}
	Amalthée	95.71 ± 0.84A^{a}	96.27 ± 1.16A^{a}	96.78 ± 0.59A^{a}	96.76 ± 0.62A^{a}	95.53 ± 0.49B^{b}	95.71 ± 0.35A^{b}
TSS (ºBrix)							
	Parador	4.35 ± 0.05 A^{a}	4.26 ± 0.02 A^{b}	3.98 ± 0 B^{c}	3.98 ± 0.02 B^{a}	3.75 ± 0.08 BC^{b}	3.59 ± 0.02 B^{c}
	Cronos	3.85 ± 0.53 AB^{a}	4.18 ± 0.14 A^{a}	4.08 ± 0.01 A^{a}	4.20 ± 0.02 A^{a}	3.69 ± 0.02 C^{b}	3.44 ± 0.03 C^{c}
	Cassiope	3.52 ± 0.19 B^{a}	3.30 ± 0.13 C^{a}	3.30 ± 0.01 D^{a}	3.80 ± 0.06 B^{b}	3.94 ± 0.02 A^{a}	3.75 ± 0 A^{b}
	Amalthée	4.05 ± 0.08 AB^{a}	3.63 ± 0.27 B^{b}	3.39 ± 0 C^{b}	3.87 ± 0.17 B^{a}	3.83 ± 0.02 B^{a}	3.74 ± 0.03 A^{a}
pH							
	Parador	6.46 ± 0.05 B^{c}	6.87 ± 0.02 A^{b}	7.06 ± 0 A^{a}	6.44 ± 0.05 B^{b}	6.95 ± 0 A^{a}	6.93 ± 0.02 A^{a}
	Cronos	6.55 ± 0.05 AB^{c}	6.65 ± 0.03 B^{b}	6.75 ± 0 C^{a}	6.48 ± 0.04 AB^{c}	6.64 ± 0.03 C^{b}	6.73 ± 0.02 C^{a}
	Cassiope	6.54 ± 0.04 AB^{c}	6.84 ± 0.02 A^{b}	7.06 ± 0 A^{a}	6.47 ± 0.02 B^{c}	6.66 ± 0.03 C^{b}	6.86 ± 0 B^{a}
	Amalthée	6.59 ± 0.05 A^{c}	6.86 ± 0.03 A^{b}	7.01 ± 0 B^{a}	6.55 ± 0.04 A^{c}	6.76 ± 0.02 B^{b}	6.88 ± 0.01 B^{a}

[a] Mean value ± standard deviation error of the means (n=3)

[b] Means followed by different letters, uppercase and lowercase for columns and rows respectively, are statistically different according LSD test at $p \leq 0.05$.

Table IV.3. 4. Effect of cultivar and maturity stage on firmness, color parameters, TSS and pH of fresh-cut zucchini slices at 6 ºC under MAP conditions (40 µm film) during 10 days.

40 µm film		Maturity stage – 1			Mature stage – 2		
		Days of storage			Days of storage		
Parameter	**Cultivar**	**0**	**5**	**10**	**0**	**5**	**10**
Firmness (N)	**Parador**	10.03 ± 0.29A[a]	7.41 ± 2.34B[a]	7.71 ± 0.44B[a]	10.18 ± 1.36A[a]	9.10 ± 1.43A[a]	9.69 ± 0.55B[a]
	Cronos	10.86 ± 1.08A[a]	9.58 ± 1.82AB[a]	9.04 ± 0.33B[a]	9.73 ± 1.54A[a]	11.12 ± 1.27A[a]	10.88 ± 0.22A[a]
	Cassiope	10.51 ± 0.82A[a]	12.01 ± 2.50A[a]	11.48 ± 1.09A[a]	9.69 ± 0.45A[a]	11.07 ± 0.83A[a]	10.66 ± 0.87AB[a]
	Amalthée	10.71 ± 0.56A[a]	9.84 ± 0.33AB[ab]	8.78 ± 0.85B[b]	10.12 ± 0.95A[a]	10.54 ± 1.42A[a]	9.97 ± 0.53AB[a]
L* value							
	Parador	84.01 ± 0.46B[a]	83.61 ± 0.85B[a]	82.74 ± 0.56B[a]	83.54 ± 0.44B[b]	85.38 ± 0.28B[a]	83.33 ± 0.72C[b]
	Cronos	85.63 ± 0.90A[a]	82.29 ± 0.94B[b]	81.51 ± 0.55C[b]	85.00 ± 0.16A[a]	85.84 ± 0.85B[a]	85.16 ± 0.94B[a]
	Cassiope	84.13 ± 0.56B[a]	85.19 ± 0.74A[a]	84.44 ± 0.33A[a]	85.19 ± 0.32A[a]	84.66 ± 0.85B[a]	83.75 ± 0.36C[b]
	Amalthée	86.36 ± 0.34A[a]	85.56 ± 0.41A[ab]	84.79 ± 0.73A[b]	85.26 ± 0.45A[b]	87.15 ± 0.40A[a]	86.99 ± 0.56A[a]
b* value							
	Parador	34.42 ± 0.82A[a]	30.39 ± 0.73A[b]	28.73 ± 0.77A[c]	26.15 ± 2.87A[a]	24.91 ± 0.96A[a]	25.47 ± 0.26A[a]
	Cronos	27.77 ± 0.68C[a]	26.45 ± 0.62B[b]	24.53 ± 0.32B[c]	22.55 ± 2.27AB[a]	18.58 ± 0.84C[b]	18.46 ± 0.25C[b]
	Cassiope	31.23 ± 1.04B[a]	24.48 ± 1.11C[b]	22.14 ± 0.82C[c]	22.53 ± 1.28AB[a]	22.15 ± 0.14B[a]	22.11 ± 0.57B[a]
	Amalthée	25.13 ± 0.51D[a]	23.63 ± 0.82C[b]	22.35 ± 0.64C[b]	19.54 ± 0.54B[a]	18.56 ± 0.89C[a]	18.20 ± 0.88C[a]
hº value							
	Parador	93.64 ± 0.79B[a]	92.31 ± 1.29C[ab]	91.48 ± 0.17D[b]	93.35 ± 0.89B[a]	91.19 ± 1.08C[b]	90.71 ± 0.09C[b]
	Cronos	95.45 ± 0.93A[a]	95.23 ± 0.66AB[a]	95.13 ± 0.37B[a]	93.76 ± 0.28B[a]	94.00 ± 0.72B[a]	93.74 ± 0.69B[a]
	Cassiope	96.79 ± 0.17A[a]	96.23 ± 0.78A[a]	95.95 ± 0.60A[a]	95.40 ± 0.92A[a]	96.53 ± 0.92AB[a]	95.33 ± 0.60A[a]
	Amalthée	95.71 ± 0.84A[a]	94.27 ± 0.92B[ab]	94.20 ± 0.24C[b]	96.76 ± 0.62A[a]	95.98 ± 0.97A[ab]	94.96 ± 0.33A[b]
TSS (ºBrix)	**Parador**	4.35 ± 0.05 A[a]	3.50 ± 0.01A[b]	3.52 ± 0.01 C[b]	3.98 ± 0.02 B[a]	3.95 ± 0.02A[a]	3.77 ± 0.02 A[b]
	Cronos	3.85 ± 0.53 AB[a]	3.47 ± 0.01 A[a]	3.66 ± 0.01 B[a]	4.20 ± 0.02 A[a]	3.41 ± 0.01 B[c]	3.47 ± 0.01 B[b]
	Cassiope	3.52 ± 0.19 B[a]	3.04 ± 0.25 B[b]	3.30 ± 0.03 D[ab]	3.80 ± 0.06 B[a]	3.31 ± 0.02 C[b]	3.38 ± 0.02 B[b]
	Amalthée	4.05 ± 0.08 AB[a]	3.65 ± 0.02 A[b]	3.75 ± 0 A[b]	3.87 ± 0.17 B[a]	3.41 ± 0.05 B[b]	3.52 ± 0.25 B[ab]
pH	**Parador**	6.46 ± 0.05 B[c]	6.92 ± 0.01 C[b]	7.05 ± 0 D[a]	6.44 ± 0.05 B[b]	7.08 ± 0.02 A[a]	7.05 ± 0 C[a]
	Cronos	6.55 ± 0.05 AB[b]	7.03 ± 0.04 B[a]	7.03 ± 0 C[a]	6.48 ± 0.04 AB[c]	6.97 ± 0.02 B[b]	7.04 ± 0.02 C[a]
	Cassiope	6.54 ± 0.04 AB[c]	7.30 ± 0.06 A[b]	7.42 ± 0.01 A[a]	6.47 ± 0.02 B[c]	7.06 ± 0.02 A[b]	7.19 ± 0.01 A[a]
	Amalthée	6.59 ± 0.05 A[c]	7.00 ± 0.03 B[b]	7.11 ± 0 B[a]	6.55 ± 0.04 A[c]	7.07 ± 0.03 A[b]	7.15 ± 0 B[a]

[a] Mean value ± standard deviation error of the means (n=3)

[b] Means followed by different letters, uppercase and lowercase for columns and rows respectively, are statistically different according LSD test at $p \leq 0.05$.

3.3. Firmness

There was a three-way interaction for L* values (cultivar x maturity stage x film packaging) **(Table IV.3.1)**, showing zucchini slices at MS1 with the highest firmness immediately after cutting (day 0) **(Table IV.3.3 and IV.3.4)**. However, zucchini slices at MS1 generally showed more susceptibility to loss of firmness when high O_2 ($r = 0.23$, $P < 0.01$) and L* values ($r = 0.19$, $P < 0.01$), and low CO_2 levels ($r = -0.24$, $P < 0.01$) were registered, meaning that firmness loss mainly took place during the first days of storage. Firmness loss is produced by the action of pectin enzymes, especially polygalacturonases, which was more obvious at MS1 on cv. 'Parador' and cv. 'Amalthée'. For example, during storage time, zucchini slices suffered firmness losses of between 11.51 and 29.37% (28.61% on cv. 'Parador', 19.98% on cv. 'Amalthée', 29.37% on cv. 'Cronos' and 11.51% on cv. 'Cassiope') when they were kept in trays sealed in 25 µm film; and from 9.22 to 23.13% (23.13% for cv. 'Parador', 18.02% for cv. 'Amalthée', 16.75% for cv. 'Cassiope' and 9.22% for cv. 'Cronos') when trays were sealed with 40 µm film. These results are in agreement with authors[37] who associated less loss of firmness with longer exposition times in high CO_2 levels (as is the case of trays sealed with 40 µm film).

On the contrary, considering the best conditions (zucchini slices at MS2 in the trays sealed with 25 µm film) **(Table IV.3.3)** firmness quality was kept during storage time in all zucchini cultivars (no statistical differences at $P < 0.05$), except on cv. 'Parador'.

3.4. Total soluble solids (TSS) and pH

A three-way interaction (cultivar x maturity stage x storage time) was found for both parameters, total soluble solids ($P < 0.001$) and pH ($P < 0.0001$) **(Table IV.3.1)**. A strong correlation between TSS and pH was found for respiration rate ($r = 0.46$, $P < 0.0001$; $r = -0.68$, $P < 0.0001$, for TSS and pH, respectively), O_2 ($r = 0.47$, $P < 0.0001$; $r = -0.84$, $P < 0.0001$ for TSS and pH, respectively) and CO_2 levels inside the trays ($r = -0.50$, $P < 0.0001$; $r = 0.88$, $P < 0.0001$ for TSS and pH, respectively). These correlations support the idea that soluble solids are used as energy reserves during respiration rate over time, so a decrease in TSS may also be related to damage caused in the cell structure.[38] On the other hand, a lower metabolism acceleration on fresh-cut zucchini slices held in trays sealed with 25 µm film was observed compared to 40 µm film **(Table IV.3.3 and IV.3.4)**. In fact, the decrease in TSS content was the highest on cv. 'Parador' (MS1), especially after overwrapping fresh-cut zucchini trays with 40 µm film (from 4.35 to 3.52 ºBrix, meaning a decrease of 19.08%), which probably might be explained by the high zucchini metabolism activity experienced.

In relation to pH, higher values were found in zucchini slices cv. 'Cassiope' at MS1 (reaching values from 6.54 to 7.06 and from 6.54 to 7.42, corresponding to an increase of 7.95% and 13.45% for 25 µm and 40 µm packaging film, respectively), which may

be associated with production of organic acids and subsequently getting superior quality fruits with delay of ripening.[39]

3.5. Total chlorophylls

There was a three-way interaction (cultivar x maturity stage x storage time) for total chlorophylls **(Table IV.3.1)**. Chlorophylls are related to green color in plants and they are mainly contained within the chloroplasts, having as their primary role the photosynthetic production of carbohydrates from carbon dioxide and water. Almost any type of food processing or storage causes some deterioration of the chlorophyll pigments, being the phenophytinisation (conversion of chlorophyll to phenophytin compound) the major change produced.[40] Therefore, it is reasonable to expect that after processing, protein denature occurs breaking down the zucchini cell walls, reducing firmness and contributing to chlorophyll pigment degradation, showing in our study a correlation of $r = 0.30$, $P < 0.001$. Indeed this chlorophyll loss seems to be more noticeable with respiration rate increases ($r = -0.24$, $P < 0.01$) **(Table IV.3.2).**

Cultivar and maturity at processing had more effect ($P < 0.0001$) on total chlorophyll content than packaging film selection ($P < 0.01$) **(Table IV.3.2)**. Thus, after 10 days of storage the highest values in total chlorophylls were monitored on cv. 'Cronos' at MS1 and cv. 'Cronos' at MS2 (9.03 vs. 5.57 mg/100 g FW; 6.69 vs. 3.80 mg/100 g FW, corresponding to 25 vs. 40 µm film, respectively), being slices from cv. 'Parador' and cv. 'Amalthée' severely affected by pigment losses (97.66 and 74.83%, respectively) **(Figure IV.3.4A and IV.3.4B)**. This fact supports the idea that when higher metabolic activity occurs (clearly found in 40 µm film) a higher sugar consumption takes place, resulting in lower TSS values and total chlorophyll content (TSS correlation with $r = 0.20$, $P < 0.01$). Hence, from our study it is logical to suppose that a decrease in total chlorophylls encouraged higher values in yellow (b* correlation with $r = -0.20$, $P < 0.01$) and lower in green color (h^o correlation with $r = 0.23$, $P < 0.01$).[41]

In general, high-permeability film (25 µm) contributed to maintaining chlorophyll pigments on minimally processed zucchini samples during storage. Thus, an increase in chlorophyll levels was observed in the best packaging film (25 µm), particularly in cv. 'Cassiope' at MS1 and cv. 'Cronos' at MS1 (exhibiting increases by 17-fold and 4-fold, respectively). These results are in accordance with other authors[42] who concluded that the notable increase in total chlorophylls observed in fresh-cut immature broccoli were associated to keep samples under low oxygen conditions (3% O_2+10% CO_2).

3.6. Sensory evaluation

A three-way interaction (cultivar x maturity stage x storage time) was found **(Table IV.3.1)**. As expected, 'overall quality score' decreased during time of storage ($P < 0.0001$) **(Table IV.3.1)** following for both packaging films the same zucchini cultivar ranking (cv. 'Cronos' > cv. 'Amalthée' > cv. 'Cassiope' > cv. 'Parador') **(Figure**

IV.3.4C and IV.3.4D). In general, MS1 had a negative impact on the sensory evaluation of zucchini slices compared to MS2, showing clear differences between maturity stages after 5 days of storage. Comparing zucchini slices at MS1 vs. MS2 at the end of the storage period, in the best packaging conditions (25 µm film), 'overall quality scores' were as follows cv. 'Cronos' (6.17 vs. 6.75) > cv. 'Amalthée' (5.70 vs. 6.0) > cv. 'Cassiope' (4.50 vs. 5.04) > cv. 'Parador' (3.04 vs. 3.14). These results confirm fresh-cut studies on melon (belonging to *'Cucurbitaceae'* family) in which the sensory quality in immature fresh-cut was lower compared to more mature fruit after 3 days of storage at 10 ºC.[43] Aditionally, our results are in agreement with Brecht[44] about the impact of variety on fresh-cut product quality.

Also gas composition within the trays affected 'overall quality' of zucchini slices. For instance, high levels in O_2 ($r = 0.75$, $P < 0.0001$) and low in CO_2 ($r = -0.77$, $P < 0.0001$) **(Table IV.3.2)** contributed to a better score in zucchini slices during sensory evaluation by panelists, offering an improvement on 'overall quality score' in the 25 µm film trays. In spite of the very low O_2 and high CO_2 levels inside the trays sealed with 40 µm film, when these trays were opened after 10 days only a slight off-odor was detected but it quickly dissipated and no off-flavors were apparent when the zucchini slices were evaluated, except for cv. 'Parador' resulting in noticeable decay symptoms falling below the limit of marketability (overall quality score of 2.5 – close to poor). This might be due on one hand to off-odor (data not shown) and zucchini slice discoloration (L* and h^o correlation of $r = 0.40$, $P < 0.0001$ and $r = 0.41$, $P < 0.0001$, respectively); and on the other hand to firmness loss ($r = 0.20$, $P < 0.01$) caused by long exposition time (from day 3 to day 10) to extremely low O_2 levels when 40 µm film was used **(Figure IV.3.4C and IV.3.4D)**. These results described are in accordance with the greatest aptitude of high-permeability films for preserving fresh-cut zucchini[18] or mushrooms.[45]

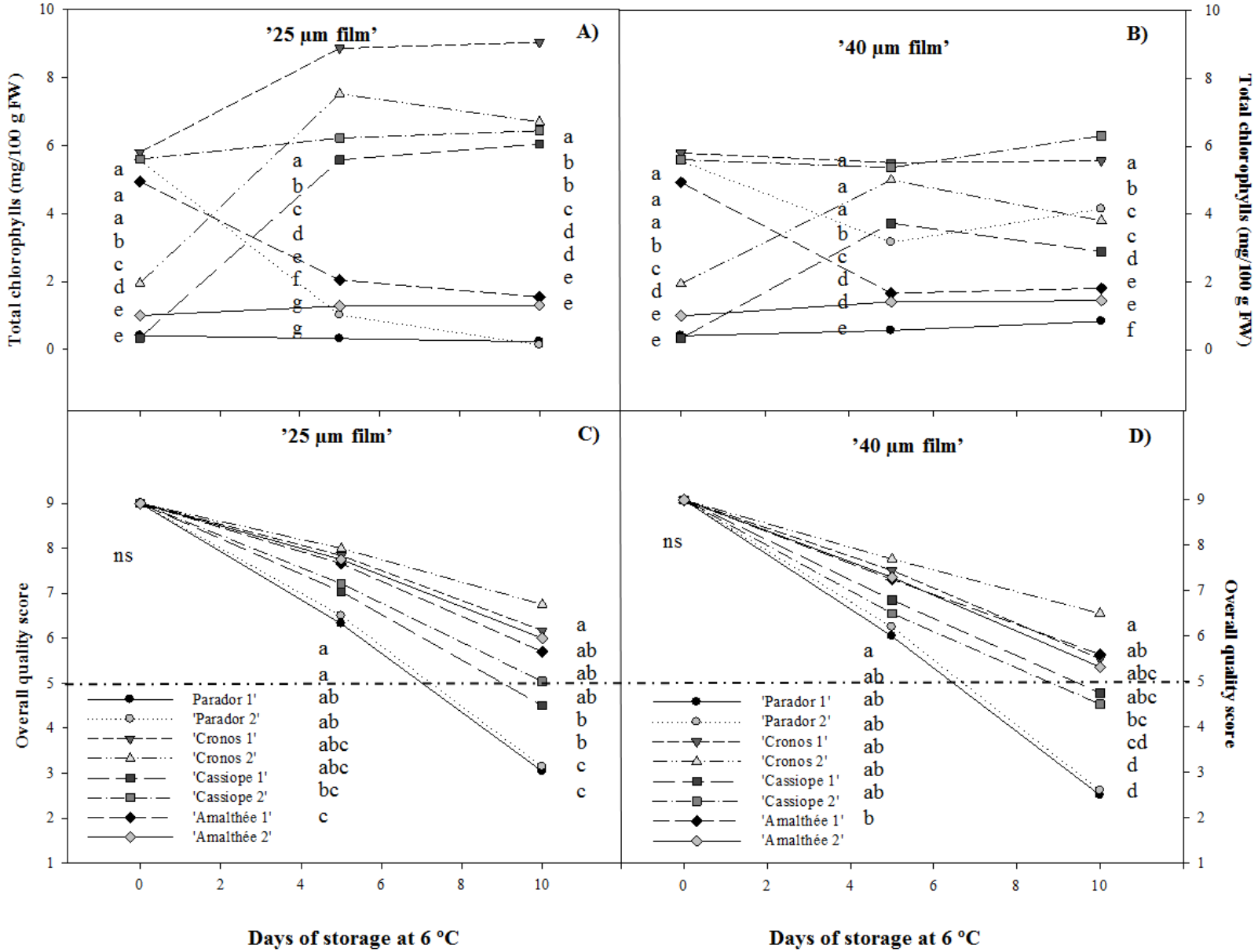


Figure IV.3. 4. Total chlorophylls of fresh-cut zucchini cultivars in trays sealed with 25 µm film (A) or with 40 µm film (B) for 10 days at 6 °C. Overall quality score of fresh-cut zucchini cultivars in trays sealed with 25 µm film (C) or with 40 µm film (D) for 10 days at 6 °C. Means followed by different letters are statistically different according to LSD test at $P \leq 0.05$.

3.7. Total phenolic content

A four-way interaction (cultivar x maturity stage x packaging film x storage time) was found **(Table IV.3.1)**. Initially, TPC in zucchini cultivars studied decreased by delayed harvest, corresponding to an increase in TPC between less mature and more mature zucchini stages around 16.65-34.29%. In fact, TPC could be decreased due to the enlargement of the fruit, being in accordance with other authors who studied maturity stages on vegetables such as sweet pepper[46] or baby leaf lettuce,[13] as well as on fruits like pears.[35] A strong correlations between TPC and different parameters O_2 ($r = -0.55$, $P < 0.0001$), CO_2 ($r = 0.62$, $P < 0.0001$), TSS ($r = -0.36$, $P < 0.0001$), pH ($r = 0.65$, $P < 0.0001$) and 'overall quality score' ($r = -0.59$, $P < 0.0001$) were found; meaning that TPC significantly ($P < 0.05$) **(Figure IV.3.5A and IV.3.5B)** increased gradually throughout the experimental period. Indeed, packaging film selection was an important factor in TPC ($P < 0.0001$), 25 µm film promoting a noticeable increase in these compounds (especially in zucchini slices at MS1). For example, after 10 days in 25 µm film TPC accumulation was higher in cv. 'Parador' > cv. 'Amalthée' > cv. 'Cronos'> cv. 'Cassiope' (172.93% and 223.36%; 94.28% and 108.40%; 105.04% and 79.58%; 36.30% and 83.71% corresponding to MS1 and MS2, respectively). The TPC increase in freshly-cut zucchini samples may be linked to enhanced oxidative stress, which induces the TPC rise involved in the repair of the damaged tissue.[13] Additionally, our results agree with Oms-Oliu *et al.*, [47] who observed a similar trend in TPC in fresh-cut melon stored under the best MAP conditions (2.5 kPa).

3.8. Vitamin C

Cultivar ($p < 0.0001$), maturity stage ($p < 0.01$), packaging film ($p < 0.0001$) and storage time ($p < 0.0001$) as well as their interactions ($p < 0.01$) affected vitamin C content **(Table IV.3.1)**. Vitamin C content in zucchini cultivars studied increased by delayed harvest **(Figure IV.3.5C and IV.3.5D)** that could be attributed in response to high intensity light reception during the period of ripening.[48] Similar behaviour was found for vitamin C compared to TPC. Thus, vitamin C content registered lower values when an increase in both respiration rate ($r = -0.55$, $P < 0.0001$) and O_2 levels ($r = -0.61$, $P < 0.0001$) and a decrease in CO_2 concentrations ($r = 0.65$, $P < 0.0001$) inside the trays were reached (more noticeable during the first five days of storage) **(Figure IV.3.5C and IV.3.5D)**. Additionally, other correlations were found, such as those with TSS ($r = -0.43$, $P < 0.0001$), pH ($r = 0.67$, $P < 0.0001$) and 'overall quality score' ($r = -0.65$, $P < 0.0001$); suggesting that both packaging conditions preserved vitamin C content **(Table IV.3.2).**

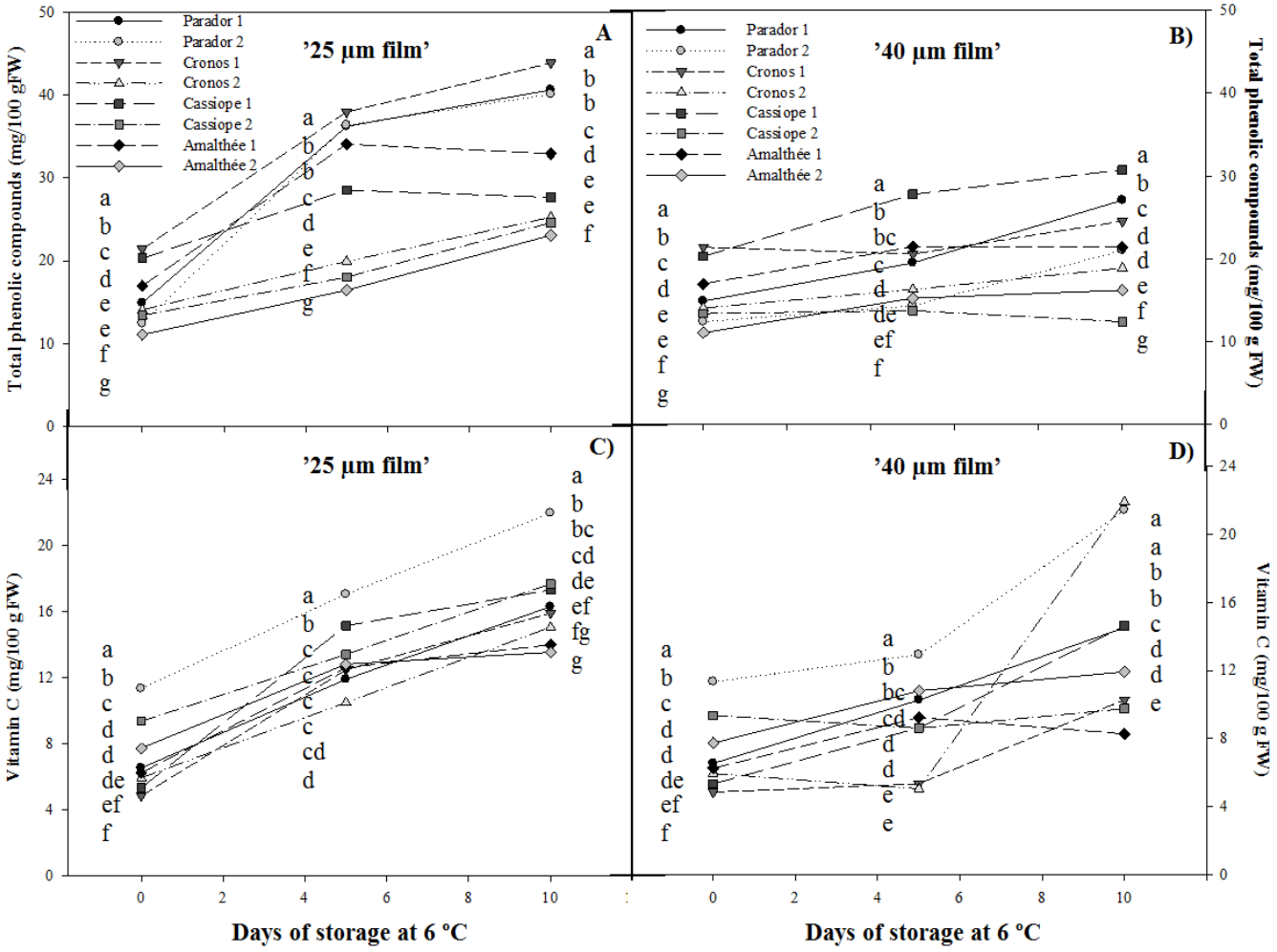


Figure IV.3. 5. Total phenolic compounds of fresh-cut zucchini cultivars in trays sealed with 25 µm film (A) or with 40 µm film (B) for 10 days at 6 °C. Total vitamin C of fresh-cut zucchini cultivars in trays sealed with 25 µm film (C) or with 40 µm film (D) for 10 days at 6 °C. Means followed by different letters are statistically different according to LSD test at P ≤ 0.05.

Higher content in vitamin C was reached when the highest permeability film was used (25µm thickness), although the values depended on cultivar selected. For example, after 5 of storage fresh-cut zucchini cv. 'Parador' contained around 2-fold increase in vitamin C content compared to cv. 'Cronos' (10.25 and 12.91 mg/100 g for cv. 'Parador', while 5.34 and 5.04 mg/100 g FW for cv. 'Cronos', corresponding to MS1 and MS2, respectively). Intermediate vitamin C values than those previously described were determined in zucchini slices cv. 'Amalthée' (9.23 and 10.80 mg/100 g FW corresponding to MS1 and MS2, respectively) and cv. 'Cassiope' (8.61 and 8.60 mg/100 g FW for MS1 and MS2, respectively); increasing cv. 'Cronos' at MS1 the initial values by 228.09% (ranging vitamin C content from 4.84 to 15.88 mg/100 g FW). These results support studies by Tudela *et al.*,[49] in which increments in vitamin C contents were found in fresh cut potato under MAP conditions and refrigeration.

On the contrary, losses in vitamin C on zucchini slices were more evident when the 40 µm film was used, that could be related to atmosphere conditions inside the trays. In this case, a long exposition time to very high CO_2 concentrations contributed to reduce vitamin C content, causing cytoplasmic acidification and, consequently oxidative damage with increasing ascorbate oxidase activity. In consequence, from a nutritional standpoint, CO_2-rich atmosphere may not be suitable for vitamin C preservation in several vegetables.[50] Our results are in agreement also with previous literature that attributed a decrease in vitamin C content when very high CO_2 levels were detected inside the bags of fresh-cut tomatoes[51] or fresh-cut apples.[52]

4. Conclusions

This is the first report on the study of cultivar selection, maturity at processing and film packaging on physicochemical, nutritional and sensory quality of fresh-cut zucchini during time of storage, supporting that studies about cultivars and maturity stages are the first step before the development new fresh-cut products. In this work the best aptitude to be minimally processed was shown by cv. 'Cronos', corresponding to cv. 'Parador' the worst response for fresh-cut zucchini processing due to a high metabolism activity and, consequently, rapid energy reserves consumption. Considering maturity at processing, there were significantly differences in respiration rate as well as in nutritional compounds and sensory evaluation, displaying more mature zucchini ('maturity stage 2') the best quality. The effectiveness of 25 µm thickness polypropylene film in fresh-cut zucchini shelf-life extension as well as antioxidant compounds preservation was observed in all treatments studied. However, our results suggest that storage time exceeding 10 days should be avoided even after packaging in the best conditions (high-permeability polypropylene film), due to yellowing, firmness loss and off-flavors in sliced zucchini. Additionally, minimal processing of zucchini is recommended as an alternative to increasing the added value of this vegetable (usually

exported as a fresh product) which should be considered by international seed companies and processing industries.

