UNIVERSIDAD DE CÓRDOBA
Departamento de Bromatología y Tecnología de los Alimentos

CARACTERIZACIÓN DE COMPONENTES BIOACTIVOS DEL ESPÁRRAGO VERDE: OBTENCIÓN DE INGREDIENTES FUNCIONALES A PARTIR DE LOS SUBPRODUCTOS GENERADOS DURANTE SU TRANSFORMACIÓN INDUSTRIAL

Memoria que presenta D. JOSÉ MARÍA FUENTES ALVENTOSA, Ldo. en Ciencia y Tecnología de los Alimentos y Ldo. en Veterinaria, para optar al Grado de Doctor por la Universidad de Córdoba.

Córdoba, 20 de junio de 2009

TITULO: *Caracterización de componentes bioactivos del espárrago verde: obtención de ingredientes funcionales a partir de los subproductos generados durante su transformación industrial*

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Dª. Rocío Rodríguez Arcos y Dª. Ana José Jiménez Araujo, directoras de la Tesis y D. Rafael Moreno Rojas, tutor, autorizamos la presentación de la Tesis Doctoral "**Caracterización de componentes bioactivos del espárrago verde: obtención de ingredientes funcionales a partir de los subproductos generados durante su transformación industrial"**, realizada por D. José María Fuentes Alventosa.

Córdoba, 20 de junio de 2009

LAS DIRECTORAS: VºBº, EL TUTOR:

Rocío Rodríguez Arcos Ana José Jiménez Araujo Rafael Moreno Rojas

“Lo importante no es el destino, sino el viaje”

Constantino P. Cavafis

A mis maravillosos padres

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ÍNDICE

1. INTRODUCCIÓN

El espárrago ha sido considerado un alimento de gran prestigio desde la antigüedad. Los primeros vestigios de espárragos aparecieron en forma de pinturas en los monumentos egipcios (3.000 a.C.). Eran dibujados atados en manojos con dos o tres ligaduras y en este caso parecían ser utilizados como ofrenda a los dioses. Los médicos griegos y romanos recomendaban su consumo por las múltiples propiedades terapéuticas encontradas en los espárragos silvestres, actualmente conocidos como trigueros. Así lo acreditan antiguos escritos latinos de Catón, Plinio el Viejo, Columel, Cayo Aparicio y Dioscórides (Espejo *et al.*, 2000). Serían los romanos quienes introdujeran este cultivo en Europa septentrional. Tras las invasiones bárbaras, su cultivo sólo se conservó en España hasta el final de la Edad Media, en que volvieron a cultivarse en el norte y centro de Europa. Los árabes utilizan los espárragos en la preparación de jarabes y pócimas para tratamientos de diabetes, insomnio y reumatismo. En la farmacopea española, al espárrago se le atribuyen propiedades medicinales como tónico estomacal, diurético, laxante y antihemorrágico; pudiendo utilizarse también para prevenir la formación de cálculos renales y biliares, e incluso como anticancerígeno.

La producción de espárragos a nivel mundial se ha constituido durante los últimos años como una actividad en auge dado el incremento de su consumo y la variedad de preparaciones, tratándose de un producto con un nivel preferencial en el mercado internacional. España es uno de los principales productores de espárrago de la Unión Europea, ocupando el quinto lugar a nivel mundial, después de China, Perú, Estados Unidos y Alemania. Dentro de nuestro país, Andalucía es la responsable de más del 50% de la producción. Los rendimientos por hectárea y la precocidad del cultivo son superiores a cualquier otra zona. El valor de la producción bruta sobrepasa los 120 millones de euros. Además es importante destacar el enorme beneficio social que el cultivo proporciona al generar 43 millones de euros en jornales de recolección y más de 30 en jornales indirectos de manipulación, transporte y comercialización. Esto supone 30.000 puestos de trabajo en el periodo de recolección. Tradicionalmente, ésta y otras comunidades, tales como Extremadura, Castilla La Mancha y Navarra, han dedicado grandes extensiones de terreno al cultivo del espárrago blanco, sin embargo, en los últimos años éste ha sido reemplazado en buena parte por el espárrago verde. Así, actualmente, de las 17000 Has de tierra destinadas al cultivo del espárrago, más del 30% se dedican a turiones verdes, mientras que 5-10 años atrás dicho porcentaje no alcanzaba el 10% de la producción total española. Las previsiones apuntan a que España

debe convertirse en uno de los principales abastecedores de espárrago verde en el mercado europeo en los próximos años (Benson, 2008; Behr, 2006; Pierron-Darbonne, 2006).

El espárrago triguero silvestre es un producto con una gran tradición en España y otros países mediterráneos. Procede de diferentes especies de plantas del género *Asparagus* que forman parte de la vegetación silvestre y no se cultivan. En España se encuentran cinco especies comestibles, no cultivadas, que son: *A. acutifolius* L., *A. albus* L., *A. aphyllus* L., *A. maritimus* L. y *A. horridus* L. También se encuadra dentro de la denominación de triguero el espárrago autóctono de Huétor Tájar (Granada), aunque en este caso se trata de un espárrago cultivado.

En las regiones donde se produce, el espárrago triguero es bastante apreciado fundamentalmente por sus características organolépticas claramente diferenciadoras. Sin embargo, en otros mercados como el norte y centro-europeo, éstas no tienen tanta importancia en la decisión de compra. Desde el punto de vista del productor de espárrago es más rentable cultivar otras variedades con más calibre y más productividad procedentes de Estados Unidos (Mary Washington, UC-157, Grande, Atlas, etc.), lo que está haciendo disminuir en los últimos años las extensiones de terreno dedicadas al espárrago de Huétor Tájar. Por otra parte, el mercado del espárrago silvestre en la actualidad es bastante limitado y de carácter local y en algunas regiones la alta recolección está poniendo incluso en peligro la propia existencia de estas especies silvestres.

Tanto en el caso del espárrago de Huétor Tájar como en el del silvestre, para poder aumentar su implantación en el mercado es necesario, además de mejorar sus características agronómicas, encontrar un valor añadido que los diferencie de los demás. Un aspecto muy interesante del espárrago y que no se ha utilizado hasta el momento como criterio en los programas de mejora, es su contenido en compuestos potencialmente beneficiosos para la salud (compuestos bioactivos). De hecho el espárrago es una hortaliza que tradicionalmente ha gozado de fama de ser muy saludable, no en vano ha sido utilizado durante milenios por distintas culturas como planta medicinal. Sin embargo, en el caso del espárrago triguero, las propiedades

beneficiosas para la salud no han sido nunca comprobadas científicamente y tampoco se han caracterizado las sustancias bioactivas responsables de ellas.

Aunque el espárrago tenga numerosas propiedades beneficiosas para la salud, éste no atrae al consumidor como planta medicinal, sino como una hortaliza de exquisito gusto, agradable aroma y textura tierna y carnosa. Debido a su riqueza en nutrientes y fibra, junto a su bajo contenido calórico, puede llegar a ser un producto de gran interés para ese amplio sector de la población demandante de alimentos que, además de cubrir sus necesidades nutricionales y dietéticas, mejoren sus condiciones de salud.

Entre los compuestos responsables de la actividad biológica del espárrago cabe destacar compuestos fenólicos, como ácidos hidroxicinámicos y flavonoides; terpenoides de tipo saponinas y esteroles; y carbohidratos como la inulina, fructo-oligosacáridos y los polisacáridos insolubles de la denominada fibra alimentaria.

Estos compuestos bioactivos pueden resultar interesantes para el desarrollo de una estrategia de aprovechamiento de los subproductos del espárrago. Para la elaboración industrial los turiones se cortan a una determinada longitud y, en algunos casos, se pelan, llegando a representar estas porciones que se descartan más del 50% del total. Estos residuos son, por tanto, un gran problema desde el punto de vista económico y medioambiental para las industrias del sector. Hasta ahora, los restos aprovechables de este proceso sólo se han utilizado para productos de escaso valor, como alimentación animal. Sin embargo, actualmente existen grandes dificultades en el aprovechamiento de los residuos del espárrago, ya que cuando se utiliza para piensos destinados al ganado bovino o caprino, se reduce considerablemente la riqueza grasa de la leche que producen estos animales. Pero muchos de los componentes bioactivos del espárrago se encuentran también en las porciones del turión que se desechan durante el procesado del mismo, presentando tanto éstas como la parte comestible una composición similar en dichas sustancias. Esto resulta de gran interés científico e industrial. Todos los compuestos bioactivos presentes en el espárrago, además de determinar la calidad organoléptica y nutricional del producto, podrían aportar un gran valor añadido a los residuos generados durante la elaboración industrial del espárrago. Así, en un trabajo de nuestro Grupo de Investigación, aplicable tanto a los turiones como a los subproductos

del espárrago, se comprobó que los extractos etanólicos de distintas variedades de espárragos trigueros de Huétor Tájar poseen una alta capacidad antioxidante (Rodríguez *et al.*, 2005a). Además de los compuestos solubles concentrados en estos extractos, tanto los espárragos como sus subproductos poseen un alto contenido en fibra que podríamos denominar "biológicamente activa", ya que se encuentra asociada a compuestos antioxidantes tales como el ácido ferúlico y sus derivados.

La presencia de compuestos potencialmente beneficiosos para la salud en el espárrago y sus subproductos es, por tanto, el punto de partida de esta Memoria de Tesis. Ha resultado de gran interés encontrar un valor añadido en un producto autóctono andaluz como es el espárrago triguero de Huétor Tájar, mediante el estudio del perfil de fitoquímicos. Y, por otra parte, es de gran interés, científico e industrial, el haber desarrollado un proceso de aprovechamiento de los subproductos generados durante la elaboración industrial.

2. ANTECEDENTES BIBLIOGRÁFICOS

2.1. ALIMENTOS FUNCIONALES Y NUTRACÉUTICOS

Tanto la comunidad científica como la industria alimentaria trabajan para aumentar la calidad de vida, y, en particular, tratar las enfermedades crónicas que aparecen con la edad (Temple, 2002). La realización de estudios epidemiológicos, investigación animal, ensayos clínicos y la investigación en bioquímica nutricional sugiere que algunos suplementos dietéticos pueden ser beneficiosos para las enfermedades coronarias, cáncer, osteoporosis y otras enfermedades crónicas y degenerativas tales como diabetes, enfermedad de Parkinson y Alzheimer. Parece necesario profundizar en el conocimiento de los compuestos bioactivos presentes en los alimentos y su función en la prevención de diversas enfermedades. De esta manera con futuras investigaciones se podrá tener un mejor conocimiento de cómo los componentes funcionales de un alimento podrían ampliar el papel de la dieta en la prevención de una enfermedad así como, incluso, determinar qué individuos de una población se beneficiarán con más probabilidad de un alimento funcional (Mandel *et al.*, 2005).

Los nutracéuticos se definen como sustancias químicas que se encuentran como componentes naturales de los alimentos, u otras formas ingeribles, en los que se ha determinado que benefician a la salud, ya que previenen una o más enfermedades o mejoran el estado fisiológico del individuo. Los nutrientes esenciales se pueden considerar nutracéuticos si proporcionan beneficios más allá de su papel esencial en el crecimiento normal o mantenimiento del cuerpo humano (Wildman, 2001). Un ejemplo son las propiedades antioxidantes de las vitaminas C y E.

El concepto de alimento funcional incluye un alimento, tanto natural como formulado, que ejerce un efecto beneficioso sobre la salud del consumidor y/o reduce el riesgo de enfermedad crónica más allá de las funciones nutricionales básicas (Huggett *et al.*, 1996). Esto ha desencadenado que estén recibiendo en la actualidad el reconocimiento internacional por sus potenciales efectos beneficiosos cuando son consumidos como parte de una dieta variada seguida habitualmente y en niveles efectivos. El mercado de alimentos está siguiendo esta tendencia y los alimentos funcionales representan la respuesta directa que las compañías de alimentos pueden dar a la petición de "salud por alimento" (Pascal, 1996; Moira, 1998; De Jong *et al.*, 2003).

La Tabla 2.1 muestra, a modo de ejemplo, algunos ingredientes para alimentos funcionales.

El término "fitoquímicos" constituye la evolución más reciente del término "alimentos funcionales" y enfatiza las fuentes vegetales de la mayoría de los compuestos preventivos de enfermedades (Vasconcellos, 2000). Estos metabolitos tienen baja potencia como compuestos bioactivos si se comparan con los medicamentos farmacéuticos, pero si son ingeridos regularmente y en cantidades significativas como parte de la dieta, pueden tener un efecto fisiológico perceptible a largo plazo (Espín *et al.,* 2007).

Por otra parte, las autoridades públicas ven a los alimentos funcionales como una posible herramienta para reducir los costes sociales de salud. Así, en Japón se llevó a cabo un proceso regulatorio para la aprobación por el Ministerio de Salud de "alimentos para uso específico de salud" ("foods for specified health use" o FOSHU), que es como se conocen en este país a los alimentos funcionales (Arai, 1996).

Finalmente hay que mencionar el Reglamento (CE) Nº 1924/2006 del Parlamento Europeo y del Consejo de 20 de diciembre de 2006 relativo a las declaraciones nutricionales y de propiedades saludables en los alimentos, que armoniza las disposiciones legales, reglamentarias o administrativas de los Estados miembros relativas a las declaraciones nutricionales y de propiedades saludables, con el fin de garantizar un funcionamiento eficaz del mercado interior a la vez que se proporciona un elevado nivel de protección de los consumidores. Este Reglamento debe aplicarse a las declaraciones nutricionales y de propiedades saludables efectuadas en las comunicaciones comerciales, ya sea en el etiquetado, la presentación o la publicidad de los alimentos que se suministren como tales al consumidor final.

Tabla 2.1

Ejemplos de ingredientes para alimentos funcionales, así como la fuente de la que se obtienen y los beneficios potenciales o atribuidos.

TIPO/COMPONENTES	FUENTE	BENEFICIOS ATRIBUIDOS O POTENCIALES*
Carotenoides		
Alpha-caroteno	zanahorias	neutraliza radicales libres que producen daños a las células
Beta-caroteno	frutas, vegetales	neutraliza radicales libres
Luteína	vegetales verdes	contribuye a la conservación de una visión saludable
Licopeno	productos derivados del tomate	puede reducir el riesgo de cáncer de próstata
Zeaxantina	huevos, cítricos, maíz	contribuye a la conservación de una visión saludable
Hidrolizado de Colágeno		
Hidrolizado de colágeno	gelatina	puede mejorar algunos síntomas de artritis ósea
Fibra Dietética		
Fibra insoluble	salvado de trigo	reduce el riesgo de cáncer de mama y/o colon
Beta glucan*	avena	reduce el riesgo de enfermedades cardiovasculares
Fibra soluble*	psyllium	reduce el riesgo de enfermedades cardiovasculares
Cereales integrales*	cereales	reduce el riesgo de enfermedades cardiovasculares
Ácidos Grasos		
Ácidos grasos Omega-3-DHA/EPA	atún, pescado, aceites marinos	reduce el riesgo de enfermedades cardiovasculares** y mejora las funciones mentales y visuales
Ácido linoleico conjugado (CLA)	queso, productos cárnicos	disminuye el riesgo de ciertos tipos de cáncer

Flavonoides		
Antocianidinas	frutas	reduce el riesgo de ciertos tipos de cáncer
Catequinas	té	reduce el riesgo de ciertos tipos de cáncer
Flavanonas	cítricos	reduce el riesgo de ciertos tipos de cáncer
Flavonas	frutas/vegetales	reduce el riesgo de ciertos tipos de cáncer
Fenoles		
Ácido cafeico, ácido ferúlico	frutas, vegetales, cítricos	reduce el riesgo de enfermedades degenerativas, enfermedades del corazón, enfermedades de los ojos
Esteroles vegetales		
Estanol ester*	maíz, soja, trigo	disminuye los niveles de colesterol en la sangre
Prebióticos/Probióticos		
Fructo-oligosacaridos (FOS)	alcachofa de Jerusalén, chalotas, cebolla en polvo	mejora la salud gastrointestinal
Lactobacillus	yogur, productos lácteos	mejora la salud gastrointestinal, reduce la diarrea de origen bacteriano**
Saponinas		
Saponinas	granos de soja, alimentos de soja y alimentos que contienen proteína de soja	disminuye el colesterol LDL
Proteína de Soja		
Proteína de soja*	granos de soja y alimentos de soja	25 g/día reducen el riesgo de enfermedades cardíacas
Fitoestrógenos		
Isoflavonas - daidzeína, genisteína	granos de soja y alimentos de soja	reduce los síntomas de la menopausia
Lignanos	linaza, centeno, vegetales	protege contra enfermedades del corazón y algunos tipos de cáncer, disminuye el colesterol LDL, colesterol total y triglicéridos

Taninos		
Proantocianidinas	productos de arándanos, chocolate	mejora la salud del tracto urinario, reduce el riesgo de enfermedades cardiovasculares

* Muchos de los componentes mencionados no han sido evaluados en estudios a largo plazo en humanos. Las afirmaciones se basan a menudo en investigaciones de laboratorio y pueden no ser válidas para todos los grupos dentro de una población.

** La FDA aprobó la afirmación establecida para el componente (en Estados Unidos) o la evidencia científica válida para la mayoría de los grupos dentro de una población.

Fuente: Departamento de Tecnología de los Alimentos de la Universidad de Wageningen (Holanda). Ejemplos de ingredientes para alimentos funcionales. En *"http://www.food-info.net/es"*.

2.2. EL ESPÁRRAGO

2.2.1. Morfología

El espárrago cultivado pertenece a la especie *Asparagus officinalis* L. Es una planta de la familia de las Liliáceas, originaria de la flora de las regiones de la cuenca del Mediterráneo, cuya parte comestible son los tallos jóvenes, carnosos y tiernos, llamados turiones. Aunque es perenne, su fase de aprovechamiento comercial es de 10 a 15 años.

La planta del espárrago está formada por los tallos aéreos ramificados y una parte subterránea constituida por raíces y yemas, que es lo que se denomina comúnmente "garra" o zarpa. El sistema radical del espárrago es muy potente. Las raíces principales son cilíndricas, gruesas y carnosas, y tienen la facultad de acumular reservas que permiten su próxima producción de turiones. De estas raíces principales nacen las raicillas o pelos absorbentes que son las encargadas de absorber el agua y las sales minerales.

El tallo está formado por un disco o cepa, sobre el que se forman las yemas que darán lugar a los turiones o espárragos, que es la parte comestible y comercializable de este producto.

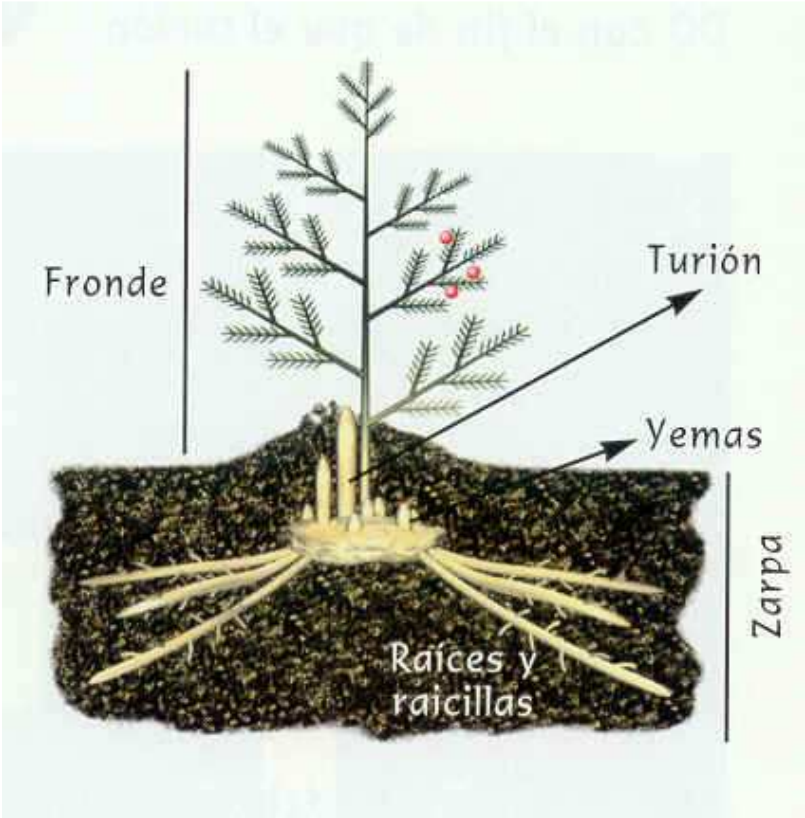
La planta del espárrago es dioica, lo que explica la diferenciación de sexos, existiendo plantas sólo con flores masculinas, plantas sólo con flores femeninas, y plantas hermafroditas, con flores masculinas y femeninas.

Los frutos son bayas de unos 6 mm de diámetro, de color rojo intenso en su maduración, con 5 ó 6 semillas de color negro. Las plantas femeninas tienen gran cantidad de bayas y, debido a su peso, son más susceptibles que las masculinas a doblarse por los fuertes vientos y caer sobre las entrelíneas. Este hecho dificulta las labores y reduce las reservas de las garras, lo que se traduce en una menor producción al año siguiente. Las plantas masculinas son más productivas en turiones, ya que no utilizan sus reservas en la formación de frutos y semillas, sino que las acumulan en las

raíces para la próxima producción de turiones. Además, son más precoces y longevas (Benages, 1990).

Imagen 2.1

Esquema de la planta de espárrago.



2.2.2. Tipos de espárragos

El espárrago se divide en dos grupos principales: el espárrago verde y el espárrago blanco.

Desde el punto de vista botánico, el espárrago blanco y verde constituyen la misma planta. La diferencia entre uno y otro surge de la forma en que ha crecido el brote. Mientras los brotes jóvenes de los espárragos están creciendo dentro de la tierra, son de color blanquecino, pero cuando emergen del suelo y se exponen a la luz, adquieren una coloración verde debido a que la misma activa la función clorofílica.

Imagen 2.2

Cultivo de espárrago blanco y verde.

El espárrago blanco, por tanto, se recolecta tan pronto como emerge de la tierra, mientras que los verdes se espera a cortarlos cuando alcanzan una altura sobre el suelo de unos 20 cm.

Desde el punto de vista agrícola, el espárrago verde tiene una mejor adaptación a diferentes tipos de suelos y condiciones geográficas, es más fácil su mecanización, son necesarias menos labores de cultivo y sus costos de producción son menores, existiendo una menor exigencia de mano de obra y una mayor elasticidad en las fechas de la recolección.

Las características organolépticas y los usos culinarios de cada tipo de espárrago son diferentes. El verde se caracteriza por tener mayor valor nutritivo, textura carnosa y firme, aroma más intenso y sabor ligeramente más dulce, mientras el blanco tiene un mayor contenido en azúcares y más fibra.

Ambos tipos de espárragos son cultivados a nivel mundial, aunque tradicionalmente el tipo blanco se ha cultivado en China y Europa, mientras que el tipo verde en Estados Unidos y, dentro de Europa, el sur de la Península Ibérica.

En España y otros países mediterráneos se consumen también otro tipo, los espárragos silvestres (espárragos trigueros) que proceden de diferentes especies de plantas del género *Asparagus*. Entre ellas, además de la especie anteriormente citada *Asparagus officinalis* L., nos encontramos: *A. acutifolius* L., *A. albus* L., *A. aphyllus* L., *A. maritimus* L. y *A. horridus* L. Estas especies forman parte de la vegetación silvestre de regiones mediterráneas como la andaluza. Los espárragos trigueros son muy apreciados en Andalucía, hasta el punto que ya forman parte de la tradición gastronómica regional. Se diferencian de los espárragos verdes cultivados en que los espárragos trigueros son más delgados, presentan púas o espolones debajo de las escamas, los tallos tienen colores más oscuros (bronce y morado), y a nivel organoléptico se caracterizan por un sabor más amargo, un fuerte aroma y una textura flexible y carnosa. Ya desde la época de los griegos, Catón reseñaba la predilección de ciertos consumidores por las propiedades organolépticas de las especies silvestres, por su ligero amargor (Espejo *et al.*, 2000).

El espárrago autóctono de Huétor Tájar se encuadra dentro del grupo de espárragos trigueros, que proceden de variedades autóctonas seleccionadas en la zona desde principios del siglo XX.

Imagen 2.3
Espárragos de Huétor Tájar.

Originariamente fue una especie silvestre que crecía en el sotobosque del río Genil en Huétor Tájar, cuyas semillas fueron recolectadas para su cultivo en las fértiles tierras de la Vega Baja del Genil de Granada. El cultivo se efectuaba en pequeños huertos familiares de la zona, bien con fines de autoconsumo o bien para su comercialización a nivel local.

Este espárrago autóctono se caracteriza por un tallo delgado, flexible, de color verde morado o bronce, más oscuro en la cabeza. Cuando se degusta el espárrago de Huétor Tájar, se observa una textura tierna, carnosa y firme, un peculiar sabor amarguidulce y un fuerte aroma, típicos de los espárragos trigueros.

Desde 1996 la zona goza de la Denominación Específica "Espárrago de Huétor Tájar", reconocida asimismo desde el año 2000 como I.G.P. (Indicación Geográfica Protegida) por la Comisión Europea. El área de influencia de la zona esparraguera de Huétor Tájar comprende las poblaciones de Huétor Tájar, Loja, Villanueva de Mesía, Salar, Moraleda de Zafayona e Íllora, todos de la provincia de Granada.

Imagen 2.4

Zona esparraguera de Huétor Tájar.

2.2.3. Valor dietético del espárrago

Existen diferencias notables en la composición nutricional del espárrago según sea blanco o verde. En general, el contenido en nutrientes es mayor en el espárrago verde que en el espárrago blanco, excepto en proteínas y azúcares que es mayor en el blanco. En la Tabla 2.2 se presentan valores medios de concentración de nutrientes según datos recopilados de varios autores. Por otro lado, aunque no se disponen de datos definitivos del valor nutricional de los espárragos trigueros, análisis efectuados en el espárrago triguero de Huétor Tájar por el Consejo Regulador, apuntan a que estos presentan un 1% menos de agua, así como unos contenidos ligeramente mayores en proteínas, azúcares, y algunos minerales que los espárragos verdes procedentes de variedades híbridas norteamericanas (Espejo *et al.*, 2000).

Desde el punto de vista nutricional, el espárrago verde proporciona un aporte calórico bajo, alrededor de 22-35 kcal/100 g, por lo que se le considera un alimento poco energético, ideal para su inclusión en dietas de adelgazamiento. El aporte medio de macronutrientes por 100 g de espárrago verde es: 2-4 g de proteínas; 3-4,5 g de hidratos de carbono, y en cuanto a lípidos, los niveles son de trazas. Cuentan con una importante presencia de minerales como potasio, fósforo, calcio y magnesio, y un alto contenido en vitaminas B_1, B_2, B_3, C, A y E (Amaro *et al.*, 1995, 1999). Contiene también ácido fólico y proteínas vegetales de gran calidad.

Tabla 2.2

Composición nutricional del espárrago fresco.

COMPOSICIÓN NUTRICIONAL DEL ESPÁRRAGO FRESCO			
	Espárrago blanco (1)	**Espárrago verde (2)**	**Espárrago triguero de Huétor Tájar (3)**
Humedad	93-94 %	92-93 %	91-92 %
Proteína	2,8 %	1,9 %	3,85 %
Azúcares totales	4 %	3 %	4,5 %
Lípidos	Trazas	Trazas	Trazas
Calorías	25 cal/100 g	22 cal/100 g	35 cal/100 g
Fibra	0,9 %	0,9 %	1,0 %
Fósforo	60 mg/100 g	75 mg/100 g	69 mg/100 g
Calcio	26 mg/100 g	40 mg/100 g	48 mg/100 g
Magnesio	16 mg/100 g	21 mg/100 g	21 mg/100 g
Sodio	4 mg/100 g	4 mg/100 g	8 mg/100 g
Potasio	360 mg/100 g	370 mg/100 g	286 mg/100 g
Cobre	0,02 mg/100 g	2,5 mg/100 g	0,21 mg/100 g
Hierro	0,03-0,2 mg/100 g	1,2-1,9 mg/100 g	0,9 mg/100 g
Manganeso	0,01 mg/100 g	0,3-0,5 mg/100 g	0,12 mg/100 g
Zinc	0,04 mg/100 g	0,7 mg/100 g	0,6 mg/100 g
Cromo	0,007 mg/100 g	0,007 mg/100 g	-
Vitamina A	50 U.I./100 g	980 U.I./100 g	-
Vitamina B1	0,11 mg/100 g	0,23 mg/100 g	-
Vitamina B2	0,08 mg/100 g	0,15 mg/100 g	-
Vitamina B3	1,1 mg/100 g	2,2 mg/100 g	-
Vitamina C	28 mg/100 g	48 mg/100 g	20 mg/100 g

Referencias bibliográficas:

(1) y (2):
- Howard F.D., Mac Gillivray J.H., Ymaguchi M. 1962. Nutrient composition of fresh California-grown vegetables. *California Agr. Exp. Sta. Bull.* 738-743.
- Departamento norteamericano de Agricultura. 1981. Science and Education Administration Nutritive Value of Foods 52-53.
- Hartmann H.D. 1989. Spargel. Grundlagen für den Anbau. *Verlag Eugen Ulmer, Stuttgart*.
- Amaro M.A. 1993. Variaciones del contenido de elementos minerales en espárrago (*Asparagus officinalis*, L.). Tesis Doctoral. Facultad de Veterinaria de la Universidad de Córdoba.

(3):
Datos analíticos realizados por el Consejo Regulador de la Denominación Específica "Espárrago de Huétor Tájar" en el laboratorio del Centro Tecnológico Nacional de la Conserva en Molina de Segura (Murcia).

2.2.4. Compuestos bioactivos en el espárrago

El espárrago, además de ser una hortaliza muy apreciada por sus características organolépticas y nutricionales, se puede considerar un alimento de gran calidad funcional ya que contiene diversos fitoquímicos que le confieren una potencial actividad biológica importante (Bast, 2005). Entre éstos hay que destacar fenoles (como ácidos hidroxicinámicos y flavonoides), saponinas, esteroles, fructanos y polisacáridos de la pared celular. Desde hace siglos, las culturas orientales milenarias han usado los extractos de espárrago como tónico, laxante, antitusivo, diurético, etc. Estudios farmacológicos más recientes (Yu *et al.*, 1996; Kamat *et al.*, 2000; Wiboonpun *et al.*, 2004) han mostrado que dichos extractos poseen diversas actividades biológicas, siendo de especial interés la capacidad antioxidante y la actividad antitumoral, si bien éstas siempre han sido medidas en ensayos *in vitro*. A estas dos actividades hay que añadir la capacidad de algunos de los componentes del espárrago, como los esteroles y las saponinas, de influir sobre el metabolismo lipídico y ayudar a disminuir los niveles de colesterol en el organismo.

2.2.4.1. Fenoles

Estudios realizados sobre composición química y actividad biológica de espárragos de distintas variedades y procedencia, han revelado que los factores genéticos y medioambientales (clima, tipo de suelo, etc.) influyen considerablemente en el contenido fenólico y en la capacidad antioxidante de los turiones. Así, se vio que los espárragos trigueros y los híbridos americanos cultivados en Huétor Tájar (Granada) poseen una mayor capacidad antioxidante que los cultivados en la provincia de Sevilla, estando esto asociado a un contenido más elevado en compuestos fenólicos. Además, comparando los híbridos americanos con los espárragos trigueros procedentes de la misma zona, se ha comprobado que estos últimos son más ricos en fenoles y poseen mayor capacidad antioxidante (Rodríguez *et al.*, 2005a). En esta misma línea, los trabajos de Martín-Belloso *et al.* (2001) sobre composición química de conservas vegetales también ponen de manifiesto la influencia del tipo de vegetal y de la zona de cultivo en la concentración de compuestos antioxidantes. Estos autores encuentran que el contenido en ácido ascórbico, tanto antes como después de la elaboración, es más alto en espárrago que en otros alimentos enlatados, como tomate, champiñón y lentejas. En

lo que a procedencia del espárrago se refiere, los cultivados en Andalucía son más ricos en vitaminas que los de Extremadura y Aragón.

Los principales fenoles del espárrago blanco son los derivados de ácidos hidroxicinámicos mientras que en el espárrago verde son los flavonoides, concretamente los glicósidos de flavonoles (Guillén *et al.*, 2008). Existen numerosos trabajos sobre el papel de los flavonoides en la prevención de distintas enfermedades, basados en la alta capacidad antioxidante que presentan estos compuestos (Harbone *et al.*, 2000; Nijveldt *et al.*, 2001). En los últimos años se ha comenzado a estudiar más en profundidad los mecanismos que controlan su metabolismo y biodisponibilidad en el organismo. Durante algún tiempo se pensó que la cantidad de flavonoides absorbida era despreciable, debido a que en la mayoría de los casos estaban en forma de glicósidos. Sin embargo, desde hace unos años se sabe que esto no es así (Hollman *et al.*, 1998; Spencer, 1999; Rechner *et al.*, 2002; Ross *et al.*, 2002). Los derivados de quercetina, que representan el grupo mayoritario dentro de los flavonoles, han sido los más estudiados hasta el momento y los resultados obtenidos han revelado que los glicósidos de quercetina son ampliamente metabolizados en nuestro organismo (Hollman, 2004) y que algunos de sus metabolitos podrían jugar un papel destacado en la prevención de enfermedades relacionadas con procesos oxidativos, tales como enfermedades cardiovasculares y tumorales (Manach *et al.*, 1998).

Los flavonoides han mostrado un efecto protector *in vitro* frente a distintos tipos de cáncer (colon, glándula mamaria, etc.). Por su capacidad antioxidante y captadora de radicales libres han mostrado poseer potenciales propiedades quimiopreventivas en distintos tipos de cáncer (Colic *et al.*, 2000; Moon *et al.*, 2006). En experimentos *in vitro* se ha confirmado el papel protector de la quercetina, la cual ejerce efectos de inhibición frente a células cancerígenas en humanos: en colon, glándula mamaria y ovario, en región gastrointestinal y en leucemia (Martínez-Flórez *et al.*, 2002).

2.2.4.2. Saponinas

Las saponinas son glicósidos de terpenoides que se distribuyen ampliamente en el reino vegetal (Kite *et al.*, 2007). Están presentes en todas las plantas del género *Asparagus* y son las principales responsables del sabor amargo-dulce característico de

este producto. Las saponinas del espárrago pertenecen al grupo de glicósidos esteroideos (Schwarzbach *et al.*, 2006). En la especie *Asparagus officinalis L.* la principal es la protodioscina ($C_{51}H_{84}O_{22}$) [Wang *et al.*, 2003].

También se han visto grandes diferencias en el perfil de saponinas de la población de espárrago triguero de Huétor Tájar con respecto al de cultivares mundiales, ya que, por un lado, se han detectado en el primero hasta seis saponinas distintas que no se han encontrado en las variedades comerciales analizadas y, por otro, solamente se detectó protodioscina en una sola de las muestras analizadas de espárrago de Huétor Tájar. Las saponinas encontradas en dicho trabajo, y que no habían sido previamente descritas en el espárrago, son derivados de la protosarsasapogenina (Fuentes-Alventosa, 2007).

Las investigaciones realizadas durante los últimos años han revelado que las saponinas procedentes de diferentes vegetales disminuyen los niveles de colesterol en plasma, tanto en animales de experimentación como en humanos (Southon *et al.*, 1988; Harwood *et al.*, 1993; Al-Habori *et al.*, 1998). Grandes micelas formadas por la interacción de saponinas con los ácidos biliares conllevan al aumento de su excreción, cuando se consumen alimentos ricos en saponinas (Oakenfull, 1986; Oakenfull *et al.*, 1990). El consecuente metabolismo acelerado del colesterol en el hígado causa una disminución del colesterol en plasma.

Recientemente, varios productos naturales que contienen saponinas en su composición, están empezando a ser considerados como posibles suplementos nutricionales, ya que estas saponinas actúan como principios activos útiles en el control de la hipercolesterolemia, hiperlipidemia, hiperglucemia y obesidad (Han *et al.*, 2002; Bramlett *et al.*, 2003).

Estudios realizados en animales han mostrado que extractos purificados de saponinas obtenidas de la semilla de soja, incorporados a la dieta, son capaces de disminuir significativamente los niveles de colesterol plasmático y hepático (Lee *et al.*, 2005).

Las saponinas han mostrado un efecto protector *in vitro* frente a distintos tipos de cáncer (colon, leucemia, etc.). Distintas saponinas esteroideas, similares a las existentes en el espárrago, han demostrado tener efecto tanto citostático como citotóxico en células HL-60 de leucemia humana (Mimaki *et al.*, 1998, 2001a; González *et al.*, 2003), así como acción antiproliferativa en la línea murina 26-L5 de carcinoma de colon y en la de fibrosarcoma humana HT-1080 (Tran *et al.*, 2001). Incluso saponinas esteroideas de otras especies de espárragos han mostrado propiedades citotóxicas frente a diversas líneas tumorales de cáncer (Koo *et al.*, 2000; Kim *et al.*, 2005).

2.2.4.3. Esteroles

Otros compuestos de interés son los esteroles. No existe mucha información en relación a su composición en distintas variedades de espárrago, aunque su actividad biológica ha sido objeto de numerosas investigaciones en otros productos, y se encuentra actualmente bien establecida. Los esteroles o fitosteroles son triterpenos que estabilizan las membranas plasmáticas vegetales, teniendo la misma función que el colesterol en los animales. Tienen capacidad de disminuir la absorción del colesterol, tanto del endógeno como del ingerido en la dieta (Moreau *et al.*, 2002). El β-sitosterol es uno de los fitosteroles más importantes en el espárrago y se encuentra ampliamente distribuido en otros vegetales. Tiene una marcada actividad antiinflamatoria (Okoli *et al.*, 2004), además de presentar efectos anticancerígenos demostrados en el caso del cáncer de colon, mama, estómago y próstata (Assmann *et al.*, 2004).

2.2.4.4. Fructanos

Al igual que en el caso de los esteroles tampoco existe mucha información en cuanto a su composición en distintas variedades de espárrago, pero su actividad biológica también está actualmente bien establecida.

El espárrago contiene distintos tipos de fructanos, como inulina y fructooligosacáridos, que ejercen un efecto beneficioso sobre la salud al actuar como prebióticos en el organismo humano (Sims, 2004). Estos componentes, no digeribles por las enzimas del intestino delgado, son susceptibles de ser degradados por los microorganismos del intestino grueso, afectando a una serie de procesos fisiológicos y

bioquímicos de nuestro organismo, lo que se traduce en una mejora del estado de salud y en la prevención de distintas enfermedades (Kaur *et al.*, 2002). Un gran número de estudios en animales muestran que la inulina tipo fructosana reduce el riesgo de cáncer de colon (Pool-Zobel, 2005). Por otro lado, investigaciones recientes han demostrado que afecta al metabolismo lipídico, principalmente porque reduce la trigliceridemia, al reducir el número de partículas VLDL del plasma. (Roberfroid, 2005; Lapointe *et al.* 2006).

2.2.4.5. Polisacáridos de la pared celular

Otro tipo de compuestos con actividad funcional son los polisacáridos de la pared celular, que son los principales constituyentes de la denominada fibra alimentaria. Éstos no son digeridos por enzimas humanas y, sin embargo, sí son parcialmente fermentados por bacterias del colon, pudiendo actuar como prebióticos. La presencia de componentes minoritarios en la pared celular como el ácido ferúlico, esterificando polisacáridos pécticos y arabinoxilanos, tiene una gran importancia en las propiedades funcionales de la fibra alimentaria. Durante la fermentación en el intestino grueso, el ácido ferúlico se libera y entra en la circulación enterohepática y por tanto puede actuar como preventivo en determinados tipos de cáncer. La fibra del espárrago, que lleva enlazados restos de ferúlico, puede considerarse como fibra antioxidante y, por lo tanto, como otro componente bioactivo del espárrago (Rodríguez *et al.*, 2004, 2005b).

2.2.5. Compuestos bioactivos en los subproductos de espárrago

Los subproductos del espárrago tienen una composición similar a la parte comestible de los turiones y representan, por tanto, una fuente prometedora de nuevos compuestos con valor añadido (fitoquímicos y fibra) (Nindo *et al.*, 2003).

Dentro del propio tallo se ha visto que existen diferencias en la concentración de compuestos bioactivos. Así, se ha descrito que la parte inferior del tallo (la que se desecha durante el procesado) contiene 100 veces más protodioscina (saponina principal del espárrago) que la punta y 30 veces más que la parte intermedia. Sin embargo la punta es la parte del espárrago que contiene más rutina (principal flavonoide del espárrago). Si bien hay que comentar que las diferencias en contenido de rutina por

zona del tallo no son tan grandes como en el caso de la protodioscina, lo cual indica que la parte que se desecha en el procesado del espárrago puede servir como fuente de ambos compuestos (Wang *et al.*, 2003).

Los principales grupos de componentes bioactivos en el espárrago y sus subproductos son:

- Flavonoides y saponinas: son los fitoquímicos mayoritarios y la mayor parte de ellos se solubilizan en los extractos acuosos y etanólicos.

- Fibra: lleva unida a ella los fitoquímicos que no se han solubilizado en la extracción correspondiente.

2.3. FLAVONOIDES

Los flavonoides son compuestos fenólicos que desempeñan un papel importante en la biología vegetal. Así, responden a la luz y controlan los niveles de las auxinas reguladoras del crecimiento y diferenciación de las plantas. Otras funciones incluyen un papel antifúngico y bactericida. Además, confieren coloración, lo que puede contribuir a los fenómenos de polinización y tienen una importante capacidad para fijar metales como hierro y cobre (Formica *et al.*, 1995).