Acknowledgements

This work was supported by the project PP.TRA2010.2012 (TRANSFORMA) and FEDER funds. Authors thank to the seed companies above mentioned for providing the material used in this study and to J. Clement Bromley for grammatical revision of the manuscript. M.T. Blanco-Díaz acknowledges the financial support from the Spanish Ministry of Economy and Competitiveness as a contracted researcher of the Program 'Researcher Training' (Formación de Personal Investigador, FPI-INIA).

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4.4 ‘Strategies to extend the shelf-life of fresh-cut zucchini’

María Teresa Blanco-Díaz, Antonio Pérez-Vicente, Rafael Font, Alicia Fayos Moltó

Department of Postharvest Technology and Food Industry- IFAPA Centro La Mojonera

Camino San Nicolás, 1, 04745 La Mojonera, Almería, Spain

In preparation

Abstract

Several variables greatly affect the minimally processed product's shelf-life. In this study different strategies including cultivar, cutting type, storage temperature and modified atmosphere packaging (MAP) conditions in fresh cut zucchini shelf-life. The treatments consisted in a 2x2x2x3 factorial combination of variety, cutting type, storage temperature and storage time studied throughout two independent experiments carried out by two different strategies: strategy 1 (with a non-microperforated film) and strategy 2 (with a microperforated film). Trays overwrapped by strategy 1 exhibited a more rapid decrease of O_2 and an abusive accumulation of CO_2, a significant reduction in the microbial spoilage but also it was developed a strong off-odor, loss of freshness as well as higher titrable acidity values.

In spite of the high number of bacterial spoilage in fresh cut zucchini from strategy 2, between 7 and 8 log cfu g−1 (especially under the most critical cutting type, sticks), the appearance, firmness, aroma as well as overall quality of samples was scored as good or very good (> 6) after 14 days of storage, except for cv. 'Natura' sticks at 10 ºC. Therefore, most of the treatment from strategy 2 was beneficial in maintaining the quality of fresh cut zucchini up to day 14 of storage, even under the highest fresh cut zucchini metabolic activity (sticks at 10 ºC) (4 days more compared to strategy 1).

Keywords: Fresh-cut zucchini, Modified Atmosphere Packaging (MAP), microbial spoilage, sensory, shelf-life.

1. Introduction

Fresh-cut vegetable market has been constantly increasing during the last ten years, as a result of the new consumer's preferences in healthier and more convenient food. Although leafy vegetables are the most popular commodities amongst this kind of fresh processed foods, other horticultural and fruit products have been introduced as technology has progressively developed.

Cucurbits are considered relevant in the daily human diet due to their high moisture and low fat contents, characteristics that makes them attractive for consumers interested in a healthy diet (Weng and Sun, 2011). Amongst cucurbits, cucumber, melon, watermelon and summer squash are the four most commonly cultivated crops in Spain, being zucchini (*Cucurbita pepo* spp. *pepo*) the main summer squash morphotype consumed and commercialized around the world (Loy, 2011). So, we consider that fresh zucchini could be an excellent raw material to be processed as a fresh cut product as long as quality and safety of the freshly-cut product could be guaranteed.

After being cut, the fruit vegetable remains physiologically active, due to the fact that tissues are living and respiring, with shifting cellular processes and interactions in response to the tissue damage inflicted by the operations (Dea et al., 2011). In particular, zucchini is considered as a susceptible vegetable due to a high degree of nutrient release promoting an intense microbiological proliferation characterized by the production of enzymes and metabolites associated with visual and textural defects and off-odors (Ragaert et al., 2011).

Usually, the quality of fresh-cut items is determined by a consistent and fresh appearance (referring to visual symptoms of deterioration on the cut surfaces), acceptable texture, microbial spoilage (due to proliferation of microorganisms) and sufficient shelf-life to survive the distribution system (Varoquaux and Wiley, 1994; Brecht, 1995; Ragaert, 2007a; Dea et al., 2011).

The effect after cutting varies greatly, depending on the cultivar, final piece size, temperature's management as well as modified atmosphere packaging (MAP); these variables have considerable influence on minimally processed product's shelf-life.

In this context, several reports have described the benefits of adequate genotype/cultivar in fresh cut processing in order to reduce mesophilic bacteria, and yeast and moulds counts in green-multi leaf and green-baby leaf fresh cut lettuce, respectively (Martínez-Sánchez et al., 2012), as optimal tomato cutting size (wedges) resulted in 1 log cfu g^{-1} lower counts in both, mesophilic bacteria and yeast populations also affecting the appearance and overall quality of the product (Aguayo et al., 2004).

Although MAP is an important factor in preserving fresh cut produce quality due to the inhibition of metabolic activity of the living plant tissue and in retarding microbial spoilage, because of the reduction of oxygen concentration, it is the storage temperature rather than the gas composition which is the most effective technique in microbiological

growth control (Ragaert et al., 2011). For example, passive MAP conditions created by packaging with different films in fresh cut asparagus only reduced significantly the proliferation of *Enterobacteriaceae* population (not reducing the proliferation of mesophilic and psychrophilic bacteria) (Simón et al., 2004). On the contrary, an evident reduction on the final counts (~3 log cfu g^{-1}) of several microbial groups in fresh cut peppers were observed when the temperature was decreased by 5 ºC (from 10 to 5 ºC), such as those from total coliforms as well as yeasts and molds (González-Aguilar et al., 2004).

However, in spite of the importance of the mentioned aspects, very few information is available concerning both, physicochemical and microbial spoilage in fresh cut zucchini held under MAP conditions during time of storage. In this respect, only one study about fresh cut zucchini is available in the literature (Lucera et al., 2010), mainly focused on the effect of cultivar and MAP on microbial population growth not covering other factors (cutting type, storage temperature and also their interactions) either on the microbiological or on the physicochemical quality parameters.

Therefore, the aim of this work was to study different strategies including cultivar, cutting type, storage temperature and MAP conditions together with their interactions for extending the fresh-cut zucchini shelf-life, carried out throughout two independent experiments.

2. Material and methods

2.1. Plant material

Zucchini squash (*Cucurbita pepo* spp. *pepo*) from different international seed companies cv. 'Natura' (Enza Zaden, Co. Ltd) and cv. 'Sinatra' (Clause, Co. Ltd) were harvested when they reached commercial size (18-21 cm) from a commercial farm in Almería (Augro Fresh Spain S.L.) and transported to the laboratory. Then, the samples were stored in darkness at 6 ± 1 ºC and 90% relative humidity (RH) for 12 h. The fruits were sorted according to the uniformity of size and homogeneous skin color, and finally those showing defects were discarded. Fruits were processed in a cold room at 6 ºC under suitable hygienic conditions before processing.

2.2. Washing, packaging and storage

Intact summer squash fruits were pre-washed in tap water for 1 min to eliminate soil and insect residues. The experimental design including both preharvest (cultivar) as well as postharvest (cutting type, storage temperature and MAP conditions) factors were carried out following different strategies described in the diagram of the zucchini experimental procedure **(Fig. IV.4.1).**

Zucchini fruits were cut into slices (0.6 $\pm$ 0.01 cm thick) and sticks (1 $\pm$ 0.01 cm thick) with commercial equipment (SAMMIC CA-300, Guipúzcoa, Spain). Zucchini slices or sticks were washed for 2 min in 150 mg L^{-1} free chlorine solution (NaOCl) adjusted to

pH 6.5 with citric acid. The excess surface water remaining on the zucchini slices or sticks was removed by centrifuging at 900 rpm for 4 min (SAMMIC ES-150, Guipúzcoa, Spain). To compare the effect of cutting types and temperatures in minimally processed zucchini cultivars, different MAP conditions were created by passive MAP conditions, controlling the weight and the tray transmission area (the most economical form to get these conditions) **(Fig. IV.4.1).** Polypropylene trays described in **Fig. IV.4.1** were overwrapped by thermosealing on the top with a low permeability film (non-microperforated polypropylene film) for strategy 1 and with a high permeability film (micro-perforated bioriented polypropylene film, BOPP, with diameter micro-perforations of 150 µm) for strategy 2. Three replicates of each cutting format, cultivar, storage temperature and day of storage were packaged in both packaging conditions (strategy 1 and strategy 2) and stored under refrigeration conditions (6 ºC and 10 ºC) for 14 days.

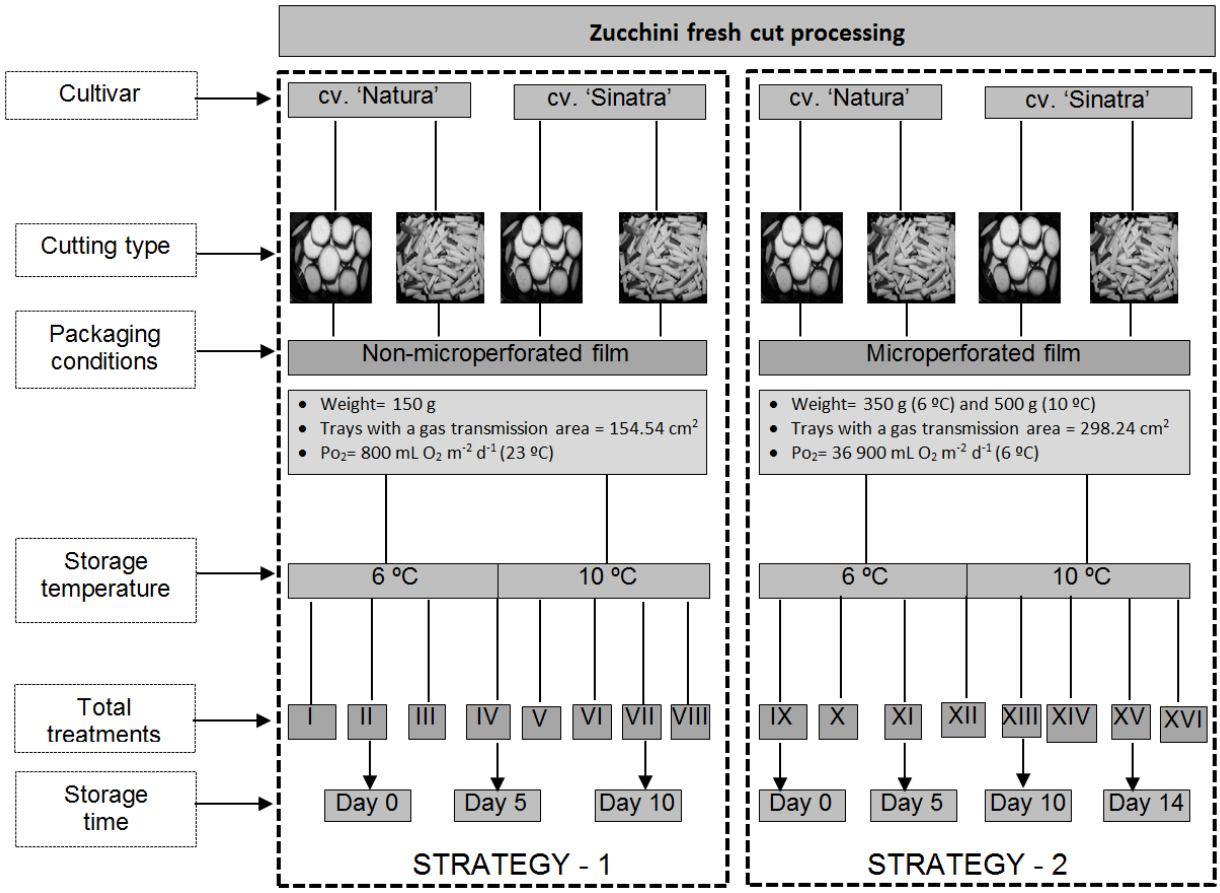

Figure IV.4. 1. Diagram of the fresh cut zucchini experimental strategies.

2.3. Gas composition within the packages

Gas composition (oxygen and carbon dioxide) of fresh-cut zucchini were measured in order to evaluate the effects of cutting types, storage temperatures and cultivars for each packaging strategy. Gas sampling was monitored daily by means of a needle connected to an O_2/CO_2 gas analyzer (CheckMate 3 PBI Dansensor, Ringsted, Denmark) with an

accuracy of 0.5% using a septum in the lid for sampling gas in the headspace at different times (Caleb et al., 2012). This gas analyzer measures the oxygen content by means of a highly stable zirconium electrochemical sensor and measures the CO_2 concentration with a mini-IR spectrophotometer detector.

2.4. Sensory evaluation

After equilibrium at room temperature, a panel of five trained judges using a 9-point scale was used to record the panelist's perceptions for visual appearance (where 1=poor and 9= excellent) and for odor (where 1= completely lacking or soft and 9= full characteristic or fresh) (Artés et al., 1999).

An objective evaluation of the fresh processed zucchini firmness was carried out by using a texture analyzer (TA-XT-Plus, Stable Micro System, Surrey, UK) calibrated with a 5-kg weight and equipped with a 4-mm diameter probe. Firmness was determined following the method previously reported in zucchini by Brew *et al.*[18] on the sliced or sticked zucchini mesocarp region. The insert distance was 3 mm, with a stroke speed of 50 $mm \cdot min^{-1}$. At each storage time, the mean of five tissue pieces (slices or sticks) was measured per package for each cultivar in the study (cv. 'Natura' or cv. 'Sinatra') and for each experimental strategy. Firmness was expressed in Newtons (N) in triplicate.

Finally, panelists were asked to base their decision on the samples 'overall quality' using a 9-point scale (9 = excellent, 7 = very good, 5 = good, limit of marketability, 3 = fair, limit of usability and 1 = poor, unusable) (Larmond, 1977). A score equal to 5 was used as the threshold for the produce acceptability. Three number codes were used to name samples in order to avoid the identification of the applied treatments.

2.5. Color, total soluble solids, pH and titrable acidity

Color was measured using a CM-700d spectrophotometer equipped with D65 illuminant source (Minolta, Ramsey, NJ, USA) taking three measurements on the mesocarp region of the sliced or sticked zucchini. The instrument was previously calibrated on a white tile at an observation angle of 0. The values were expressed using the CIE *L*a*b** system, Chroma (C*) and hue angle (h^o) were also determined. At each storage time, the mean of five tissues pieces (slices or sticks) were measured per tray for each MAP treatment (non microperforated and microperforated films), storage temperature (6 or 10 ^oC) and cultivar (cv. 'Natura' and cv. 'Sinatra').

Three replicates of fresh-cut zucchini from each treatment were homogenized using a commercial blender (Moulinex, Barcelona, Spain). Total soluble solids (TSS) of the juice was measured with a hand refractometer (SMART-1, Atago, Japón) and expressed as ^{o}Brix at 20 ^oC. Then, the pH of the juice was measured using a pH-meter (GLP 21+, Crison, Barcelona, Spain), while for titrable acidity (TA), 10 ml of juice was titrated with 0.1 N NaOH to an endpoint of pH 8.1 (AOAC, 1984) and expressed as g of malic

acid per 100 ml of juice (% malic acid), the most predominant acid in this vegetable (Wang and Buta, 1999).

2.6. Microbiological analysis

Microbiological analysis was performed immediately before packing and along storage time. Zucchini samples (10 g) were diluted (1:10) in Buffered Peptone Water (BPW) (Oxoid Limited, Wade Road, Basingstoke, Hampshire RG24 8PW, England) and homogenized for 2 min in a stomacher (Smasher, AES Laboratoire, Combourg, France). Subsequently, a decimal dilution series was made in BPW and enumeration of the epiphytic microbiota was performed by the appropriate sample dilution on specific culture media: (1) Plate Count Agar (Oxoid, Hampshire, United Kingdom) incubated aerobically at 30 ºC for 72 h for total mesophilic aerobic microorganisms and at 7 ºC for 10 days for total psychrophiles; (2) Chromogenic medium REBECCA TM (Biolife, Italiana S.r.l., Milano, Italy) incubated at 37 ºC for 24 h for *Enterobacteriaceae*; (3) Rose Bengal Agar Chloramphenicol (Biolife, Italiana S.r.l., Milano, Italy) incubated at 25 ºC for 5 days for yeasts and moulds. Experiments and sample analysis were performed in triplicate and results were reported as log cfu g^{-1} of tissue.

2.7. Statistical analysis

For each strategy proposed, three replications were evaluated per cultivar, cutting type, temperature of storage and evaluation period. Experiments were performed using a completely randomized design. Analysis of variance (ANOVA) was performed by four-way (cultivar x cutting type x temperature x storage time) with type III sums of squares using the GLM (General Linear Model) procedure of the LSD (Least Significant Difference test) to compare means, and significance was accepted at $P < 0.05$ level. Additionally, parameters evaluated were subject to principal component analysis (PCA) to compare strategies presented. Statistical analysis was performed using SAS for Windows and Statgraphics plus 5.0 (Statistical Graphics Corp., Rockville, MD, USA).

3. Results and discussion

Atmosphere within the tray, color, soluble solids, pH, titrable acidity, microbial spoilage (aerobic mesophilic and psychrophilic bacteria, *Enterobacteriaceae* populations and yeast and moulds counts) as well as sensorial evaluation (appearance, firmness, odor and overall quality) were evaluated in the two applied strategies. Quality attributes were determined at initial moment (0 day), 5, 10 and 14 days of storage.

3.1. Effects on packages atmospheres

The effects on package atmospheres for non-microperforated (strategy 1) and microperforated films (strategy 2) are shown in **Fig. IV.4.2a** and **Fig. IV.4.2b**, respectively. Differences between strategies primarily affected to the evolution of the

gas composition inside the packages, being shelf-life in strategy 1 up to 10 days due to the high CO_2 levels and the low O_2 levels registered (4 less days in shelf-life than the proposed strategy 2).

In non-microperforated film overwrapped trays (strategy 1) O_2 concentrations decreased rapidly during the first 4-5 days until values close to zero (0.1 KPa), while abusive CO_2 levels (~ 30 KPa) were observed in packages at 10 ºC **(Fig. IV.4.2a)**. In this strategy, gas composition was mainly affected by temperature, while cutting type or zucchini cultivar showed to have less influence on this parameter (**Fig. IV.4.2a and Fig. IV.4.2b**). In more detail, the initial O_2 content inside the packages decreased along the storage time registering at the end of the storage values ranging between 0.05 KPa (for cv. 'Sinatra' sticks at 10 ºC) and 0.1 KPa (corresponding to cv. 'Sinatra' sticks at 6 ºC), while a pronounced CO_2 accumulation was found ranging from 18.15 KPa to 30.85 KPa (found in cv. 'Natura' sticks at 6 ºC and cv. 'Natura' slices at 10 ºC, respectively).

On the contrary, MAP conditions created by strategy 2 seem to have a positive effect in establishing a steady-state in the MAP packages after 14 days **(Fig. IV.4.2b)**. In fact, O_2 levels inside the packages decreased at a slightly slower rate during the first 5 days, reaching an apparent equilibrium in the gas atmosphere at an O_2 concentration of 0.90 KPa and 1.86 KPa (for cv. 'Natura' sticks at 6 ºC and cv. 'Sinatra' slices at 10 ºC, respectively). In contrast, CO_2 concentrations increased gradually during storage until day 6, in which levels remained around 19.20 KPa and 20.06 KPa (for cv. 'Natura' slices at 6 ºC and cv. 'Natura' sticks at 6 ºC, respectively).

Considering both cutting types, and evaluating the response of zucchini cultivars from both strategies cv. 'Sinatra' registered the slowest atmosphere evolution as well as the lowest CO_2 and the highest O_2 contents inside the packages. In addition very high CO_2 (23.33 KPa) and very low O_2 (0.38 KPa) levels were detected in cv. 'Natura' sticks held at 10 ºC at day 10 of storage.

Passive MAP conditions described in the present study were in general lower in CO_2 and higher in O_2 levels than those created in zucchini slices at 5 ºC using biodegradable co-extruded polyester (COEX thickness 35 µm) and oriented-polypropylene (OPP thickness 90 µm) films (Lucera et al., 2010); while O_2 concentrations previously described in strategy 2 were similar to those recommended for preserving fresh cut zucchini quality (O_2 = ~0.5-1 KPa) (Izumi et al. 1996, Gorny, 1997).

3.2. Effects on sensory quality

As sensory evaluation is one of the most limiting factors affecting to the fresh-cut products quality (Robertson, 2006; Lawless and Heymann, 2010), this parameter was

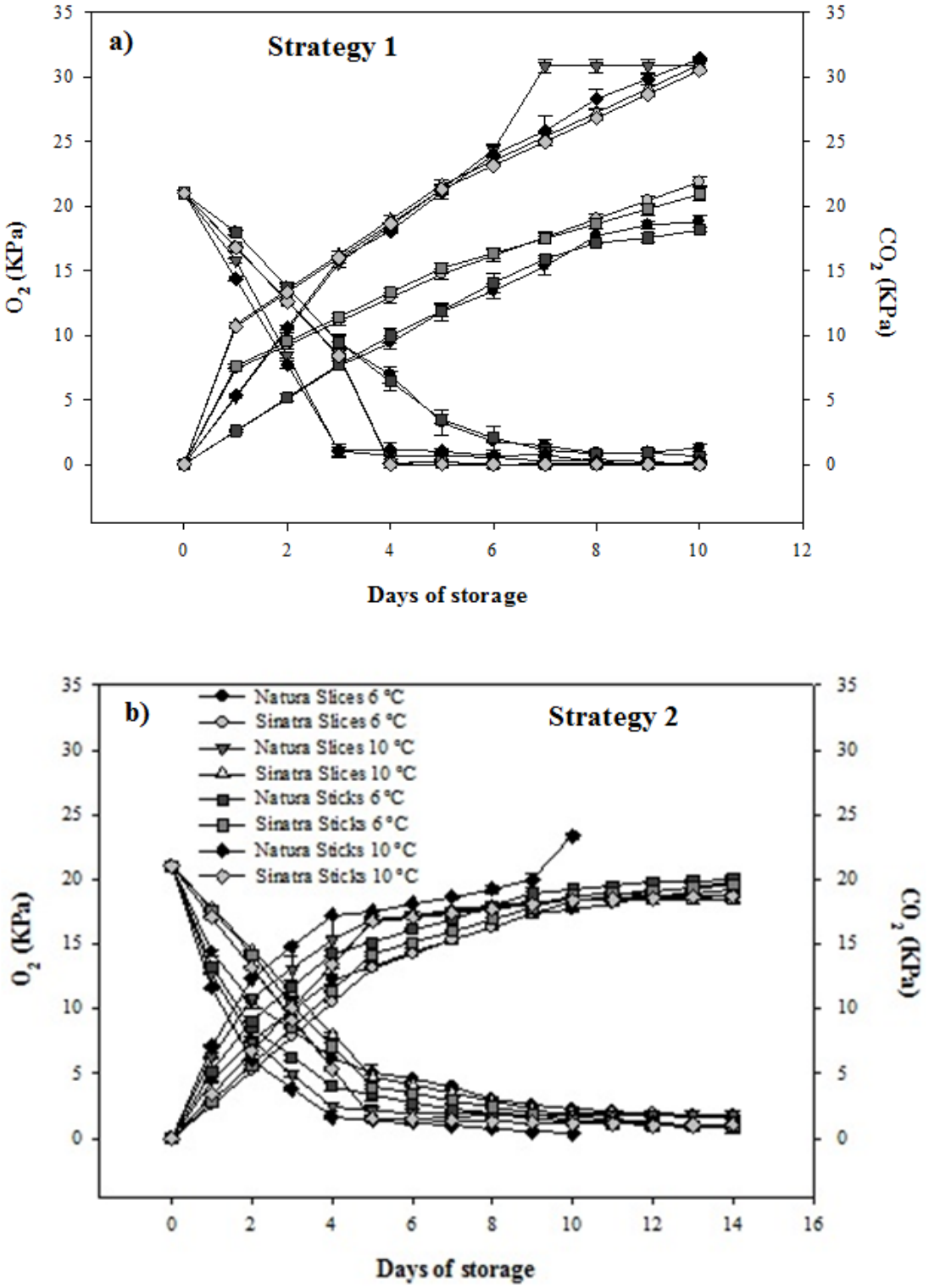

Figure IV.4. 2. Changes in headspace partial pressure of O_2 and CO_2 (KPa) of fresh-cut zucchini (slices or sticks) cv. 'Natura' and cv. 'Sinatra' during 14 days of storage at 6 ºC and 10 ºC for strategy 1 (a) and for strategy 2 (b).

the primary factor considered in this study to establish the shelf-life of fresh-cut zucchini samples.

The effects of the two different strategies on fresh-cut zucchini sensory evaluation are shown in **Table IV.4.1** and **Fig. IV.4.3a and IV.4.3c** (for non-microperforated film, strategy 1) and in **Table IV.4.2 and Fig. IV.4.3b and IV.4.3d** (for microperforated film, strategy 2).

Texture was an important attribute in the sensory quality evaluation, both strategies presenting a four-way interaction (cultivar x cutting type x temperature x storage time) in this parameter, $P < 0.0001$ for strategy 1 and $P < 0.001$ for strategy 2 **(Table IV.4.1 and Table IV.4.2).** At day 0, no statistical differences between cultivars and cutting types were observed, but showing slightly higher mean scores in overall quality in cv. 'Sinatra' sticks due to the higher freshness of freshly cut zucchini aroma **(Fig. IV.4.3c and IV.4.3d)**.

In general, the aroma was the most affected quality factor along storage time, falling below marketability limits in most of the cases when overwrapped with non-microperforated film (strategy 1) after 10 days **(Fig. IV.4.3c)**. Moreover, long exposition time to high CO_2 levels (strategy 1) resulted in loss of the quality appearance, probably due to stress response in the freshly cut zucchini tissue **(Fig. IV.4.3a and IV.4.3b)** as well as strong off-odors (data not shown). In addition, as reported by Izumi et al. (1996), Gorny (1997) and confirmed in our experiment, fresh cut zucchini sensorial quality increases when low O_2 levels close to 1 KPa occur (strategy 2). However, when O_2 conditions drop below these levels (after 4 days in strategy 1), it results in the development of an off-odor and the loss of the appearance of the cut zucchini freshness.

On the contrary, microperforated film (strategy 2) showed significantly better performances in prolonging the sensorial shelf-life of the zucchini samples increasing up to 14 days the shelf-life of the fresh cut zucchini (except for cv. 'Natura' sticks at 10 ºC in which very low O_2 and high CO_2 levels, 0.38 KPa and 23.33 KPa, respectively were registered) **(Fig. IV.4.3b)**.

In general, in our study it could be suggested that better results in terms of shelf-life were obtained for cv. 'Sinatra' compared to cv. 'Natura', especially under the most critical situation (sticks at 10 ºC).

Additionally, considering all sensorial quality parameters evaluated in this work, the shelf-life in all cases for strategy 1 was established in 10 days (mainly due to the strong off-odors detected after long time CO_2 exposition), while for strategy 2 the shelf-life was set in 14 days (4 more days than strategy 1).

Table IV.4. 1. Four-way ANOVA in color, TSS and sensory parameters affected by cultivar, cutting type, temperature and storage time for strategy 1 (non-microperforated film).

Cultivar	Cut type	Temperature (ºC)	Color					TSS	Appearance	Texture	Aroma	Overall Quality
			L*	a*	b*	C*	hº					
Natura	Slices	6	86.02 a	-1.06 a	21.64 bc	21.74 cd	92.95 d	4.46 ab	6.91 a	14.35 a	6.40 a	6.92 ab
	Slices	10	85.41 ab	-0.98 a	23.55 ab	23.86 abc	92.23 e	4.38 bc	6.33 a	15.24 a	5.51 a	6.20 ab
	Sticks	6	82.64 d	-1.66 b	20.04 c	20.43 d	94.85 b	4.42 ab	6.30 a	11.49 b	6.28 a	6.28 ab
	Sticks	10	82.34 d	-1.55 b	19.42 c	19.77 d	94.70 b	4.24 bc	5.25 a	11.70 b	4.72 b	5.11 b
Sinatra	Slices	6	84.26 bc	-1.98 bc	26.19 a	26.34 a	94.42 b	4.67 a	6.97 a	8.78 c	6.58 a	7.22 a
	Slices	10	84.14 c	-1.67 b	25.88 a	25.54 ab	93.69 c	4.25 bc	6.80 a	8.10 c	6.18 a	6.01 ab
	Sticks	6	84.18 c	-2.57 d	24.88 a	24.33 ab	95.74 a	4.35 bc	6.52 a	7.78 c	6.30 a	6.92 a
	Sticks	10	84.17 c	-2.44 cd	24.19 ab	23.52 bc	95.61 a	4.17 c	5.30 a	5.97 d	4.74 b	5.96 ab
Interactions												
Cultivar (Cv)			****	****	****	****	****	ns	ns	****	ns	****
Cutting type (C)			****	****	****	****	****	****	****	****	****	****
Temperature (T)			ns	**	ns	ns	***	****	****	*	****	****
Storage time (S)			****	****	****	****	**	****	****	***	****	****
Cv x C			ns	ns	****	ns	**	ns	ns	****	ns	ns
Cv x T			ns	ns	***	***	ns	****	ns	****	*	ns
Cv x S			ns	****	****	****	****	****	ns	****	***	ns
C x T			ns	ns	****	***	**	***	**	**	****	**
C x S			****	ns	****	****	**	****	****	****	****	**
T x S			ns	ns	***	**	*	****	****	ns	****	***
Cv x C x T			ns	ns	***	***	ns	ns	ns	ns	*	ns
Cv x C x S			ns	***	****	***	**	ns	ns	***	**	ns
Cv x T x S			ns	***	*	*	****	***	ns	***	*	*
C x T x S			ns	ns	****	****	*	*	**	*	**	****
Cv x C x T x S			ns	****	*	*	**	****	ns	****	ns	ns

ns= non-significant
* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$
**** $P \leq 0.0001$

Table IV.4. 2. Four-way ANOVA in color, TSS and sensory parameters affected by cultivar, cutting type, temperature and storage time for strategy 2 (microperforated film).