Son constituyentes de la parte no energética de la dieta humana pero nuestro organismo no puede sintetizarlos y deben, por tanto, obtenerse mediante la alimentación (frutas, verduras y diversas bebidas como vino, cerveza y té) o en forma de suplementos.

Aunque los hábitos alimentarios son muy diversos en el mundo, el valor medio de ingesta de flavonoides se estima en 23 mg/día, siendo la quercetina el predominante con un valor medio de 16 mg/día. Excede, por tanto, a la de otros antioxidantes en la dieta, tales como el beta-caroteno (2-3 mg/día) y la vitamina E (7-10 mg/día) y es igual aproximadamente a un tercio de la vitamina C (70-100 mg/día) (Martínez-Florez *et al.*, 2002). Los flavonoides representan, pues, una contribución importante al potencial antioxidante de la dieta humana (Rice-Evans *et al.*, 1998).

2.3.1. Estructura química

Los flavonoides son compuestos de bajo peso molecular que comparten un esqueleto común de difenilpiranos (C6-C3-C6), compuesto por dos anillos de fenilos (A y B) ligados a través de un anillo C de pirano (heterocíclico) con diferente número de grupos hidroxilo unidos a las estructuras de los anillos. Los átomos de carbono en los anillos C y A se numeran del 2 al 8, y los del anillo B desde el 2' al 6' (Kühnau**,** 1976), según puede verse en la Fig. 2.1. La actividad de los flavonoides como antioxidantes depende de las propiedades redox de sus grupos hidroxifenólicos y de la relación estructural entre las diferentes partes de la estructura química (Bors *et al.,* 1990). Esta estructura básica permite una multitud de patrones de sustitución y variaciones en el anillo C. En función de sus características estructurales se pueden clasificar en:

1. **Flavanoles**, como la catequina, con un grupo -OH en posición 3 del anillo C.

2. **Flavonoles**, representados por la quercetina, que posee un grupo carbonilo en posición 4 y un grupo -OH en posición 3 del anillo C.

3. **Flavonas**, como la diosmetina, que poseen un grupo carbonilo en posición 4 del anillo C y carecen del grupo –OH en posición 3 del anillo C.

4. **Antocianidinas**, que tienen el grupo -OH en posición 3 pero además poseen un doble enlace entre los carbonos 3 y 4 del anillo C.

Tres características estructurales son importantes para su función:

a) La presencia en el anillo B de la estructura catecol u o-dihidroxi;

b) La presencia de un doble enlace en posición 2,3;

c) La presencia de grupos hidroxilo en posición 3 y 5 (Letan, 1966).

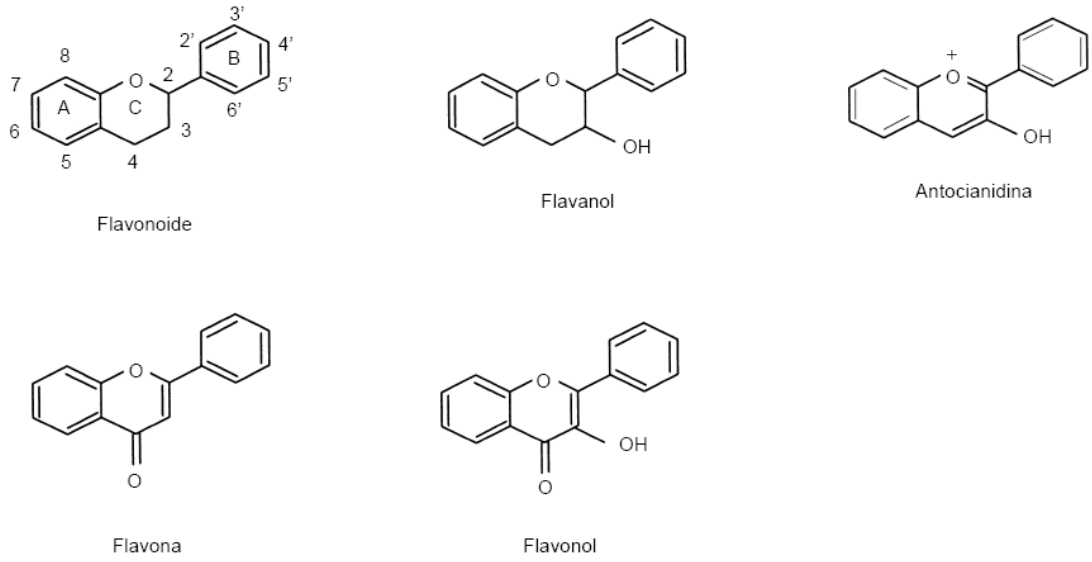
La mayoría de estos compuestos se encuentran conjugados con azúcares, que pueden ser hexosas, desoxihexosas o pentosas y, en algunos casos, ácido glucurónico (Stobiecki, 2000). Se han descrito desde flavonoides monoglicosídicos hasta pentaglicosídicos. Esta sustitución de azúcar, generalmente como O-glicósidos, tiene lugar principalmente en los hidroxilos de las posiciones 3-, 7- y 4'- del núcleo del flavonoide (Ferreres *et al.*, 2004). Así, a los flavonoles y las flavonas se unen azúcares, preferentemente a la posición C3 y con menor frecuencia al C7 del anillo A, de forma que estos compuestos se encuentran comúnmente como O-glicósidos, siendo la D-glucosa el residuo de azúcar más frecuente. Otros residuos de azúcares son la D-galactosa, la L-ramnosa, la L-arabinosa, la D-xilosa, así como el ácido D-glucurónico. Asociaciones con otros compuestos tales como ácidos carboxílicos y orgánicos, aminas y lípidos y enlaces con otros fenoles son también comunes.

La parte sin azúcares de la molécula flavonoide se llama aglicona. Los glicósidos son más solubles en agua y menos reactivos frente a radicales libres que su aglicona respectiva.

Investigaciones recientes sobre la relación entre estructura y actividad han revelado que pequeñas diferencias en la estructura de un flavonoide conducen a cambios significativos en sus actividades biológicas (Ablajan *et al.*, 2006).

Figura 2.1

Flavonoides. Estructura básica y tipos.



2.3.2. Actividades biológicas

2.3.2.1. Acción antioxidante de los flavonoides

El creciente interés en los flavonoides se debe principalmente a su actividad antioxidante, la cual está estrechamente relacionada con su estructura (Hvattum *et al.*, 2003).

Los criterios químicos para establecer la capacidad antioxidante de los flavonoides (Bors *et al.*, 1990) son:

- Presencia de estructura o-dihidroxi en el anillo B; que confiere una mayor estabilidad a la forma radical y participa en la deslocalización de los electrones.

- Doble enlace conjugado con la función 4-oxo del anillo C (Rice-Evans *et al.*, 1996; Cody *et al.*, 1998).

- Grupos 3- y 5-OH con función 4-oxo en los anillos A y C necesarios para ejercer el máximo potencial antioxidante.

Siguiendo estos criterios, el flavonoide quercetina es el que mejor reúne los requisitos para ejercer una efectiva función antioxidante. Su capacidad antioxidante medida como equivalentes Trolox es de 4,7 mM, lo que resulta ser 5 veces mayor al demostrado por las vitaminas E y C y tiene una hidrosolubilidad similar a la de la vitamina E (Merck, 2000). La función antioxidante de la quercetina muestra efectos sinérgicos con la vitamina C. El ácido ascórbico reduce la oxidación de la quercetina, de manera tal que combinado con ella el flavonoide mantiene sus funciones antioxidantes durante más tiempo. Por otra parte, la quercetina protege de la oxidación a la vitamina E, con lo cual también presentan efectos sinérgicos. También se ha demostrado que el flavonoide inhibe la fotooxidación de la vitamina E en la membrana celular de las células sanguíneas en presencia de hematoporfirina como fotosensibilizador (Pace-Asciak *et al.*, 1995).

Los flavonoides retiran oxígeno reactivo especialmente en forma de aniones superóxidos, radicales hidroxilos, peróxidos lipídicos o hidroperóxidos. De esta manera bloquean la acción nociva de dichas sustancias sobre las células. Sus efectos citoprotectores son, por ejemplo, bien patentes en fibroblastos de la piel humana, queratinocitos, células endoteliales y ganglios sensoriales cultivados en presencia de sulfoxina-butionina, un inhibidor irreversible de la glutatión sintetasa (Merck, 2000). Diversos flavonoides han mostrado su eficacia para eliminar los procesos de peroxidación lipídica del ácido linoleico o de los fosfolípidos de las membranas, la peroxidación de los glóbulos rojos o la autooxidación de los homogeneizados de cerebro (Laughton *et al.*, 1989; Ursini *et al.*, 1994). Asimismo, se ha comprobado su potente capacidad de inhibir *in vitro* la oxidación de las lipoproteínas de baja densidad

(LDL) por los macrófagos y reducir la citotoxicidad de las LDL oxidadas (Hirano *et al.*, 2001; Terao *et al.*, 2001). De hecho, las poblaciones que consumen productos ricos en flavonoides estadísticamente presentan menores riesgos de afecciones cardiovasculares (Hertog *et al.*, 1996; Geleijnse *et al.*, 2002)

2.3.2.2. Acción anticarcinogénica

Los flavonoides han demostrado poseer efectos antimutagénicos y anticarcinogénicos. Diversos datos experimentales han demostrado la acción antiproliferativa y anticarcinogénica, así como el papel de agente quimiopreventivo de los flavonoides (Hardigree *et al.*, 1978; Stavric, 1994; Birt *et al.*, 2001).

Entre los numerosos fenómenos que tienen lugar durante el proceso carcinogénico y que ofrecen opción para la modulación mediante factores externos, se encuentran la formación de metabolitos carcinógenos, que se forman por la acción de enzimas citosólicas y microsómicas. Estas enzimas controlan este paso crítico en el proceso carcinógeno. Estudios *in vivo* e *in vitro* han demostrado que los flavonoides pueden modular su actividad. Una posible explicación a estos efectos anticancerígenos podría derivarse del incremento que algunos flavonoides producen en las concentraciones intracelulares de glutatión a través de la regulación de la expresión de la enzima limitante en su síntesis (Myhrstad *et al.*, 2002). Las mismas propiedades que caracterizan su actividad antioxidante, determinan que puedan presentar efectos prooxidantes. Debe destacarse que las propiedades prooxidantes y mutagénicas de los flavonoides se hallan unidas a la acción de eliminar radicales libres que tienen estos compuestos. Sin embargo, lo que determina el carácter antioxidante o prooxidante de esta reacción inicial es, como ya se mencionó previamente, la estabilidad/labilidad redox del compuesto radical formado a partir del flavonoide original. Ahora bien, dichas acciones prooxidantes sólo parecen producirse cuando las dosis de flavonoides utilizadas son muy altas (Laughton *et al.*, 1991; Da Silva *et al.*, 2002).

2.3.2.3. Otras actividades biológicas

El creciente interés en los flavonoides se debe a la apreciación de su amplia actividad farmacológica. Pueden unirse a los polímeros biológicos, tales como enzimas, transportadores de hormonas, y ADN; quelar iones metálicos transitorios, tales como Fe^{2+}, Cu^{2+}, Zn^{2+}; catalizar el transporte de electrones, y depurar radicales libres (Saskia *et al.*, 1998). Debido a este hecho se han descrito efectos protectores en patologías tales como diabetes mellitus, cáncer, cardiopatías, infecciones víricas, úlcera estomacal y duodenal, e inflamaciones (Saskia *et al.*, 1998). Otras actividades que merecen ser destacadas son sus acciones antivirales y antialérgicas (Vrijsen *et al.*, 1988), así como sus propiedades antitrombótica y antiinflamatoria (Swies *et al.*, 1984; Gryglewki *et al.*, 1987; Alcázar *et al.*, 1988; Brasseur *et al.*, 1989).

Además, los flavonoides presentan otras propiedades que incluyen la estimulación de las comunicaciones a través de las uniones en hendidura, el impacto sobre la regulación del crecimiento celular y la inducción de enzimas de detoxificación, tales como las monooxigenasas dependientes de citocromo P-450, entre otras (Stahl *et al.*, 2002).

2.4. SAPONINAS

Las saponinas son glicósidos de terpenoides que se distribuyen ampliamente en el reino vegetal (Kite *et al.*, 2007). De hecho son el grupo de metabolitos secundarios que se han encontrado en mayor número de especies vegetales, muchas de ellas usadas en medicina tradicional. También se han descrito en algunos organismos marinos (Oleszek y Bialy, 2006).

Se encuentran en gran variedad de alimentos incluyendo espárragos, judías, guisantes, moras, patatas, remolacha azucarera y té (Dini *et al.*, 2001). Se piensa que el papel natural de estos compuestos en las plantas es proteger contra el ataque de patógenos potenciales (Osbourn, 2003).

Poseen propiedades surfactantes y cuando se agitan en soluciones acuosas producen una espuma jabonosa estable (Vincken *et al.*, 2007). De acuerdo con Haralampidis *et al.* (2002), se conoce muy poco sobre las enzimas y rutas bioquímicas implicadas en la biosíntesis de saponinas en las plantas.

2.4.1. Clasificación y estructura

Las saponinas son moléculas complejas consistentes en una aglicona apolar unida a uno o más monosacáridos (Oleszek, 2002). Se pueden clasificar en dos grupos basados en la naturaleza de su aglicona (Bruneton, 1995):

- **Saponinas esteroideas:** están casi exclusivamente en las plantas angiospermas monocotiledóneas. A este grupo pertenecen las saponinas del espárrago.

- **Saponinas triterpénicas:** son las más comunes y aparecen principalmente en las angiospermas dicotiledóneas.

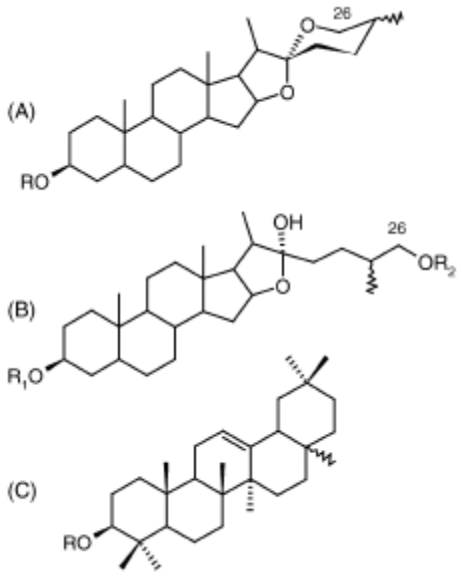
Sin embargo, hay que destacar que algunos autores distinguen un tercer grupo llamado aminas esteroideas, clasificadas por otros autores como alcaloides esteroideos (Bruneton, 1995).

Las saponinas esteroideas pueden ser de tipo espirostano y furostano. Las primeras consisten en una aglicona esteroidea, una estructura de espirostano de C_{27}, constando generalmente de una estructura de 6 anillos (fig. 2.2-A). En las segundas el grupo hidroxilo de la posición 26 es ocupado por un enlace glicosídico, y así la estructura de la aglicona permanece pentacíclica (fig. 2.2-B). Las saponinas triterpénicas constan de una aglicona triterpénica, que tiene una estructura de C_{30} pentacíclica (fig. 2.2-C).

Las agliconas en el caso de las saponinas reciben el nombre de geninas y las saponinas sin azúcares se denominan sapogeninas.

Figura 2.2

Estructura de las geninas de (A) saponinas esteroideas, tipo espirostano, (B) saponinas esteroideas, tipo furostano y (C) saponinas triterpénicas. R= azúcares.



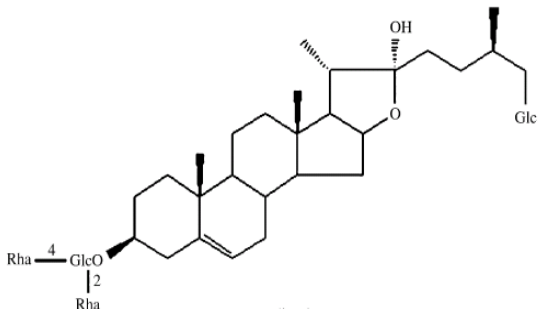
Ambos tipos de geninas pueden presentar un número diferente de sustituyentes (-H, -COOH, $-CH_3$). Las cadenas de azúcares pueden constar de uno, dos o tres monosacáridos (pentosas, hexosas o ácido glucurónico). El número de sustituyentes y las diferentes posibilidades de composición y de unión de las cadenas de azúcares producen una gran diversidad natural de estructuras de saponinas. Incluso en diferentes

partes de una misma planta (raíz, tallo, hoja) se pueden encontrar saponinas con estructuras distintas (Oleszek y Bialy, 2006).

Los glicósidos de furostanol constituyen un gran grupo de saponinas esteroideas. A este grupo pertenece la protodioscina (3-O-[α-L-ramnopiranosil-(1→2)-{α-L-ramnopiranosil-(1→4)}-β-D-glucopiranosil]-26-O-[β-D-glucopiranosil]-(25R)-furost-5-ene-3β,26-diol), que se ha descrito como la principal saponina del espárrago (Wang *et al.*, 2003). Se trata de una diosgenina glicosilada (ver Figura 2.3) con cuatro residuos de azúcares consistentes en dos desoxihexosas, concretamente ramnosas, y dos hexosas, concretamente glucosas.

Figura 2.3

Estructura de la protodioscina.



En Fuentes-Alventosa (2007) se realizó un estudio de saponinas en variedades comerciales de espárrago verde y de espárrago triguero de Huétor Tájar. Se informa de que las saponinas encontradas en el espárrago triguero de Huétor Tájar son, fundamentalmente, derivados de la sarsasapogenina (Figura 2.4). De hecho, se detectaron ocho saponinas diferentes en las muestras de la población Huétor Tájar analizadas en aquel estudio (Tabla 2.3).

Figura 2.4

Estructura de la sarsasapogenina.

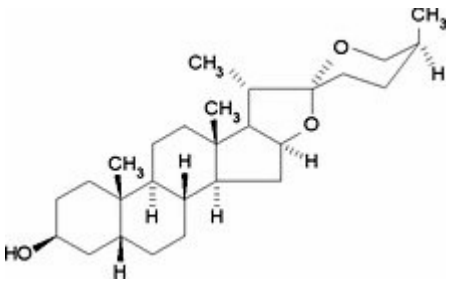


Tabla 2.3

Pesos moleculares, geninas y azúcares obtenidos a partir de los espectros de masas de las distintas saponinas identificadas en muestras de espárrago triguero de Huétor Tájar.

Saponina	Peso Molecular	Genina	Azúcares en la estructura
Protodioscina	1048	diosgenina	2 ramnosas 2 glucosas
Saponina 1	1052	sarsasapogenina	1 xilosa 3 glucosas
Saponina 2	920	sarsasapogenina	3 glucosas
Saponina 3	1022	sarsasapogenina	2 xilosas 2 glucosas
Saponina 4	890	sarsasapogenina	1 xilosa 2 glucosas
Saponina 5	1050	sarsasapogenina	2 ramnosas 2 glucosas
Saponina 6	890	sarsasapogenina	1 xilosa 2 glucosas
Saponina 7	904	sarsasapogenina	1 ramnosa 2 glucosas

Fuente: Fuentes-Alventosa, J.M. 2007. Caracterización de flavonoides y saponinas en el espárrago triguero de Huétor Tájar (Granada). Trabajo de Investigación correspondiente al Máster en Agroalimentación 2006/2007. Universidad de Córdoba.

El análisis HPLC-MS (Tabla 2.4) reveló que en todas las muestras de cultivares mundiales había protodioscina. Además se detectó en dos variedades una saponina cuya genina es la sarsasapogenina.

Sin embargo, el perfil de las saponinas encontrado en las muestras de espárrago triguero de Huétor Tájar es muy distinto al de los cultivares mundiales. En primer lugar, la protodioscina sólo se detectó en una de las diez muestras analizadas (HT-1) y por otra parte se han encontrado otras siete nuevas saponinas, incluida la mencionada en los cultivares mundiales.

Tabla 2.4

Porcentaje de saponinas[a] encontradas en las muestras de espárrago triguero de Huétor Tájar y de cultivares mundiales, calculado a partir del área de los picos obtenidos con HPLC-MS.

	%S1	%S2	%S3	%S4	%Proto.	%S5	%S6	%S7
HT-1	20	7	0	0	40	0	0	32
HT-2	25	0	0	0	0	13	0	62
HT-3	23	0	0	0	0	20	0	57
HT-4	19	15	23	16	0	0	0	26
HT-5	21	6	24	0	0	13	16	20
HT-6	22	0	61	0	0	0	0	17
HT-7	33	7	0	0	0	19	0	41
HT-8	11	5	27	15	0	0	0	42
HT-9	50	11	9	0	0	0	0	30
HT-10	0	0	46	28	0	0	26	0
APOLLO	0	0	0	0	61	0	0	39
BACKLIM	0	0	0	0	100	0	0	0
ERCOLE	0	0	0	0	100	0	0	0
GUELP	0	0	0	0	100	0	0	0
JIUC-1	0	0	0	0	100	0	0	0
NJ956	0	0	0	0	76	0	0	24
PLAVERD	0	0	0	0	100	0	0	0
RAVEL	0	0	0	0	100	0	0	0
SOLAR	0	0	0	0	100	0	0	0
SUPREME	0	0	0	0	100	0	0	0

[a] *Proto.: protodioscina y S1-S7: saponinas 1 a 7*

Fuente: Fuentes-Alventosa, J.M. 2007. Caracterización de flavonoides y saponinas en el espárrago triguero de Huétor Tájar (Granada). Trabajo de Investigación correspondiente al Máster en Agroalimentación 2006/2007. Universidad de Córdoba.

Como ya se ha comentado anteriormente, se ha establecido que las propiedades bioactivas de las saponinas dependen de su estructura. Pequeñas variaciones en ella dan lugar a variaciones sustanciales en las propiedades físicas, químicas y biológicas (Oakenfull, 1981). Así, por ejemplo, Mimaki *et al.* (2001a) concluyeron que la estructura de la parte de azúcar de las saponinas esteroideas jugaba un papel importante en la citotoxicidad específica frente a células tumorales. De este modo, la realización de estudios más profundos en el aislamiento y caracterización estructural de saponinas de espárrago triguero permitirán establecer relaciones entre estos compuestos y acciones

beneficiosas específicas asociadas al espárrago. Este distinto perfil de saponinas puede ser un factor de revalorización de la población triguero de Huétor Tájar así como para la autenticación frente a las variedades comerciales.

2.4.2. Actividades biológicas de las saponinas

La amplia diversidad química tanto de las saponinas triterpénicas como esteroideas ha dado lugar a un renovado interés y a investigaciones de estos compuestos en los últimos años, particularmente como potenciales agentes quimioterapéuticos.

Entre las actividades biológicas de las saponinas destacan las siguientes (Sparg *et al.*, 2004):

2.4.2.1. Actividad antibacteriana

Los tetraglicósidos de saponinas tienen una actividad antibacteriana más fuerte que los triglicósidos de saponinas (Konishi *et al.*, 1998). Si bien hay que indicar que muchos estudios muestran que la actividad antibacteriana es débil.

2.4.2.2. Actividad antifúngica/antilevadura

La actividad antifúngica es mayor en las saponinas que en las sapogeninas y saponinas acetiladas, estando esta actividad altamente influenciada por el número de componentes monosacáridos y su secuencia (Mahato *et al.*, 1982). Se ha visto inhibición del crecimiento de levaduras que deterioran alimentos, levaduras formadoras de velo y hongos y levaduras dermatofíticos (Miyakoshi *et al.*, 2000).

2.4.2.3. Actividad citotóxica y antitumoral

Existen estudios sobre las destacadas propiedades citotóxicas de muchas saponinas. Sin embargo para su uso como agentes antitumorales hay que tener en cuenta que, aunque tengan una alta citotoxicidad, no siempre poseen propiedades antitumorales. Mimaki *et al.* (2001b), en estudios de actividad citotóxica realizados con saponinas esteroideas aisladas de la dama de noche (*Cestrum nocturnum*) contra células

escamosas del carcinoma oral humano (HSC-2) y fibroblastos gingivales humanos normales, concluyeron que la estructura del resto de azúcar de estas saponinas parece jugar un papel importante en la citotoxicidad específica contra las células tumorales. Mimaki *et al.* (2001a) y Yokosuka *et al.* (2002) comprobaron en ensayos con saponinas esteroideas aisladas de plantas de la familia de las Liliáceas contra células promielocíticas de leucemia humana HL-60, que la actividad citotóxica estaba relacionada con los monosacáridos constituyentes de los restos de azúcar y sus secuencias, así como con la estructura de las agliconas. Incluso ligeras diferencias estructurales pueden afectar a dicha actividad. Marquina *et al.* (2001) informaron de la actividad citotóxica de una mezcla de saponinas, que no presentaban sus distintos componentes por separado.

2.4.2.4. Actividad hipocolesterolémica

Saponinas de especies del género *Allium* han mostrado que descienden los niveles de colesterol total en plasma (Matsuura, 2001), afirmándose que las saponinas esteroideas son las responsables de los efectos de disminución del colesterol en el ajo. En una revisión bibliográfica sobre productos naturales y sus actividades biológicas se clasificaron a las saponinas como compuestos con actividad hipocolesterolémica (Wang y Ng, 1999).

2.4.2.5. Otras actividades

Las saponinas también han mostrado tener actividad antiinflamatoria, antiviral, antiparasitaria, antigenotóxica, antiulcerogénica, inmunoestimulante, efectos hepatoprotectores y neuroprotectores, molusquicida, hemolítica, afrodisiaca y de prevención y terapia de algunos efectos adversos de la nicotina.

2.5. FIBRA ALIMENTARIA

La definición de fibra alimentaria o dietética es frecuentemente controvertida ya que entre los especialistas en este tema no termina de existir unanimidad de criterios. La razón estriba en que no se trata de un solo componente de los alimentos, sino de un conglomerado de varios, y no está delimitada la inclusión o exclusión de algunos. Incluso desde una perspectiva más amplia, existen materiales fibrosos de plantas, tales como algodón, madera, etc. que son completamente indigeribles por el hombre y no son fibra alimentaria humana, aunque son materiales fibrosos, en apariencia (Heredia Moreno *et al.*, 2002).

El concepto más generalizado de fibra alimentaria es el que se refiere al conjunto de polisacáridos y lignina de la dieta que no son digeridos por las secreciones endógenas del tracto digestivo humano (Trowell *et al.*, 1976). Esta definición significa que la pared celular de frutas, verduras, legumbres y cereales constituye casi toda la fibra de la dieta (Selvendran y Robertson, 1994; McDougall *et al.*, 1996).

Sin embargo, muy recientemente se acordó una definición de fibra alimentaria en la 30ª reunión del Comité del Codex sobre Nutrición y Alimentos para Regímenes Especiales (Codex Committee for Nutrition and Foods for Special Dietary Uses – CCNFSDU), celebrada del 3 al 7 de noviembre de 2008 en El Cabo (Sudáfrica), que dice así:

"Se entenderá por fibra dietética los polímeros de hidratos de carbono[1] con diez o más unidades monoméricas[2], que no son hidrolizados por las enzimas endógenas del intestino delgado humano y que pertenecen a las categorías siguientes:

- *polímeros de carbohidratos comestibles que se encuentran naturalmente en los alimentos en la forma en que se consumen;*

- *polímeros de carbohidratos obtenidos de materia prima alimentaria por medios físicos, enzimáticos o químicos, y que se haya demostrado que tienen un efecto fisiológico beneficioso para la salud mediante pruebas científicas generalmente aceptadas aportadas a las autoridades competentes;*

- *polímeros de carbohidratos sintéticos que se haya demostrado que tienen un efecto fisiológico beneficioso para la salud mediante pruebas científicas generalmente aceptadas aportadas a las autoridades competentes."*

1 La fibra dietética, si es de origen vegetal, puede incluir fracciones de lignina y/u otros compuestos cuando están asociados a los polisacáridos en la pared celular vegetal y si tales compuestos se han cuantificado mediante el método de análisis gravimétrico de la AOAC para el análisis de la fibra dietética: las fracciones de lignina y los otros compuestos (fracciones proteínicas, compuestos fenólicos, ceras, saponinas, fitatos, cutina, fitosteroles, etc.) íntimamente "asociados" a los polisacáridos vegetales, suelen extraerse con los polisacáridos según el método AOAC 991.43. Estas sustancias quedan incluidas en la definición de fibra por cuanto están efectivamente asociadas con la fracción polisacárida u oligosacarídica de la fibra. Sin embargo, no pueden ser definidas como fibra dietética si se extraen o incluso si se reintroducen en un alimento que contiene polisacáridos no digeribles. Al combinarse con polisacáridos, estas sustancias asociadas pueden aportar efectos beneficiosos complementarios (pendiente de la adopción de la sección sobre los métodos de análisis y muestreo).

2 La decisión sobre si se deben incluir los carbohidratos con entre tres y nueve unidades monoméricas debe recaer en las autoridades nacionales.

2.5.1. Fibra y pared celular

La pared celular es uno de los componentes fundamentales de las células vegetales. Presenta una estructura semirrígida que se encuentra rodeando a la membrana plasmática, y constituye la capa más externa de la célula. Está formada por polisacáridos, lignina, otros compuestos fenólicos y pequeñas cantidades de glicoproteínas, contrastando con la membrana plasmática, de menor espesor, en la que fosfolípidos y proteínas son los componentes principales.

- Estructura de la pared celular.

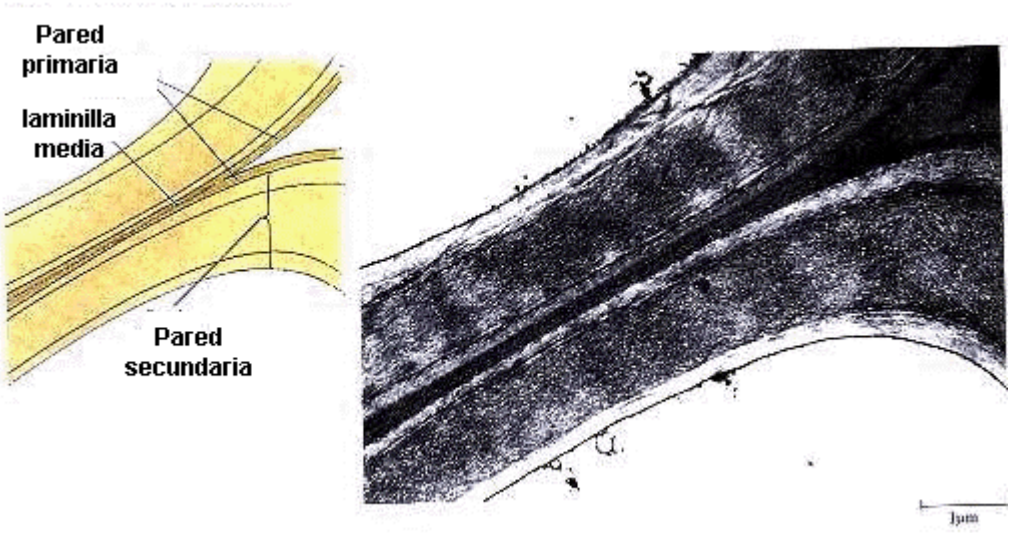
Se encuentra formada por tres capas, que son el resultado de la evolución de una serie de etapas de diferenciación celular (Imagen 2.5).

La lámina media es la más externa, común a dos células contiguas, de las que constituye una especie de tabique de separación. Es la primera que se forma en la célula

durante la división del citoplasma. Es coloidal y ópticamente inactiva y está compuesta principalmente por sustancias pécticas, particularmente por sales cálcicas de pectinas.

Imagen 2.5

Capas de la pared celular vegetal.



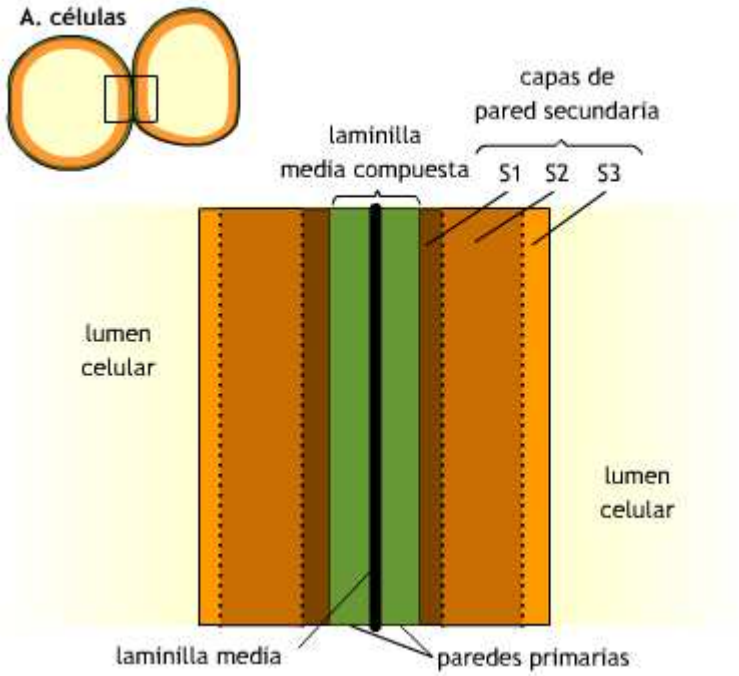
Tras la formación de la lámina media, comienzan a depositarse carbohidratos a ambos lados de la misma, así como algunas glicoproteínas y compuestos fenólicos, originándose la pared primaria que es la capa más externa de la pared celular y que continúa su engrosamiento mientras dura el crecimiento celular. Los carbohidratos de esta pared, fundamentalmente celulosa, se sintetizan en la propia superficie de la membrana citoplasmática y pueden ser segregados al exterior del plasmalema por exocitosis. La pared celular primaria controla el crecimiento celular y forma la base estructural del esqueleto de la planta.

Muchas células tienen pared celular formada únicamente por estas dos capas, aunque existen algunas células especializadas que, después de completarse la expansión celular, pueden continuar sintetizando polímeros y originarse la pared secundaria (Wilson, 1993), considerada como pared suplementaria, cuya principal función es mecánica (Monties, 1980) y su aparición origina cambios en la composición de la lámina media y pared primaria, tales como lignificación (Jiménez, 1993; Heredia *et al.*, 1993; 1995). Generalmente comprende tres capas, conocidas de fuera adentro como S_1, S_2 y S_3 que se distinguen ultraestructuralmente por la diferente orientación de las

microfibrillas de celulosa (Harris, 1990). Lo más común es que la lignificación se inicie en la lámina media y pared primaria cuando cesa la expansión, y se extienda a la pared secundaria, que es la que llega a tener la mayor cantidad de la lignina presente en la planta. Las paredes celulares con pared secundaria llegan a tener un espesor entre 1 y 3 micras o más, proporcionando a la célula su capacidad para resistir presiones externas.

Imagen 2.6

Porción de pared celular entre dos células.



- Composición química de la pared celular.

a) Celulosa.

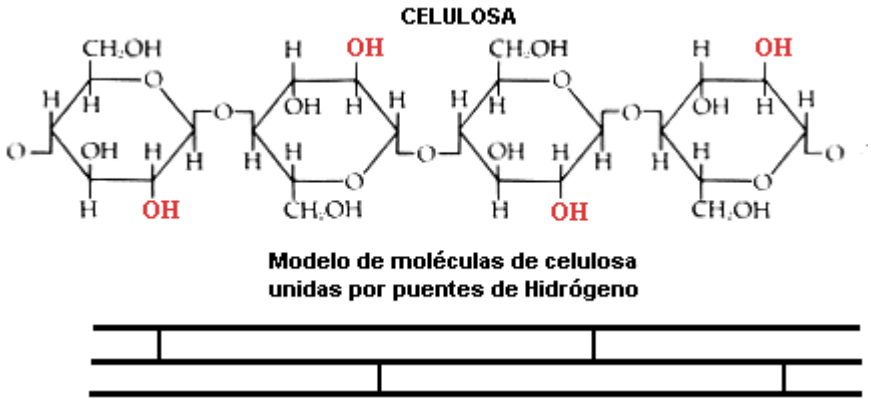
Es el componente mayoritario de los materiales lignocelulósicos, siendo la base estructural de células vegetales. Actualmente supone la base de muchos productos de interés industrial (papel, fibras, aditivos, etc.).

La celulosa, de fórmula $(C_6H_{10}O_5)_n$, es un homopolímero lineal (*β-1,4-glucana*) constituido por unidades de β–glucosa unidas entre sí por enlaces 1-4. Es de alto peso molecular (0,5-1 x 10^6 Da) y con grado de polimerización superior a 15.000 en paredes secundarias, e inferior en primarias. Estas moléculas se pueden hidrolizar con dificultad

en medios catalizados por ácidos. Entre las principales propiedades físico-químicas de la celulosa se encuentran el índice o grado de polimerización, la cristalinidad y la porosidad (Browning, 1967; Stone y Scallaham, 1967; Sjöstrtröm, 1981). La cadena de celulosa es alargada y las unidades de glucosa están dispuestas en un solo plano debido a la presencia del anillo glicosídico y a su conformación (Fengel y Wegener, 1984) (Figura 2.5). La configuración más estable es en forma de silla con los grupos hidroxilos en posición ecuatorial. Los grupos -OH que se encuentran en los dos extremos de la cadena muestran un comportamiento diferente. Mientras que el grupo -OH del C1 que se encuentra en uno de los extremos es un grupo aldehído y, por tanto, con propiedades reductoras, el grupo -OH del C4 que está situado en el extremo opuesto de la cadena es un grupo hidroxil alcohol y, en consecuencia, no reductor.

Figura 2.5

Estructura de la cadena de celulosa.



b) Hemicelulosas.

Se engloban dentro de esta denominación el resto de polisacáridos que se encuentran en los materiales lignocelulósicos además de la celulosa. Las hemicelulosas, a diferencia de la celulosa, están compuestas de diferentes azúcares formando cadenas más cortas y con ramificaciones. Los azúcares que forman las hemicelulosas se pueden dividir en diferentes grupos como las pentosas (xilosa y arabinosa), hexosas (glucosa, manosa y galactosa), ácidos hexurónicos (ácido glucurónico, metilglucurónico y galacturónico) y desoxihexosas (ramnosa y fucosa) (Figura 2.6). La cadena principal de una hemicelulosa puede consistir en una sola unidad que se repite (homopolímero),

como, por ejemplo, los xilanos, o en dos o más unidades (heteropolímero), como, por ejemplo, los glucomananos. La mayoría de las hemicelulosas son heteropolisacáridos complejos que contienen entre dos y cuatro tipos de azúcares (Southgate, 1990).

La Tabla 2.5 da una relación de los principales polisacáridos incluidos en este grupo.

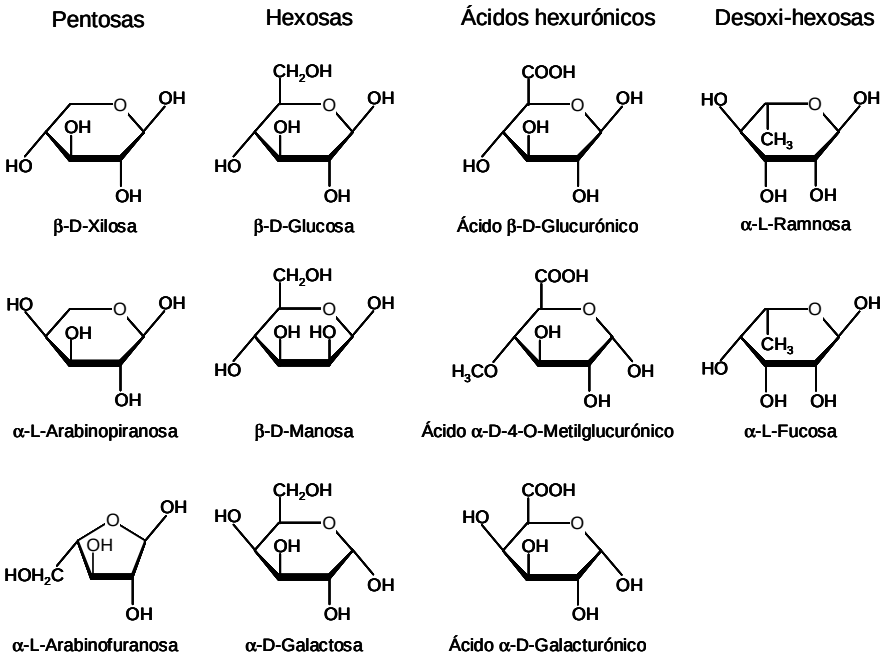
Tabla 2.5

Cuadro resumen de las distintas hemicelulosas que forman la pared celular.

HEMICELULOSA	UNIDADES	FUENTES
1. Xilana	β-1,4-xilosa	Esparto, centeno, madera, salvado de trigo, tomate, aceituna, abeto, grama
2. Glucomanana Galactoglucomanana	β-1,4-glucosa β-1,4-manosa α-1,6-galactosa	Semilla del espárrago, zarzamora
3. Manana Galactomanana	β-1,4-manosa α-1,6-galactosa	Semilla de café, girasol, remolacha, semillas de acacia
4. Glucuromanana	α-1,2-manosa β-1,4-glucurónico	Tomate, gomas
5. Xiloglucana	β-1,4-glucosa α-1,6-xilosa β-1,2-galactosa α-1,2-fucosa	Abeto, judía, col, rosa, calabaza, cebolla
6. β-1,3-glucana	β-1,3-glucosa	Tubos polínicos
7. β-1,3-b-1,4-glucana	β-1,3-glucosa β-1,4-glucosa	Trigo, cebolla
8. Arabinogalactana II	β-1,3-galactosa β-1,6-galactosa 1,3-arabinosa 1,6-arabinosa	Madera de coníferas, semilla de café, mucílago de aloe

Figura 2.6

Monómeros precursores de las hemicelulosas (Fenge y Wegener, 1984).



c) Sustancias pécticas.