Cultivar	Cut type	Temperature (ºC)	Color					TSS	Appearance	Texture	Aroma	Overall quality
			L*	a*	b*	C*	hº					
Natura	Slices	6	85.17 a	-0.76 a	22.96 c	23.26 cd	91.89 d	5.44 a	7.10 ab	14.10 a	6.87 a	7.06 ab
	Slices	10	84.90 a	-0.63 a	24.36 bc	23.45 bcd	91.54 d	5.38 a	6.48 ab	13.67 a	6.35 ab	6.35 abc
	Sticks	6	84.25 a	-1.02 a	24.10 bc	22.56 de	92.11 c	5.07 b	6.50 ab	11.01 b	6.80 a	6.45 abc
	Sticks	10	73.81 b	-0.80 a	25.14 ab	20.96 e	91.28 d	4.83 cd	6.16 b	10.75 b	5.54 b	5.66 c
Sinatra	Slices	6	83.98 a	-1.58 b	23.47 bc	25.44 ab	93.78 ab	5.03 b	7.53 a	8.41 c	7.06 a	7.57 a
	Slices	10	83.79 a	-1.68 b	26.51 ab	27.06 a	93.46 b	4.90 cd	6.76 ab	8.62 c	6.68 ab	6.48 abc
	Sticks	6	82.62 ab	-2.24 c	24.51 bc	24.78 bc	94.48 a	4.62 d	6.80 ab	7.40 d	6.78 a	7.14 ab
	Sticks	10	82.89 ab	-2.22 c	27.43 a	24.95 abc	93.65 b	4.58 d	6.30 b	7.52 d	6.26 ab	6.16 abc
Interactions												
Cultivar (Cv)			****	****	****	****	****	****	***	****	****	****
Cutting type (C)			****	****	****	****	***	****	****	****	****	****
Temperature (T)			****	**	****	**	****	****	****	****	****	****
Storage time (S)			****	****	****	****	****	****	****	****	****	****
Cv x C			****	**	ns	ns	ns	ns	ns	****	ns	ns
Cv x T			****	***	****	****	ns	****	ns	****	**	ns
Cv x S			****	ns	****	****	ns	****	*	****	*	ns
C x T			****	ns	ns	***	ns	****	ns	****	**	ns
C x S			****	**	****	****	ns	****	***	****	*	****
T x S			****	*	****	****	***	****	***	****	***	**
Cv x C x T			****	ns	ns	ns	ns	ns	ns	***	**	ns
Cv x C x S			****	***	ns	****	*	ns	ns	****	ns	ns
Cv x T x S			****	***	***	****	**	***	ns	****	ns	ns
C x T x S			****	ns	**	***	*	****	ns	**	ns	ns
Cv x C x T x S			****	ns	ns	ns	ns	ns	*	**	ns	ns

ns= non-significant
* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$
**** $P \leq 0.0001$

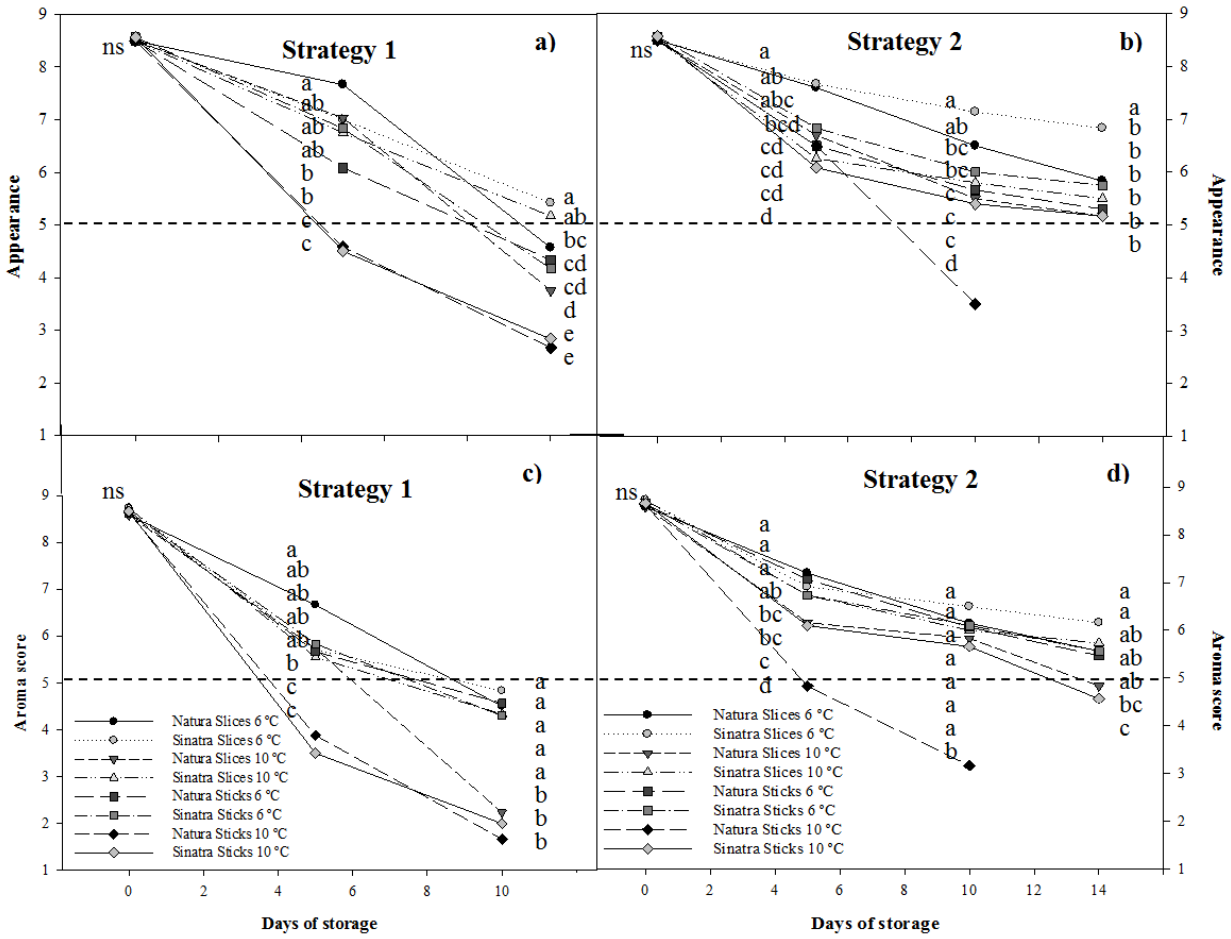

Figure IV.4. 3. Effect of cutting types and temperature of storage in the appearance of fresh-cut zucchini cv. 'Natura' and cv. 'Sinatra' during storage for strategy 1 (a) and for strategy 2 (b); and in the aroma score of fresh-cut zucchini cv. 'Natura' and cv. 'Sinatra' during storage for strategy 1 (c) and for strategy 2 (d). Means followed by different letters are statistically different according to LSD test at P < 0.05.

3.3. Effects on physicochemical quality

All factors studied (cultivar x cutting type x temperature storage x storage time) presented a high influence on the TSS content in samples from strategy 1 ($P < 0.0001$) **(Table IV.4.1)**. On the contrary, in strategy 2 factors studied had less importance on the the TSS content (cultivar x storage time and cultivar x temperature, both with $P < 0.001$) **(Table IV.4.2)**.

Under higher tissue metabolism activity (sticks at 10 ºC) higher sugar consumption occurs contributing to reduce the TSS values, but two different trends were observed in fresh cut zucchini depending on the selected strategy. On one hand strategy 1 showed a higher influence of storage temperature ($P < 0.0001$) in the consumption of TSS in both zucchini cultivars resulting in a higher means of TSS in those samples held at a lower storage temperature ($TSS_{slices\ 6ºC} > TSS_{sticks\ 6\ ºC} > TSS_{slices\ 10\ ºC} > TSS_{sticks\ 10\ ºC}$) **(Table IV.4.1)**; on the other hand, in strategy 2 temperature showed also a high influence in in both zucchini cultivar´s and TSS content ($P < 0.001$), but being the TSS means classified as follows: $TSS_{slices\ 6ºC} > TSS_{slices\ 10\ ºC} > TSS_{sticks\ 6\ ºC} > TSS_{sticks\ 10\ ºC}$ **(Table IV.4.2).**

Regarding the pH and titrable acidity (TA) interactions, also strategy 2 reduced the high impact on the studied cases **(Table IV.4.1 and Table IV.4.2)**. Changes in pH and TA values depended also on the fresh-cut zucchini cultivar studied **(Fig. IV.4.4a and IV.4.4b)**. Both strategies showed variations in these parameters along the storage time, being more severe in sticks at 10 ºC with independence of the strategy developed. Negatively for sensorial quality, it was found in strategy 1 higher values in TA **(Fig. IV.4.4a)**, whereas strategy 2 contributed to maintain initial values in several of the cases until day 10th of storage **(Fig. IV.4.4b)**. These results are in agreement with other authors that observed higher variations in pH and TA in the most stressing storage conditions (diced onions at 10 ºC and wedged tomatoes at 5 ºC), due to higher metabolic tissue activity (Aguayo et al., 2004; Dallocca-Berno et al., 2014).

In accordance with previous results, strategy 2 decreased the significance level of factors studied in much of the color parameters **(Table IV.4.1 and Table IV.4.2)**. In general, the higher O_2 content inside the packages for strategy 2 could be related to a higher fresh-cut zucchini tissue oxidation (lower L* and hue values as well as higher b* and chroma values) **(Fig. IV.4.4c and IV.4.4d)**, meanwhile the low O_2 levels for strategy 1 would contribute to reduce changes in color (Lanciotti et al., 1999). However, although lower oxygen concentration values inside the packages from strategy 1 contributed to decrease the alterations in fresh-cut zucchini color, the negative effect on fresh-cut zucchini sensorial quality was more evident after long time under very high CO_2 levels exposition (~ 30 KPa).

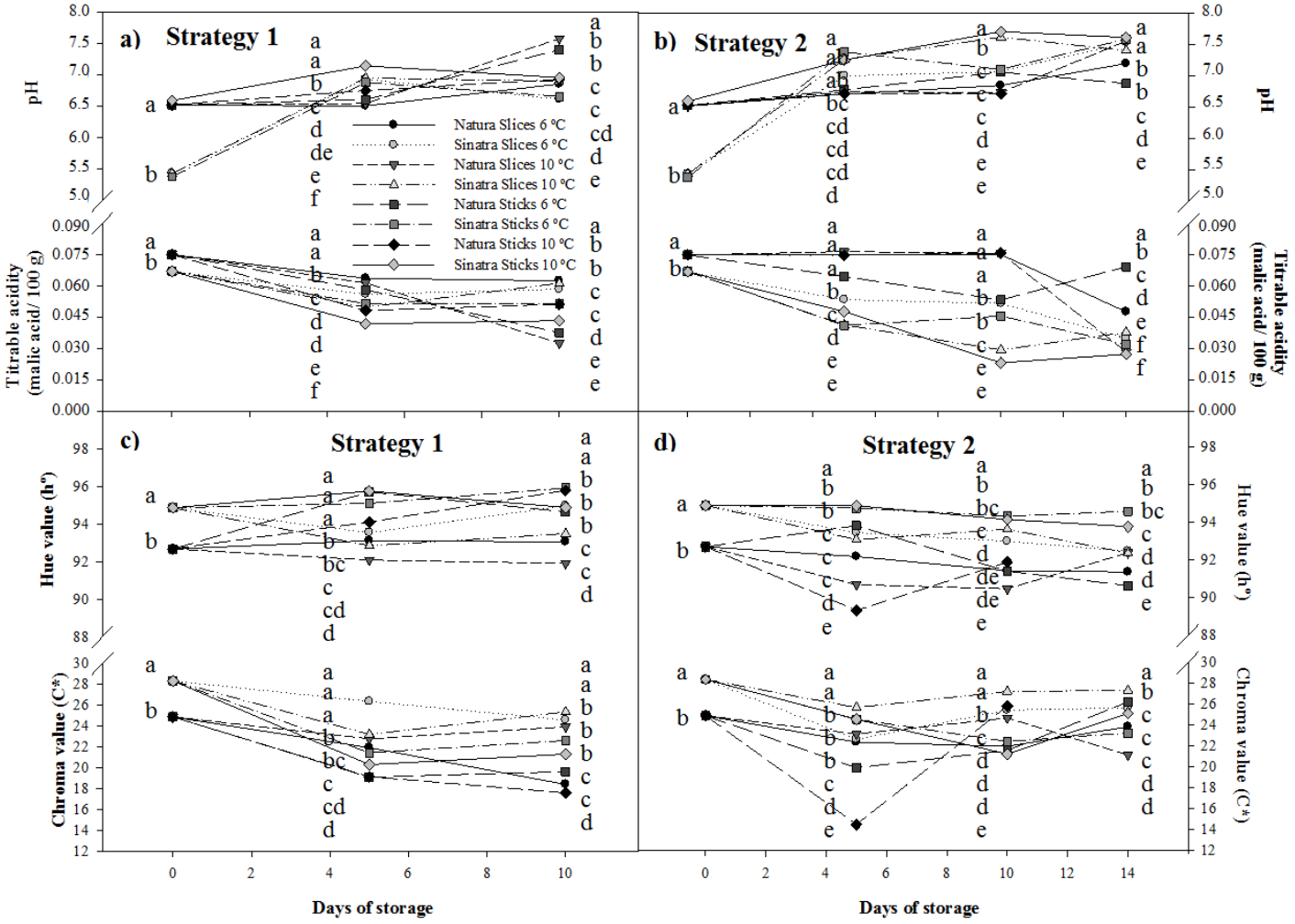

Figure IV.4. 4. Effect of cutting types and temperature of storage in the pH and titrable acidity of fresh-cut zucchini cv. 'Natura' and cv. 'Sinatra' during storage for strategy 1 (a) and for strategy 2 (b); and in the hue (h°) and Chroma (C*) values of fresh-cut zucchini cv. 'Natura' and cv. 'Sinatra' during storage for strategy 1 (c) and for strategy 2 (d). Means followed by different letters are statistically different according to LSD test at P < 0.05.

3.4. Effects on epiphytic microbiota

In both strategies, microbial spoilage showed a gradual increase along time of storage ($P < 0.0001$) **(Table IV.4.3 and Table IV.4.4)**, being the microbial load concentration in freshly cut zucchini similar to other cucurbits (Erkan et al., 2001; Jacxsens et el., 2002).

In general, levels in both mesophilic and psychrophilic aerobic growth **(Fig. IV.4.5a and IV.4.5b, Fig. IV.4.5c and Fig. IV.4.5d**, respectively) as well as *Enterobacteriaceae* populations **(Fig. IV.4.6a and Fig. IV.4.6b)** were affected significantly by cultivar and cutting size. For all microbial populations, zucchini sticks registered at day 0 the highest counts probably due to that it was the most aggressive cutting process. Differences between zucchini cutting types could be due to the ratio of cutting area to volume (A/V), [sticks (0.4) > slices (0.3)] as well as a consequence of vascular fluids leakage that in the case of the sticks are higher than in slices, promoting the quickest spoilage increase (Cyprowski et al., 2014). This is in agreement with different authors, who found microbial reductions in the microbial count populations in the log units when comparing slices vs. sticks in different fresh cut commodities including carrots (Izumi et al., 1995). On the other hand, initial differences between cultivars were found to be from 0.3 to 0.6 log units, which could be related to factors as postharvest handling procedure, presence of soil accompanying the product, hygienic conditions during processing (Pla et al., 2005), or natural variability of the product (Ponce et al., 2002).

Water activity, sugar content and intracellular medium-high pH values of vegetable tissues allow the growth of microorganisms in fresh cut vegetable products, especially when nutrients and fluids are released as a consequence of cutting process, being *Enterobacteriaceae* spp. one of the dominating genera among vegetable microflora which includes mesophilic and psychrotrophic groups (Ragaert et al., 2007b).

Several authors report microbial counts in freshly processed vegetables ranging from 3 to 9 log cfu g^{-1} and recognize that visual appearance of these products can be acceptable despite of such high microbial concentrations (Nguyen-the and Carlin, 1994; Brackett, 1999).

Accordingly to these authors, in our study, although microbial counts in zucchini samples reached 7-8 log cfu g^{-1} (beyond 7 log cfu g^{-1} recommended by former Spanish legislation) such high microbial concentrations did not result in the rejection of the fresh cut zucchini samples during the sensory evaluation. As we established the shelf-life limit based on the sensory evaluation, it has shown to be of 10 days in all treatments for strategy 1. However, for strategy 2 the shelf-life was set in 14 days in all cases, except for cv. 'Natura' sticks at 10 ºC in which 10 days of shelf-life was determined.

Table IV.4. 3. Four-way ANOVA in microbial spoilage population, pH and titrable acidity parameters affected by cultivar, cutting type, temperature and storage time for strategy 1 (non-microperforated film).

Cultivar	Cut type	Temperature (ºC)	Mesophilic bacteria	Psycrophilic bacteria	*Enterobacteriaceae spp*	Yeast	pH	Titrable acidity
Natura	Slices	6	2.80 a	3.75 d	2.53 e	2.32 d	6.62 ab	0.067 a
	Slices	10	3.62 cd	3.68 d	3.20 de	2.37 d	6.89 a	0.056 ab
	Sticks	6	4.70 bc	4.55 cd	4.11 cd	3.29 bcd	6.84 ab	0.057 ab
	Sticks	10	5.52 ab	5.69 abc	5.27 bc	3.43 bc	6.73 ab	0.058 ab
Sinatra	Slices	6	4.54 bc	5.54 abc	3.22 de	3.11 bcd	6.32 ab	0.064 a
	Slices	10	4.21 c	4.94 bcd	3.60 de	2.75 cd	6.43 ab	0.047 ab
	Sticks	6	6.17 a	5.99 ab	5.79 ab	4.47 a	6.30 b	0.059 ab
	Sticks	10	6.30 a	6.65 a	6.65 a	3.85 ab	6.49 ab	0.053 ab
Interactions								
Cultivar (Cv)			****	****	****	****	****	****
Cutting type (C)			****	****	****	****	ns	****
Temperature (T)			****	***	****	ns	****	****
Storage time (S)			****	****	****	****	****	****
Cv x C			ns	*	***	ns	ns	****
Cv x T			****	*	ns	*	*	****
Cv x S			****	****	****	*	****	****
C x T			ns	****	****	ns	****	****
C x S			****	ns	***	****	**	****
T x S			***	**	****	ns	****	****
Cv x C x T			*	ns	****	ns	****	****
Cv x C x S			***	ns	ns	*	*	****
Cv x T x S			****	*	ns	ns	ns	****
C x T x S			*	****	***	ns	****	****
Cv x C x T x S			**	ns	****	ns	****	****

ns= non-significant
* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$
**** $P \leq 0.0001$

Table IV.4. 4. Four-way ANOVA in microbial spoilage population, pH and titrable acidity parameters affected by cultivar, cutting type, temperature and storage time for strategy 2 (microperforated film).

Cultivar	Cut type	Temperature (ºC)	Mesophilic bacteria	Psycrophilic bacteria	*Enterobacteriaceae spp*	Yeast	pH	Titrable acidity
Natura	Slices	6	5.23 c	4.58 b	3.73 d	4.16 b	6.82 cd	0.068 a
	Slices	10	5.25 c	4.76 b	4.44 cd	4.19 b	6.88 bcd	0.064 a
	Sticks	6	7.02 a	6.18 a	5.89 abc	5.86 a	6.81 cd	0.065 a
	Sticks	10	6.75 ab	6.26 a	5.97 ab	6.20 a	6.65 d	0.075 a
Sinatra	Slices	6	5.50 bc	5.56 ab	4.42 cd	3.72 b	7.04 abc	0.052 b
	Slices	10	5.45 c	5.52 ab	4.45 bcd	3.65 b	7.21 a	0.044 bc
	Sticks	6	6.80 a	6.79 a	6.29 a	4.13 b	7.16 ab	0.045 bc
	Sticks	10	6.92 a	6.64 a	6.30 a	4.09 b	7.29 a	0.040 c
Interactions								
Cultivar (Cv)			****	****	****	****	ns	****
Cutting type (C)			***	****	*	****	****	****
Temperature (T)			ns	****	**	ns	****	****
Storage time (S)			****	****	****	****	****	****
Cv x T			*	****	ns	ns	ns	****
Cv x C			ns	****	****	****	**	****
Cv x S			*	****	****	****	ns	****
C x S			****	****	***	****	****	****
T x S			**	****	ns	ns	**	****
T x C			ns	****	***	*	****	****
Cv x C x T			ns	****	ns	ns	ns	**
Cv x C x S			ns	****	**	****	*	****
Cv x T x S			*	***	ns	ns	*	****
C x T x S			ns	****	**	ns	****	****
Cv x C x T x S			****	****	ns	ns	*	****

ns= non-significant
* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$
**** $P \leq 0.0001$

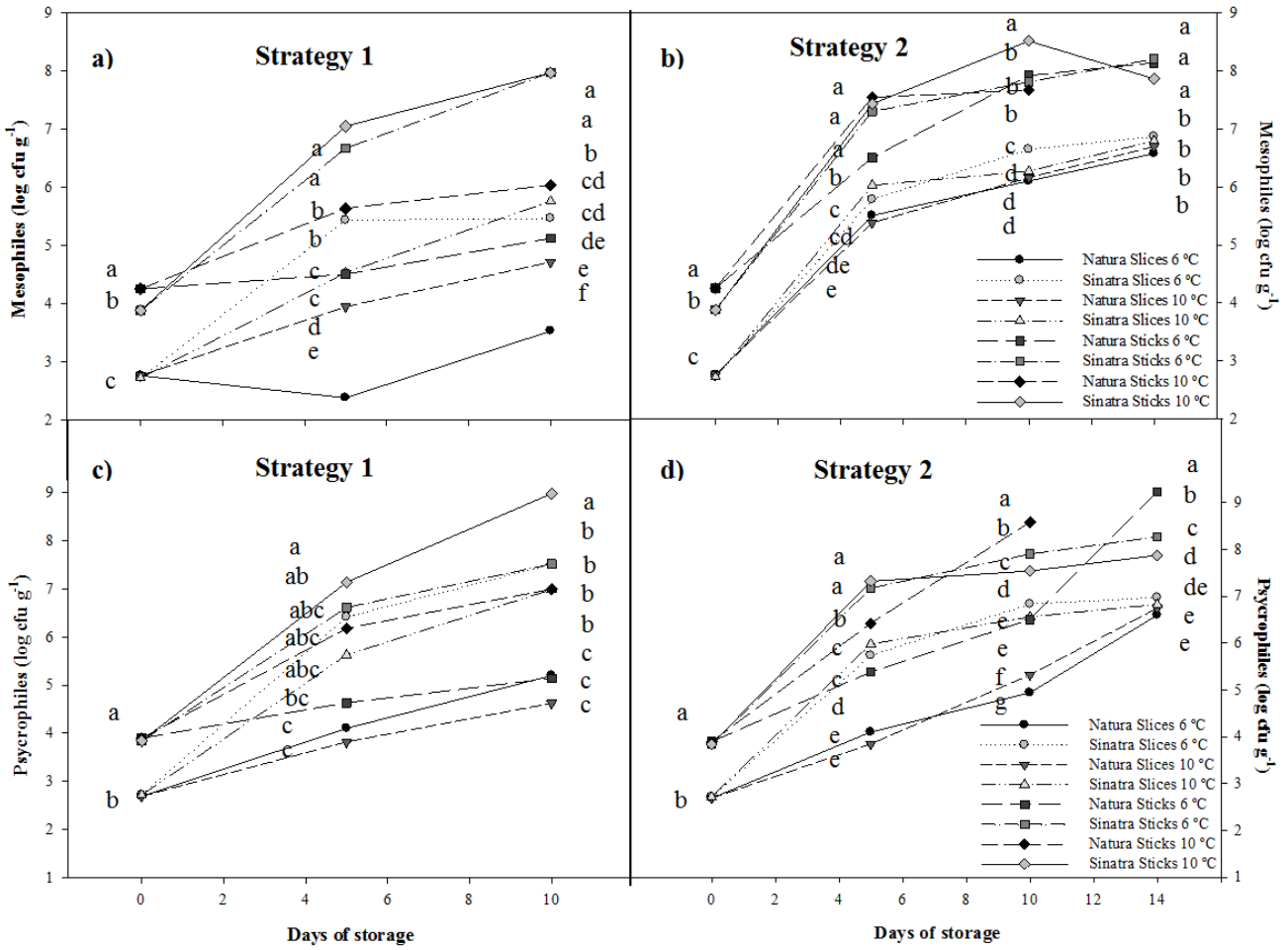

Figure IV.4. 5. Effect of cutting types and temperature of storage in the total mesophilic aerobic bacteria of fresh-cut zucchini cv. 'Natura' and cv. 'Sinatra' during storage for strategy 1 (a) and for strategy 2 (b); and in the total psychrophilic aerobic bacteria of fresh-cut zucchini cv. 'Natura' and cv. 'Sinatra' during storage for strategy 1 (c) and for strategy 2 (d). Means followed by different letters are statistically different according to LSD test at $P < 0.05$.

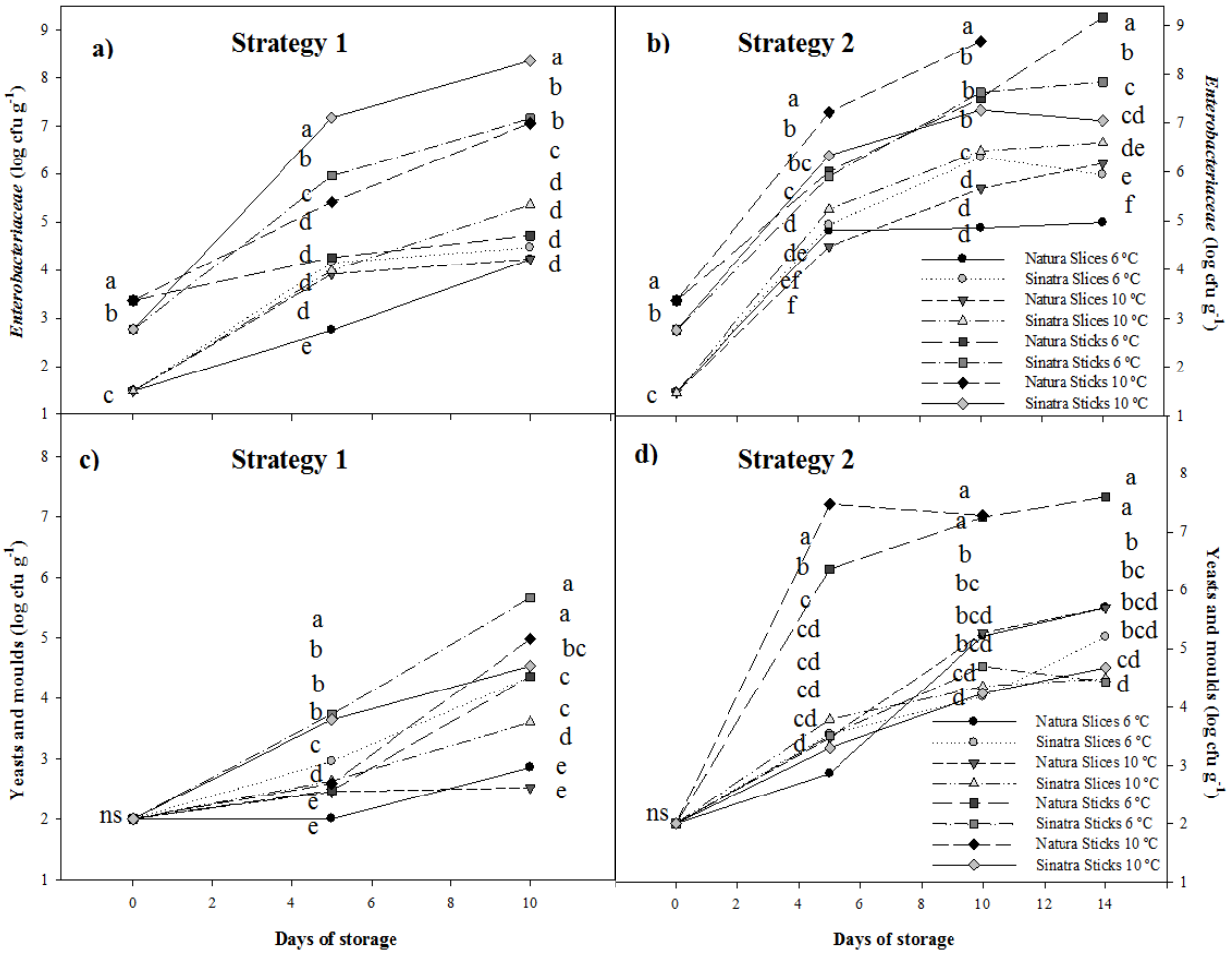

Figure IV.4. 6. Effect of cutting types and temperature of storage in the *Enterobacteriaceae* spp. population of fresh-cut zucchini cv. 'Natura' and cv. 'Sinatra' during storage for strategy 1 (a) and for strategy 2 (b); and in the yeast and moulds counts of fresh-cut zucchini cv. 'Natura' and cv. 'Sinatra' during storage for strategy 1 (c) and for strategy 2 (d). Means followed by different letters are statistically different according to LSD test at P < 0.05.

Considering the mesophilic population, temperature was an important factor for strategy 1 **(Table IV.4.3)** (showing the highest counts in 'Sinatra' sticks for both temperatures at day 10, ~8 log cfu g^{-1}). On the contrary, for strategy 2, storage temperature did not affect to mesophilic counts **(Table IV.4.4)**, cutting type having more effect on final counts. In fact, during storage two well established cutting types-groups were identified in mesophilic counts, $load_{sticks}$ (~8 log cfu g^{-1}) > $load_{slices}$ (~7 log cfu g^{-1}) at day 14.

According to psychrophilic aerobic populations, a three-way interaction (cutting type x temperature x storage time) ($P < 0.0001$) was found for strategy 1 **(Table IV.4.3)**, while for strategy 2 a four-way interaction (cultivar x cutting type x temperature x storage time) ($P < 0.0001$) was observed **(Table IV.4.4)**. For *Enterobacteriaceae* spp. population, interaction between cultivar x cutting type x temperature x storage time significantly affected to strategy 1 **(Table IV.4.3)**, while in strategy 2 less influence of cutting type and storage temperature was observed on this population **(Table IV.4.4)**. Both microbial counts (psychrophilic aerobic populations and *Enterobacterciaeae* spp.) registered similar trend during storage when comparing both strategies. In more detail, for strategy 1 at the end of the shelf-life (day 10), the highest total psychrophilic bacteria and *Enterobacteriaceae* spp. populations were found in cv. 'Sinatra' sticks at 10 ºC (~ 8 log cfu g^{-1}) **(Fig. IV.4.5c and Fig. IV.4.6a)**. For strategy 2, cv. 'Natura' (sticks at 6 ºC and at 10 ºC) reached the highest counts (~9 log cfu g^{-1}) in psychrophilic aerobic bacteria and *Enterobactericaceae* spp. population **(Fig. IV.4.5d and Fig. IV.4.6b)**. This fact could be due to the increase of the bacterial spoilage with the more evident decrease in the TSS content in strategy 1 (cv. 'Sinatra' sticks at 10 ºC showing the lowest TSS levels) (Asrar et al., 2012; Behera et al., 2012). For strategy 2, as in other cucurbits, the increases in the microbial spoilage could be linked to high pH values along storage time **(Fig. IV.4.4b)** (Perkins-Veazie et al., 2012).

As occurs in other fresh-cut vegetables due to the intrinsic zucchini properties (neutral pH), moulds counts were less important favouring the growth of other populations such as bacteria and yeasts (Giménez et al., 2003; Tournas, 2005). In fact, moulds population was not found in any of the zucchini samples studied thorough the storage period.