Están estrechamente ligadas a la estructura de la pared celular y, en consecuencia, a la textura de productos vegetales. Constituyen una mezcla compleja de polisacáridos coloidales. Los de alto grado de esterificación tienen poca capacidad de interacción con otros componentes de la pared celular y, por ello, pueden extraerse de la misma con agua. Los de medio o bajo grado de esterificación están estabilizados por enlaces iónicos con calcio, formando geles con estructura "caja de huevos" y pueden solubilizarse en agentes quelantes. Otros pueden estar enlazados por enlaces covalentes a hemicelulosas, celulosa o incluso a proteínas y se separan sólo por tratamiento con álcali o con ácido diluido (Van Buren, 1979). Son constituyentes fundamentales de la lámina media y lo más característico de estas sustancias son los residuos de ácido anhidrogalacturónico y, además, poseen restos de ramnosa, arabinosa y galactosa. Generalmente la ramnosa forma parte de la cadena principal, en tanto que la arabinosa y galactosa se encuentran en cadenas laterales.

Los polisacáridos que constituyen las sustancias pécticas aparecen en la Tabla 2.6.

Tabla 2.6

Cuadro resumen de las distintas pectinas que forman la pared celular.

PECTINA	UNIDADES	FUENTES
1. Homogalacturonana	α-1,4-galacturónico	Girasol, abeto, cebolla, manzana, zanahoria, aceituna
2. Ramnogalacturonanas I y II	α-1,4-galacturónico 1,2-ramnosa 1,3-ramnosa	Sicamoro, tomate, maíz, mijo
3. Arabinana	α-1,5-arabinosa α-1,3-arabinosa	Garbanzo rojo, pastinaca
4. Galactana	β-1,4-galactosa	Altramuz
5. Arabinogalactana I	β-1,4-galactosa α-1,5-arabinosa	Apio

d) Lignina y otros compuestos fenólicos.

La lignina es de gran importancia en vegetales en general, por cuanto su presencia y naturaleza determina las características de la fibra. Su presencia en la pared es la responsable fundamental de la dificultad de degradar la fibra, ya que forma una especie de pantalla que dificulta enormemente la accesibidad de agentes químicos y/o enzimáticos a los otros componentes de la fibra. Esto constituye un factor importante a efectos de alimentación animal. Así, resulta que la fracción más digerible de la pared son las sustancias pécticas, en tanto que las hemicelulosas y celulosa están protegidas por la barrera de lignina, posiblemente unidas directamente a ella (Wilson *et al.*, 1989).

Además de la lignina, pueden estar presentes otros compuestos fenólicos, siendo el más notable el ácido ferúlico que se encuentra esterificando a arabinosa y galactosa de sustancias pécticas y hemicelulosas. La mayoría de los derivados ferúlicos se encuentran presentes en forma de dímeros, pudiendo éstos contribuir al mantenimiento de la textura del vegetal y aportar su consumo dietético beneficios para la salud (Parr *et al.*, 1997). Por el contrario, el ácido cumárico se encuentra fundamentalmente esterificado y su concentración aumenta con la maduración (Iiyama *et al.*, 1990).

Se han propuesto diversos tipos de unión entre lignina y carbohidratos de pared, entre los que se encuentran la unión entre azúcares y grupos hidroxilos de cadenas laterales de lignina, los enlaces glicosídicos vía fenoles, las uniones éster entre grupos carbonilo de ácidos urónicos y grupos hidroxilos de lignina, etc. (Iiyama *et al.*, 1993).

e) Proteínas.

Actualmente está en fase de discusión si las proteínas resistentes se han de considerar como parte de la fibra. Todas las paredes celulares en desarrollo contienen glicoproteínas, con un 67% de carbohidratos como máximo. Las más conocidas son las extensinas, que aparecen glicosiladas con cadenas de arabinosa y son ricas en el aminoácido hidroxiprolina, conteniendo también lisina, tirosina, valina y serina (Showalter, 1993). Están distribuidas de manera uniforme en la pared y pueden formar entramados celulares a través de enlaces con otros polímeros de la misma. Otro tipo de glicoproteínas son las arabino-galactana-proteínas, ricas en hidroxiprolina, serina, alanina y glicina (Fincher *et al.*, 1983).

f) Otros polisacáridos de la fibra.

Además de los principales constituyentes de la pared celular, la fibra incluye un grupo heterogéneo de polisacáridos no estructurales (Asp y Johansson, 1984; Heredia y Guillén, 1986) que son indigeribles por los enzimas digestivos.

Se consideran también componentes de la fibra una serie de compuestos que, o bien forman parte del propio producto natural, o bien se añaden al alimento con el fin de aumentar su contenido en fibra. Son hidrocoloides que se emplean en los alimentos para aumentar su viscosidad, o como gelificantes o estabilizantes. Generalmente derivan de extractos de algas, exudados de plantas, semillas y fuentes microbianas (Lineback *et al.*, 1995). Se consideran como componentes de la fibra y no son digeridos por los enzimas del sistema gastrointestinal humano. Se han de destacar las gomas, mucílagos, sustancias cuticulares (cutina y suberina) y polisacáridos de algas (tales como alginatos).

También hay que mencionar como componente de la fibra alimentaria a la inulina, que es un carbohidrato de reserva que se encuentra generalmente en las raíces, tubérculos y rizomas de ciertas plantas fanerógamas (diente de león, achicoria, ajo, alcachofa, cebolla, espárrago, etc.). Es soluble en agua y no es digerible por los enzimas digestivos, sino por los microorganimos intestinales (especialmente las bifidobacterias), promoviendo su asentamiento y desarrollo. Por ello se dice que la inulina tiene un efecto bifidogénico (Kaur y Gupta, 2002) y actividad prebiótica (Roberfroid *et al.*, 2005).

2.5.2. Fuentes de fibra

Las principales fuentes de fibra son los frutos, vegetales y cereales. Las que poseen mayor concentración de fibra son los granos, especialmente el salvado de trigo. Dentro de ellos, los alimentos sin almidón aportan 20-35 g de fibra/100 g de su peso seco y los que contienen almidón, alrededor de 10 g/100 g de peso seco; la contribución de frutos y vegetales se sitúa en un máximo de 5 g/100 g de peso fresco, aunque una gran mayoría se sitúan entre 1,5-2,5 g/100 g de peso seco (Selvendran y Robertson, 1994). Distribuida por tipo de alimentos, el 45%-50% proviene de cereales, el 30%-40% de vegetales, 16% de frutos y 3% de otras fuentes (Gregory *et al.*, 1990; Cummings, 1996).

Las aportaciones de estos tipos de alimentos se podría resumir como sigue (Johnson y Southgate, 1994):

- Cereales: la cantidad de fibra que aportan depende de la forma de extracción del cereal. Así, el contenido de fibra de trigo puede variar desde un valor de 2,5 g/100 g en harina muy refinida, hasta 12 g/100 g en harina procedente de trigo completo. La mayor parte de esta fibra es fibra insoluble y es la que principalmente se pierde con el incremento de la refinación.

- Vegetales: debido a su elevado contenido en agua, su concentración es baja, oscilando entre 0,5% y 3%, lo que puede representar, en algunos vegetales, hasta un 28%-30% de la materia seca. No obstante, las

legumbres tienen valores muy superiores; por ejemplo, habichuela blanca 17%, habichuela roja 15,2%, nabo 5,7% (referido a producto original), etc.

- Frutos: al igual que los vegetales en general, contienen alto porcentaje de agua y poca cantidad de tejidos vasculares lignificados, y su pared celular es delgada. Sus valores, referidos a fruto fresco, oscilan entre 1% y 3,5%.

2.5.3. Fibra y salud

La fibra alimentaria tiene efectos beneficiosos sobre la salud humana, así que es recomendable, y se ha de promover por los profesionales de la alimentación y de la nutrición, el consumo de alimentos ricos en fibra tales como los cereales, legumbres, frutas y verduras. Las poblaciones que consumen más fibra alimentaria sufren menos enfermedades crónicas, ya que ésta tiene efectos beneficiosos sobre los factores de riesgo que desarrollan algunas de las mismas. Se recomienda el consumo de 14 g de fibra dietética por cada 1000 kcal, o 25 g al día para mujeres adultas y 38 g para hombres adultos, datos basados en estudios epidemiológicos que mostraron protección frente a enfermedades cardiovasculares (American Dietetic Association, 2008).

- ***Papel fisiológico.***

Un factor de interés en relación con el valor nutritivo de la fibra es que, si bien la mayor parte de los componentes son por sí mismos indigeribles, por fermentación bacteriana se convierten parcialmente en ácidos grasos volátiles que pueden ser absorbidos por el intestino (Heredia y Fernández, 1979).

La fibra alimentaria afecta a muchos procesos del sistema gastrointestinal, desde la ingestión hasta la excreción, pero es en el intestino delgado donde más se manifiesta. En general, los polisacáridos más viscosos, como las pectinas y gomas, reducen la absorción de nutrientes, mientras que los componentes más insolubles tienen poco efecto en la misma. La masa bacteriana que se produce a partir de las fibras fermentables (sustancias pécticas), unida a los residuos de las poco fermentables (celulosa y hemicelulosas) y al agua retenida por las mismas, son las que originan el aumento de la masa fecal (Madar y Odes, 1990; Lefebvre y Thébaudin, 2002). En

consecuencia, la fibra puede modificar muy directamente y disminuir la digestibilidad de las proteínas, junto con lípidos y ciertos minerales (Kritchevsky, 1988).

Existen evidencias de que los polisacáridos de la fibra ejercen una influencia sustancial en la absorción de lípidos por el organismo. Estudios *in vitro* han demostrado que la fibra alimentaria puede ser un factor limitante de la absorción de grasa, debido a su efecto inhibidor de la actividad de la lipasa pancreática (Schneeman, 1978; Dunaif y Schneeman, 1981). Por otro lado, disminuye el colesterol total y la lipoproteína de baja densidad en el plasma sanguíneo, lo que podría ser consecuencia de una menor absorción de los lípidos y colesterol producido por la dilución y excreción de ácidos biliares (Gallaer *et al.*, 1992).

La fibra puede ejercer su acción en la disponibilidad de carbohidratos en el tracto intestinal, y su efecto directo en la absorción de carbohidratos se ha puesto de manifiesto en individuos diabéticos alimentados con dietas ricas en fibra, especialmente fibras viscosas que han experimentado un descenso de los niveles de glucosa en sangre. Las fibras de hortalizas regulan el tránsito intestinal por la acción que ejercen sobre el volumen de las heces y también actúan sobre el metabolismo de glúcidos y lípidos, estando frecuentemente asociadas con otros componentes, como pueden ser flavonoides y carotenoides, que presentan propiedades beneficiosas para la salud (Lefebvre y Thebaudin, 2002).

- ***Relación con enfermedades.***

Existe un creciente interés por el estudio de la fibra, por su efecto protector frente a enfermedades cardiovasculares, diverticulosis, obesidad, cáncer de colon y diabetes (Mann y Cummings, 2009), patologías que son más frecuentes con un consumo bajo de fibra.

Estudios epidemiólogicos permiten deducir que existe una relación entre el consumo de fibra y la reducción de enfermedades cardiovasculares (Bazzano *et al.*, 2003). Hay evidencia de que un alto consumo de fibra de cereales pueden prevenir enfermedades cardiovasculares (Jensen *et al.*, 2004) y cáncer (Mendez *et al.*, 2007).

La fibra alimentaria comprende dos fracciones, insoluble y soluble. La primera estaría relacionada con la regulación intestinal, mientras que la soluble ejerce cierto efecto en la disminución de colesterol y absorción intestinal de glucosa (Scheneeman, 1987).

Kaline *et al.* (2007) revisaron la importancia y el efecto de la fibra alimentaria en la prevención de diabetes. Sugieren que los cereales son especialmente efectivos en la prevención de diabetes mellitus de tipo 2 y recomiendan un consumo diario de 30 g/día de fibra alimentaria como protección.

Las relaciones entre fibra alimentaria y cáncer de colon, aún siendo de naturaleza compleja, están ampliamente documentadas, habiéndose encontrado una relación inversa entre consumo de la primera e incidencia de esta enfermedad y dentro de los alimentos ricos en fibra se incluyen de manera preferente verduras, frutas, granos y legumbres, al contener éstos una amplia gama de compuestos anticarcinogénicos (Steinmetz y Potter, 1991; Slavin, 2001). Estudios epidemiológicos sugieren que un elevado consumo de fibra puede llevar a una reducción de este tipo de cáncer (Potter, 1997; Bingham *et al.*, 2003).

De manera análoga, existen estudios que encuentran una relación positiva entre cáncer de próstata y dietas con bajo contenido en fibra (Ross *et al.*, 1990), aunque al igual que en el caso anterior se necesita continuar investigando antes de llegar a conclusiones más definitivas.

- ***Posibles efectos de la fibra sobre el balance mineral.***

Un efecto a considerar es la adsorción de minerales en la fibra, lo que puede tener un efecto negativo en su disponibilidad para el organismo. Esto puede ser debido a la afinidad de los polisacáridos ácidos por cationes mono y divalentes, lo que puede originar un incremento de la excreción fecal de varios minerales y electrolitos. La asociación entre un alto contenido de fibra alimentaria y sus efectos adversos sobre la biodisponibilidad de minerales se debe a la facilidad de la fibra de formar quelatos *in vitro*, que raramente ocurre de manera uniforme, sino que afecta a minerales específicos o a grupos de minerales (Harland y Naruda, 2001).

Por ejemplo, se encontró que la fibra de trigo puede disminuir los niveles de hierro y calcio (Widdowson y McCance, 1942; Heaton y Pomare, 1974), así como la biodisponibilidad de zinc y manganeso (Reinhold *et al.*, 1973, 1976), aunque este efecto puede estar parcialmente relacionado con el contenido en fitatos.

Sin embargo, aunque se piense que la fibra alimentaria descienda la absorción de minerales se ha visto que las fibras prebióticas, la inulina y la oligofructosa aumentan la absorción de calcio y los niveles de magnesio en mujeres postmenopáusicas (Salovaara *et al.*, 2007).

La fibra de frutas, verduras, legumbres y cereales es una parte esencial de la dieta, bien como componente natural, o como ingrediente añadido para suplementar dietas, lo que constituye una incuestionable contribución a la nutrición y la salud humana. Por tanto, hay que asumir y contrarrestar el efecto adverso que representa en cuanto a una posible disminución en la biodisponibidad de ciertos minerales.

2.6. LOS SUBPRODUCTOS VEGETALES COMO FUENTE DE INGREDIENTES FUNCIONALES

La actividad agrícola lleva asociada, en la actualidad, una mayor oferta de productos hortofrutícolas, ya sean sin procesar, procesados en forma de zumos, conservas, etc.; o en forma de productos mínimamente procesados, como, por ejemplo, ensaladas preparadas listas para su consumo (Llorach *et al.*, 2003). Pero las envasadoras de productos vegetales producen grandes cantidades de residuos (hojas, tallos, peladuras, etc.), que en ocasiones pueden alcanzar el 50% del material recolectado. Estos residuos son un material muy perecedero y difícil de gestionar, por lo que minimizar su impacto ambiental ha sido una preocupación creciente en los últimos años. Este material, si no se le da una salida útil, podría ser un factor limitante en la comercialización de dichos productos, ya que al constituir un porcentaje importante del material fresco, encarece notablemente su precio de mercado (Schieber *et al.*, 2001).

Se puede considerar subproducto a todo aquel residuo que es obtenido tras un procesado industrial. En este sentido, los residuos derivados tanto de la manipulación como de la transformación de los alimentos vegetales pueden considerarse como subproductos (Llorach *et al.*, 2003).

Las partes más externas de las plantas son los principales residuos generados durante la manipulación y procesado de frutas y verduras, constituyendo una buena fuente para la extracción de fitoquímicos. Los fitoquímicos se biosintetizan preferentemente en los tejidos externos de las plantas, debido al papel que desempeñan en ellas (protección frente a la radiación ultravioleta, protección contra insectos, atrayentes de animales para asegurar la polinización y la dispersión de semillas, etc.).

Pero los subproductos vegetales además de ser una buena fuente de fitoquímicos también lo son de fibra, que puede tener un gran valor en la preparación de alimentos funcionales. Las fuentes usuales de fibra alimentaria para adicionar a los alimentos del tipo zumos, productos lácteos y de repostería son los cereales. Sin embargo, actualmente se está incrementando la demanda de los subproductos de frutas y verduras como fuentes de fibra alimentaria, debido a que presentan una mayor calidad nutricional, cantidades más altas de fibra total y soluble, menor contenido calórico,

mayor capacidad antioxidante y niveles más altos de fermentabilidad y retención de agua (Rodríguez *et al.*, 2006).

En los últimos años se han publicado algunos estudios que ponen de manifiesto el interés del uso de los subproductos vegetales como fuente de compuestos beneficiosos para la salud, principalmente polifenoles (Peschel *et al.*, 2006; Balasundram *et al.*, 2006).

A continuación se va a mencionar algunos ejemplos encontrados en la bibliografía sobre diversos subproductos vegetales:

- La industria del vino genera un cuantioso producto de desecho, si bien los subproductos de la uva son una buena fuente de fibra alimentaria y tienen propiedades antioxidantes (Llobera *et al.*, 2007). De hecho, los residuos de la producción de vino contienen cantidades considerables de fenoles antirradicales. Esto es de gran importancia para esta industria, ya que se está incrementando el uso de los extractos de estos subproductos como sustancias activas para productos de cosmética y farmacéuticos (Pinelo *et al.*, 2005).

- Lario *et al.* (2004) usaron subproductos de la elaboración de zumo del limón para obtener fibra alimentaria con unas buenas características funcionales, físico-químicas y calidad microbiológica adecuada para su empleo en la formulación de diversos alimentos.

- Mandalari *et al.* (2006) demostraron que la cáscara de la bergamota contiene cantidades significativas de flavonoides y pectinas. Incluso detectaron flavonoides que no han sido descritos en cáscaras de otros cítricos como el limón o la naranja. Estos residuos son una fuente potencial de flavonoides (encontrados a más bajos niveles en otros cítricos) para aplicaciones fitofarmacéuticas y como aditivos naturales para alimentos funcionales.

- Figuerola *et al.* (2005) concluyeron en su estudio de concentrados de fibra obtenida a partir de piel de manzana y cáscara de cítricos, que son una fuente interesante para la elaboración de alimentos ricos en fibra, ya que presentaban buenas propiedades funcionales y un alto porcentaje de fibra alimentaria (entre 44,2 y 89,2 g en 100 g de materia seca), con una alta proporción de fibra insoluble.

- La fibra recuperada de la piel de mango puede contribuir también a la suplementación de dietas. Sudhakar y Maini (2000) estandarizaron un método para recuperar de ella una pectina altamente esterificada.

- El concentrado en fibra alimentaria obtenido a partir del subproducto del melocotón se mostró no sólo como una excelente fuente de la misma sino como un ingrediente apropiado para la industria alimentaria, debido a su alta afinidad por el agua (9,12-12,09 g agua/g fibra) y baja energía (3,723-3,494 kcal/g) (Grigelmo-Miguel *et al.*, 1999).

- También el producto de desecho del kiwi es una buena fuente de fibra, con un porcentaje aproximado del 25% referido a materia seca (Martín-Cabrejas *et al.*, 1995).

- La piña, aunque se consume en fresco, mayoritariamente es procesada. El subproducto o material de desecho de este fruto tropical representa hasta un 35% de la materia original. Se han hecho diversos ensayos de aprovechamiento, entre los que figura un estudio para caracterizar su cáscara como una nueva fuente de fibra, habiéndose encontrado que tiene un elevado porcentaje de fibra insoluble (del orden del 70% de la total), compuesta fundamentalmente por azúcares neutros, de los que son mayoritarios la xilosa y la glucosa. Su composición es análoga a la fibra de la pulpa fresca y, además, presenta actividad antioxidante. Por todo ello puede resultar adecuada para suplementar dietas (Salvi y Rajput, 1995; Larrauri *et al.*, 1997a).

- La guayaba tropical (*Psidium guajava*) es una fruta tropical que se produce fundamentalmente en Brasil, Colombia, México y Venezuela. Tiene un pericarpio carnoso y aloja en su interior una pulpa carnosa y numerosas semillas pequeñas. Aunque se consume fresca, también se procesa para su adición a gelatinas, siropes, productos deshidratados, etc. La piel y la pulpa contienen elevadas cantidades de fibra que, al estar asociada a componentes antioxidantes naturales, puede constituir un suplemento dietético importante (Jiménez-Escribano *et al.*, 2001).

- La industria del cacao es muy importante a nivel mundial y del mismo se generan cantidades abundantes de subproducto, fundamentalmente cáscara. Lecumberri *et al.* (2007) vieron que la capacidad antioxidante y propiedades físico-químicas de la fibra obtenida a partir de la cáscara del cacao, la convierten en un ingrediente adecuado de usarse en la preparación de alimentos bajos en calorías y ricos en fibra.

- La aceituna se destina en su mayor parte a la extracción del aceite, quedando un subproducto rico en distintos componentes, entre los que se encuentran el hidroxitirosol, de un gran poder antioxidante (Fernández-Bolaños *et al.*, 1998).

- Finalmente, con relación a los subproductos de espárrago, asumiendo que tienen una composición similar a la de la parte comestible de los turiones, representarían una fuente prometedora de compuestos con alto valor añadido (fitoquímicos y fibra) (Nindo *et al.*, 2003). De hecho los subproductos de espárrago tienen una actividad antioxidante importante y sus extractos secos podrían sustituir a otros antioxidantes en la industria alimentaria y de cosmética (Rodríguez *et al.*, 2005). Sanz *et al.* (2008) al considerar a los subproductos del espárrago ricos en fibra y compuestos bioactivos, enriquecieron el yogur con fibra obtenida de la parte no comestible de los tallos de espárrago.

Por tanto, como se puede observar, los residuos vegetales pueden contener muchas sustancias reutilizables de alto valor. Con el uso de una tecnología adecuada

este material residual se puede revalorizar como fuente de ingredientes funcionales de nuevos productos. Esto es lo que se ha pretendido conseguir en esta Memoria de Tesis, pero en el caso concreto de los subproductos de espárrago.

3. OBJETIVOS

Como OBJETIVOS de esta Memoria de Tesis se enumeran los siguientes:

- GENERALES:

1. La caracterización de componentes funcionales del espárrago verde.

2. El aprovechamiento integral de los subproductos del espárrago mediante procesos simples y de fácil adaptación industrial que permitan obtener ingredientes funcionales a partir de estos residuos de la industria alimentaria.

- ESPECÍFICOS:

1. Caracterización de flavonoides del espárrago triguero de Huétor Tájar.

2. Comparación de la composición en flavonoides del espárrago triguero de Huétor Tájar con la de variedades comerciales de espárrago verde.

3. Obtención de extractos funcionales de subproductos de espárrago.

4. Desarrollo de un sistema de aislamiento y purificación de los distintos componentes bioactivos.

5. Obtención y caracterización de la fibra funcional presente en los subproductos.

4. IDENTIFICATION OF FLAVONOID DIGLYCOSIDES IN SEVERAL GENOTYPES OF ASPARAGUS FROM THE HUÉTOR-TÁJAR POPULATION VARIETY

IDENTIFICATION OF FLAVONOID DIGLYCOSIDES IN SEVERAL GENOTYPES OF ASPARAGUS FROM THE HUÉTOR-TÁJAR POPULATION VARIETY

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Abstract

The qualitative and quantitative composition of flavonoids from the Huétor-Tájar population variety of asparagus (commonly known as "*triguero*") was investigated. Flavonoids were analyzed by reversed-phase high-performance liquid chromatography–diode array detection (HPLC–DAD). Liquid chromatography–mass spectrometry (LC–MS) under identical HPLC conditions was used to verify the identities of the flavonoid glycosides from *triguero* asparagus. The quantities of asparagus flavonoids were calculated according to concentration curves constructed with authentic standards. Total flavonoid contents, calculated as the sum of individual compounds, were determined and ranged from 400 to 700 mg/kg fresh weight. The most abundant was rutin, which represented 55–98% of the total flavonoid complement. *Triguero* asparagus were revealed to be an important source of not only quercetin derivatives but also kaempferol and isorhamnetin glycosides. Significant differences ($p < 0.05$) in the content and relative composition of flavonoids were found among the spears of the distinct asparagus genotypes from the Huétor-Tájar population variety.

4.1. INTRODUCTION

Flavonoids represent the most common and widely distributed group of plant-food phenolics, and their contents and compositions have been related to the antioxidant properties of different fruits and vegetables (Hollman & Katan, 1999; Harborne & Williams, 2000). The beneficial effects of the consumption of vegetables, such as broccoli (Vallejo *et al.*, 2004), spinach (Kuti & Konoru, 2004), onion, and asparagus (Makris & Rossiter, 2001), on human health can, at least partly, be explained by their flavonoid content.

Asparagus is a vegetable that has traditionally been very appreciated for its organoleptic and nutritional characteristics, but this product is also a good source of bioactive compounds that may contribute to enhancing its cultivation and consumption. It has been established that, among the most commonly consumed vegetables, asparagus has the highest antioxidant capacity (Vinson *et al.*, 1998; Pellegrini *et al.*, 2003), and this property is associated to a great extent to its total phenolic content (Salvatore *et al.*, 2005; Rodríguez *et al.*, 2005a).

Scarce information is available regarding asparagus spear phenolic characterization, but we have recently investigated the phenolic profile of both white and green asparagus, and the results revealed that, whereas white spears mainly contained hydroxycinnamic acid derivatives, flavonoids were the major phenolics in green asparagus (Guillén *et al.*, 2007). In agreement with Maeda *et al.* (2005), who reported that rutin represented 60–80% of the total phenolic content of purple and green asparagus extracts, we have found that rutin constituted more than 70% of the total phenolic content of asparagus from commercial hybrids. This flavonoid glycoside has been shown to act as a strong free-radical scavenger and may have a protective role in carcinogenesis and cardiovascular diseases (Harborne & Williams, 2000). The high content of rutin of green asparagus could be directly related to its antioxidant properties. However, other flavonoids that are much more abundant in triguero asparagus than in commercial hybrids (Guillén *et al.*, 2007) may contribute to that activity and have not been characterized yet.

Wang *et al.* (2003) developed a liquid chromatography–mass spectrometry (LC–MS) method for the characterization of the main bioactive compounds, including saponins and flavonoids, in asparagus spears. These authors detected only two flavonoid compounds in asparagus extracts; the major compound was identified as rutin, and the other peak appeared to be a rutin-type flavonoid that should have an extra sugar molecule on its structure.

Several recent studies have been conducted with a focus on the influence of genetics and cultivation area on the phenolic profile of plant foods. Significant differences have been found not only in the total content but also in the flavonoid profile from different varieties of several plant foods, such as strawberries (Aaby *et al.*, 2005), grapes (Cantos *et al.*, 2002), spinach (Bergquist *et al.*, 2005), and Brassica species (Romani *et al.*, 2006; Rochfort *et al.*, 2006).

We have previously reported that the flavonoid profile of green asparagus is determined by sample origin and variety (Guillén *et al.*, 2007). The main objective of the present study was the quantitative determination of the different flavonoid glycosides detected and identified from several genotypes of asparagus from the Huétor-Tájar population variety. Those flavonoids, which are not found in most commercial varieties of green asparagus, were identified and quantified by high-performance liquid chromatography–diode array detection (HPLC–DAD), and LC–MS was used to confirm their structures.

4.2. MATERIALS AND METHODS

4.2.1. Plant material

The samples investigated consisted of spears from 10 native lines of triguero asparagus from the Huétor-Tájar population variety and a sample of commercial green asparagus. Triguero asparagus are tetraploid subspecies that come from wild asparagus, autochthonous to the Huétor-Tájar area. Asparagus spears were harvested from 10 lines of triguero asparagus (HT-1, HT-2, HT-3, HT-4, HT-5, HT-6, HT-7, HT-8, HT-9, and HT-10) from Huétor-Tájar, Granada, Spain, in the spring of 2005 and 2006. These asparagus have been developed and cloned in Las Torres Agricultural Research Center, Alcalá del Río, Sevilla, Spain, during the last year, and they have been classified and selected by their agronomic characteristics. Their chemical characterization, on the basis of flavonoid profiles, may help to establish new criteria for the selection of these triguero asparagus.

The spears investigated in this work were harvested from experimental fields of Huétor-Tájar (Granada, Spain). On harvest day, asparagus spears were transported to the laboratory and then weighed, frozen at −20 °C, and freeze-dried. This plant tissue was ground into a fine powder and stored at −20 °C for further analysis.

4.2.2. Phenolic extraction

Each sample, consisting of 2.5 g of freeze-dried material, was extracted with 100 mL of 80% ethanol (EtOH). The samples were blended in a Sorvall Omnimixer, Model 17106 (Du Pont Co., Newtown, CT), at maximum speed for 1 min, and then filtered through filter paper. Ethanolic extracts were stored at −20 °C until analysis by HPLC. The method was optimized in terms of the extraction of the solvent, sample size, volume, and concentration of ethanol for asparagus spears. All extractions were made in duplicate.

4.2.3. Acid hydrolysis

The free flavonoid aglycones were released by acidic hydrolysis as follows: 2.5 g of freeze-dried material were extracted with 80 mL of 80% EtOH as described above. A total of 20 mL of 6 M HCl was added, and the solution was incubated for 2 h, with constant mixing, at 90 °C. The extract was filtered through filter paper and made to 100 mL with 80% ethanol. The extracts were stored at −20 °C until analysis.

4.2.4. Qualitative analysis of flavonoids by HPLC–DAD

Phenolic compounds were detected and quantified by HPLC using a Synergi 4 μm Hydro-RP80A reverse-phase column (25 cm × 4.6 mm i.d., 4 μm; Phenomenex, Macclesfield, Cheshire, U.K.). The gradient profile for the separation of flavonoids was formed using solvent A [10% (v/v) aqueous acetonitrile plus 2 mL/L acetic acid] and solvent B (40% methanol, 40% acetonitrile, and 20% water plus 2 mL/L acetic acid) in the following program: the proportion of B was increased from 10 to 42.5% B for the first 17 min, then to 70% B over the next 6 min, maintained at 70% B for 3.5 min, then to 100% B over the next 5 min, maintained at 100% B for 5 min, and finally returned to the initial conditions. The flow rate was 1 mL/min, and the column temperature was 30 °C. Phenolic compounds were detected using a Jasco-LC-Net II ADC liquid chromatograph system equipped with DAD and a Rheodyne injection valve (20 μL loop). Spectra from all peaks were recorded in the 200–600 nm range, and the chromatograms were acquired at 360 nm.

4.2.5. Isolation of the new flavonoids identified in triguero asparagus

A HPLC method similar to that described above but using a semipreparative Synergi 4 μm Hydro-RP80A reverse-phase column (25 cm × 46 mm i.d., 4 μm; Phenomenex, Macclesfield, Cheshire, U.K.) was developed for the isolation of the new flavonoids. The flow rate was maintained at 10 mL/min, and the injection volume was 400 μL. Elution was monitored by UV at 360 nm, and the flavonoids were manually collected after the UV detector. The two fractions containing each individual compound were then reinjected onto the analytical column, to purify the two isolated flavonoids. Those were concentrated under nitrogen prior to lyophilization.

4.2.6. Characterization of flavonoids by HPLC–DAD–MS

Rutin and the "new flavonoids" detected in asparagus were separated by HPLC, as described above, and identified by their electron impact mass data collected on a quadrupole mass analyzer (ZMD4, Micromass, Waters, Inc., Manchester, U.K.). Electrospray ionization (ESI) mass spectra were obtained at ionization energies of 50 and 100 eV (negative mode) and 50 eV (positive mode), with MS scans from m/z 100 to 1000. Capillary voltage was 3 kV; desolvation temperature was 200 °C; source temperature was 100 °C; and extractor voltage was 12 V. The flow was maintained at 1 mL min^{-1}.

4.2.7. HPLC–DAD–MS system for quantitative analysis

Flavonoid compound quantification was achieved by integration of peak areas, with reference to calibrations made using known amounts of pure compounds.

Results were calculated from the mean of three replicates. Comparisons among samples were done by the analysis of variation (ANOVA) test and the least-square differences (LSD) method at a 95% confidence level.

4.2.8. Validation of the method of HPLC–DAD–MS for quantitative analysis

Calibration curves were established on 8 data points that covered a concentration range of 5–250 μg/mL for each flavonoid glycoside. The linearity response of rutin, kaempferol-3-O-rutinoside, and isorhamnetion-3-O-rutinoside was determined using standards purchased from Megazyme. Eight concentrations of the mixed standard 80% ethanol solution were injected in duplicate. The calibration curves were constructed by plotting the mean peak area versus the concentration of standards. The limits of detection (LOD) and quantification (LOQ) were determined at a signal-to-noise ratio (S/N) of 3 and 10, respectively.

The precision of the method was evaluated from the measurement of intra- and interday variability. For this purpose, the same mixed standard 80% ethanol solution was analyzed 3 times within the same day and then for 3 consecutive days. The assays

were realized by triplicate, and the relative standard deviation (RSD) was taken as a measure of precision.

A recovery test was used to evaluate the accuracy of the method. Accurate amounts of the three standards were added to 2.5 g of freeze-dried asparagus (plant material) and then extracted and analyzed as described above. The average percentage recoveries were calculated as the ratio of detected amount versus added amount. The recovery experiment was performed with three replicates at two concentration levels.

4.3. RESULTS AND DISCUSSION

4.3.1. Extraction procedure

Prior to achieving the detailed analysis of the phenolic profile of green asparagus, the influence of different process conditions (raw material, solvent type, solvent/solid ratio, simple or sequential extraction, time of extraction) on the phenolics extraction efficiency was studied. The results (Tables 4.1–4.3) showed the following:

1) The yield of total soluble phenolics was equivalent from fresh asparagus and freeze-dried material. The water content of the spears was 90%, and the values of the distinct samples investigated were not significantly different ($p < 0.05$).

2) The yield of phenolics was also equivalent when using methanol or ethanol aqueous solutions and higher than that reached using water as the extraction solvent. It is noteworthy that the highest results were attained when the alcohol concentration was ≥70%.

3) The solvent/solid ratio greatly influenced the phenolic yield. Preliminary assays were conducted by extracting 25 g of fresh sample and 2.5 g of freeze-dried material with 25–50 and 100 mL of 80% ethanol or water, respectively, and the results revealed that the higher the solvent/solid ratio, the higher the total amount of phenolics solubilized. Extraction volumes higher than 100 mL did not increase the amount of phenolics released, which revealed that the optimal solvent/solid ratio was 1:4 (g of fresh sample/mL of ethanol).

4) Sequential extraction of asparagus samples, using 4 × 25 or 4 × 100 mL aliquots of extraction solvent, did not result in a higher yield of phenolics compared to simple extraction with 100 mL of 80% ethanol.

5) In the same way, increasing the time of extraction did not have a positive effect on extraction efficiency, because extraction for 1, 2, and 24 h with constant mixing did not make a significant difference in the amount of solubilized phenolics compared to that obtained by extracting the samples for 1–2 min.

Table 4.1

Effect of the solvent type and solvent/solid ratio on the flavonoid contents extracted from triguero asparagus[a].

	mg/kg fresh weight			
	ratio of 1:1	**ratio of 1:2**	**ratio of 1:4**	**ratio of 1:8**
water	214 ± 15	227 ± 9	252 ± 6	250 ± 9
80% ethanol	233 ± 4	545 ± 8	591 ± 6	594 ± 15
80% methanol	443 ± 6	514 ± 17	583 ± 13	594 ± 9

[a] *Data are the means of three replicates.*

Table 4.2

Effect of sequential extraction on the flavonoid contents extracted from triguero asparagus[a].

	mg/kg fresh weight			
	1st extraction	**2nd extraction**	**3rd extraction**	**4th extraction**
1 × 25 mL of 80% EtOH	233 ± 4			
1 × 100 mL of 80% EtOH	591 ± 6			
4 × 25 mL of 80% EtOH	233 ± 4	155 ± 21	71 ± 5	0
4 × 100 mL of 80% EtOH	591 ± 6	9 ± 1	0	0

[a] *Data are the means of three replicates.*

Table 4.3

Effect of the extraction time on the flavonoid contents extracted from triguero asparagus[a].

	mg/kg fresh weight			
	1 min	**1 h**	**2 h**	**16 h**
1 × 25 mL of 80% EtOH	233 ± 4	237 ± 6	230 ± 4	235 ± 9
1 × 100 mL of 80% EtOH	591 ± 6	588 ± 11	605 ± 8	593 ± 8

[a] *Data are the means of three replicates.*

4.3.2. Characterization of flavonoid glycosides from triguero asparagus

A representative chromatogram of the ethanolic extract from triguero asparagus is shown in Figure 4.1. From the HPLC–DAD data, the major peak was identified as rutin, because its retention time and UV spectrum were identical to those of the rutin standard purchased from Sigma. In addition to rutin, there were two significant peaks on the chromatogram of the ethanolic extract from triguero asparagus (Figure 4.1A). From the HPLC–DAD data, those peaks were tentatively identified as flavonoid glycosides, because the UV spectra from peak 1 (264, 280sh, and 348) and peak 2 (252, 280, and 356) were similar to that from rutin (255, 279sh, and 355). The chromatogram from the ethanolic extract of commercial green asparagus was also recorded (inset of Figure 4.1A), and it consisted of a very prominent peak of rutin, which was only accompanied by one or two other minor peaks of flavonoids that, in many cases, were only detected in trace amounts.

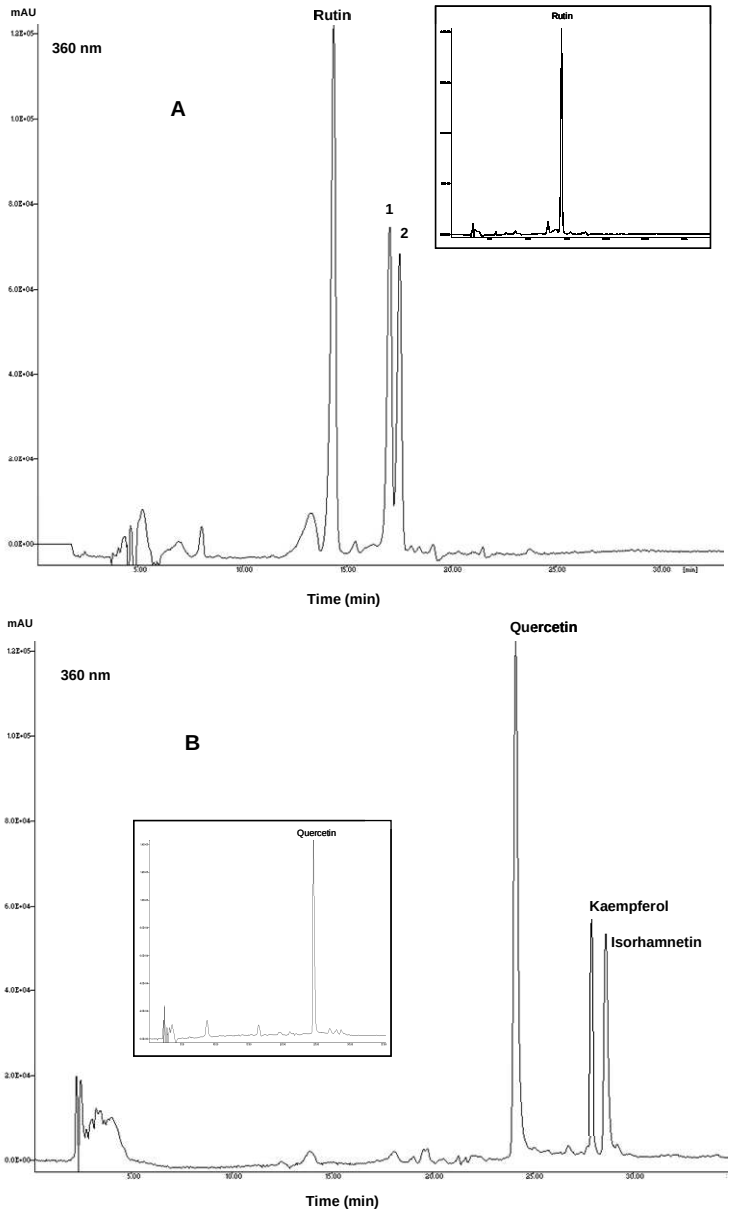
After acid hydrolysis of the triguero and commercial green asparagus ethanolic extracts, three different flavonol aglycones were detected for the former (Figure 4.1B), while a unique peak was found for the latter (inset of Figure 4.1B). The aglycones were identified by a comparison of retention times, DAD information, and co-injection with standards. The results revealed that commercial asparagus only yielded quercetin. Analysis of the hydrolyzate from triguero asparagus revealed that quercetin was the most prominent aglycone as expected, because rutin represented more than 50% of the total flavonoid complement in all samples investigated in this study. However, it has been revealed that triguero asparagus is also a good source of glycosides from kaempferol and isorhamnetin, flavonols that are not found in most varieties of green asparagus. By a comparison of the UV spectra from the new flavonoid glycosides with those from the aglycones, it can be proposed that peak 1 must be a kaempferol (264, 280sh, and 364) derivative, while peak 2 could derive from quercetin (252, 284sh, and 372) or isorhamnetin (252, 284sh, and 368).

Figure 4.1

Chromatographic profiles acquired by HPLC–DAD (360 nm) of the 80% ethanolic extract of triguero asparagus (A) and its acid hydrolyzate (B).

(Insets)

Chromatograpahic profiles acquired by HPLC–DAD (360 nm) of the 80% ethanolic extract of commercial asparagus (A) and its acid hydrolyzate (B).