Considering all cases studied (cultivar, cutting type, storage temperature and time), in strategy 1 yeast growth was in lower counts (~3.5 log cfu g^{-1}) than in strategy 2 (~5 log cfu g^{-1}) **(Fig. IV.4.6c and Fig. IV.4.6d**, respectively). This could be due to the highest CO_2 concentration inside the packages in the non-microperforated film (strategy 1) that contributes to decrease yeast populations (Gil et al., 2002), decreasing in 2.77 and 0.24 log units (corresponding to cv. 'Natura' sticks at 10 ºC and cv. 'Sinatra' sticks at 10 ºC, respectively).

Finally, it is important to note that in general, lower spoilage counts (mesophilic and psychrophilic aerobic growth, *Enterobacteriaceae* spp. populations and yeast) have been found in non-perforated film packages (strategy 1) compared to micro-perforated

film packages (strategy 2). This could be linked to the antimicrobial activity of CO_2 at high concentrations registered inside the packages (Devlieghere et al., 2000; Allende et al., 2004). So as expected, due to that cv. 'Natura' registered a faster CO_2 production and higher CO_2 accumulation inside the overwrapped trays, microbial spoilage of cv. 'Natura' (for all treatments) was lower than in cv. 'Sinatra'.

3.5. Principal component analysis on evaluated parameters

The effect of the different pre-harvest (cultivar) and post-harvest (cutting type, film packaging, storage time and temperature) on the evaluated parameters was confirmed by PCA plot **(Fig. IV.4.7a and IV.4.7b)**. Three main principal components described fresh cut zucchini parameters evaluated explaining the 89.60 % of the total variance for non-microperforated film (strategy 1) **(Fig. IV.4.7a)** and the 94.07 % of the total variance for microperforated film (strategy 2) **(Fig. IV.4.7b).**

In relation to strategy 1, principal component 1 (PC1) accounted for 47.70 %, principal component 2 (PC2) for 30.11 % and component 3 (PC3) for 11.74 %. Referring to strategy 2, relations between PC1, PC2 and PC3 explained the 48.28 %, 36.43 % and 9.35 % of the total variation, respectively. PC1 describes the differences in terms of a*, and hº for both strategies developed **(Fig. IV.4.7a and Fig. IV.4.7b)**. Nevertheless, some specific microbial spoilage parameters (mesophilic and psychrophilic bacteria, *Enterobacteriaceae* spp, yeast) were the most important factors in determining the quality on zucchini samples from strategy 1, due to they were responsible for the PC1 separation. For strategy 2, a higher importance was observed for the physicochemical parameters in particular for those associated to the PC1 separation (pH, TA and b*).

On the other hand, for both strategies chroma (C*) as well as sensory parameters (aroma and appearance) contributed to divide the PC2. However, only parameters related to color (b* and C*) and overall quality contributed to divide PC2 for strategy 1 **(Fig. IV.4.7a)**, while for strategy 2 microbial spoilage (mesophilic and psychrophilic bacteria, *Enterobacteriaceae* spp, yeast and moulds) as well as TSS were responsible for the PC2 separation **(Fig. IV.4.7b)**. Finally, the parameters with lower impact on the fresh cut zucchini quality were TA and TSS (for strategy 1) and overall quality (for strategy 2), due to the contributed to divide the PC3.

In the PCA from strategy 1 **(Fig. IV.4.7a)** two well defined groups were observed: on the right hand the zucchini slices (with higher sensory quality) and on the left side the group represented by the zucchini sticks (corresponding to higher microbial spoilage counts). On the contrary in the PCA plot from strategy 2 **(Fig. IV.4.7b)** the groups are established as follows: on the right hand samples from cv. 'Natura' (with higher TSS, TA and a*) and on the left side samples from cv. 'Sinatra' (corresponding with higher microbial spoilage counts). Interestingly in those samples (including both cutting types) held under the lowest storage temperature (6 ºC) are located upper the plot (with higher appearance, aroma and overall quality), while those at 10 ºC (with higher pH,

Enterobacteriaceae spp. and psychrophiles populations) are located in the bottom of the plot.

Additionally, positive values for PC1 from both strategies **(Fig. IV.4.7a)** indicated fresh cut zucchini samples with higher L* and texture (cv. 'Natura' slices at 6 ºC and cv. 'Sinatra' slices at 6 ºC). Moreover, positive values for PC2 corresponded to samples with high aroma and appearance (for both strategies), but low in microbial spoilage counts (mesopholic and psychrophilic bacteria, *Enterobacteriaceae* spp. as well as yeast and moulds) for strategy 2. Finally, it is interesting to observe that for both PCA plots **(Fig. IV.4.7a and IV.4.7b)** the a* value content were negatively correlated with hº value and microbial spoilage, but positively correlated with texture, TSS, aroma, appearance as well as overall quality score.

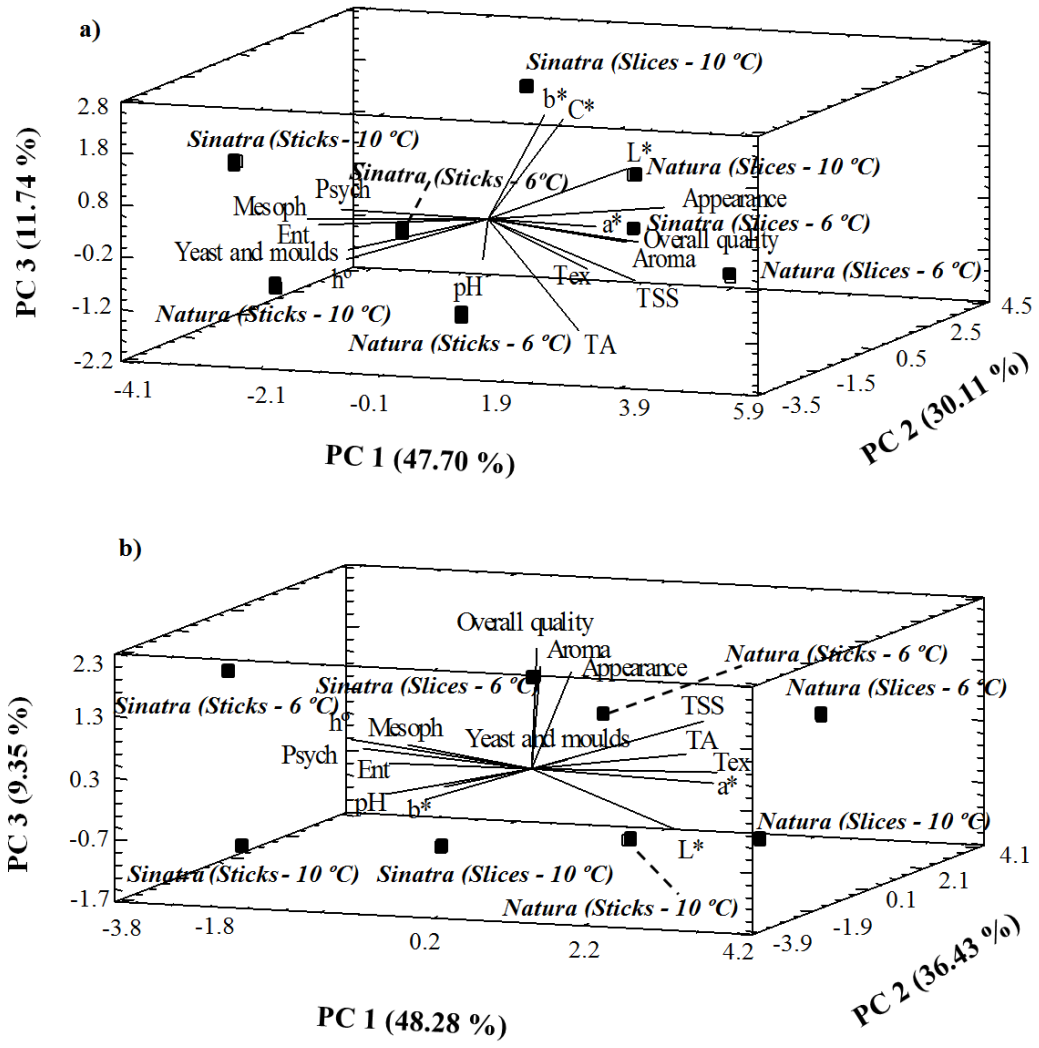

Figure IV.4. 7. Principal component analysis (PCA) plot of cutting types and temperature of storage of fresh-cut zucchini cv. 'Natura' and cv. 'Sinatra' during storage for strategy 1 (a) and for strategy 2 (b).

4. Conclusions

Pre-harvest (cultivar) and post-harvest (cutting type, film permeability, storage temperature and time) factors significantly affected the fresh cut zucchini shelf-life. Significant impacts resulted in changes in the O_2 and CO_2 compositions, color, sensorial quality and microbial counts depending on the combinations of both preharvest and postharvest factors studied.

Zucchini cultivar was an important factor, contributing higher amounts of TSS (such as those observed in cv. 'Natura') to a faster deterioration of the product. For this reason zucchini cultivar selection based on in a low TSS could be more appropriate for presenting a lower metabolism activity and consequently higher shelf-life of the product. With independence of the cultivar studied, sticks presented in general higher spoilage population than slices, while storage temperature seems not have a significant effect on the yeast population.

Referring to film packaging, the non-microperforated OPP film (strategy 1) significantly reduced aerobic mesophilic (~2.43 log units) and psycrophilic (~1.63 log units) bacterial growth, yeast (~2.77 log units) and *Enterobacteriaceae* spp. populations (~1.78 log units), but induced a strong off-odor and loss of freshness due to the combination of extremely low O_2 and high CO_2, while an increase in the titrable acidity values. On the other hand, O_2 and CO_2 concentrations inside the fresh cut zucchini trays overwrapped with micro-perforated film (strategy 2) were higher and lower, respectively, than from strategy 1 packages, contributing to better sensory quality of the product even with higher microbial growth on the samples (7-8 log cfu g^{-1}).

From evaluated results after the different pre-harvest and post-harvest combinations it could be concluded that high permeability film carried out by micro-perforated film (strategy 2) could be an economical alternative to preserve the fresh cut zucchini shelf-life up to day 14, establishing in most of treatment combinations a steady-state in the MAP inside the packages during this period of time.

Acknowledgements

This work was supported by the project RTA2009-00036-00-00 and FEDER funds. Authors thank to Augro Fresh Spain S.L for providing zucchini fruits and to Nicholas Davies for grammatical revision of the manuscript. M.T. Blanco-Díaz acknowledges the financial support from the Spanish Ministry of Economy and Competitiveness as a contracted researcher of the Program 'Researcher Training' (Formación de Personal Investigador, FPI-INIA).

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4.5.

'Influence of cutting type and temperature on the physicochemical, sensory and bioactive compounds of fresh-cut zucchini cultivars stored under modified atmosphere packaging conditions'

María Teresa Blanco-Díaz, Antonio Pérez-Vicente, Alicia Fayos-Moltó, Rafael Font

Department of Postharvest Technology and Food Industry. IFAPA Centro La Mojonera.

Camino San Nicolás, 1, 04745 La Mojonera, Almería, Spain

In preparation

Abstract

BACKGROUND: Zucchini is the most common summer squash morphotype used by the food industry. This is the first study reporting the effect during storage of different cutting types (slices and sticks) and storage temperatures (6 and 10 ºC) on the quality of fresh cut zucchini cultivars ('Natura' and 'Sinatra') under modified atmosphere packaging conditions.

RESULTS: Respiration rate in zucchini sticks at 10 ºC was the highest, increasing around ~41% compared to 6 ºC. In spite of weight loss was more evident on cv. 'Sinatra', 'overall quality score' on this cultivar was the highest. The most severe firmness loss was found on cv. 'Natura' sticks at 6 ºC (~ 30%), being the sticks color very sensitive with temperature increase. At day 14 in both cultivars, slices at 6 ºC retained the highest content in chlorophylls and carotenoids, while slices at 10 ºC reached the highest values in phenolics. At the end, significant differences in vitamin C were only found between cut formats.

CONCLUSION: A storage temperature of 6 ºC (in slices and sticks) as well as 10 ºC (only in slices) is appropriate for maintaining quality of fresh-cut zucchini for up to 14 days, thus reducing wounding stress and preserving bioactive properties. However, sticks stored at 10 ºC have a marketable period up to 10 days.

Keywords: Minimally processed zucchini; cutting stress; temperature; Modified Atmosphere Packaging (MAP); quality.

1. Introduction

The minimally processed vegetable industry has experienced a record growth during the past few decades for adapting ready-to-eat products to consumer needs and lifestyles. This is due in one hand to new consumer's occupations and on the other hand to satisfy quality consumer expectations, being the freshness of the product one of the main goals for the fresh-cut industry. The increasing popularity of minimally processed vegetables has been attributed to the health benefits associated with fresh produce, ensuring food safety while maintaining nutritional and sensorial quality.[1]

Summer squash (*Cucurbita pepo* spp. *pepo*) is one of the seasonal vegetables that it is a part of healthy nutrition due to being low in calories having a high nutritional content and medical value.[2,3] Zucchini is the most common morphotype used by the food industry due to its size and form convenience. In relation to the main area of vegetable production in Europe, as it is Almería (Southern Spain), zucchini is the third most important exported vegetable, fresh zucchini being the main commercialized type.

Otherwise, consumers are looking for zucchini products that reduce time preparation avoiding unnecessary steps such as raw material selection, washing and cutting. Thus, as fresh-cut products are important for reducing time preparation and increasing sales by adding value to the raw agricultural material.

However, fresh-cut products are highly perishable commodities due to after processing a large proportion of the surface area lacks of epidermis.[4] The processes involving fresh-cut produce preparation (such as cutting, chopping and slicing) induce mechanical injury on the tissue and consequently changes in its physiology. The main physiological manifestations after wounding tissue contribute to an increase in respiration as well as membrane degradation leading to a cellular disruption.[5,6] For this reason to understand the effect of cutting formats on physicochemical properties is essential to ensure fresh-cut product quality.[7,8]

Despite of storage temperature playing an important role in preserving wounded tissue, a combination with other techniques like modified atmosphere packaging (MAP) is widely used for prolonging the shelf-life of minimally processed vegetables. Based on the fact that fresh-cut vegetables are respiring products, the permeability selection (for O_2 and CO_2) of the packaging film is an important factor to establish an equilibrated modified atmosphere in the package, thus contributing to increase the shelf-life of the product.[9] Different studies have been carried out about the best modified atmosphere packaging conditions to preserve fresh-cut zucchini quality. On the basis of these studies, Izumi *et al.*[10] and Gorny[11] concluded that MAPs with low O_2 levels (close to 1-0.5 kPa) and medium CO_2 levels were the best gas concentrations for extending zucchini shelf-life, thus reducing in 40% the weight loss and suppressing severity the browning/decay of the damaged tissue.

In recent years, the effect of cutting types on nutritional composition of fresh-cut vegetables such as onion slices/dices; lemon wedges/slices, melon slices/trapezoids/cylinders or shredded radish has been reported by different authors.[12,13,14,15] To the best of our knowledge only one study is available concerning the effect of wounding tissue on nutritional zucchini compounds (antioxidant capacity, total soluble phenolics, ascorbic acid, total carotenoids and total anthocyanins). However, this study was carried out after processing,[16] not including quality characterization of the processed product during storage time. Hence, this is the first report including physicochemical and nutritional quality changes experienced on fresh-cut zucchini during storage under refrigeration combined with MAP conditions. Therefore, the objective of this work was to study the effects of different cutting types (slices and sticks) and refrigeration temperatures (6 ºC and 10ºC) on two fresh-cut zucchini cultivars held under optimum MAP conditions during 14 days. Respiration rate, weight loss, color, firmness, soluble solids and total chlorophylls were considered as physicochemical parameters, while sensorial evaluation and bioactive compounds (total carotenoids, total phenolic content and total vitamin C) were also evaluated. Correlation studies between cited parameters were also conducted.

2. Material and methods

2.1. Plant material

Zucchini squash (*Cucurbita pepo* spp. *pepo*) from different internationals seeds companies cv. 'Natura' (Enza Zaden, Co. Ltd) and cv. 'Sinatra' (Clause, Co. Ltd) were harvested when they reached commercial size (18-21 cm) from a commercial farm in Almería (Augro Fresh Spain S.L.) and transported to the laboratory. Then, the samples were stored in darkness at 6 ± 1 ºC and 90% relative humidity (RH) for 12 h. The fruits were sorted according to the uniformity of size and homogeneous skin color, and finally those showing defects were discarded. Fruits were processed in a cold room at 6 ºC under suitable hygienic conditions before processing.

2.2. Washing, packaging and storage

Intact summer squash fruits were pre-washed in tap water for 1 min to eliminate soil and insect residues and were cut into slices (0.6 $\pm$ 0.01 cm thick) and sticks (1 $\pm$ 0.01 cm thick) with a commercial equipment (SAMMIC CA-300, Guipúzcoa, Spain). Zucchini slices or sticks were washed for 2 min in 150 mg L^{-1} free chlorine solution (NaOCl) adjusted to pH 6.5 with citric acid. The excess surface water remaining on the zucchini slices or sticks was removed by centrifuging at 900 rpm for 4 min (SAMMIC ES-150, Guipúzcoa, Spain). To compare the effect of cutting types and temperatures in minimally processed zucchini cultivars, optimum MAP conditions in this product (O_2 close to 1 kPa and medium CO_2 levels)[10,11] were created in all treatments by passive MAP conditions, controlling the weight and the tray transmission area (the most

economical form to get these conditions). For that, weight on the trays was adjusted during several preliminary experiments (data not shown) to verify the benefit of these MAP conditions, concluding that the optimum weight to get them was 500 and 350 g for 6 and 10 ºC, respectively. Polypropylene trays used in this study had a gas transmission area of 298.24 cm^2 (1/2 GA, cod. G40B, Ilpra Systems, Mataró, Spain) and they were overwrapping by thermosealed on the top with a micro-perforated bioriented polypropylene film (BOPP) (diameter micro-perforations of 150 µm) (Acsa, Valencia, Spain). The micro-perforated film had a O_2 permeance of 36901 mL O_2 m^{-2} d^{-1} atm^{-1} and CO_2 permeance of 29672 mL CO_2 m^{-2} d^{-1} atm^{-1} (6 ºC and 95% RH) and with O_2 permeance of 41078 mL O_2 m^{-2} d^{-1} atm^{-1} and CO_2 permeance of 34285 mL CO_2 m^{-2} d^{-1} atm^{-1} (10 ºC and 95 % HR). Three replicates of each cutting format, temperature storage, cultivar and day of storage were packaged and stored under refrigeration conditions (6 and 10 ºC).

Respiration rate, gas composition in the trays, physicochemical (weight loss, color, firmness, total soluble solids, and total chlorophylls), sensory evaluation and bioactive compounds were evaluated on different cutting shapes (slices or sticks) for both cultivars stored at various temperatures of refrigeration (6 and 10 ºC). Quality attributes were determined at initial moment (0 day), 5, 10 and 14 days of storage. After physicochemical and sensory evaluation, fresh-cut zucchini employed in this study were immediately frozen in individual plastic bags at -80 ºC. About 100 g of fresh-cut samples of each package were freeze-dried (Telstar, Terrasa, Spain) until constant weight. Then, samples were ground with an industrial milled and stored at -80 ºC until chemical analyses in dark conditions.

2.3. Physicochemical analyses

2.3.1. Respiration rate and gas composition within the tray

Respiration rate (mL CO_2 kg^{-1} h^{-1}) was measured using a closed system. Whole zucchini fruits (3 fruits, about 450 g) and fresh-cut (sliced and sticked) zucchini (about 250 g) were placed in 3.65 L hermetic glass jars with a septum in the lid for sampling gas in the headspace at different times. Three replicates were analysed for each, whole or cutting zucchini at each temperature of storage and cultivar. The jars were stored in different cold-rooms at 6 and 10 ºC. Gas sampling was monitored daily by means of a needle connected to an O_2/CO_2 gas analyser (CheckMate 3 PBI Dansensor, Ringsted, Denmark) with an accuracy of 0.5% using a septum in the lid for sampling gas in the headspace at different times.[17] This head space gas analyser is based on a highly stable zirconium electrochemical sensor to record the O_2 content and a mini-IR spectrophotometer detector to record the CO_2 content. The respiration rate was calculated considering the weight of the whole fruits or fresh-cut zucchini, residual volume of the container and time.

Gas composition (oxygen and carbon dioxide) of fresh-cut zucchini were measured in order to evaluate the effects of both cutting types and storage temperature on both cultivars. Immediately before opening the trays the gas composition (O_2, CO_2) inside was measured through a septum by using a needle connected to the O_2/CO_2 gas analyzer. All measurements were made in triplicates.

2.3.2. Weight loss

Weight loss was calculated as percent variation in relation to the initial fresh weight. All measurements were made in triplicates.

2.3.3. Color, firmness and total soluble solids

Color was measured using a CM-700d spectrophotometer equipped with D65 illuminant source (Minolta, Ramsey, NJ, USA) taking three measurements on the mesocarp region of the sliced or sticked zucchini. The instrument was previously calibrated on a white tile at an observation angle of 0. The values were expressed using the CIE *L*a*b** system, hue angle (h^o) was also determined. At each storage time, the mean of five tissues pieces (slices or sticks) were measured per tray for each storage temperature (6 or 10 ºC) and cultivar (cv. 'Natura' and cv. 'Sinatra'). All measurements were made in triplicates.

Firmness was determined following the method previously reported on zucchini by Brew *et al.*[18] The use of a texture analyzer (TA-XT-Plus, Stable Micro System, Surrey, UK) calibrated with a 5-kg weight and equipped with a 4-mm diameter probe was used to assess the firmness on sliced or sticked zucchini mesocarp region. The insert distance was 3 mm, with a stroke speed of 50 $mm \cdot min^{-1}$. At each storage time, the mean of five tissues pieces (slices or sticks) were measured per tray for each cultivar on study (cv. 'Natura' or cv. 'Sinatra'), and the firmness was expressed in Newtons (N). All measurements were made in triplicates.

Each treatment was homogenized using a commercial blender (Moulinex, Barcelona, Spain). TSS of the juice was measured with a hand refractometer (SMART-1, Atago, Japón) and expressed as ºBrix at 20 ºC. All measurements were made in triplicates.

2.3.4. Sensory evaluation

After equilibrium at room temperature, a panel of five trained judges using a 9-point scale (9 = excellent, 7 = very good, 5 = good, limit of marketability, 3 = fair, limit of usability and 1 = poor, unusable)[19] in this study to quantitatively determined the 'overall quality' of the fresh-cut samples. Panelists were asked to base their decision on the samples 'overall quality' taking into account their color, odor, and firmness. A score equal to 5 was used as the threshold for the produce acceptability. Three number codes were used to name samples in order to avoid the identification of the applied treatments.

2.3.5. Total chlorophylls

The extraction and analysis of the chlorophylls pigments were carried out simultaneously, in order to avoid pigment degradation. Chlorophyll pigments were

determined using a UV–Visible spectrometer (Thermo Fisher Scientific, Madison, Wisconsin, USA) and both chlorophylls *a* and *b* were determined according to the equations reported by Lichtenthaler and Wellburn[20] using the method described by Blanco-Díaz et al.[21]; while total chlorophylls were calculated as chlorophyll *a* + chlorophyll *b*, and expressed as mg per 100 g fresh weigh (FW). All measurements were made in triplicates.

2.4. Bioactive compounds

2.4.1. Total carotenoids

Carotenoids were determined using UV–Visible spectrometer and calculate according equations described by Lichtenthaler and Wellburn[20] with minor modifications.[21]

The carotenoids were extracted from the rehydrated of 150 mg of freeze-dried sample with 8 ml acetone containing 1 mg/mL butylated hydroxytoluene (BHT). Samples were shaken by vigorous vortexing followed by ultrasonic bath during 30 min and finally centrifugated at 4000 rpm (Megafuge 1.0R, Heraeus Instruments, DJB Labcare, UK) for 10 min. All steps were carried out in darkness. This procedure was repeated until the sample became colorless. Then, an aliquot was taken from the supernatant for measurement of optical density at 470 nm in the spectrophotometer using quartz cuvettes with a cell path length of 1.0 cm. Finally, total carotenoids were expressed as mg per 100 g fresh weight (FW). All measurements were made in triplicates.

2.4.2. Total phenolic content

The concentrations of total phenolic content (TPC) in the fresh-cut zucchini samples were obtained following by the Folin-Ciocalteu reagent method using gallic acid as external standard[22] with slightly modifications.[21] Finally, results were expressed as mg per 100 g fresh weigh (FW). All measurements were made in triplicates.

2.4.3. Vitamin C

The total vitamin C analysis (ascorbic acid + dehydroascorbic acid) of the fresh-cut zucchini samples were carried out according to the method described by Rahman-Khan *et al*.,[23] with minors modifications. A ground freeze-dried sample of 200 mg or standard solution of ascorbic acid was mixed using 25 ml volumetric flask with 10 mL of filtered solution 5% metaphosforic acid-10% acetic acid and was shaken by vigorous vortexing followed by centrifugation during 10 min at 4000 rpm (Megafuge 1.0R, Heraeus Instruments, DJB Labcare, UK). Five drops of bromine water were added to the collection of the supernatant to oxidaze the ascorbic acid to dehydroascorbic acid. Then five drops of 10% thiourea were added to it to remove the excess of bromine until obtain a clear solution with constant stirring. Furthermore, 1 mL of 2, 4 – dinitrophenil hydrazine dye (DNPH) solution was added thoroughly to complete the reaction to determine content of total vitamin C in fresh-cut zucchini. For the completion of the reaction, all the standards, samples and blank solution were kept at 37 ºC for 3 hours in a water bath. After this incubation, all the standards, samples and blank were cooled in

an ice bath for 10 min and 10 mL of 85% H_2SO_4 were added with constant stirring. As a result, the absorbance of the colored solution was read at 521 nm against a blank solution using quartz cuvettes with a cell path length of 1.0 cm (Thermo Fisher Scientific, Madison, Wisconsin, USA). The regression equation and the regression coefficient (r^2=0.9964) values were obtained for the ascorbic acid. Finally, results were expressed as mg per 100 g fresh weigh (FW). All measurements were made in triplicates.

2.5. Statistical analysis

Experiments were performed using a completely randomized design. Analysis of variance (ANOVA) was performed by four-way (cultivar x cutting type x temperature x storage time) with type III sums of squares using the GLM (General Linear Model) procedure of the LSD (Least Significant Difference test) to compare means, and significance was accepted at $P \leq 0.05$ level. Additionally, Pearson correlation analysis was performed to corroborate relationships between parameters. Statistical analysis was performed using SAS for Windows and Statgraphics plus 5.0 (Statistical Graphics Corp., Rockville, MD, USA).

3. Results and discussion

3.1. Respiration rate and gas composition within the tray

There was a four-way interaction considering both intrinsic and extrinsic factors (cultivar x cutting type x temperature x storage time) for respiration rate **(Table IV.5.1).** In relation to the evolution of respiration rate during storage, differences in fresh-cut zucchini stored at 6 ºC were higher during the first 5 days (from 9.32 to 16.68 mL CO_2 kg^{-1} h^{-1} and from 8.87 to 17.11 mL CO_2 kg^{-1} h^{-1}, for cv. 'Natura' and cv. 'Sinatra', respectively) **(Fig. IV.5.1A and IV.5.1B)**, whereas for treatments held at 10 ºC changes in the respiration rate were noticeable until day 8 of storage (which ranged between 13.84 and 24.30 mL CO_2 kg^{-1} h^{-1} and between 15.33 and 23.28 mL CO_2 kg^{-1} h^{-1}, for cv. 'Natura' and cv. 'Sinatra', respectively) **(Fig. IV.5.1C and IV.5.1D).** These results agree with Fonseca et al.[24] (2002), who described that respiration rate in fresh-cut commodities may gradually increase over time until a maximum value is reached and then, start decreasing again to

Table IV.5. 1. Four-way ANOVA in physicochemical and nutritional quality parameters affected by cultivar, cutting type, temperature and storage time.

	Respiration rate ($mLCO_2Kg^{-1}h^{-1}$)	Color			Firmness (N)	Weight loss (%)	TSS (ºBrix)	Overall quality	Total chlorophylls (mg/100gFW)	Total carotenoids (mg/100gFW)	Total phenolic compounds (mg/100gFW)	Vitamin C content (mg/100gFW)
		L*	b*	hº								
Cultivar (Cv)	***	****	****	****	****	****	****	****	****	****	****	****
Cutting type (C)	****	****	****	***	****	****	****	****	****	****	****	****
Temperature (T)	****	****	****	****	****	***	****	****	**	NS	****	NS
Storage time (S)	****	****	****	****	****	****	****	****	****	****	****	****
Cv x T	NS	****	****	NS	****	NS	****	NS	****	****	NS	NS
Cv x C	**	****	NS	NS	****	NS	NS	NS	NS	**	****	NS
Cv x S	**	****	****	NS	****	NS	****	NS	****	****	****	****
C x S	****	****	****	NS	****	****	****	****	****	****	****	***
T x S	****	****	****	***	****	**	****	**	****	****	****	***
T x C	NS	****	NS	NS	****	NS	****	NS	****	****	NS	NS
Cv x C x T	NS	****	NS	NS	***	NS	NS	NS	NS	****	*	NS
Cv x C x S	****	****	NS	*	****	NS	NS	NS	****	****	****	NS
Cv x T x S	****	****	***	**	****	NS	***	NS	****	****	NS	NS
C x T x S	****	****	**	*	**	NS	****	NS	****	****	****	NS
Cv x C x T x S	****	****	NS	NS	**	NS	NS	NS	***	**	****	***

NS= non-significant
* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$
**** $P \leq 0.0001$

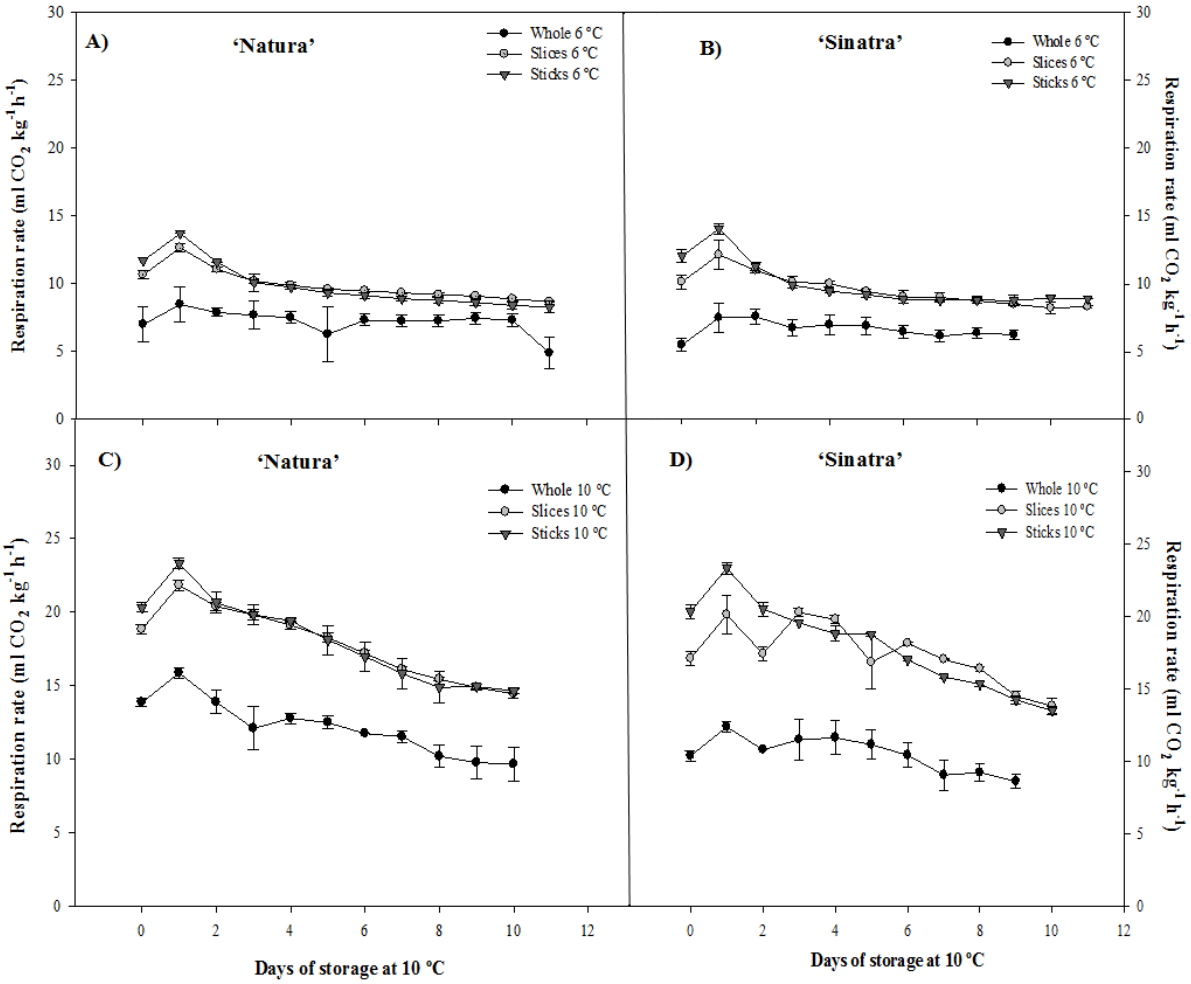

Figure IV.5. 1. Effect of cutting shapes (whole, slices and sticks) on respiration rate (RCO_2) for whole and fresh-cut zucchini cv. 'Natura' (A) and cv. 'Sinatra' (B) stored at 6 ºC; and for whole and fresh-cut zucchini cv. 'Natura' (C) and cv. 'Sinatra' (D) stored at 10 ºC.

either the value before the wounding or to a higher value; being the differences between zucchini cultivars similar to those described by other authors.[25]

Additionally, zucchini sticks at 10 ºC **(Fig. IV.5.1C and Fig. IV.5.1D)** showed the highest values in respiration rate, corresponding to an increase of 41.24% and 39.70% (for cv. 'Natura' and cv. 'Sinatra', respectively) compared to the same cutting shape maintained at 6 ºC. On the contrary, whole zucchini fruits at 6 ºC displayed the lowest respiration rate (7.40 and 6.66 mL CO_2 kg^{-1} h^{-1}, for cv. 'Natura' and cv. 'Sinatra', respectively).This could be explained by the ratio between cutting area to volume (A/V) [sticks (0.4) > slices (0.3) > whole (0)] after wounding zucchini cells. These results are in accordance with other authors concluding that respiration rate differences among cutting shapes on carrots (slices and sticks) were greater when they increase by 5 ºC the storage temperature (from 5 to 10 ºC).[10]

Passive MAP conditions created were similar in all treatments, reaching O_2 levels around 0.90-1.63 kPa and 0.91-1.86 kPa, while CO_2 levels were close to 19.20-20.06 kPa and 19.56-19.80 kPa, for cv. 'Natura' and cv. 'Sinatra' respectively **(Fig. IV.5.2A and IV.5.2B).** In general, the gas composition inside the package suffered less variation in cv. 'Sinatra' than in cv. 'Natura', being the shelf-life in cv.'Natura' sticks up to 10 days for 10 ºC due to very low O_2 levels (0.38 kPa). This is in agreement with other authors,[26] who determined that O_2 concentrations below 0.5 kPa are not recommended for fresh-cut zucchini because they are associated with exudate accumulation and decay susceptibility. On the basis of these results, controlling the weight of fresh-cut zucchini packaged was an economical alternative to holding equilibrated gas atmosphere close to 1 kPa O_2 during 14 days in most of treatments, avoiding an abusive CO_2 emission as a consequence of high respiration rate caused by increasing the storage temperature. A correlation ($r = 0.44$, $P < 0.001$) was observed between the respiration rate and the O_2 level **(Table IV.5.2)**, confirming that modified atmosphere packaging with low O_2 decrease respiration rate preserving fresh-cut products quality[27] and especially on zucchini, where low O_2 levels are desired.[10,11]

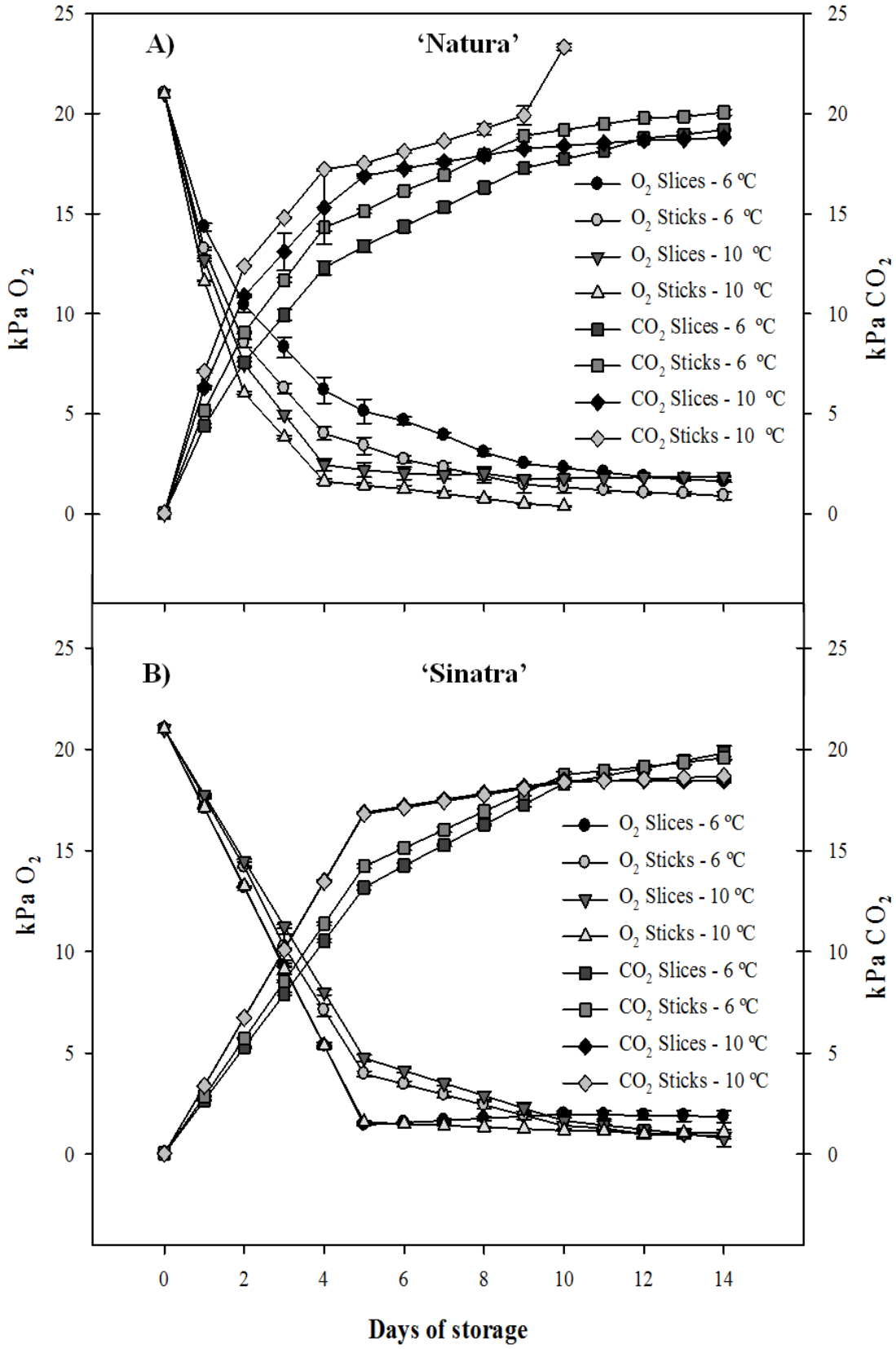

Figure IV.5. 2. Changes in headspace partial pressure of O_2 and CO_2 (kPa) of fresh-cut zucchini (slices or sticks) cv. 'Natura' (A) and cv. 'Sinatra' (B) during 14 days of storage at 6 ºC and 10 ºC.

Table IV.5. 2. Pearson's correlation coefficients and significance for physicochemical properties and bioactive compounds of fresh-cut zucchini.

	1.	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**	**10.**	**11.**	**12.**	**13.**	**14.**
1. Respiration rate	1													
2. O_2	0.44 ***	1												
3. CO_2	NS	-0.88 ****	1											
4. Weight loss	0.59 ****	NS	NS	1										
5. L* value	NS	0.43 **	-0.66 ****	0.32 *	1									
6. b* value	0.77 ****	-0.47 ****	NS	-0.30 *	NS	1								
7. hº value	-0.34 **	NS	-0.31 **	0.45 ***	NS	NS	1							
8. Firmness	NS	NS	NS	-0.45 ***	NS	NS	-0.77 ****	1						
9. TSS	NS	0.45 ***	-0.38 **	NS	0.36 **	-0.33 **	-0.30 *	0.49 ***	1					
10. Overall quality	-0.31 *	0.65 ****	-0.79 ****	NS	0.70 ****	-0.29 *	0.34 **	NS	NS	1				
11. Total chlorophylls	NS	-0.44 ***	0.46 ***	0.46 ***	NS	-0.66 *	0.49 **	-0.32 *	NS	-0.31 *	1			
12. Total carotenoids	NS	NS	NS	NS	NS	0.32 *	0.28 *	-0.60 ****	NS	NS	0.68 ****	1		
13. Total phenolic compounds	0.30 *	NS	NS	NS	NS	0.32 *	0.28 *	-0.48 ***	NS	NS	0.49 ***	0.77 ****	1	
14. Vitamin C	-0.42 **	NS	NS	-0.58 ****	NS	-0.28 *	0.33 *	-0.50 ****	NS	NS	0.36 **	0.42 **	0.36 **	1

NS= non-significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$.

3.2. Weight loss and sensory evaluation

Weight loss was affected by two-way interaction (cutting type x time of storage) **(Table IV.5.1)**. A positive correlation ($r = 0.59$, $P < 0.0001$) **(Table IV.5.2)** was observed between the weight loss and respiration rate supporting that when higher weight loss occurred an increase in respiration rate was observed **(Fig. IV.5.3A and IV.5.3B).** In general, cutting types from cv. 'Sinatra' showed more accentuated dehydration symptoms than those from cv. 'Natura'. These results agree with other authors who demonstrated that weight loss on whole zucchini cv. 'Sinatra' was more obvious than on cv. 'Natura'.[28] In addition, weight loss was more severe on sticks at the end of storage time (close to 0.73% and 1.03% for cv. 'Natura' and cv. 'Sinatra', respectively), which could be due to a higher ratio between cutting area to wounded tissues volume (A/V) (A/V_{slices}= 0.3 and A/V_{sticks}= 0.4).[9]

Also there were (cutting type x time of storage) interactions for overall quality **(Table IV.5.1).** Initially and regarding zucchini cutting type, panelists preferred slices to sticks, probably due to slices having a 'cleaner' cut than sticks and consequently were evaluated with a higher 'overall quality score' (8.6 vs. 8.0; 8.6 vs. 8.5 for cv. 'Natura' and cv. 'Sinatra' respectively) **(Fig. IV.5.3C and IV.5.3D).** A weak correlation between 'overall quality' and different parameters were found, in particular very high with L* values ($r = 0.70$, $P < 0.0001$), O_2 ($r = 0.65$, $P < 0.0001$) and CO_2 levels ($r = -0.79$, $P < 0.0001$) **(Table IV.5.2),** decreasing the score with temperature and time of storage. After 10 days of storage, sticks showed lower overall quality score than slices (4.41 vs. 6.16 and 5.95 vs. 6.82 considering both temperatures for cv. 'Natura' and cv. 'Sinatra', respectively), showing cv. 'Sinatra' the highest overall quality scores during the sensory evaluation. The effect of zucchini cutting type on the 'overall quality score' dramatically decreased at 10 ºC as observed in cv. 'Natura' at day 10, falling below the limit of marketability (overall quality score of '3' – limit of usability) probably associated with fermentative processes (O_2 ~0.3 kPa and CO_2 ~23 kPa) contributing to off-flavors and decay symptoms.

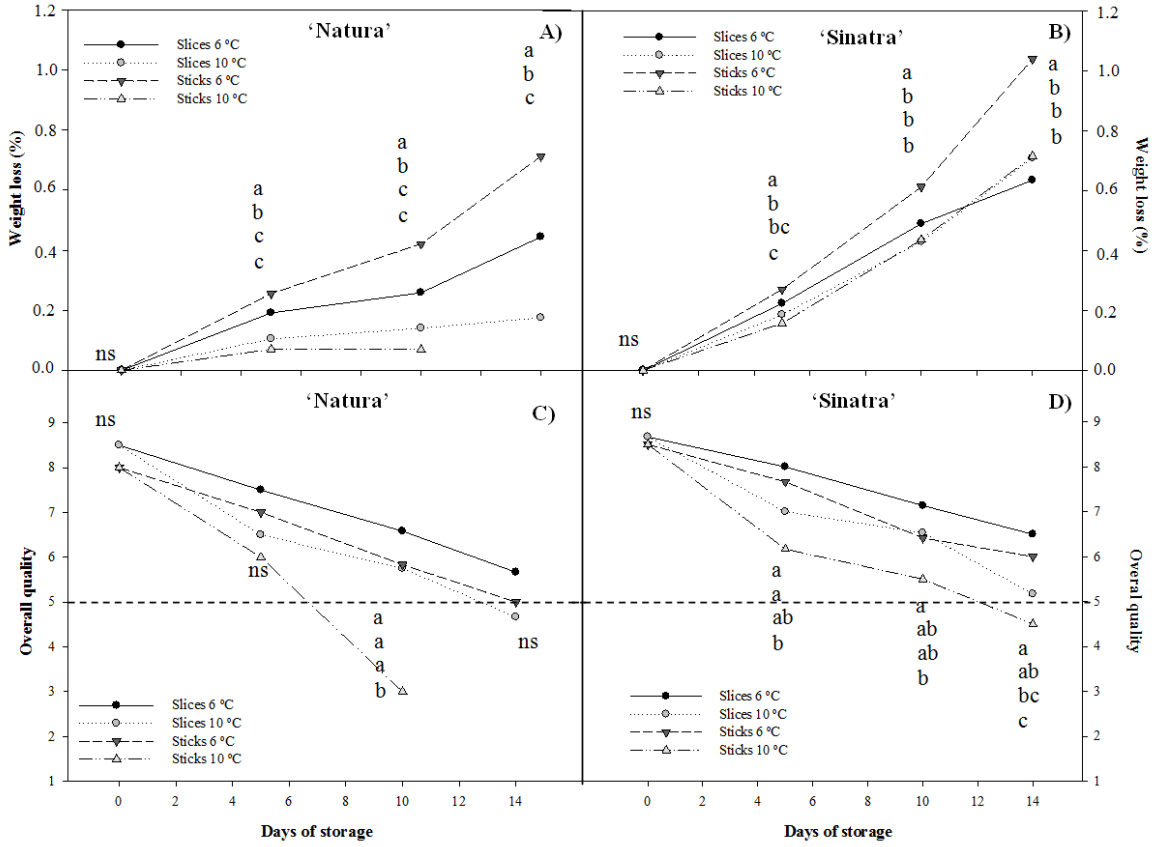

Figure IV.5. 3. Effect of cutting shapes and temperature of storage in weight loss of fresh-cut zucchini cv. 'Natura' (A) and cv. 'Sinatra' (B) during storage; and in overall quality score of fresh-cut zucchini cv. 'Natura' (C) and cv. 'Sinatra' (D) during storage. Means followed by different letters are statistically different according to LSD test at $P \leq 0.05$.

3.3. Color, firmness and total soluble solids

There was four-way interaction (cultivar x cutting types x temperature x time of storage) for L* values **(Table IV.5.1)**. A strong correlation between the L* values and the production of CO_2 levels ($r = -0.66$, $P < 0.0001$) was observed, meaning than the greater the accumulation of CO_2 inside the trays the lower the lightness values on zucchini fresh-cut **(Table IV.5.2).** On the other hand, storage temperature significantly influenced the changes in L* values, showing the samples higher variations at 10 ºC than at 6 ºC, especially in sticks on cv. 'Natura' (decreasing initial values around 0.81 vs. 33%, referring to sticks at 6 ºC vs. sticks at 10 ºC) **(Table IV.5.3).** Similar results were described on fresh-cut carrots by Bolin and Huxsoll[29] and Howard and Griffin[30] who attributed the changes in L* values to the activation of phenylpropanoids metabolism, inducing the lignification process on the cut surface.[10]

There were cultivar x temperature x storage time interactions for b* and hº values. Also for these parameters zucchini sticks were the most affected by temperature increase and storage time showing higher b* values and lower hue angles **(Table IV.5.3).** These results are in agreement with different studies on fresh-cut onions[15] or fresh-cut lemons[13] who concluded that during storage the samples suffered a rapid yellowing of the tissues, that in our study could be more severe with high cutting area formats (A/V) ratio ($A/V_{slices}= 0.3$; $A/V_{sticks}= 0.4$), like in spheres of fresh-cut papaya.[31]

A four-way interaction (cultivar x cutting type x temperature x time of storage) was found for firmness quality loss **(Table IV.5.1).** As expected, there was firmness differences between slices and sticks at day 0, being slices the format exhibiting the highest values in both cultivars (14.38 N vs. 10.70 N; 9.23 N vs. 8.65 N for slices vs. sticks of cv. 'Natura' and cv. 'Sinatra', respectively) **(Table IV.5.3).** In addition, although the effect of cutting type and the increase of storage temperature (from 6 to 10 ºC) was higher on fresh-cut zucchini cv. 'Natura' **(Table IV.5.1),** similar decreases on firmness values were observed after 14 days in both zucchini cultivars (around 21% and 20% as an average of all zucchini cutting types for cv. 'Natura' and cv. 'Sinatra', respectively). Thus, firmness values along the storage time showed an inverse correlation with the weight loss ($r = -0.45$, $P < 0.001$) **(Table IV.5.2).** In fact, water loss has been considered by different authors as a mechanism to firmness reduction,[32,33] which is consistent with results presented in this work. Metabolism activity in fresh-cut zucchini after wounding tissue also was manifested by changes in TSS having three-way interaction (cutting type x temperature x storage time) **(Table IV.5.1).** In our study, changes in TSS content throughout the storage period were correlated to O_2 concentration ($r = 0.45$, $P < 0.001$) and CO_2 ($r = -0.38$, $P < 0.01$) inside the trays **(Table IV.5.2).** These results suggest that the highest storage temperature (10 ºC) and cutting intensity (sticks) determined lower TSS content, cv. 'Natura' showing the highest TSS differences compared to day 0. At 10 ºC, TSS was less affected in zucchini

Table IV.5. 3. Effect of cutting shape (slices or sticks) and temperature of storage (6 ºC or 10 ºC) on firmness and color parameters of fresh-cut zucchini cultivars ('Natura' or 'Sinatra') in MAP conditions during 14 days.

Parameter	Conditions	Zucchini cv. 'Natura'				Zucchini cv. 'Sinatra'			
		Days of storage				Days of storage			
		0	5	10	14	0	5	10	14
L* value	S-6	85.20 ± 0.06A^{b}	85.70 ± 0.15A^{a}	85.10 ± 0.31A^{b}	84.68 ± 0.15A^{c}	84.57 ± 0.58A^{a}	83.77 ± 0.37A^{a}	83.54 ±0.78AB^{a}	84.04 ± 0.58A^{a}
	S-10	85.20 ± 0.06A^{b}	85.02 ± 0.29A^{a}	84.61 ± 0.39A^{c}	84.80 ± 0.28A^{bc}	84.57 ± 0.58A^{a}	83.04 ± 0.77A^{b}	83.95 ± 0.16A^{ab}	83.60 ± 0.42AB^{ab}
	T-6	85.20 ± 0.06A^{a}	83.39 ± 0.27B^{d}	83.91 ± 0.32B^{c}	84.51 ± 0.12A^{b}	84.57 ± 0.58A^{a}	81.76 ± 0.45B^{b}	81.80 ± 0.45C^{b}	82.36 ± 0.35C^{b}
	T-10	85.20 ± 0.06A^{a}	79.18 ± 0.73C^{b}	57.06 ± 0.46C^{c}		84.57 ± 0.58A^{a}	81.39 ± 0.82B^{c}	82.79 ± 0.30B^{b}	82.83 ± 0.52BC^{b}
b* value									
	S-6	24.85 ± 0.37A^{a}	22.64 ± 0.38C^{b}	22.33 ± 0.46B^{b}	22.03 ± 0.34B^{b}	28.25 ± 0.87A^{a}	21.73 ± 0.17D^{b}	22.02 ± 0.88B^{b}	21.91 ± 0.12C^{b}
	S-10	24.85 ± 0.37A^{a}	24.84 ± 0.07B^{a}	24.69 ± 0.32A^{a}	23.09 ± 0.46A^{b}	28.25 ± 0.87A^{a}	26.97 ± 0.96B^{ab}	25.53 ± 0.70AB^{bc}	25.29 ± 0.47B^{c}
	T-6	24.85 ± 0.37A^{a}	24.38 ± 0.37B^{ab}	23.99 ± 0.49A^{b}	23.20 ± 0.21A^{c}	28.25 ± 0.87A^{a}	24.43 ± 0.31C^{b}	23.08 ± 0.71B^{c}	22.31 ± 0.27C^{c}
	T-10	24.85 ± 0.37A^{b}	25.90 ± 0.41A^{a}	24.67 ± 0.61A^{b}		28.25 ± 0.87A^{a}	28.22 ± 0.54A^{a}	26.38 ± 0.83A^{b}	26.90 ± 0.09A^{b}
h^{o} value									
	S-6	92.68 ± 0.32Aa	92.16 ± 0.16B^{a}	91.40 ± 0.38A^{b}	91.34 ± 0.32B^{b}	94.88 ± 0.70A^{a}	93.66 ± 0.32B^{b}	93.59 ± 0.37A^{b}	93.02 ± 0.71B^{b}
	S-10	92.68 ± 0.32Aa	90.67 ± 0.46C^{b}	90.45 ± 0.11B^{b}	92.37 ± 0.69A^{a}	94.88 ± 0.70A^{a}	93.27 ± 0.67B^{b}	93.14 ± 0.51A^{b}	92.58 ± 0.13B^{b}
	T-6	92.68 ± 0.32Aa	93.79 ± 0.68A^{a}	91.38 ± 0.47A^{b}	90.62 ± 0.37B^{b}	94.88 ± 0.70A^{a}	94.83 ± 0.52A^{a}	93.89 ± 0.62A^{a}	94.35 ± 0.20A^{a}
	T-10	92.68 ± 0.32Aa	89.30 ± 0.44C^{b}	91.87 ± 0.08A^{ab}		94.88 ± 0.70A^{a}	93.27 ± 0.42A^{b}	93.50 ± 0.41A^{b}	92.98 ± 0.64B^{b}
Firmness (N)									
	S-6	14.38 ± 0.42A^{ab}	14.75 ± 0.12AB^{a}	13.62 ± 0.31B^{b}	13.64 ± 0.76A^{b}	9.23 ± 0.80A^{a}	8.80 ± 0.47A^{a}	8.41 ± 0.43A^{a}	7.18 ± 0.36B^{b}
	S-10	14.38 ± 0.42A^{a}	15.34 ± 1.04A^{a}	14.40 ± 0.03A^{a}	10.58 ± 0.75B^{b}	9.23 ± 0.80A^{a}	8.98 ± 0.20A^{ab}	8.30 ± 0.21A^{ab}	7.97 ± 0.67A^{b}
	T-6	10.70 ± 0.40B^{b}	13.41 ± 0.25BC^{a}	12.42 ± 0.24C^{a}	7.50 ± 0.97C^{c}	8.65 ± 0.72A^{a}	7.19 ± 0.58B^{b}	6.88 ± 0.50B^{b}	6.91 ± 0.23B^{b}
	T-10	10.70 ± 0.40B^{b}	12.48 ± 1.16C^{a}	9.07 ± 0.37D^{c}		8.65 ± 0.72A^{a}	7.89 ± 0.28B^{a}	7.03 ± 0.32B^{b}	6.51 ± 0.07B^{b}
TSS (ºBrix)									
	S-6	5.71 + 0.17A^{a}	5.58 ± 0.01A^{a}	5.35 ± 0.02A^{a}	5.12 ± 0.68A^{a}	5.21 ± 0.05A^{a}	5.16 ± 0.07A^{a}	5.07 ± 0.02A^{b}	4.69 ± 0.02A^{c}
	S-10	5.71 + 0.17A^{a}	5.52 ± 0.02B^{b}	5.33 ± 0.04A^{c}	4.99 ± 0.07AB^{d}	5.21 ± 0.05A^{a}	5.10 ± 0.01A^{b}	4.90 ± 0.07B^{c}	4.39 ± 0.05B^{d}
	T-6	5.71 + 0.17A^{a}	5.38 ± 0.03C^{a}	4.74 ± 0.39B^{b}	4.45 ± 0.02B^{b}	5.21 ± 0.05A^{a}	4.48 ± 0.04B^{b}	4.43 ± 0.11C^{bc}	4.36 ± 0.05B^{c}
	T-10	5.71 + 0.17A^{a}	4.43 ± 0.04D^{b}	4.37 ± 0.13B^{b}		5.21 ± 0.05A^{a}	4.41 ± 0.04B^{b}	4.40 ± 0.02C^{b}	4.30 ± 0C^{c}

S-6= zucchini slices stored at 6 ºC; S-10= zucchini slices stored at 10 ºC; T-6= zucchini sticks stored at 6 ºC; T-10= zucchini sticks stored at 10 ºC.

[a] Mean value ± standard deviation error of the means (n=3).

[b] Means followed by different letters, uppercase and lowercase for columns and rows respectively, are statistically different according LSD test at $p \leq 0.05$.

slices than in sticks ('Natura'$_{slices}$= 6.30% vs. 'Natura'$_{sticks}$= 23.46% and for 'Sinatra'$_{slices}$= 5.95% vs. 'Sinatra'$_{sticks=}$ 15.54%) **(Table IV.5.3),** that could be attributed to the lowest respiration activity of this format cut. Thus, cv. 'Sinatra' seems to have experienced less cutting stress than cv. 'Natura' in accordance with data from respiration rate, color, firmness, TSS and sensory evaluation previously described.

3.4. Total chlorophylls and carotenoids

Four-way interaction (cultivar x cutting type x temperature x time of storage) was found for both total chlorophylls and carotenoids **(Table IV.5.1).** Loss of chlorophyll mainly took place within the first five days of storage when higher O_2 ($r = -0.44$, $P < 0.001$) and lower CO_2 ($r = 0.46$, $P < 0.001$) levels were registered, being chlorophyll decreases more noticeable on cv. 'Natura' slices at 10 ºC (38.40%) **(Fig. IV.5.4A and IV.5.4B).** This might be produced by the high respiration rate observed during the same period of time, inducing enzymes involved in chlorophyll degradation or with the repression of enzymes involved in chlorophyll production regulating the color[34] (h^o correlation with $r = 0.49$, $P < 0.01$) as reported by Able *et al.*[35] After 5 days of storage, when the atmosphere stabilized at optimum MAP levels (around 1 and 19 kPa, for O_2 and CO_2 levels) total chlorophylls increased in 2.90% and 30.18% for cv. 'Natura' and cv. 'Sinatra', respectively **(Fig. IV.5.4A and IV.5.4B).** In fact, this increase in total chlorophylls could be also correlated to low O_2 levels ($r = -0.44$, $P < 0.001$); and directly correlated to CO_2 concentrations ($r = 0.46$, $P < 0.001$) inside the trays **(Table IV.5.2).**

Referring to carotenoids, great correlations were created with total chlorophylls ($r = 0.68$, $P < 0.0001$) **(Table IV.5.2)**, which might be linked to carotene pigments due to they are the most important photosynthetic pigments preserving to chlorophylls from the damage of absorbed energy by peroxidation.[36] In plant cells, carotenoids are located intracellularly in cell organelles and they are surrounded by various barriers (cell wall, cell membrane, organelle membrane, lipoprotein sheet).[37] After zucchini minimally processing, protein denaturation occurs and breaks down the cell walls, reducing firmness and making easier the release of carotenoids from the zucchini matrix, showing in our study a correlation of $r = -0.60$, $P < 0.0001$ **(Table IV.5.2)**. For color parameters correlations ($P < 0.05$) could be due to the relationship between lutein and β-carotene,[38] the major carotenoids in zucchini, with b* and h^o values.[39,40]

At the end of storage (day 14), similar trend in both total chlorophylls **(Fig. IV.5.4A and IV.5.4B)** and carotenoids **(Fig. IV.5.4C and IV.5.4D)** were found in all treatments studied. Slices at 6 ºC displayed the highest contents in total chlorophylls (5.95 vs. 4.83 mg/100 g FW in cv. 'Natura' and cv. 'Sinatra', respectively) and total carotenoids (1.23 vs. 1.48 mg/100 g FW in cv. 'Natura' and cv. 'Sinatra', respectively).