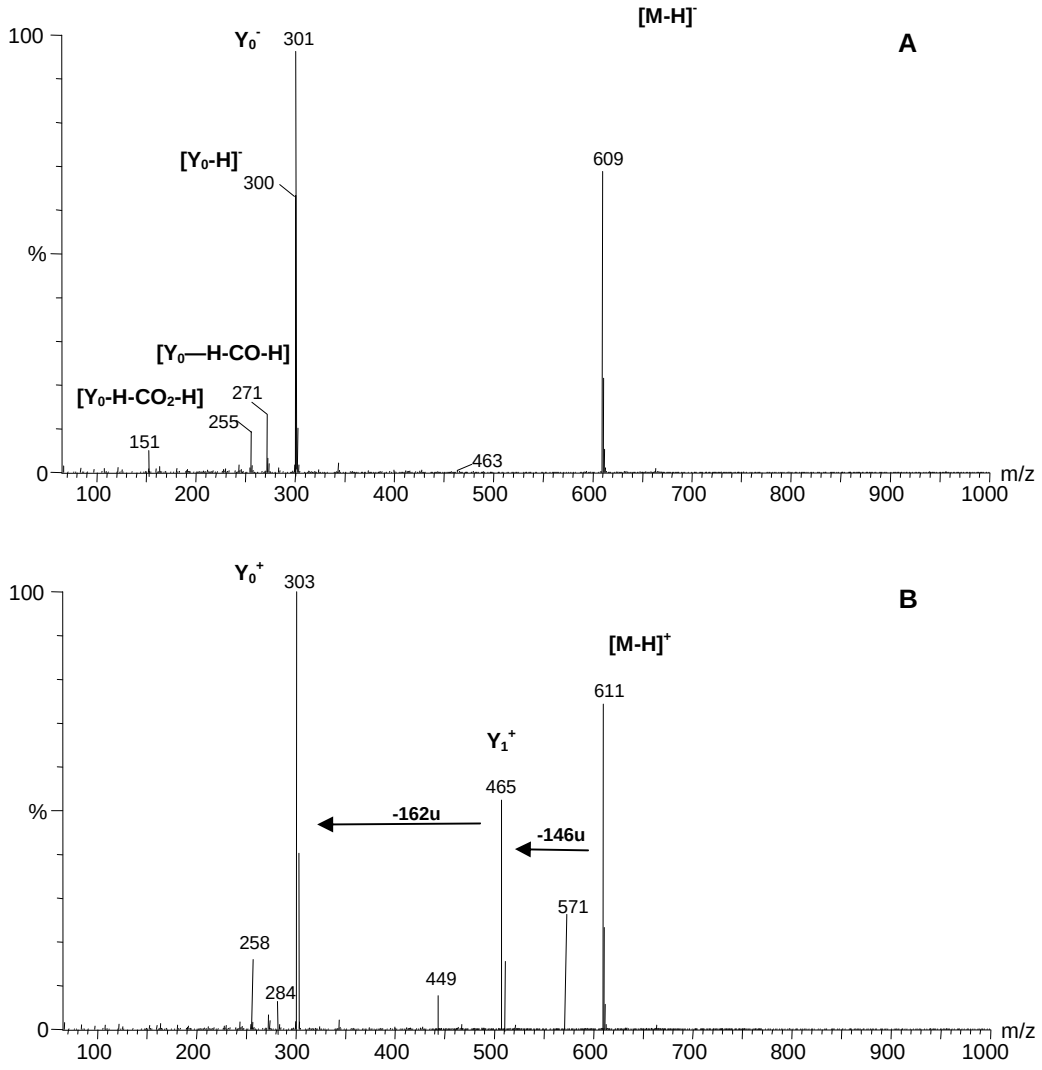


Because UV spectra were not enough to identify the flavonoids, a HPLC–MS method for recording the MS spectra from both aglycones and flavonoid glycosides was developed. The ESI–HPLC–MS analyses allowed the first structure hypotheses to be established. The use of alternating positive/negative ionization modes during recording was preferred to ensure the assignment of the molecular weights.

Figure 4.2A shows the negative-ion MS spectrum of rutin (m/z 609). It can be observed that, apart from the molecular ion, the main product ions were at m/z 301, 300, 271, 255, and 151. As reported previously (Hvattum & Ekeberg, 2003), deprotonated flavonoid-O-glycosides, such as rutin (quercetin-3-O-rhamnoglucoside), provide both a radical aglycone anion $(Y_0 - H)^{-\bullet}$ at m/z 301 and an aglycone product ion (Y_0^-) at m/z 300.

Figure 4.2

ESI spectra of rutin in negative (A) and positive (B) modes.



The $[M - H]^-$ product ion spectra of rutin also reveals an abundant $[Y_0 - H - CO - H]^-$ ion at m/z 271 and a $[Y_0 - H - CO_2 - H]^-$ ion at m/z 255. These ions provide structural information for isomeric differentiation and determination of the glycosylation position (Ablajan *et al.*, 2006), because their presence is indicative of 3-

O-glycosylation, while 7-O-glycosylated flavonoids would provide an abundant $[Y_0 - CO]^-$ ion at m/z 273, which was not detected in this case.

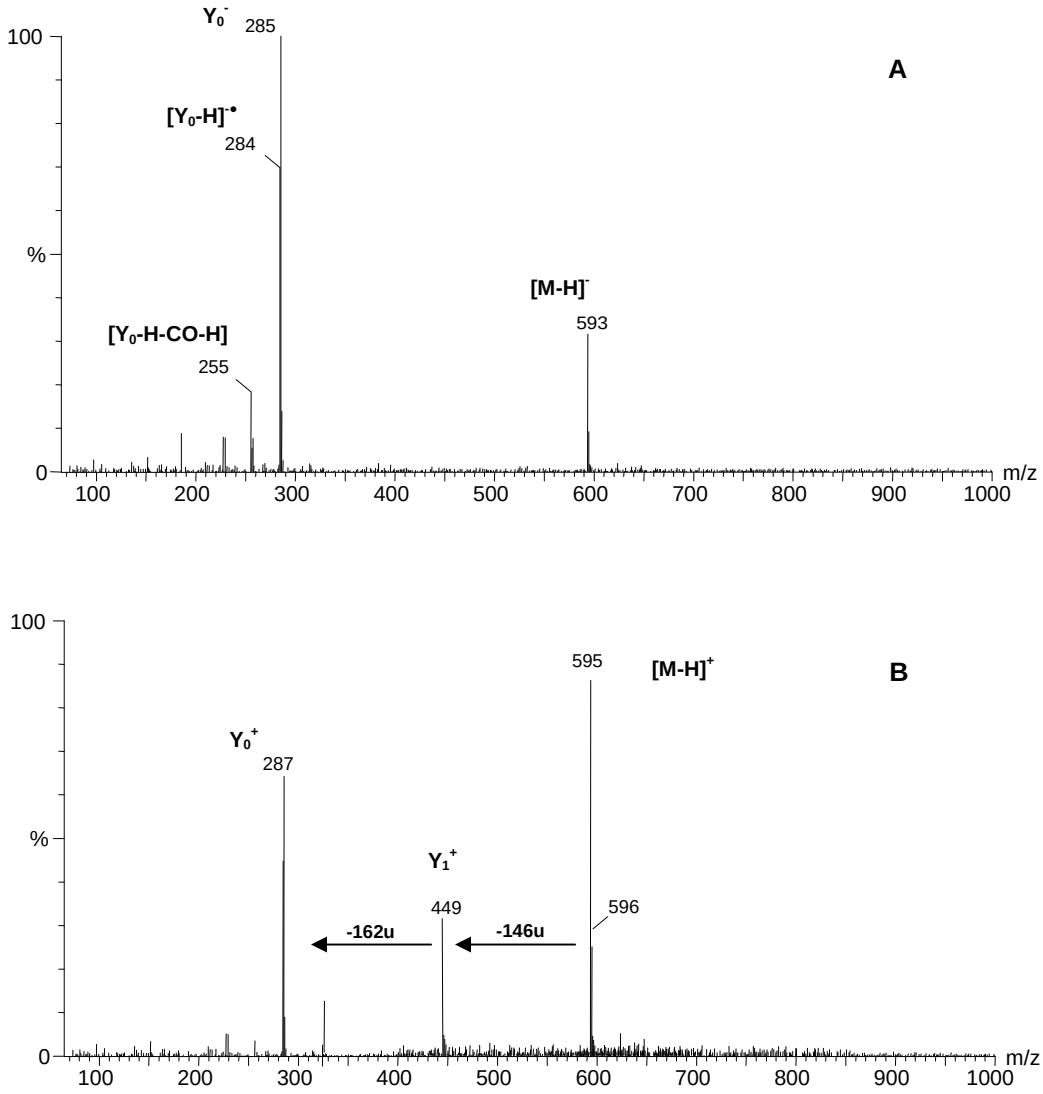
The relative abundances of the Y_0- and $[Y_0 - H]^{-\bullet}$ ions have been proposed to be related to the flavonoid glycosylation position. According to Ferreres *et al.* (2004), the Y_0^- ion was the base peak for the flavonoid O-diglycosides, whereas it was represented about 30% relative abundance for the flavonoid di-O-glycosides. In agreement with these findings, Figure 4.2A shows that rutin, with the two sugars linked in position 3, yielded Y_0^- as the main ion in the negative mode.

The positive-ion spectrum of flavonoid glycosides provides additional and complementary information about structural characteristics, mainly on those aspects related to the position of the sugars. As observed in Figure 4.2B, the ESI spectra of the $[M - H]^+$ ion of rutin, at m/z 611, showed two main product ions corresponding to two successive losses of sugar residues. The first loss corresponded to a rhamnose residue (146 units), yielding the Y_1^+ ion at m/z 465, and this was followed by the elimination of glucose (162 units), giving the Y_0^+ ion at m/z 303.

On the basis of the hypothesis illustrated above by the characterization of rutin with the application of ESI–MS techniques in positive- and negative-ion modes, it has been possible to determine the tentative structures of the two new flavonoid diglycosides detected in triguero asparagus. The MS analysis of compound 1 showed a molecular ion at m/z 593 (Figure 4.3). Its $[M - H]^-$ product ion spectrum gave rise to the Y_0- [M – H – 308] at m/z 285 as the base peak and also revealed an abundant $[Y_0 -$ H – CO – H] ion at m/z 255. This fragmentation pattern indicates that compound 1 is a kaempferol glycosylated with two sugar residues, consisting of a hexose, likely glucose (162 units), and a deoxyhexose, likely rhamnose (146 units). According to Ferreres *et al.* (2004), the flavonoid diglycosides with sugar moieties linked to different phenolic positions of the flavonoid nucleus provided a Y_1^- [M – H – 162] ion, which is formed by a loss of a glucosyl from the $[M - H]^-$ as the base peak, and the Y_0^- represented

Figure 4.3

ESI spectra of kaempferol diglycoside in negative (A) and positive (B) mode.



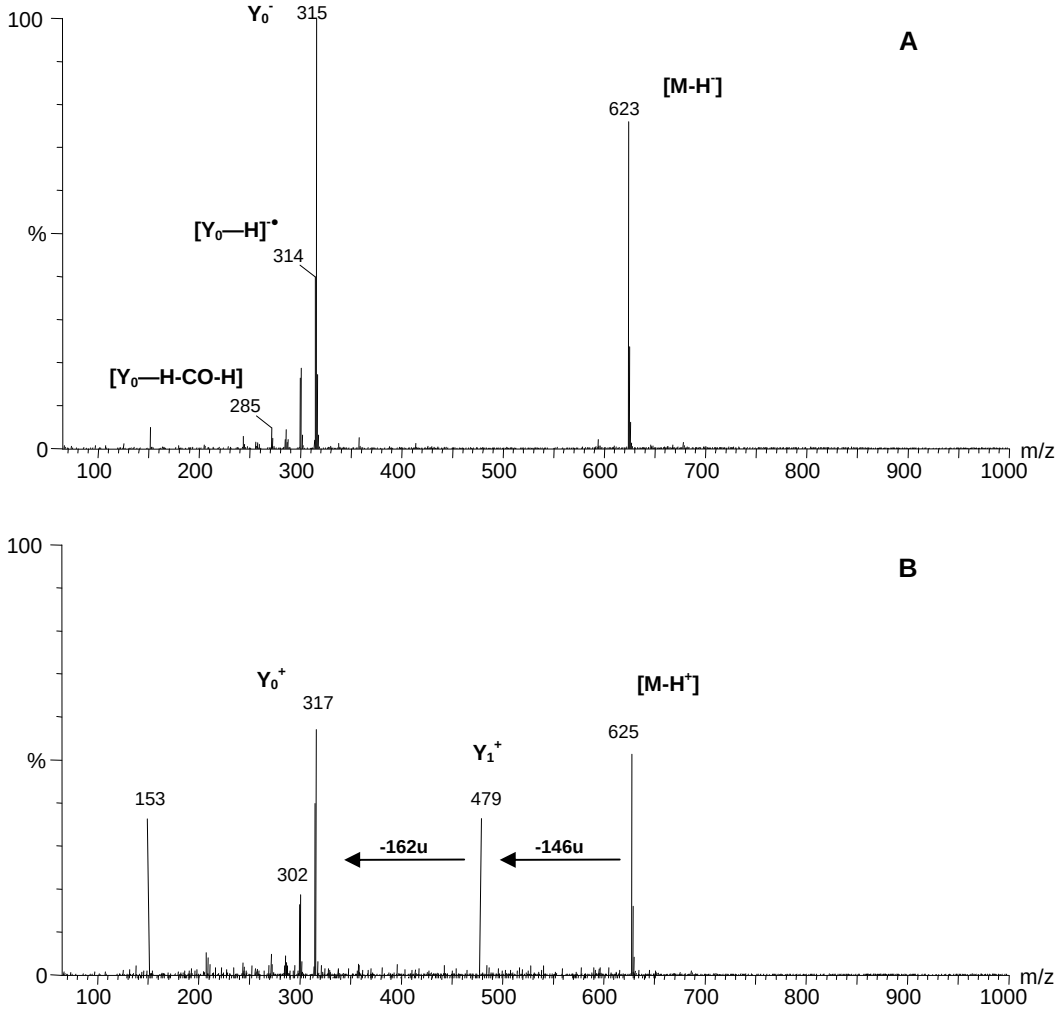
around 30% relative abundance, whereas the flavonoid diglycosides with two sugar moieties linked to the same phenolic position yielded the aglycone ion product (Y_0^-) as the base peak. As described above for rutin, an aglycone product ion is the base peak for compound 1, because it can be observed in its negative-ion spectrum (Figure 4.3A). It has also been established that the glycosylation position significantly influences the fragmentation behavior of flavonoid O-glycosides and has been shown to affect the relative abundances of radical aglycone ions, which are most pronounced for flavonol 3-O-glycosides. The presence of an abundant $[Y_0 - H]^{-\bullet}$ ion at m/z 284 and a significant $[Y_0 - H - CO - H]$ ion at m/z 255 supports the fact that the rhamnose and glucose residues are located at the 3-O positions. In addition to these findings, the fact that the

characteristic $[Y_0 - CO]^-$ ion of the 7-O-glycosides was not detected is consistent with the two sugar residues being located at the 3-O position. Detailed analysis of the positive-ion MS spectrum allowed for the confirmation of the nature and position of the sugars linked to this kaempferol derivative. The ESI spectrum of the $[M - H]^+$ ion at m/z 595 from compound 1 (Figure 4.3B) showed two main product ions, indicating two losses of sugar residues. The first loss corresponded to a rhamnose (146 units), giving Y_1^+ at m/z 449, and then the loss of glucose (162 units) yielded the Y_0^+ ion at m/z 287, which was assigned as protonated kaempferol. These results are in agreement with the fragmentation behavior of a 3-O-rutinoside flavonol, which, in this case, would be the kaempferol-3-O-rutinoside. This compound, known as nicotiflorin, has not been previously detected in asparagus, but its presence has been reported in other plant food, such as quince fruit (Silva *et al.*, 2002).

The mass spectra of compound 2 revealed that this was also a flavonoid diglycoside, whose fragmentation pattern was similar to that of rutin and compound 1. Figure 4.4 shows the negative and positive spectra of compound 2. After ensuring the assignment of its molecular weight (623), as well as that of the corresponding aglycone (315), by analyzing the fragments issued from the $[MH]^-$ ion at m/z 623 (Figure 4.4A), the nature and position of the two sugar residues present in this compound were determined from the information generated by the fragmentation of the $[M - H]^+$ ion at m/z 625 (Figure 4.4B). Following the premises established for the characterization of compound 1, the other flavonoid detected in triguero asparagus has been tentatively assigned as isorhamnetin-3-O-rutinoside. This compound, which is also present in significant quantities in the triguero cultivars investigated in this work, had not been previously described in asparagus, because it is not present in the green spears from commercial hybrids. To our knowledge, almonds are the unique plant food that contain isorhamnetin-3-O-rutinoside as a predominant flavonoid (Milbury *et al.*, 2006).

Figure 4.4

ESI spectra of isorhamnetin diglycoside in negative (A) and positive (B) mode.



The identities of kaempferol-3-O-rutinoside and isorhamnetin-3-O-rutinoside were confirmed by injection of authentic standards purchased from Extrasynthese. Authentic compounds were injected alone and with their corresponding flavonoids isolated from triguero asparagus extracts. Retention times and UV and MS profiles were the same for pure kaempferol-3-O-rutinoside and isorhamnetin-3-O-rutinoside as for those of peaks 1 and 2.

4.3.3. Validation of the method for quantitative analysis of flavonoids from triguero asparagus

To ensure the accurate assessment of the contents of the three flavonoid glycosides found in triguero asparagus, the HPLC–DAD–MS method was validated

prior to its application for the quantitative analysis of different asparagus cultivars. The calibration curves of rutin, nicotiflorin, and narcissin showed good linear regression within test ranges, as observed in Table 4.4. The limit of detection, defined as the lowest sample concentration that can be detected (signal-to-noise-ratio = 3), was 4.61 μg/mL for rutin, 1.89 μg/mL for nicotiflorin, and 2.90 μg/mL for narcissin, and the limit of quantification, defined as the lowest sample concentration that can be quantitatively determined with suitable precision and accuracy (signal-to-noise ratio = 10), was 15.39 μg/mL for rutin, 6.31 μg/mL for nicotiflorin, and 9.67 μg/mL for narcissin. As shown in Table 4.5, the developed analytical method provided good precision and stability, because the overall intra- and interday variations were less than 4.1% for all flavonoids. To test the recovery of the method, one asparagus sample was added to known quantities of each of the reference flavonoids. The samples were analyzed before and after the additions in triplicate. Results showed that 105% of rutin, 93% of kaempferol-3-O-rutinoside, and 95% of isorhamnetin-3-O-rutinoside were recovered (Table 4.5).

Table 4.4

Calibration curves, LODs, and LOQs of the three flavonoid diglycosides[a].

flavonoid diglycoside	calibration curve	r^2	test range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
rutin	y = 25 074x + 126 136	0.9978	25–250	4.61	15.39
k-3-O-rutinoside	y = 27 162x − 55 839	0.9996	5–250	1.89	6.31
i-3-O-rutinoside	y = 29 329x + 32 576	0.9991	5–250	2.90	9.67

[a] *Data are the means of three replicates.*

Table 4.5

Precisions and recoveries of the three flavonoid diglycosides[a].

flavonoid diglycoside	precision		recovery (n = 3)	
	intraday RSD (%)	interday RSD (%)	recovery (%)	RSD (%)
rutin	0.45	0.46	104.93	2.80
k-3-O-rutinoside	0.42	1.33	92.97	4.09
i-3-O-rutinoside	0.43	0.54	95.94	1.67

[a] *Data are the means of three replicates.*

Analytical characteristics of the calibration graphs as well as the precision and accuracy of the method were satisfactory and comparable to those reported by other authors that have developed HPLC–DAD–MS analytical methods for the determination

of flavonoid compounds from several plant materials (Escarpa *et al.*, 2000; Lu *et al.*, 2006; Qian *et al.*, 2007). It can be concluded that the recommended method is reliable and accurate for the qualitative and quantitative determination of flavonoid diglycosides from asparagus.

4.3.4. Quantification of flavonoids from 10 varieties of triguero asparagus

The main flavonoid glycosides found in all of the asparagus varieties were rutin, kaempferol-3-O-rutinoside, and isorhamnetin-3-O-rutinoside, but significant differences were found in the total quantities and the relative compositions of the distinct asparagus varieties (Table 4.6). The flavonoid content of the 10 varieties investigated varied between 400 and 700 mg/kg fresh weight, which was within the range of the values reported for green asparagus from commercial varieties (Wang *et al.*, 2003; Sun *et al.*, 2007). Rutin has been reported as the main phenolic compound in green asparagus, and it represents more than 80% of the total phenolic complement of commercial hybrids (Maeda *et al.*, 2005; Guillén *et al.*, 2007). Therefore, this has been described as the main flavonoid related to the antioxidant activity of ethanolic extracts from different varieties of green asparagus (Fuleki, 1999; Maeda *et al.*, 2005; Sun *et al.*, 2007), and although the presence of other related flavonoids accompanying rutin has recently been reported (Wang *et al.*, 2003), they have not been characterized yet. As observed in Table 4.6, there are several varieties of triguero asparagus, such as HT-1, HT-2, and HT-8, whose flavonoid profiles are similar to that found in commercial hybrids (Figure 4.1A). However, there is another group of triguero varieties, such as HT-5, HT-6, and HT-9, in which kaempferol-3-O-rutinoside and isorhamnetin-3-O-rutinoside represent nearly 50% of the total flavonoid content. Fuleki (1999) has reported that rutin is the only flavonoid present in green asparagus spears, and this author suggested that other minor peaks detected in the chromatogram from asparagus methanolic extracts were impurities, because most of them were even detected in commercial rutin used as a standard. From the results of the present paper, it can be proposed that some of those peaks did not correspond to impurities but to the kaempferol-3-O-rutinoside and isorhamnetin-3-O-rutinoside, which are present in significantly greater quantities in triguero asparagus than other green varieties. These findings may explain why these compounds had not been previously described in green asparagus, because of the fact they are not found or are present in very low quantities in most commercial varieties.