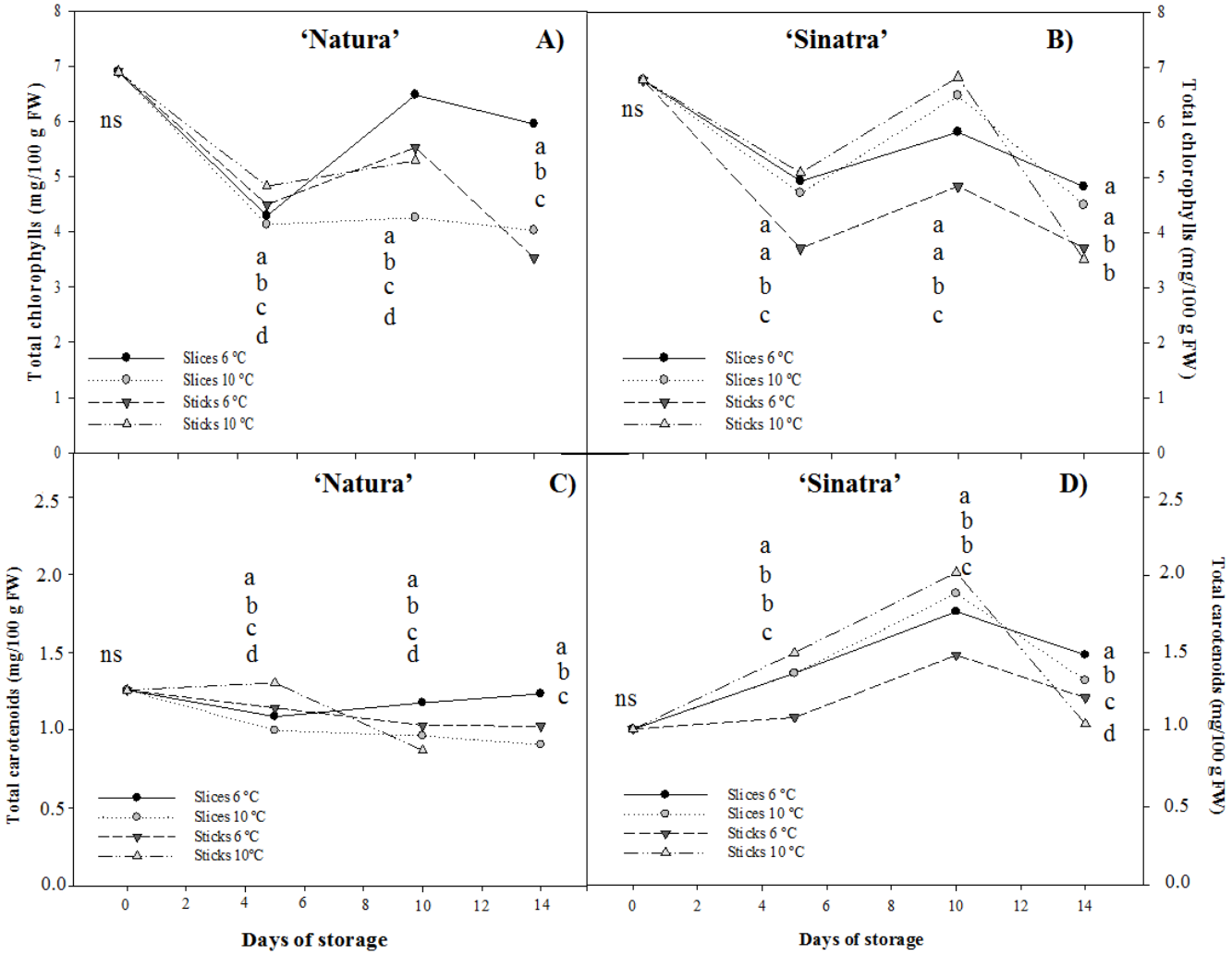

Figure IV.5. 4. Effect of cutting shapes and temperature of storage in total chlorophylls of fresh-cut zucchini cv. 'Natura' (A) and cv. 'Sinatra' (B) during storage; and in total carotenoids of fresh-cut zucchini cv. 'Natura' (C) and cv. 'Sinatra' (D) during storage. Means followed by different letters are statistically different according to LSD test at $P \leq 0.05$.

Changes found in the chlorophylls contents in fresh-cut zucchini along the storage period could be influenced by two different mechanisms. In one hand by weight loss ($r = 0.46$, $P < 0.001$) that tends to concentrate chlorophylls pigments, more obvious in 'Sinatra' at day 10 **(Fig. IV.5.4A and IV.5.4B)**; and on the other hand, by the strong action of different enzymatic mechanisms such as chlorophyllase and magnesium dechelatase (Type I) or chlorophyll oxidase and lipoxygenase (Type II)[41] which are involved in total chlorophylls degradation during time of storage ($P < 0.0001$) **(Table IV.5.1),** clearly found in both cultivars at the end of the storage.

3.5. Total phenolic content and vitamin C

There were cultivar x cutting type x temperature x storage time interactions for both TPC and vitamin C **(Table IV.5.1).** Wounding stimulated phenolic accumulation 1.2- and 1.3 -fold during storage time. This increase was observed in all treatments when less firmness was registered ($r = -0.48$, $P < 0.001$), being more evident in samples kept at 10 ºC **(Fig. IV.5.5A and IV.5.5B).** Thus, during the first 5 days of storage, a significant rise in TPC was observed in all treatments, especially on those zucchini cutting types preserved at 10 ºC ('Natura'$_{slices}$= 14.99% vs. 'Natura'$_{sticks}$= 20.41% and for 'Sinatra'$_{slices}$= 12.04% vs. 'Sinatra'$_{sticks=}$ 2.36%) **(Fig. IV.5.5A and IV.5.5B).** Changes in TPC could be related to enhanced oxidative stress induced by higher respiration rate ($r = 0.30$, $P < 0.05$) and the activity of phenylalanine lyase (PAL) that uses phenylalanine to synthesize phenolic compounds.[42] These results are in agreement with other authors that observed a significant increase in TPC related to storage temperature on fresh-cut tomato,[43] fresh-cut onions[15] or fresh-cut lemon.[13]

Immediately after cutting vitamin C content on cv. 'Natura' was lower than those found on cv. 'Sinatra' (4.83 vs. 9.09 mg/100 g FW, respectively) **(Fig. IV.5.5C and IV.5.5D)**, registering similar values than other fresh-cut zucchini cultivars (~5.9 mg/100 g FW).[16]

Vitamin C contents increased during time of storage (when lower respiration rate and firmness were observed, with correlations of $r = -0.42$, $P < 0.01$ and $r = -0.50$, $P < 0.0001$, respectively). These results support studies by Tudela *et al.*[44] and Gil *et al.*[45] in which increments in vitamin C contents (in fresh-cut potato and pineapple) or ascorbic acid (in fresh-cut cantaloupe and strawberry) were reported when samples were held at 4 ºC or 5 ºC. In our case, cv. 'Natura' exhibited less weight loss than cv. 'Sinatra', contributing the higher RH levels inside the trays of cv. 'Natura' to maintain vitamin C contents.[46]

In both zucchini cultivars at the end of storage period, similar trend was observed for TPC and vitamin C content. The highest TPC and vitamin C values were found in slices at 10 ºC (24.10 and 30.94 mg/ 100g FW for TPC in cv. 'Natura' and cv. 'Sinatra') **(Fig. IV.5.5A and IV.5.5B)** (9.72 vs. 9.06 mg/100 g FW for Vitamin C in cv. 'Natura' and cv. 'Sinatra') **(Fig. IV.5.5C and IV.5.5D).** This fact could be due to plant response to wounding with a rise in phenolic content involved in the repair of damaged tissue,[47]

while the great correlation between TPC and carotenoid content in wounding zucchini tissue ($r = 0.77$, $P < 0.0001$) could be due to oxidation mechanisms caused by polyphenoloxidases or lipoxygenases.[48] Our results also support the practice of keeping fresh-cut commodities at low temperatures for reducing its metabolism activity and, consequently, the changes in the content of these compounds.[49]

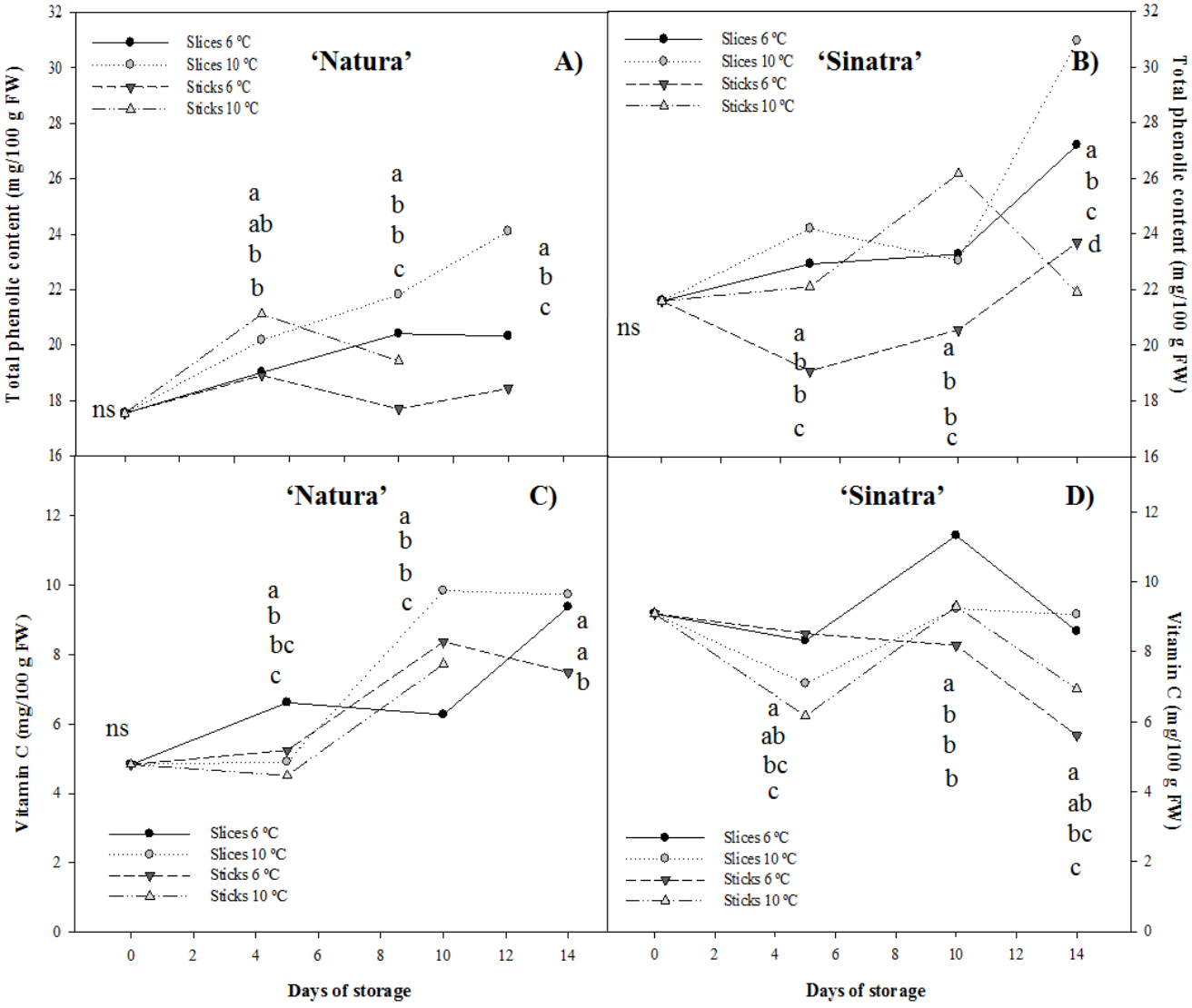

Figure IV.5. 5. Effect of cutting shapes and temperature of storage in total phenolic compounds of fresh-cut zucchini cv. 'Natura' (A) and cv. 'Sinatra' (B) during storage; and in total vitamin C content of fresh-cut zucchini cv. 'Natura' (C) and cv. 'Sinatra' (D) during storage. Means followed by different letters are statistically different according to LSD test at $P \leq 0.05$.

4. Conclusion

It has been demonstrated in this study that cultivar, cutting type, temperature and storage had a significant effect on physicochemical quality (respiration rate, weight loss, color, firmness and total chlorophylls) as well as sensory (overall quality score) and bioactive compounds (total carotenoids, total phenolic content and vitamin C) of fresh-cut zucchini stored under MAP. Fresh-cut zucchini changes after processing and storage were intensified with the temperature increase and by the ratio between cutting area to volume (A/V), showing sticks held at 10 ºC the highest decay of all formats and storage conditions studied. Therefore, it can be concluded that to increase the shelf-life period, zucchini (slices and sticks) should be kept at 6 ºC under optimum modified atmosphere (close to 1 kPa and medium CO_2 levels) which can be developed by passive MAP. However, slices and sticks stored at temperatures up to 10 ºC have marketable quality for at least 10 days, cv. 'Sinatra' being the only one to exceed this period of time.

Acknowledgements

This work was supported by the project RTA2009-00036-00-00 and FEDER funds. Authors thank to Augro Fresh Spain S.L for providing zucchini fruits and to Nicholas Davies for grammatical revision of the manuscript. M.T. Blanco-Díaz acknowledges the financial support from the Spanish Ministry of Economy and Competitiveness as a contracted researcher of the Program 'Researcher Training' (Formación de Personal Investigador, FPI-INIA).

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4.6

'Effect of edible coatings to preserve physico-chemical and sensory quality for fresh and cooked zucchini products'

María Teresa Blanco-Díaz[a], Anne Plotto[b], Jinhe Bai[b], Jan Narciso[b], Rafael Font[a], E.A. Baldwin[b]

[a] Department of Postharvest Technology and Food Industry. Instituto de Investigación y Formación Agraria y Pesquera (IFAPA) - La Mojonera, Camino San Nicolás 1, La Mojonera, 04745, Almería, Spain

[b] USDA-ARS Horticultural Research Laboratory, Fort Pierce, FL, 34945, United States

In preparation

Abstract

This research studied different edible coatings for quality preservation of zucchini slices destined for fresh-cut consumption or for cooking. In the first experiment, antioxidants including calcium ascorbate (CAA), cysteine (CYS) or ethanol (ET) in edible coatings made from chitosan (CHIT), chitosan + glucose (CHIT+GLUC), whey protein concentrate (WPC), soy protein isolate (SPI), carboxymethyl cellulose (CMC), hydroxypropyl methylcellulose (HPMC) and soy bean oil (SB) were tested for effects on appearance and weight loss of the zucchini slices stored at 6 ºC and 20 ºC. In a second experiment, the best coatings (CHIT, HPMC, SPI and SB including CAA+ET as antioxidants) were tested for effects on color of fresh-cut zucchini slices stored at two different temperatures (6 ºC and 12 ºC). The effectiveness of CHIT, SPI, with or without antioxidants, was observed and HPMC and SB were eliminated from further testing. The use of antioxidants contributed to higher L* values (lightness) and h^{o}_{ab} (hue angle) while reducing b* values (yellowness-blueness) as an indication of less discoloration than for water-treated controls. Finally, selected coatings were tested in a third experiment monitoring firmness and sensory quality before and after boiling, steaming, griddling, frying and microwaving. The use of CAA+ET, SPI and CHIT increased firmness of zucchini slices after boiling and steaming (CAA+ET and SPI only). SPI and antioxidants promoted an increase in overall color and visual linking for fresh samples, while SPI increased zucchini flavor, general flavor and overall liking of boiled and steamed samples. Both, SPI and CHIT increased color quality and visual liking for microwaved zucchini samples.

Keywords: edible coating, fresh-cut zucchini, quality

1. Introduction

During the past decades the industrialized countries have been experienced an evolution in the consumer's lifestyle, being the development of innovative food conservation techniques to ensure the quality of the product throughout the shelf-life one of the main goals for the agrifood industry.

Fresh-cut products are highly perishable commodities due to tissue wounding which results in browning, weight loss or tissue softening, and subsequent increase in susceptibility to microbial spoilage (Gonzalez-Aguilar et al., 2010). This research seeks to develop economical and effective strategies to reducing wounding stress after cutting, and preserve final fresh-cut quality.

Recent methods have been reported to improve quality of fresh-cut fruits and vegetables by controlling moisture transfer, gas exchange, oxidation processes, microbial growth and color changes. Among them are the use of modified atmosphere packaging (MAP), the use of active packaging including ethylene or humidity absorbers/carbon dioxide and the development of edible coatings incorporating natural essential oils or chemical ingredients containing antibrowning agents in their formulation (Aggarwal and Kaur, 2012; Dhall, 2013; Mehyar and Han, 2010; Valencia-Chamorro et al., 2011; Oms-Oliu, et al., 2010), with various degrees of success.

In recent years, the use of edible coatings, antioxidant reagents and essential oils for improving the quality, such as shelf-life and safety in fresh-cut melon, carotenoid retention in pumpkin or freshness retention in minimally processed melon, all fruits belonging to *Cucurbitaceae* family, have been reported by different authors (Raybaudi-Massilia et al., 2008; Lago-Vanzela et al., 2013; Moreira et al., 2014). Summer squash (*Cucurbita pepo* spp. *pepo*) is characterized for showing a high morphotype variability (8 morphotypes) (Paris, 2001), with zucchini being the most common morphotype used by the food industry due to the size and convenience of elongated shape.

According to the literature, there is little reported for use of edible coatings on whole zucchini to preserve whole fruit quality (De Jesús Avena-Bustillos et al., 1994), and no reports for use of edible coating (with or without antioxidants) to improve the fresh-cut zucchini quality.

Therefore, the main purpose of this research was to study the effect of different edible coatings for preserving quality of zucchini slices for fresh-cut consumption and after 5 subsequent cooking methods.

2. Material and methods

2.1. Plant material and sample processing

Zucchini squash (*Cucurbita pepo* spp. *pepo*. cv. 'Blackbeauty') was harvested from a commercial farm in Fort Pierce (Florida) and transported to the USDA Horticultural Research Laboratory where samples were stored in darkness at 10 ºC and 90% relative

humidity (RH) for 12 h. The fruits were sorted according to uniformity of the size, green skin color, and freedom from defects or decay. Samples were processed in a sanitized cold room at 10 ºC using suitable hygienic conditions. Whole fruit were washed for 2 min in 100 mg L^{-1} chlorine solution (NaOCl) adjusted to pH 6.5 with citric acid to disinfect and eliminate soil and insect residue. Whole zucchini fruits were cut into slices of 6 mm in thickness, mixed to obtain a homogeneous sample. Zucchini slices, around 200 g, were dipped into each treatment solution for 2 min, then packed into vented plastic clamshells (polyethylene terephthalate made [PET #1], Packaging Plus, Yakima, Wash., U.S.A.).

2.3. Coating solutions preparation, application and quality parameters

In the first experiment, antioxidants including calcium ascorbate (CAA) (Fluka, New-Ulm, Germany), acetyl-L-cysteine (CYS) (Spectrum Chemical Group, Gardena, CA) or ethanol (ET) (Pharmco-Aaper, Brookfield, Ct.) in coating formulations from chitosan (CHIT) (medium MW, Brookfield viscosity = 200.000 cps) (Aldrich Chemical Co., Inc., Milwaukee, Wis.), chitosan + glucose (CHIT+GLUC) (Sigma Chemical Co., St. Louis, Mo.), whey protein concentrate (WPC) (DMV International, Fraser, NY), soy protein isolate (SPI) (Supro 120, Solae LLC, St. Louis, Mo.), carboxymethyl cellulose (CMC) (1% CMC sodium salt, medium viscosity, Aqualon, Wilmington, Del.), hydroxypropyl methylcellulose (HPMC) (Sigma Chemical Company, St. Louis, Mo.), and soy bean oil (SB) (Vegetable oil, Publix Supermarket, Ft. Pierce, FL) were tested for effects on appearance and weight loss of the zucchini slices after 9 days of storage at either 6 ºC or at ambient temperature.

In a second experiment, the coatings that retarded weight loss with the best visual appearance (CHIT, HPMC, SPI and SB including CAA+ET as antioxidants) were tested for effects on color of fresh-cut zucchini slices at two different storage temperatures (6 ºC and 12 ºC). Both, antioxidant reagents and edible coating concentrations were selected after testing in preliminary experiments. Potassium sorbate (PS) (Sigma Chemical Co., St. Louis, Mo) was applied as an antimicrobial reagent, and Tween 80 (Sigma Chemical Co., St. Louis, Mo.) was added as a surfactant when required. The aqueous formulations (expressed as percentage by weight) were prepared using magnetic stirring at 25 ºC as following: 1) control (CONT = distilled water); 2) antibrowning reagents (CAA+ET = 0.5 % calcium ascorbate + 0.5% ethanol + 0.1% PS; 3) chitosan, prepared by dispersing chitosan powder in an acetic acid solution (CHIT= 2% chitosan + 1.25% acetic acid solution + CAA+ET) hydroxypropyl methylcellulose (HPMC= 2% hydroxypropyl methylcellulose + CAA+ ET); 5) soy protein isolate, prepared using glycerol as plasticizer to obtain flexible coatings with reduced cracking or flaking (SPI = 2% soy protein isolate + 0.5% glycerol + CAA+ET); and 6) soybean oil, prepared using Tween 80 as surfactant in order to reduce its surface tension and

enhance its wettability to improve adhesion to zucchini slices, and soy protein isolate as a protein additive (SB = 2% soy bean oil + 0.5% glycerol + CAA+ET). Antibrowning reagents, edible coatings and their concentrations were selected after testing in preliminary experiments (data not shown). Fifteen replicates were included in each treatment group, and subsequently every 3 or 4 days (day 0, 4, 7, 11 and 14), three replicates from each treatment group were analyzed. For color, two measurements on sliced fruit in the mesocarp region were taken using Minolta CR-300 Chroma Meter (Minolta, Tokyo, Japan) calibrated to a white plate using the CIE *L**, *a**, and *b** system. The mean of five zucchini slices stored in a clamshell represented one replication (n= 3).

Finally, selected coatings were tested in a third experiment monitoring texture and sensory quality before and after boiling, steaming, griddling, frying and microwaving at day 4 of storage at 10 ºC. Cooking conditions were determined, with a preliminary experiment and were carried out as following: 1) UNCOOKED (fresh-cut); 2) BOILING (for 5 min in 200 mL tap water that had just come to a boil); 3) STEAMING (for 5 min in 200 mL tap water that had just come to a boil); 4) GRIDDLING (in a griddle pan for 5 min with no oil); 5) FRYING (in frying pan with 200 mL hot vegetable oil for 5 min at 170 ◦C) and 6) MICROWAVING (in an uncovered glass container using a commercial, 1000 W microwave oven for 5 min and 5 min standing). All fresh and cooking treatments were repeated three times for three replications (n= 3) developed, using of 200 g zucchini slices for each application.

Texture was determined following the method previously reported for summer squash slices by Brew et al., 2006. A texture analyzer (XT2i, Stable Micro Systems, England) calibrated with a 5-kg weight and equipped with a 3-mm diameter probe was used to assess the firmness on sliced fruit in the mesocarp region. The insert distance was 3 mm, with a stroke speed of 50 $mm \cdot min^{-1}$. Five slices were measured per replication (n= 3), and the texture was expressed in Newtons (N).

2.4. Sensory analysis

In experiment one and two, zucchini slices were only evaluated for visual quality in an open room under white lighting (daylight type). Overall appearance (from poor to excellent), odor (from undesirable off-odor to good/fresh zucchini odor), color (from brown to white), dehydration (evidence of shriveling, flaccid to turgid) and firmness (evaluated by the give of zucchini slices under light finger pressure), were rated on a 10 cm line scale with a mark every two cm with the numbers 1 to 5, and the anchor words at the extremes for each descriptor. For overall appearance, a value of 3 was considered to be the commercial acceptability threshold. Thirteen assessors participated in the visual quality.

For cooked zucchini, taste evaluations were conducted in individual sensory booths, equipped with positive air pressure to avoid odor cross-contamination under red lighting to disguise sample color. Two slices were placed in 120 soufflé cups with lids (SOLO® Cup Company, Urbana, IL) right after cooking and kept at 50 °C until served within one hour of preparation, except for the uncooked slices which were maintained and served at room temperature. Four 2-digit coded samples (including uncoated control) were presented to the panelists together with a reference sample (uncoated control).

Panelists were instructed to open the lid and smell, then taste the samples and reference and rate odor, texture, flavor and overall liking on an unstructured line scale (-100 to + 100) in comparison with the reference. A mark in the middle indicated "same as the reference", and the anchor words at the extremes were "worse"/"better" for overall odor, texture and flavor, and "less/more" for zucchini odor, firmness and zucchini flavor intensity. A final question asked of the panelists was "overall liking". Samples were presented in a randomized order following a Williams design, and data were collected using Compusense five (Compusense Inc., Guelph, ON, Canada).

After tasting the samples, panelists were directed to a room with daylight lighting where they evaluated visual quality. Samples were presented in the 1-pound clamshell with open lids. The uncoated (control) sample was presented as a reference. Panelists rated overall visual quality (worse/better) and color (darker/lighter) in comparison with the reference using a 10 cm unstructured line scale.Twelve panelists participated in this test.

2.5. Statistical analysis

STATISTIX Version 8.0 (Analytical Software, Tallahassee, FL, USA) was used for analysis variance (ANOVA) of quality parameters (color, weight loss and firmness). Mean separation was determined by least significant difference (LSD) test ($p \leq 0.05$). For visual data of experiment one and two, data were analyzed by ANOVA using SENPAQ Version 5.01 (Qi Statistics Ltd., Berkshire, UK) ($p \leq 0.05$). For sensorial data of the cooked samples (experiment three), -100 to +100 data were transformed to 0-200. ANOVA and principal components analysis (PCA) were performed using SENPAQ.

3. Results and discussion

The efficacy of antioxidants, combined or not with different edible coatings, on zucchini slices for weight loss and appearance **(Table IV.6.1)** showed that the combination of CAA+ET were one of the most effective treatments. However, other edible coating formulations that included antioxidants (CHIT or SPI) were useful for reduction of decrease weight loss as well as to preserve visual quality. These results agreed with other authors (Pushkala et al., 2013), who reported that radish shreds treated with chitosan exhibited a lower degree of weight loss, whereas SPI is the most studied coating material for fresh-cut products (Dea et al., 2011; Montero-Calderón and Cerdas-Araya, 2011).

Table IV.6. 1. Effect of antioxidants combined or not with different edible coatings on weight loss (%) and appearance evaluation at day 9 of storage.

	Evaluation at day 9 of storage			
	Zucchini slices stored at 6 ºC		Zucchini slices stored at 20 ºC	
	Weight loss	Good visual quality	Weight loss	Good visual quality
Control	5.91	X	4.53	X
CAA+ CYS	4.23		9.27	
CAA+ ET	2.38	X	9.25	
CHIT + CAA + CYS	5.15		12.71	
CHIT + CAA + ET	5.31	X	11.46	X
CHIT + GLU + CAA + CYS	3.99		12.09	
CHIT + GLU + CAA + ET	3.31		7.42	
WPC + CAA + CYS	2.75		12.56	
WPC + CAA + ET	3.68		9.82	
SPI + CAA + CYS	6.68		9.16	
SPI + CAA + ET	5.50	X	7.77	X
CMC + CAA + CYS	8.22		-	
CMC + CAA + ET	8.59		-	
HPMC + CAA + CYS	8.24		-	
HPMC + CAA + ET	8.13	X	-	
SB + CAA + CYS	8.59		-	
SB + CAA + ET	7.39	X	-	

CAA= 0.5% Calcium ascorbate; CYS= 0.5% Cysteine; ET= 0.5% ethanol; - = bad visual appearance at day 9 of storage.

In general, the application of edible coatings had a positive influence for maintaining fresh-cut quality when the zucchini slices were stored under refrigeration (6 ºC) compared to storage at abusive temperatures for fresh-cut commodities (20 ºC). HPMC and SB were selected for presenting good visual quality at 6 ºC.

The effectiveness of antioxidants and edible coatings on color quality of fresh-cut zucchini slices was affected by the temperature of storage at 6 °C and 12 °C **(Fig. IV.6.1** and **Fig. IV.6.2,** respectively). In this study the effect for preserving color quality was greater when the zucchini slices were stored at 6 ºC than at 12 ºC, probably due to the higher temperature inducing stress and decay that could not be controlled by the treatments. Considering both temperatures, the edible coatings formulations preserved lighter color (higher L* values, **Fig. IV.6.1a** and **Fig. IV.6.2a**) also reflected as lower hº (**Fig. IV.6.1c** and **Fig. IV.6.2c**) and lower b* values (**Fig. IV.6.1b** and **Fig. IV.6.2b**), with the L* value, representing lightness of the sample, being most altered by the coating treatments. This confirmed previous studies that showed the effect of edible coatings to preserve quality of lightly (and slightly) processed products (Baldwin et al., 1995). For the samples stored at 6 ºC, antioxidants combined with or without t SPI coatings resulted in the best treatments to maintain color quality until day 7, with the antioxidants alone extending color quality until day 14. For samples stored at 12 ºC, the main color parameter influenced by the stress of the temperature was the b* value (yellowness-blueness) with these samples more browning-yellowness symptoms. These results are in accordance with other authors (Shon and Haque, 2007) that reported the potential of SPI to reduce loss of lightness, and reduce browning, while providing a moisture barrier effect on fresh cut apples, potatoes, carrots, and onions. Despite that some authors found HMPC coatings preserved color changes in fresh-cut carrot slices (due to an increase in the L* value) (Villalobos-Carvajal et al., 2009) and SB coatings improved from dehydration and surface appearance (Kester and Fennema, 1986; Hall, 2011), in our study HPMC and SB were eliminated for not improving the quality on fresh-cut zucchini slices.

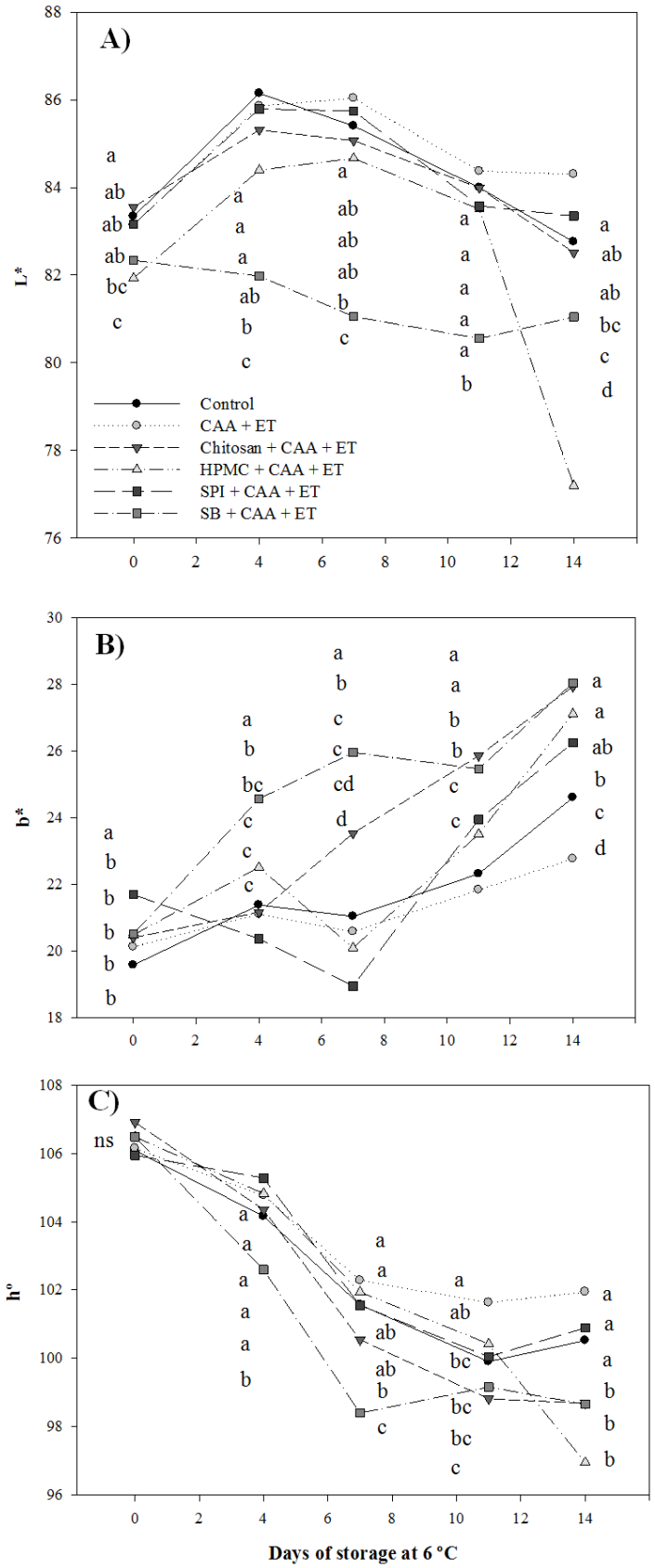

Figure IV.6. 1. Effect of antioxidants combined or not with different edible coatings on color parameters (lightness, L* (A); yellowness-blueness, b* value (B) and hue angle, h° (C)) of zucchini slices during storage at 6 ºC. Values are the mean (five zucchini slices per replication). Different letters for the same day of storage are statistically different according to LSD test at $p \leq 0.05$.

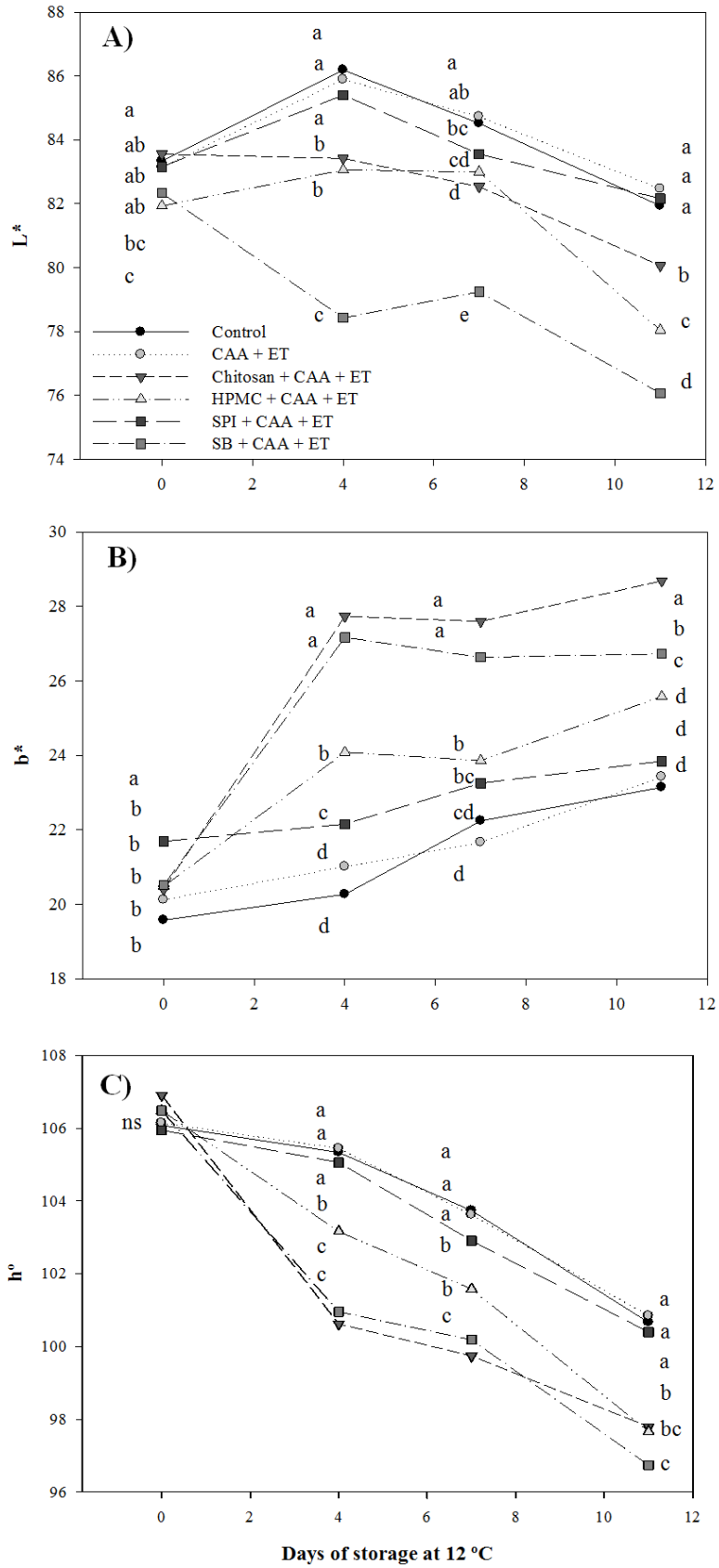

Figure IV.6. 2. Effect of antioxidants combined or not with different edible coatings on color parameters (lightness, L* (A); yellowness-blueness, b* value (B) and hue angle, hº (C)) of zucchini slices during storage at 12 ºC. Values are the mean ± standard deviation of three replicates (five zucchini slices per replication). Different letters for the same day of storage are statistically different according to LSD test at $p \leq 0.05$.

The role of antioxidants and edible coatings to preserve texture on fresh-cut uncooked and after cooking was influenced by the cooking method **(Table IV.6.2)**. While the formulation including only antioxidants (calcium ascorbate and ethanol) was able to improve the firmness after 4 days of storage at 10 ºC on uncooked samples, some edible coatings including antioxidants (Chitosan, SPI) as well as antioxidants alone contributed to reduced tissue softening after some cooking methods (boiling and steaming). These cooking methods have the common characteristic that the vapor goes around the zucchini slices, and suggest the importance of the hydrophobic substances from the coating that might have provided barriers to moisture migration (Avena-Bustillos and McHugh, 2011; Morillon et al., 2002; Hernandez, 1994). The edible coating effect on firmness was not apparent in those cooking methods in which a higher temperature was applied (griddling, frying and microwave), which was probably attributed to the aggressive cooking technique removing the coating.

Table IV.6. 2. Firmness evaluation (Newton) of zucchini slices treated with antioxidants with or without different edible coatings before cooking (uncooked/fresh-cut) and after five different cooking methods at day 4 of storage at 10 ºC. Values are the mean ± standard deviation of three replicates (five zucchini slices per replication). Different letters for the same cooking method (rows) mean significant difference by LSD test at $p \leq 0.05$.

	Firmness (N) at day 4 of storage at 10 ºC			
	Water (Control)	**Antioxidants (CAA + ET)**	**Chitosan CAA + ET**	**SPI + CAA + ET**
Uncooked	8.06 ± 0.37 b	9.80 ± 1.27 a	8.38 ± 0.82 ab	7.88 ± 0.66 b
Boiling	0.51 ± 0.03 b	0.86 ± 0.05 a	0.78 ± 0.06 a	0.96 ± 0.17 a
Steaming	0.67 ± 0.03 b	1.07 ± 0.20 a	0.89 ± 0.06 ab	0.93 ± 0.03 a
Griddling	2.64 ± 0.17 a	2.47 ± 0.30 a	2.61 ± 0.55 a	3.10 ± 0.39 a
Frying	0.59 ± 0.11 a	0.52 ± 0.07 a	0.62 ± 0.13 a	0.53 ± 0.04 a
Microwave	0.82 ± 0.19 a	1.13 ± 0.54 a	0.80 ± 0.31 a	0.79 ± 0.10 a

CAA= 0.5% Calcium ascorbate; ET= 0.5% ethanol.

Sensory analysis on uncooked zucchini samples, water treated controls, showed high scores for natural/fresh zucchini flavor, while chitosan gave higher scores for texture **(Fig. IV.6.3a)**. However, SPI and antioxidants promoted color, odor and visual liking in zucchini fresh-cut slices, confirming the results obtained in this study during the second zucchini experiment. On the other hand, the effect of water-treated samples (control) had a negative influence on the appearance (dark color) showing that the edible coatings provided a protection barrier to preserve the visual quality after boiling **(Fig. IV.6.3b)**. Both, antioxidants, with or without the SPI coating, increased general odor, visual liking and color quality after steaming (data not shown) and boiling. These results agree with studies that reported SPI, alone or with additives, improved the sensory quality of fresh-cut cantaloupe and apple (Eswaranandam et al., 2006).

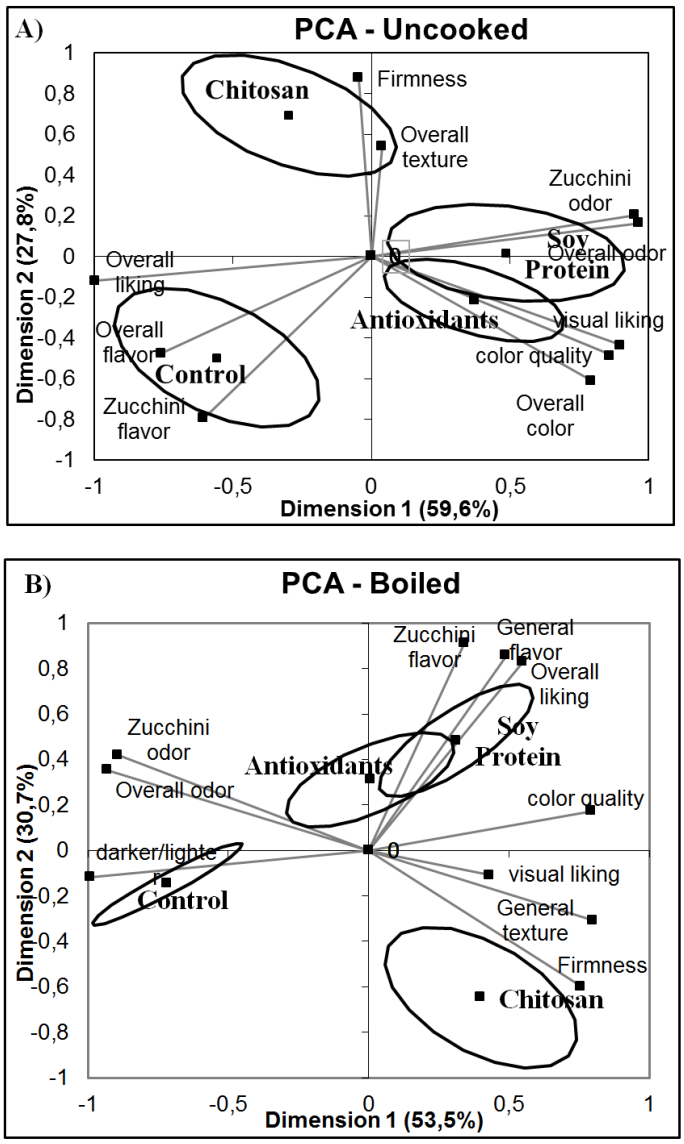

Figure IV.6. 3. Principal component analysis plot of sensorial analysis of uncooked (A) and boiled (B) zucchini slices with antioxidants including or not different edible coatings after 4 days of storage at 10 ºC.

4. Conclusion

In conclusion, the most promising film formers for both fresh and cooked zucchini slices were CHIT and SPI with CAA, ET and PS as antioxidants and antimicrobials. These treatments improved both color in fresh cut as well as color, texture and or flavor in some cooking methods.

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4.7

'Use of visible and near-infrared spectroscopy for predicting antioxidant compounds in summer squash (Cucurbita pepo ssp pepo)'

María Teresa Blanco-Díaz[a], Mercedes Del Río-Celestino[b], Damián Martínez-Valdivieso[b], Rafael Font[a]

[a] Department of Postharvest Technology and Food Industry, IFAPA Center La Mojonera Camino San Nicolás, 1, 04745 La Mojonera, Almería, Spain
[b] Department of Plant Breeding and Crop Biotechnology, IFAPA Center ***La Mojonera,*** Camino San Nicolás, 1, 04745 La Mojonera, Almería, Spain

DOI: 10.1016/j.foodchem.2014.05.019

Food Chemistry, 2014; 164:301–308. (Impact Factor: 3.26) – Published

Abstract

The food industry and plant breeding programs require fast, clean and low-cost screening techniques for nutritional compounds determination in food matrices. This is the first report on the study of the potential of near-infrared spectroscopy (NIRS) for the prediction of antioxidant compounds in summer squash tissues collected since 2009 to 2012. Modified partial least-squares (MPLS) regression was used to correlate spectral information and the different antioxidant compounds in the samples. The coefficients of determination in the external validation (r^2_{ev}) obtained were for ascorbic acid (0.77 and 0.86), chlorophyll *a* (0.79 and 0.66), chlorophyll *b* (0.86 and 0.79) and total phenolic compounds (0.65 and 0.68) in exocarp and mesocarp tissues, respectively, supporting that NIRS is able to predict in a rapid way these components for screening purposes. Major wavelengths influencing the calibration equations showed that chromophores as well as fiber components of the fruits highly participated in developing the NIR equations.

Keywords: Summer squash, near-infrared spectroscopy (NIRS), ascorbic acid, total phenolic content, chlorophylls.

1. Introduction

Fruits and vegetables ingestion has proven to play a key role in preventing diseases due to their potent antioxidant activity, being summer squash one of the seasonal vegetables that takes part of healthy nutrition due to his low in calories and high nutritional contains and medical value (Menéndez, Capó, Menéndez-Castillo, González, Domínguez & Sanabria, 2006; Shokrzadeh, Azadbakht, Ahangar, Hashemi & Saeedi-Sarav, 2010). This vegetable is originated in the tropical and subtropical regions of America, although it could be considered as widespread growing under different climate conditions. In relation to the main area of production of vegetables in Europe, as it is Almeria (Southern Spain), summer squash is the third more important exporting vegetable.

The main components of summer squash are carbohydrates which represent the 85-90% of the total dry matter. Other minor substances found in this vegetable as carotenoids, chlorophylls pigments (*a* and *b*), total phenolic compounds (TPC) and ascorbic acid (AA) have been attributed beneficial properties due to its antioxidant/anti-radical, anti-carcinogenic, anti-inflammatory, antiviral, and antimicrobial activities (Møller & Loft, 2004; Oloyede, Agbaje, Obuotor & Obisesan, 2012). The action mechanisms of these molecules are in the way of delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals reducing oxidative damage to the human body.

Although it is possible to find six different chlorophylls derivates in vegetables (chlorophyll pigments *a* and *b*, pheophytin *a* and *b* and pheophorbide *a* and *b*) (Lanfer-Marquez, Barros & Sinnecker, 2005), chlorophylls pigments, *a* (Chl-A) and *b* (Chl-B) are the most common in summer squash fruits being chlorophyll *a* the pigment with the highest antioxidant activity (Khattab, Goldberg, Lin & Thiyam, 2010). Also some phytochemicals such as AA and TPC are also good sources of antioxidant nutrients. TPC are synthesized in plants partly as a response to ecological and physiological pressures such as pathogen and insect attack, UV radiation and wounding (Kennedy & Wightman, 2011; Zulak, Liscombe, Ashihara & Facchini, 2006). On the other hand, AA is a water-soluble vitamin which is required for a range of essential metabolic reactions; in addition it plays an important role for prevention of scurvy, maintenance of healthy skin, gums and blood vessels. This vitamin is also an effective antioxidant and protects proteins, lipids and carbohydrates from damage by free radicals and reactive oxygen species (Davey et al., 2000).

Different analytical methods have been reported for the quantification of the antioxidant activity in vegetables. Among them are spectrophotometric or chromatographic techniques such as high performance liquid chromatography (HPLC), high speed counter current chromatography or supercritical fluid chromatography. These

techniques are time-consuming due to multiple steps are needed and usually hazardous reactive are used.

In recent years, the use of multivariate spectroscopic, chemical data and chemiometrics models for predicting some particular components of organic matrices belonging to *Cucurbitaceae* family, such as sugar content in melon (Sugiyama, 1999), nitrogen in marrow seeds (El-Shora & Ali, 2011) or oil in pumpkin seed (Lankmayr et al., 2004) have been reported by different authors. However, no references have been found in the scientific literature to near-infrared reflectance spectroscopy (NIRS) for determining antioxidant compounds in summer squash fruits, in spite of being an important issue for breeding programs and food industry.

Therefore, the objectives of the present work were to study the potential of the NIRS technology for predicting Chl-A and Chl-B, TPC and AA in summer squash exocarp and mesocarp tissues, being these compounds some of the main responsible molecules of the antioxidant properties in this vegetable.

2. Material and methods

In this study summer squash belonging to different morphotypes (vegetable marrow, zucchini, pumpkin and cocozelle) were grown during three seasons from 2009 to 2012 in the greenhouses of the Centre IFAPA La Mojonera (Almería, Spain) (36º 46' N, 2º 48' O) and collected when they reached a commercial size. These morphotypes covered a high variability representing some of the most important commercial and traditional cultivars in the production zones of the Iberian Peninsula.

2.1. Summer squash samples

Summer squash seeds were germinated and then they were transplanted into rockwool cubes (Grodan BV, 6040KD Roermond, NL) in greenhouses following standard local cultural practices for both, plant nutrition and insect pests and diseases control. The vegetables used in this work showed a good visual appearance without surface defects, matching the generally accepted commercial standards for this product. Each summer squash employed in this study was manually peeling to separate exocarp from mesocarp and these were immediately frozen in individual plastic bags at -80 ºC. About 100 g of fresh fruit were freeze-dried (Telstar, Terrasa, Spain) until constant weight. Then, samples were ground with an industrial milled and stored at -80 ºC until chemical analysis in dark conditions.

2.2. Acid ascorbic analyses

The reference values for AA were obtained using an automatic titration (Metrohm, 862 Compact Titrosampler, Metrohm, US) by the iodine titration method (Suntornsuk, Gritsanapun, Nilkamhank, & Paochom, 2002) with minor modification. Thus, 250 mg of freeze-dried sample was mixed with distilled deioniser water until final weight of 50 g and treated with 2 mL glyoxal solution (40%), stirred briefly and allowed to stand for

5 min. After the addition of 5 mL sulfuric acid (25%), it was titrated with iodine (0.01 mol/L) up to the endpoint (EP1). The linearity of the method was determined using AA as an external standard. Triplicate titrations were made for each standard solution. The regression equation and the regression coefficient (r^2=0.9997) values were obtained. Finally, the ascorbic acid content was expressed as mg/g dry weight (DW).

2.3. Chlorophylls pigments a and b

The extraction and analysis of chlorophylls pigments were carried out simultaneously, in order to avoid pigment degradation. Then, chlorophyll pigments were determined using a UV–Visible spectrometer (Thermo Fisher Scientific, Madison, Wisconsin, USA) and calculated according to the chlorophylls equations reported by Lichtenthaler & Wellbur (1983).

Chlorophyll *a* =11.75 A_{662} - 2.35 A_{645}

Chlorophyll *b* =18.61 A_{645} - 3.96 $A_{662,}$ where A is absorbance

Eight mL of acetone containing butylated hydroxytoluene (BHT) (1 mg/ml) were added to 150 mg of freeze-dried sample and were shaken by vigorous vortexing followed by ultrasonic bath during 30 min and finally centrifugated at 4000 rpm (Megafuge 1.0R, Heraeus Instruments, DJB Labcare, UK) for 10 min. This procedure was repeated until the sample became colorless. Then, an aliquot was taken from the supernatant for measurement of optical density at 662 and 645 nm in the spectrophotometer using quartz cuvettes with a cell path length of 1.0 cm. Finally, chlorophylls pigments *a* and *b* were expressed as µg/ g DW.

2.4. Total phenolic content determination

The concentrations of TPC in the different samples were obtained by the Folin-Ciocalteu reagent method using gallic acid as external standard (Singleton & Rossi, 1965). To do this, 150 mg of freeze-dried sample was mixed with 5 ml of methanol (80%) and was shaken by vigorous vortexing followed by centrifugation during 10 min at 4000 rpm. Then, 1 ml of the supernatant was mixed with 9 ml of distilled water in a falcon flask. An aliquot (1 ml) of extracts or standard solution of gallic acid (18, 28, 37, 56, 75, 112, 150, 188 mg/l) was added to 25 ml volumetric flask, containing 9 ml of distilled deionised water. Then, 1 ml of Folin–Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to volume (25 ml) with distilled deionised water. The solution was incubated at room temperature in the dark for 90 min, and the absorbance was read at 750 nm against a blank solution using quartz cuvettes with a cell path length of 1.0 cm. The regression equation and the regression coefficient (r^2=0.9984) values were obtained for the gallic acid. Finally, results were reported in gallic acid equivalents (mg/g DW).

2.5. NIRS analysis

Visible and NIR spectra were recorded on a VIS–NIR spectrometer model 6500 (Foss-NIRSystems, Inc., Silver Spring, MD, USA) in reflectance mode equipped with a transport module. The monochromator 6500 consists of a tungsten bulb and a rapid scanning holographic grating with detectors positioned for transmission or reflectance measurements.

To produce a reflectance spectrum, a ceramic standard is placed in the radiant beam, and the diffusely reflected energy is measured at each wavelength. The actual absorbance of the ceramic is very consistent across wavelengths.

Freeze-dried, ground summer squash exocarp and mesocarp samples were placed in the sample holder (3 cm diameter, 10 mL volume approximately) until it was full (sample weight 3.50 g), and were then scanned. Their spectra were acquired at 2 nm wavelength resolutions as log 1/R (where R is reflectance) over a wavelength range from 400 to 2500 nm (visible and near-infrared regions).

To minimise sampling error, spectral readings were taken in quadruplicate from each sample, and was obtained as an average of 32 scans over each sample, plus 16 scans over the ceramic standard before and after scanning the sample. The ceramic and the sample spectra were used to generate the final log (1/R, where R is reflectance) spectrum.

The whole sets of spectra and their corresponding laboratory values for exocarp and mesocarp tissues were split into two subsets, one for calibration and other for an external validation. Two thirds of the spectra formed the calibration subset and were used for performing the different calibration equations. The rest of spectra formed the validation subset and were used for testing the predictive ability of each of the calibration equations obtained. Calibration and external validation groups were randomly selected but they were adjusted so that their content standard deviations were similar to ensure that the range and distribution of the two groups were comparable.

For the statistical analysis, the WinISI II version 1.50 (Intrasoft International, LLC) was used.

2.6. Calibration

Calibration equations were computed using the raw optical data (log 1/R, where R is reflectance), or first, second or fourth derivatives of the log 1/R data, with several combinations of derivative (gap) sizes and smoothing (i.e. (1,4,4,1); derivative order, segment of the derivative, first smooth, second smooth); (0,0,1,1); (2,5,5,1); (2,10,5,1); (4,5,5,1). Wavelengths from 400 to 2500 nm every 8 nm were used to perform the different calibration equations. Modified partial least square (MPLS) regression was used to correlate spectral information and the different antioxidant compounds from summer squash exocarp and mesocarp. This regression method constructs a number of factors as linear combinations of the original spectral data, performing a regression on

the factor scores to derive a prediction equation (Shenk & Westerhaus, 1996). The final objective of the statistical procedure was to reduce the high number of spectral data points (absorbance values from 400 to 2500 nm every 2 nm, i.e. 1050 data) and to eliminate the correlation of absorbance values presented by neighbouring wavelengths (Shenk & Westerhaus, 1996). Standard normal variate and de-trend transformations (SNV+DT) (Barnes, Dhanoa & Lister, 1989) and weighted multiplicative scatter correction (MSC) were used to correct baseline offset due to scattering effects (differences in particle size among samples).

In the first step, the performances of the different calibration equations obtained for the different antioxidant compounds in exocarp and mesocarp summer squash tissues were determined from cross-validation (Martens & Naes, 1989). Cross-validation was also used for determining the best number of terms to use in the equation (Shenk, Workman & Westerhaus, 2001) and to identify those samples being chemical *(t)* or spectral *(H)* outliers which were eliminated in two passes (Shenk & Westerhaus, 1996).

The external validation was conducted with equations developed in the calibration process. These equations were tested on an external group of samples not included in the calibration set. The prediction ability of the different equations obtained was determined on the basis of the optimum combination of the following statistics: high coefficient of determination in the external validation (r^2_{ev}), and also high ratios of the standard deviation (SD) to standard error of performance (SEP) (RPD_{ev}), and of the range to SEP (RER) (Williams & Sobering, 1996). As these authors report the RPD ratio is desired to be greater than 3 for a good calibration, while the RER ratio should be as high as possible, being the mathematical expressions (Eq.1) and (Eq.2):

$$RPD = SD\langle[(\sum_{i=1}^{n}(y_i - \hat{y}_i)^2)(n - K - 1)^{-1}]^{0.5}\rangle^{-1} \quad \text{(Eq.1)}$$

where y_i = lab reference value for the ith sample; $\hat{y}_i$ = NIR measured value; n = number of samples, K = number of wavelengths used in an equation; SD = standard deviation of the chemical data.

$$RER = range\langle[(\sum_{i=1}^{n}(y_i - \hat{y}_i)^2)(n - K - 1)^{-1}]^{0.5}\rangle^{-1} \quad \text{(Eq.2)}$$

3. Results and discussion

3.1. Antioxidant compounds in summer squash exocarp and mesocarp tissues

The descriptive analysis (mean, minimum, maximum, standard deviation and coefficient variation) and the frequency distribution of the antioxidant compounds in the summer squash samples of the fruits used in this work are summarized in the **Table IV.7.1** and **Fig. IV.7.1**, respectively. The coefficient of variation (CV) values reported in **Table IV.7.1** were in all cases (except for TPC in both tissues) higher than 40 % due

to this parameter is a function of the wide variability shown as a result of the different ripening stages of the summer squash fruits studied.

Table IV.7. 1. Reference chemical data of the antioxidant attributes of summer squash exocarp (104 ≤ n ≤ 126) and mesocarp (89 ≤ n ≤ 154) tissues of the fruits used in this work.

Antioxidant	Tissue	Range	Mean	SD	CV (%)
AA (mg/ g DW)	**Exocarp**	0.15-6.53	1.60	1.32	82.50
	Mesocarp	0.28-4.79	1.44	1.12	77.74
Chl-A (µg/ g DW)	**Exocarp**	8.96-6888.17	1421.59	1380.32	97.09
	Mesocarp	4.97-947.03	87.18	151.48	173.75
Chl-B (µg/ g DW)	**Exocarp**	9.52-6981.13	964.06	1195.30	123.98
	Mesocarp	2.35-577.35	69.82	94.95	135.99
TPC(mg/ g DW)	**Exocarp**	0.43-8.28	3.87	1.36	35.14
	Mesocarp	1.34-8.11	3.61	1.46	40.44

SD = standard deviation
CV = coefficient of variation

Summer squash exocarp and mesocarp showed similar means and SD for AA (mg/g DW) and TPC (mg/g DW) (AA: 1.60 vs. 1.44; TPC: 3.87 vs. 3.61; AA: 1.32 vs. 1.12; TPC: 1.36 vs. 1.46), respectively, but significant differences were obtained between tissues for Chl-A and Chl-B (Chl-A: 1421.59 vs. 87.18; Chl-B: 964.06 vs. 69.82; Chl-A: 1380.32 vs. 151.48; Chl-B: 1195.30 vs. 94.95).

Values of AA described in our work were slightly higher than those found in zucchini fruits by other authors (Reyes, Villarreal & Cisneros-Zevallos, 2007) but similar to others reported in winter squash fruits (*Cucurbita moschata*) (Roura, Moreira & Del Valle, 2004; Roura, Del Valle, Aguero & Davidovich, 2007).

On the other hand, the contents of Chl-A and Chl-B found in exocarp fruits in this work were lower than those described in cucumber fruits by other authors (Costache, Campeanu & Neata, 2012), but higher than those obtained in the mesocarp by the same authors.

In relation to TPC, values yielded in this study were similar to those observed in pumpkin by Oloyede, Agbaje, Obuotor & Obisesan (2012). However, others authors (Mongkolsilp, Pongbupakit, Sae-Lee & Sitthithaworn, 2004) have concluded that the TPC in others *Cucurbitaceae* fruits were slightly higher than TPC in summer squash fruits.

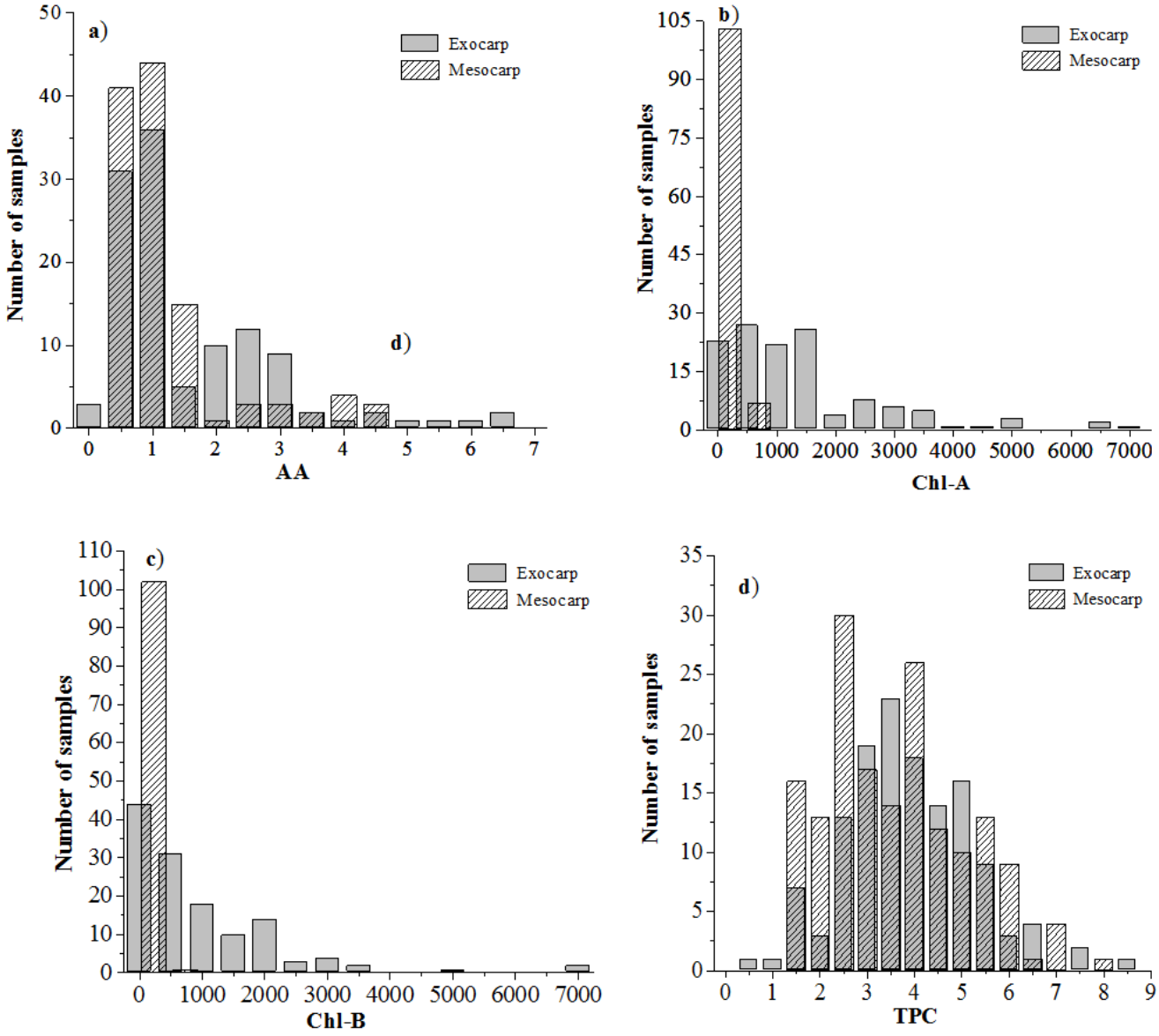

Figure IV.7. 1. Frequency distribution of AA (a), Chl-A (b), Chl-B (c) and TPC (d) in exocarp and mesocarp tissues of the summer squash fruits used in this study.

3.2. NIR spectra information

Average spectrum of exocarp and mesocarp tissues showed bands exhibiting absorption maxima at 440, 542, 670, 1456, 1476, 1934, 2148, 2278 and 2346 nm (exocarp) and at 444, 670, 1449, 1936, 2110, 2272, 2346 nm (mesocarp). Exocarp and mesocarp summer squash spectra were mainly characterized by the presence of two prominent maxima, at 430-445 nm and at 670 nm, both attributable to Chl-A absorptions (Lichtenthaler & Buschmann, 2005), the most extended pigment in green vegetables. Those pigments originated a light absorption at these wavelengths as a consequence of electronic transitions taking place in the photoactive part of the molecule (chromophore). Others pigments as anthocyanins have been reported (Giusti & Wrolstad, 2005) as showing absorption bands that match those bands found from 445 to 670 nm in the exocarp spectrum.

Several carotenoids reported in maize have been described as the responsible molecules for the absorption bands at 1458 nm and at 2348 nm (Brenna & Berardo, 2004), due to mainly zeaxanthin and lutein. This fact could explain the occurrence of the weak absorption bands of these carotenoids at 1456 nm and 2346 nm in summer squash exocarp and mesocarp. Also, absorption bands between 1449 and 1936 nm in the spectra of exocarp and mesocarp are due to water bands (second and first –OH overtone, respectively) (Shenk & Westerhaus, 1996).

3.3. Calibration and Prediction capacity of the NIR calibration models

As shown in **Table IV.7.2** the lowest standard error of calibration (SEC) and higher coefficient of determination in the calibration (R^2_{cal}) were obtained transforming the raw optical data into the second derivative, except for Chl-A y Chl-B, in which non derivative (for Chl-A and Chl-B in mesocarp) or fourth derivative (for Chl-B in exocarp) were the best mathematical treatments.

Table IV.7. 2. Calibration and cross-validation statistics for the different antioxidant compounds in the exocarp and mesocarp tissues for the selected equations performed in the range 400 to 2500 nm.

Antioxidant	Tissue	Mathematic treatment	n	Range	Mean	SD	nt	Calibration		Cross-Validation		
								SEC	R^2_{cal}	SECV	r^2_{cval}	RPD_{cval}
AA (mg/ g DW)	**Exocarp**	SNV+DT (2,10,5,1)	68	0.15-3.37	1.27	0.83	6	0.43	0.73	0.50	0.64	1.70
	Mesocarp	SNV+DT (2,10,5,1)	73	0.28-3.64	1.13	0.81	7	0.22	0.92	0.29	0.88	2.80
Chl-A (µg/ g DW)	**Exocarp**	SNV+DT (2,5,5,1)	78	8.96-6346.19	1337.52	1247.76	7	329.50	0.94	509.72	0.85	2.45
	Mesocarp	Weighted+MSC (0,0,1,1)	60	5.54-126.1	42.63	29.60	4	12.33	0.83	14.55	0.76	2.03
Chl-B (µg/ g DW)	**Exocarp**	SNV+DT (4,5,5,1)	78	9.52-3431.3	849.10	862.64	8	123.49	0.98	282.91	0.90	3.04
	Mesocarp	Weighted+MSC (0,0,1,1)	58	2.35-266.2	50.24	58.80	5	20.35	0.88	32.13	0.71	1.83
TPC (mg/ g DW)	**Exocarp**	SNV+DT (2,10,5,1)	82	0.97-8.28	3.90	1.36	8	0.72	0.71	0.87	0.61	1.60
	Mesocarp	SNV+DT (2,10,5,1)	100	1.34-7.11	3.50	1.37	8	0.62	0.79	0.67	0.76	2.04

Mathematic treatment: SNV+DT= standard normal variate plus de-trend transformations; Weighted+MSC= weighted plus multiplicative scatter correction; (derivative order, segment of the derivative, first smooth, second smooth).
n= number of samples.
SD= standard deviation.
nt= number of terms in the calibration model.
SEC= standard error of calibration.
R^2_{cal}= coefficient of determination in the calibration.
r^2_{cval}= coefficient of determination in the cross-validation.
SECV= standard error of cross-validation.
RPD_{cval}= ratio of the standard deviation to standard error of cross-validation.

In most of the cases the combination of SNV+DT was used to obtain the best prediction equations except for Chl-A and Chl-B in mesocarp in which case Weighted+MSC was applied to get the best results. As a result of the calibration process between 2 and 5 samples were identified as being '*t*' outliers which were eliminated from the calibration set in two elimination passes (Shenk & Westerhaus, 1996). The equations obtained displayed r^2_{cval} values in cross-validation that ranged from 0.61 (TPC in exocarp) to 0.90 (Chl-B in exocarp), which also showed the lowest (1.60) and highest (3.04) RPD_{cval} values, respectively **(Table IV.7.2).** The equations with the best predictive ability in cross-validation on the basis of their r^2_{cval} and RPD_{cval} values were then validated on an external set of samples. For these mathematical models, the coefficients of determination in the external validation (r^2_{ev}) and standard error of prediction (SEP) **(Table IV.7.3)** ranged from 0.65 (meaning that 65% of the chemical variability in the data was explained) (SEP= 0.74) corresponding to TPC in exocarp and Chl-A in mesocarp (SEP= 18.58) **(Fig. IV.7.2a and IV.7.2b),** which was indicative of equations useful for correct separation of the samples with low, medium and high contents, to 0.86 for AA in mesocarp (SEP= 0.33) and Chl-B in exocarp (SEP= 345.00) **(Fig. IV.7.2c and IV.7.2d),** which was indicative of good precision equations (Shenk & Westerhaus, 1996).

Table IV.7. 3. External validation statistics for antioxidant compounds in summer squash exocarp and mesocarp for the selected equations performed in the range 400 to 2500 nm.

Antioxidant	Tissue	Mathematic treatment	n	Range	Mean	SD	External validation			
							SEP	r^2_{ev}	RPD_{ev}	RER
AA (mg/ g DW)	**Exocarp**	SNV+ DNT (2,10,5,1)	34	0.234-3.106	1.26	0.81	0.39	0.77	2.08	7.36
	Mesocarp	SNV+ DNT (2,10,5,1)	38	0.37-3.4	2.18	0.87	0.33	0.86	2.64	9.18
Chl-A (µg/ g DW)	**Exocarp**	SNV+ DNT (2,5,5,1)	36	11.34-5020.74	1359.76	1187.38	580.79	0.79	2.04	8.63
	Mesocarp	Weighted + MSC (0,0,1,1)	30	6.1-127.04	44.36	32.23	18.58	0.66	1.73	6.51
Chl-B (µg/ g DW)	**Exocarp**	SNV+ DNT (4,5,5,1)	34	15.62-3085.56	791.72	813.60	345.00	0.86	2.36	8.90
	Mesocarp	Weighted + MSC (0,0,1,1)	29	4.08-288.68	48.48	62.22	28.92	0.79	2.15	9.84
TPC (mg/ g DW)	**Exocarp**	SNV+ DNT (2,10,5,1)	40	1.52-7.41	4.03	1.26	0.74	0.65	1.70	8.00
	Mesocarp	SNV+ DNT (2,10,5,1)	49	1.4-6.13	3.45	1.29	0.73	0.68	1.77	6.48

n= number of samples.
SD= standard deviation.
SEP= standard error of prediction in the external validation.
r^2_{ev}= coefficient of determination in the external validation.
RPD_{ev}= ratio of the standard deviation to standard error of prediction in the external validation.
RER= ratio of the range to standard error of prediction in the external validation.

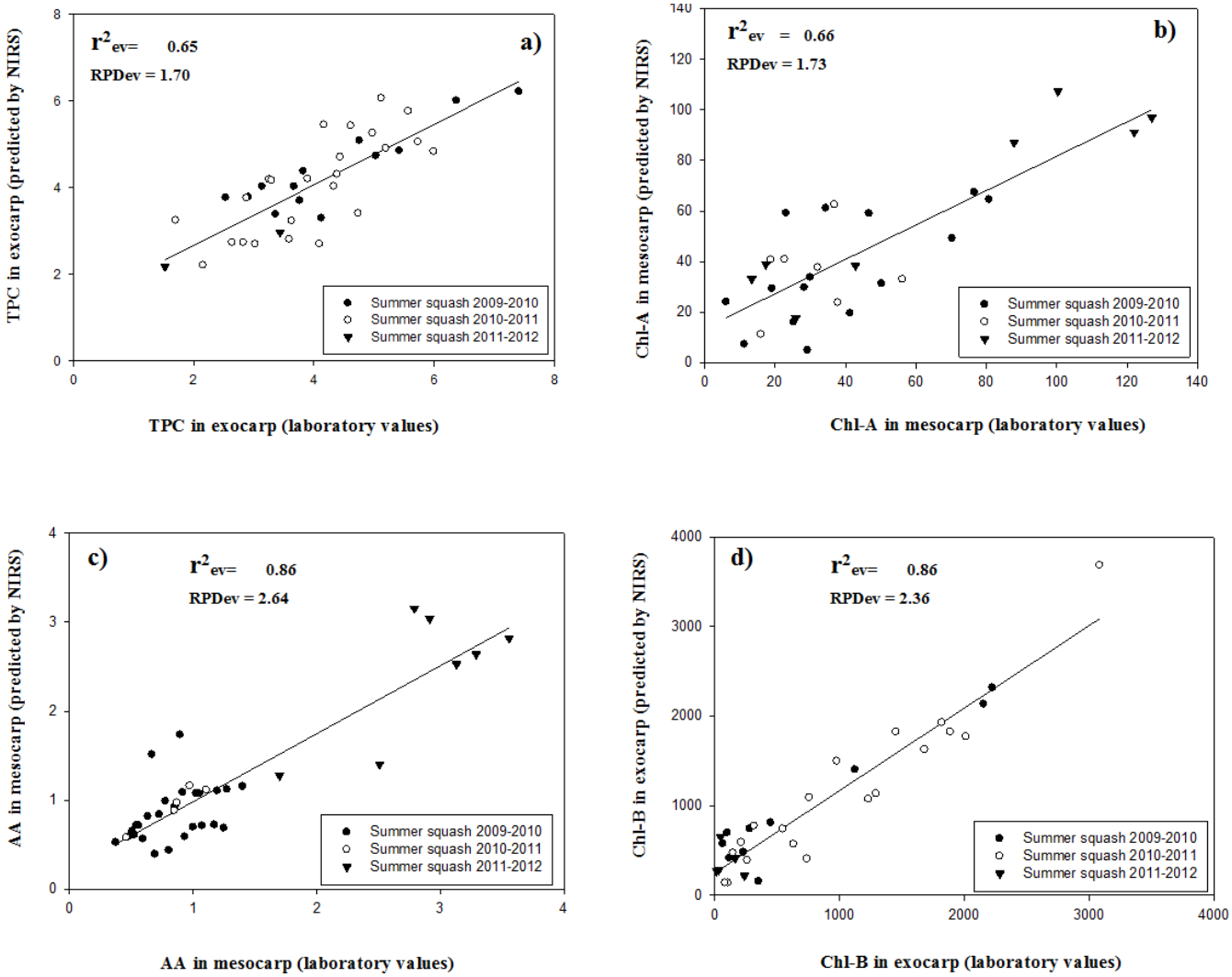

Figure IV.7. 2. External validation scatter plots of laboratory vs. predicted values by NIRS for TPC in exocarp (a), Chl-A in mesocarp (b), AA in mesocarp (c) and, Chl-B in exocarp (d) of summer squash fruits used in this study.

These r^2_{ev} values are in concordance with the prediction of antioxidant compounds reported in other fruits not belonging to the *Cucurbitaceae* family, such as blueberries (Sinelli, Spinardi, Di Egidio, Mignani & Casiraghi, 2008), apples (Zude-Sasse, Truppel & Herol, 2002) and quinoa (Wells-Moncada, González-Martín, Escuredo, Fischer & Montserrat-Míguez, 2013).

The RPD_{ev} and RER statistics in the external validation are measurements of the ability of an NIRS model to predict a constituent and enables comparison of model performance across populations with different standard deviations (Cozzolino et al., 2004) and ranges, respectively. In accordance with the limits for RPD recommended by Williams & Sobering (1996) and Davey, Saeys, Hof, Ramon, Swennen & Keulemans (2009), RPD values below 1.5 are considered unusable, values of between 1.5-2.0 can be used for rough predictions, those between 2.0 and 2.5 allow approximate quantitative predictions to be made, while values above 2.5 and 3.0 are considered to be good and excellent predictive models, respectively, whereas for RER values as high as possible are recommended.

The RPD_{ev} statistics performed in this work ranged from 1.70 for TPC in exocarp (acceptable and suitable for the rough screening) (RER= 8.00) **(Fig. IV.7.2a)** to 2.64 for AA in mesocarp (displaying good accuracy of the developed VIS/NIRS model) (RER= 9.18) **(Fig. IV.7.2c).** These RPDs for the different antioxidant compounds obtained in the external validation in summer squash tissues analyzed **(Table IV.7.3)** agreed with those reported by other authors in blueberries (Sinelli, Spinardi, Di Egidio, Mignani & Casiraghi, 2008), apples (Zude-Sasse, Truppel & Herol, 2002), pepper (Ignat, Schmilovitch, Fefoldi, Steiner & Alkalai-Tuvia, 2012) and cotton (Liu, Gamble & Thibodeaux, 2010), who reported RPD values between 2 and 2.3 for the same components studied in this work.

3.4. Partial least squares modified loadings for antioxidant compounds in summer squash tissues

Modified partial least-squares (MPLS) regression reduces the spectral information of the samples by creating a much smaller number of new orthogonal variables (factors), which are combinations of the original data, and which retain the essential information needed to predict the composition. The role played by the VIS spectrum where light absorption by pigments dominates the reflectance spectrum (these absorptions being due to electronic transitions taking place in the photoactive part of the molecule or chromophore) and NIR absorbers (organic and inorganic molecules) presented in summer squash tissues in modeling the calibration equations for antioxidant compounds is interpreted by studying the bands of the MPLS factors (loading plots). **Fig. IV.7.3** displays the loading plots which show the regression coefficients of each wavelength related to the element AA, Chl-A, Chl-B or TPC being calibrated, for each factor of the equation.

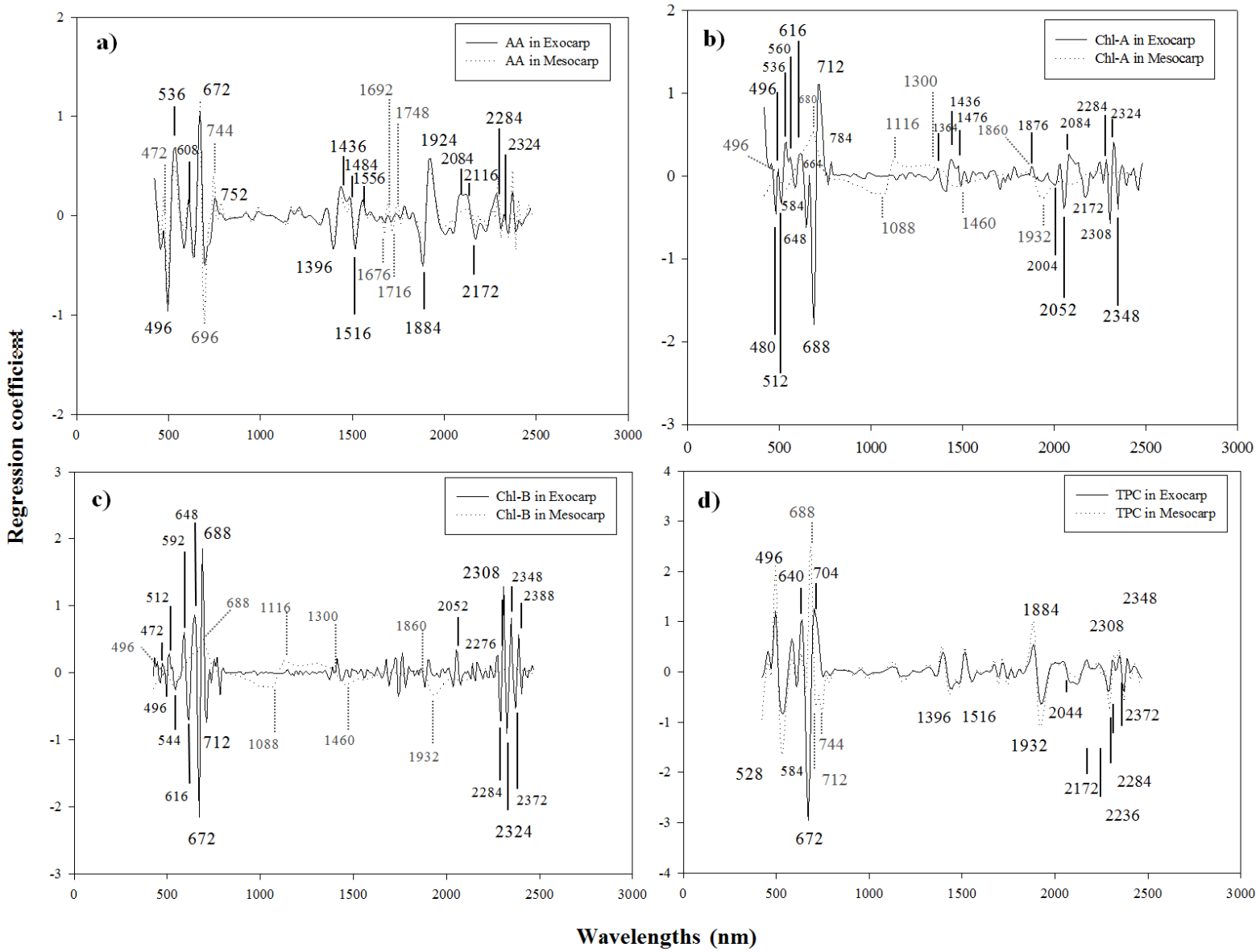

Figure IV.7. 3. First MPLS loading spectra for antioxidant compounds in summer squash exocarp and mesocarp using the best mathematical treatment for AA (a), Chl-A (b), Chl-B (c) and TPC (d).

In this Figure the wavelengths in the loading plots more highly participating in the development of each factor are those that have greater spectral variation and better correlation with the element in the calibration set.

Thus, loading plots for AA in exocarp and mesocarp **(Fig. IV.7.3a)** displayed spectral peaks with a high weight which correspond to absorptions by chromophores located in the range from 496 to 696 nm. There are at least five intense peaks located at 496 nm (electronic transitions, green), 536 nm (electronic transitions, green), 608 nm (electronic transitions, orange), 672 nm (electronic transitions, red) and 696 nm (electronic transitions, red) in the visible region, and five shown at 1396 nm (combination band C-H), 1516 nm (first overtone of the N-H stretching vibrations in protein) , 1692 nm (first overtone of the C-H stretching vibrations), 1884 nm (combination bands of stretching-bending) and 1924 nm (second overtones of C=O stretching modes and O-H stretching vibrations) in the NIR region (Osborne, Fearn & Hindle 1993). These results are in accordance with previous studies concluding that NIR spectrum provides sufficient information on hydroxyl (O–H), amino (N–H) and C–H groups for the study of ascorbic acid (Pissard, Fernandez-Pierna, Baeten, Sinnaeve, Lognay, Mouteau, Dupont, Rondia, & Lateur 2013).

Loading plots for Chl-A **(Fig. IV.7.3b)** showed peaks that match absorptions by chromophores at 496 nm (electronic transitions, green), 648 nm (electronic transitions, red), 680 nm (electronic transitions, red) and 712 nm in the visible region and, others six peaks located at 1088 nm (transition between VIS and NIR), 1116 nm (combination bands of stretching-bending), 1932 nm (O-H stretching vibrations in cellulose), 2284 nm (C-H stretching vibrations), 2308 nm (second overtones of C-H bending vibrations) and 2348 nm (C-O stretching and bending vibrations) in the NIR region.

Regarding Chl-B loading graphics **(Fig. IV.7.3c)** some peaks that showed a higher weight are common to those displayed in Chl-A regression plot previously cited. Thus, 496 nm, 680-688 nm and 712 nm wavelengths correspond to chromophores located at the visible region, and four wavelengths are also common at 1088 nm, 1116 nm (combination bands of stretching-bending), 1932 nm (O-H stretching vibrations in cellulose) and 2308 nm (second overtones of C-H bending vibrations), while specific peaks appeared at 2052 nm (N-H stretching vibrations in protein) and 2324 nm (C-H stretching vibrations) in the NIR region. The study of the MPLS loadings for Chl-A and Chl-B showed that main wavelengths used for developing calibration models for these compounds at the shortest range of the spectrum were similar to those previously assigned to carotene absorptions (from 400 to 500 nm) (Britton, 1995). In particular, absorption at 496 nm might be due to γ-carotene, which is a bright monocyclic carotene showing 11 conjugated doubled bounds (Rodriguez-Amaya, 2001).

In the red region of the spectrum, electronic transitions are experienced by the chlorophyll *a/b* protein, showing absorption bands located at 652 and 670 nm (Britton,

1995; Ignat, Schmilovitch, Fefoldi, Steiner & Alkalai-Tuvia, 2012) that might explain the occurrence of wavelengths at 648 nm and 672 nm for Chl-A and Chl-B, respectively.

Taking into consideration TPC in exocarp and mesocarp summer squash tissues **(Fig. IV.7.3d),** there are six peaks corresponding to chromophores located at 496 nm (electronic transitions, green), 528 nm (electronic transitions, green), 640 nm (electronic transitions, orange), 672 nm (electronic transitions, red), 688 nm (electronic transitions, red) and 704 nm (transition between VIS and NIR) in the visible region and four positioned at 1396 nm (C-H stretching and bending vibrations), 1516 nm (first overtone of N-H streaching in protein and urea), 1884 nm (combination bands of stretching-bending) and 1932 nm (O-H stretching vibrations in cellulose) in the NIR region. Among them, peaks at both VIS (496 nm, 672 nm) and NIR (1396 nm, 1516 nm, 1884 nm) regions are common to those found in the AA loading plots, while peaks at 680-688 nm and 1932 nm match those of the Chl-A and Chl-B. This confirms previous studies that showed phenolics to be compounds possessing one or more aromatic rings with one or more hydroxyl groups, mainly related to combination bands of the -OH functional group, symmetric and anti-symmetric stretching, also related to C-H aromatic second overtones and C-H third overtones (Osborne, Fearn, & Hindle, 1993) that reported the main bands in the regions from 1415 to 1512 nm and from 1512 to 2035 nm.

4. Conclusions

The results obtained in this work support the idea that NIRS is suitable for the evaluation of antioxidant compounds in summer squash fruits, predicting several traits simultaneously, and offering a promising method for quickly characterize individual fruits in plant breeding programs in which thousands of fruits are generated.

In addition, NIR technology could be applicable to the summer squash industry, this work being the first report which includes the use of this method in the main antioxidants compounds in this product.

The best prediction models which were obtained for the total value of ascorbic acid and chlorophyll pigments *b* showed a high ability of prediction. Furthermore, chlorophyll pigments *a* and total phenolic compounds allowed approximate quantitative predictions to be made, being useful for lowering the analytical laboratory and industrial input.

Acknowledgements

This work was supported by the projects RTA2009-00036-00-00 and RTA2009-00039-00-00 (INIA) and FEDER funds. The authors thank Dr. J.M. Moreno and Dr. F. Peña for the NIR spectra acquisition assistance. M.T. Blanco-Díaz acknowledges the financial support from the Spanish Ministry of Economy and Competitiveness as a

contracted researcher of the Program 'Researcher Training' (Formación de Personal Investigador, FPI-INIA).

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Capítulo V. Conclusiones

Chapter V. Conclusions

V. CONCLUSIONES

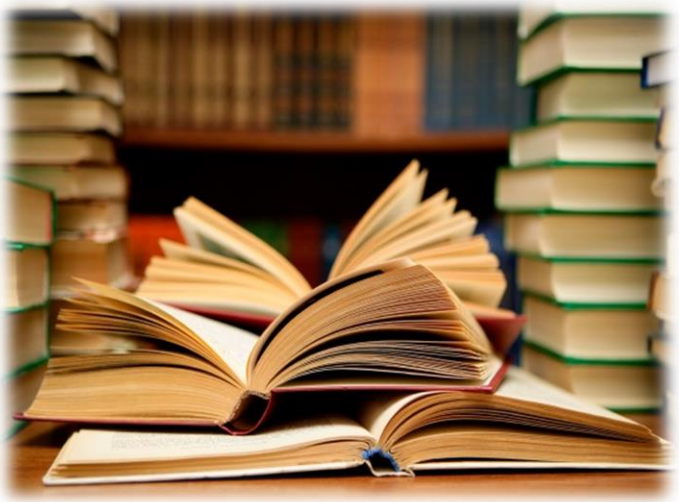
Los resultados obtenidos en los diferentes capítulos de este Trabajo de Investigación, han permitido establecer las siguientes conclusiones:

1. El calabacín *(Cucurbita pepo)* presenta una enorme variabilidad tanto en pigmentos naturales como en compuestos antioxidantes, siendo el morfotipo 'zucchini' el que reúne las características más idóneas para su posterior procesado en IV Gama.
2. Entre las variables precosecha estudiadas (variedad, estado de madurez y momento de recolección) fue la variedad la que mayor efecto tuvo en la calidad del calabacín fresco, obteniéndose una mayor calidad fisicoquímica en variedades de color verde claro, mientras que los compuestos antioxidantes y clorofilas predominaron en variedades de calabacín de color amarillo y verde oscuro; aumentando las concentraciones de éstos compuestos en los estados de madurez más jóvenes. Además se observó que períodos de recolección tempranos y medios en el ciclo de primavera (mediados de April a mediados de Mayo) fueron los más adecuados desde el punto de vista de la calidad.
3. De las cuatro variedades de calabacín estudiadas para su procesado mínimo ('Cronos', 'Parador', 'Cassiope', 'Amalthée'), fue 'Cronos' la variedad que presentó mejor conservación postcosecha, mientras que en la variedad 'Parador' se observó una mayor actividad metabólica tras el procesado. Tras 10 días de conservación a 6 ºC, todas las variedades presentaron una mejor calidad físico-química, nutricional y sensorial cuando se procesaron los frutos de mayor estado de madurez (18-21 cm). La atmósfera que le proporcionó al calabacín una vida útil de hasta 10 días fue aquella de concentraciones próximas a 0.5 KPa en O_2 y a 18 KPa en CO_2.
4. La calidad del calabacín mínimamente procesado se vio beneficiada tras su conservación en atmósferas bajas de O_2 (1.5 KPa) y medias de CO_2 (19 KPa), incrementándose la vida útil del mismo hasta los 14 días.
5. La variedad, el formato de corte, la temperatura así como el tiempo de conservación afectaron significativamente a la calidad fisicoquímica, contenido de compuestos bioactivos y análisis sensorial del calabacín. Obteniéndose los mejores resultados de conservación tras 14 días en rodajas de la variedad 'Sinatra' conservadas a 6 ºC.
6. La eficacia de los tratamientos de etanol y ascorbato cálcico junto a otros recubrimientos comestibles (chitosán y la proteína de soja) redujeron significativamente las alteraciones en el color del calabacín IV Gama. Estos tratamientos mejoraron la firmeza y además incrementaron el aroma y la calidad visual de rodajas de calabacín tanto en crudo como tras ser cocinado (especialmente en los métodos de cocción menos agresivos: hervido y al vapor).
7. Las ecuaciones de predicción NIR desarrolladas en calabacín, demostraron el elevado potencial de la tecnología para la predicción de ácido ascórbico y clorofila *b*, mientras que las ecuaciones desarrolladas para clorofila *a* y compuestos fenólicos totales fueron de moderada capacidad predictiva.

V. CONCLUSIONS

The main conclusions that can be drawn based on the research carried out and the results obtained are:

1. Summer squash genotypes *(Cucurbita pepo)* showed a high variability of natural pigments and phytochemical compounds, 'zucchini' being the most suitable morphotype to be processed as a fresh-cut product.
2. Among preharvest handling parameters studied (variety, ripening stage and harvest date), the variety was the main factor affecting the major evaluated parameters. In the light green zucchini the physical properties were the highest, while dark green and yellow zucchini were identified as a good source of chlorophylls and health-promoting compounds, respectively; decreasing antioxidant compounds with the ripening stage in all varieties tested. In addition, early and middle harvest dates in the spring season (from the middle of April to the middle of May) were the best from a zucchini quality point of view.
3. Considering the 4 zucchini cultivars studied ('Cronos', 'Parador', 'Cassiope', 'Amalthée'), 'Cronos' was the cultivar that presented the best aptitude to be minimally processed. 'Parador' showed the worst response for fresh-cut zucchini processing due to a high metabolic activity. After 10 days of storage at 6 ºC, more mature zucchini cultivars (18-21 cm) presented the best physico-chemical, nutritional and sensory quality. In general, MAP conditions close to 0.5 KPa in O_2 and 18 KPa in CO_2 preserved zucchini fresh-cut quality until day 10 of storage.
4. Benefits in zucchini fresh-cut quality were observed when MAP conditions were low in O_2 (1.5 KPa) and medium in CO_2 (19 KPa) extending the shelf-life of the product up to 14 days.
5. Cultivar, cutting type, storage temperature as well as time of storage significantly affected physicochemical, bioactive compounds and sensory analysis. 'Sinatra' slices at 6 ºC presented the best quality results after 14 days.
6. The effectiveness of ethanol and calcium ascorbate including or not edible coatings (chitosan and soy protein isolate) was observed in the color quality of zucchini slices. These treatments also contributed to reduce tissue softening as well as an increase in the general odor and visual aspect in zucchini raw slices and after some cooking methods (especially in those less aggressive cooking techniques: boiling and steaming).
7. The NIRS prediction models developed in summer squash, demonstrated that ascorbic acid and chlorophyll *b* showed a high ability of prediction, while chlorophyll *a* and total phenolic compounds allowed approximate quantitative predictions.



Capítulo VI. Bibliografía

Chapter VI. References

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