Table 4.6

Triguero asparagus flavonoids identified by HPLC–DAD and HPLC–MS[a].

asparagus line	mg/kg fresh weight						
	rutin	(%)	k-3-O-rutinoside	(%)	i-3-O-rutinoside	(%)	total flavonoids
HT-1	336±15	(83)	5±0	(1)	66± 0	(16)	407±13
HT-2	476±30	(86)	13±1	(2)	65± 1	(12)	553±44
HT-3	332±16	(69)	39±4	(8)	111±18	(23)	481±37
HT-4	401±13	(78)	6±0	(1)	108± 3	(21)	515±16
HT-5	382±28	(55)	108±2	(16)	203±16	(29)	692±47
HT-6	368±15	(67)	35±1	(6)	147± 2	(27)	549±14
HT-7	498±12	(72)	34±1	(5)	162± 4	(23)	694±18
HT-8	548± 8	(97)	5±0	(1)	009± 4	(2)	562± 5
HT-9	230± 5	(55)	31±1	(7)	157± 3	(38)	418± 9
HT-10	411± 4	(75)	42±2	(8)	97± 4	(18)	549± 6

[a] *Data are the means of three replicates. Data in parentheses represent the relative percent.*

A recent study about the characterization of antioxidant components of some Italian edible wild greens (Salvatore *et al.*, 2005) showed that wild asparagus species, such as Asparagus acutifolius, have a considerable antioxidant capacity that seems to be related to their flavonoid content. The detailed analysis of their flavonoid profiles revealed that, apart from rutin and other quercetin glycosides, those asparagus contain significant amounts of kaempferol and isorhamnetin derivatives. Because the samples were analyzed after acidic hydrolysis, just the aglycones were determined and flavonoid content was quantified as quercetin equivalents, because that was the most abundant aglycone. These data are in agreement with our results and support the fact that triguero asparagus may come from wild species distinct from Asparagus officinalis, which gives them a characteristic phytochemical profile that can be used for differentiating and revalorizating native asparagus cultivars. It has been established that the bioactive properties of flavonoid compounds are dependent upon their structure and that minor differences in the number and position of –OH and sugar residues linked to the flavonol skeleton may lead to great differences in the bioactive properties of the individual compounds (Seyoum *et al.*, 2006). Thus, deeper studies on the isolation and structural characterization of flavonoids and other bioactive compounds from triguero asparagus will establish relationships between individual components and specific beneficial actions associated with asparagus, mainly derived from its antioxidant capacity.

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5. FLAVONOID PROFILE OF GREEN ASPARAGUS GENOTYPES

FLAVONOID PROFILE OF GREEN ASPARAGUS GENOTYPES

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Abstract

The determination of flavonoid profiles from different genotypes of *triguero* asparagus and their comparison to those from green asparagus commercial hybrids was the main goal of this study. The samples consisted of 32 commercial hybrids and 65 genotypes from the Huétor-Tájar population variety (*triguero*). The analysis of individual flavonoids by HPLC-DAD-MS has allowed the determination of eight naturally occurring flavonol derivatives in several genotypes of *triguero* asparagus. Those compounds included mono-, di-, and triglycosides of three flavonols, that is, quercetin, isorhamnetin, and kaempferol. The detailed analysis of the flavonoid profiles revealed significant differences among the distinct genotypes. These have been classified in three distinct groups as the result of a *k*-means clustering analysis, two of them containing both commercial hybrids and *triguero* asparagus and another cluster constituted by 21 genotypes of *triguero* asparagus, which contain several key flavonol derivatives able to differentiate them. Hence, the triglycosides tentatively identified as quercetin-3-rhamnosyl-rutinoside, isorhamnetin-3-rhamnosyl-rutinoside, and isorhamnetin-3-*O*-glucoside have been detected only in the genotypes grouped in the above-mentioned cluster. On the other hand, the compound tentatively identified as isorhamnetin-3-glucosyl-rutinoside was present in most genotypes of triguero *asparagus*, whereas it has not been detected in any of the commercial hybrids.

5.1. INTRODUCTION

Asparagus known as triguero are tetraploid subspecies from Huétor-Tájar, Granada, which proceed from wild species that have traditionally been collected and consumed throughout southern Spain. These green-purple asparagus are very appreciated for their organoleptic and nutritional properties, but their cultivation is being replaced by commercial hybrids, which yield more homogeneous production and spears of greater caliber.

Because health-promoting characteristics in food are increasingly demanded and included in the purchase decision by the discriminating consumer, the investigation of the bioactive compounds responsible of the beneficial effects associated with a specific product is of great interest for its revalorization.

Experimental evidence has demonstrated that each plant species is characterized by a limited number of phytochemicals; and within the same species, the nature of those compounds can vary from organ to organ but is constant enough toward several other factors (Proestos *et al.*, 2006; Lako *et al.*, 2007). Therefore, the characterization of fruits and vegetables can be made from their phytochemical profile, and it may be used, for example, to differentiate varieties of a plant food (Martínez-Sánchez *et al.*, 2007; Mertz *et al.*, 2007; Slimestad *et al.*, 2007).

We have previously reported that there is high correlation between antioxidant capacity and total phenol content in triguero asparagus (Rodríguez *et al.*, 2005a), which suggests that phenols could be mainly responsible for that activity as happens for other plant-derived products (Gardner *et al.*, 2000; Gil *et al.*, 2000; Wang & Lin, 2000; Gorinstein *et al.*, 2004). It has also been established that flavonoids are the most abundant phenolics in green asparagus and that their profile is significantly different from that found in green spears from commercial hybrids (Guillén *et al.*, 2008; Fuentes-Alventosa *et al.*, 2007). The composition of flavonoids in plants is influenced by both genetic factors and environmental conditions. The former seem to be the most determinant factor because significant differences were found between triguero native spears and commercial hybrids both cultivated in Huétor-Tájar (Fuentes-Alventosa *et al.*, 2007). The determination of the flavonoid profiles from different genotypes of

triguero asparagus and their comparison to those from green asparagus hybrids developed in recent years by major international asparagus programs was the main goal of this study. The chemical characterization of those native spears may allow new criteria to be established for selection and contribute to the promotion of the cultivation and consumption of a very high quality product that is still poorly known.

5.2. MATERIALS AND METHODS

5.2.1. Plant material

The samples investigated consisted of spears from 32 green asparagus hybrids developed in recent years by major international asparagus programs and 65 different native lines of triguero asparagus from the Huétor-Tájar population variety. The first were cultivated in Las Torres Agricultural Research Center, Alcalá del Río, Sevilla, Spain, and the triguero asparagus samples were collected from Huétor-Tájar, Granada, Spain.

The asparagus samples were collected over a 2 year period and from experimental fields under controlled conditions. The spears were harvested at the same point of the harvest period (april−may 2005 and 2006), cut to the same length, and kept under refrigerated conditions (T^a = 4 °C) from the field to the laboratory to minimize the influence of environmental and storage conditions on the flavonoid profile.

Prior to being analyzed, the spears were washed with sodium hypochlorite solution (50 ppm of active Cl_2) and cut to a distance of 20 cm from the tip. Asparagus samples were weighed, frozen at −20 °C, and freeze-dried. This plant tissue was ground into a fine powder and stored at −20 °C for further analysis.

5.2.2. Chemicals and reagents

Authentic standards of quercetin (Q), kaempferol (K), isorhamnetin (IR), and rutin (quercetin 3-O-rutinoside) were purchased from Sigma-Aldrich Quimica (Madrid, Spain); kaempferol-3-O-rutinoside (nicotiflorin), isorhamnetin 3-O-rutinoside (narcissin), and isorhamnetin 3-O-glucoside were purchased from Extrasynthese (Genay, France).

All solvents were of HPLC grade purity (Romyl, Teknokroma, Barcelona, Spain). All sample solutions were prepared using Milli-Q water.

5.2.3. Flavonoids extraction

Phenolic compounds, mainly flavonoids, were extracted as described in Fuentes-Alventosa *et al.* (2007). Each sample, consisting of 2.5 g of freeze-dried material, was extracted with 100 mL of 80% ethanol (EtOH). The samples were blended in a Sorvall Omnimixer, model 17106 (DuPont Co., Newtown, CT), at maximum speed for 1 min and then filtered through filter paper. Ethanolic extracts were stored at −20 °C until analysis by HPLC. All extractions were made in duplicate.

5.2.4. Acid hydrolysis

The free flavonoid aglycones were released by acidic hydrolysis as follows: 2.5 g of freeze-dried material was extracted with 80 mL of 80% EtOH as described above. Twenty milliliters of 6 M HCl was added, and the solution was incubated for 2 h, with constant mixing, at 90 °C. The extract was filtered through filter paper and made up to 100 mL with 80% ethanol. The extracts were stored at −20 °C until analysis.

5.2.5. HPLC-DAD analysis

Analyses of flavonoids were carried out using a Jasco-LC-Net II ADC liquid chromatograph system equipped with a diode array detector (DAD). Flavonoid compounds were separated by using a Synergi 4 μm Hydro-RP80A reverse phase column (25 cm × 4.6 mm i.d., 4 μm particle size; Phenomenex, Macclesfield, Cheshire, U.K.). The gradient profile for the separation of flavonoids was formed using solvent A [10% (v/v) aqueous acetonitrile plus 2 mL/L acetic acid] and solvent B (40% methanol, 40% acetonitrile, 20% water plus 2 mL/L acetic acid) in the following program: the proportion of B was increased from 10 to 42.5% B for the first 17 min, then to 70% B over the next 6 min, maintained at 70% B for 3.5 min, then to 100% B over the next 5 min, maintained at 100% B for 5 min, and finally returned to the initial conditions. The flow rate was 1 mL/min, and the column temperature was set at 30 °C. Spectra from all peaks were recorded in the 200−600 nm range, and the chromatograms were acquired at 360 nm for flavonoid glycosides and at 370 nm for their aglycones.

5.2.6. Isolation of the new flavonoids identified in green asparagus

A HPLC method similar to that described above, but using a semipreparative Synergi 4 μm Hydro-RP80A reverse phase column (25 cm × 46 mm i.d., 4 μm; Phenomenex), was developed for the isolation of the new flavonoids. The flow rate was maintained at 10 mL/min, and the injection volume was 400 μL. Elution was monitored by UV at 360 nm, and the flavonoids were manually collected after the UV detector. The four fractions containing each individual compound were then re-injected onto the analytical column to purify the four isolated flavonoids. Those were concentrated under nitrogen prior to lyophilization.

5.2.7. Characterization of flavonoids by HPLC-DAD-MS

Flavonoid glycosides detected in green asparagus were separated by HPLC as described above and identified by their electron impact mass data collected on a quadrupole mass analyzer (ZMD4, Micromass, Waters Inc., Manchester, U.K.). Electrospray ionization (ESI) mass spectra were obtained at ionization energies of 50 and 100 eV (negative mode) and 50 eV (positive mode), with MS scans from m/z 100 to 1000. Capillary voltage was 3 kV, desolvation temperature was 200 °C, source temperature was 100 °C, and extractor voltage was 12 V. The flow was maintained at 1 mL min^{-1}.

5.2.8. Identification and quantification of individual flavonoids

Quantitative evaluation of flavonoid content was carried out as described by Fuentes-Alventosa *et al.* (2007). Identification of individual flavonoid glycosides was carried out using their retention times and both spectroscopic and mass spectrometric data. Quantification of individual flavonoid monoglycosides and flavonoid diglycosides was directly performed by HPLC-DAD using an eight-point regression curve in the range of 0−250 μg on the basis of standards. When standards were not available, as in the case of the new flavonid triglycosides described in the present work, quantification was based on an average value for that class of compound, because responses were essentially similar within classes. Results were calculated from the mean of three

replicates. Comparisons among samples were done by the ANOVA test and the LSD method at 95% confidence level.

5.3. RESULTS AND DISCUSSION

5.3.1. Determination of flavonoids from green asparagus

The separation of flavonol glycosides in a triguero asparagus ethanolic extract is presented in Figure 5.1. As can be seen, the analytical method allowed the determination of eight flavonol glycosides. From HPLC-DAD data, all flavonoids are glycosylated derivatives of three flavonols, that is, quercetin (252, 267sh, 372), kaempferol (264, 296sh, 364), and isorhamnetin (252, 268sh, 368). No free aglycones were detected in the ethanolic extracts from triguero asparagus, but acid hydrolysis of the samples confirmed that triguero asparagus flavonoids are derivatives of three different aglycones, quercetin (Q) being the major flavonol, followed by isorhamnetin (IR) and kaempferol (K) (Figure 5.2). The identities of these aglycones were confirmed by the comparison of the HPLC-DAD-MS data of the hydrolysate to those of commercial standards.

Figure 5.1

Chromatograpahic profile acquired by HPLC-DAD (360 nm) of a triguero asparagus ethanolic extract.

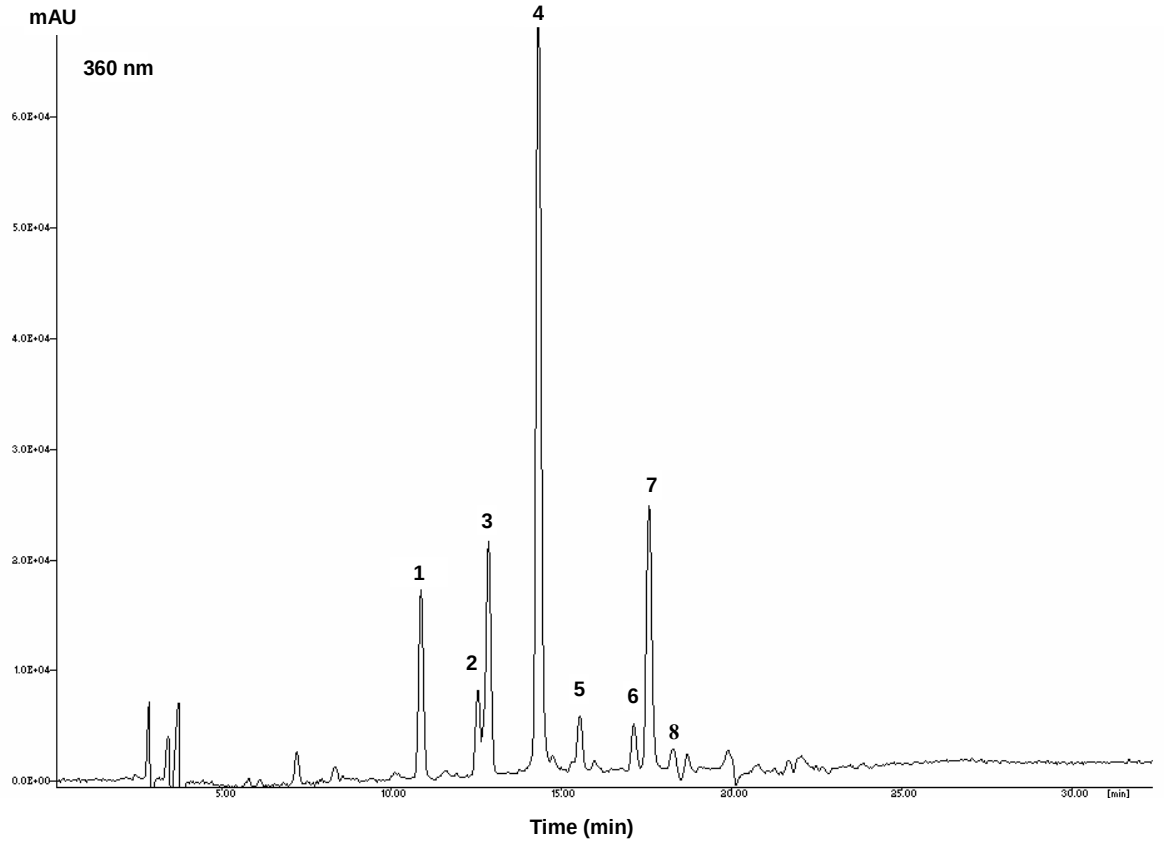
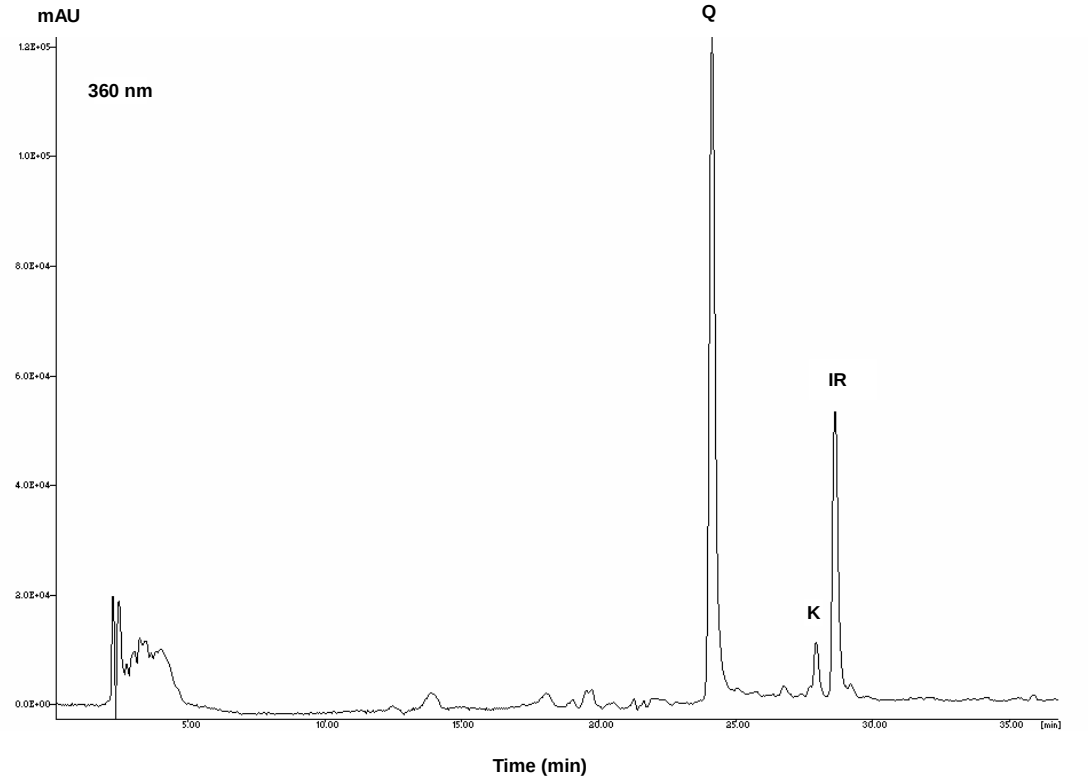


Figure 5.2

HPLC-DAD chromatogram at 370 nm of the hydrolysate extract of triguero asparagus.



Peaks 4, 6, 7, and 8 were respectively identified as rutin (quercetin-3-O-rutinoside), nicotiflorin (kaempferol-3-O-rutinoside), narcissin (isorhamnetin-3-O-rutinoside), and isorhamnetin-3-O-glucoside, on the basis of their spectral characteristics and comparison to standards. The above three flavonol diglycosides had been previously described in triguero asparagus (Fuentes-Alventosa *et al.*, 2007), and the isorhamnetin-3-O-glucoside is the first flavonol monoglycoside detected in green asparagus. This compound has been described in other plant tissues, such as grapes (Dopico-García *et al.*, 2007), calendula (Matysik *et al.*, 2005), and turnip tops (Romani *et al.*, 2006).

The four flavonol triglycosides, which had not been previously described in asparagus, were tentatively identified by means of a combination of the retention times (t_R), UV, and mass spectra obtained by HPLC-DAD-MS. Peaks 1 and 2 were tentatively identified as quercetin derivatives and peaks 3 and 5 as isorhamnetin derivatives.

As the UV spectra from all of the flavonol glycosides are very similar, they were not very useful for identification. MS fragmentation patterns of these compounds were

used to obtain more information about their molecular masses and structural characteristics. Table 5.1 shows the values obtained for each of the detected compounds (numbered from 1 to 8 following their retention times).

Table 5.1

Peak numbers, retention times (t_R), assigned structures, UV, and main ion species observed during HPLC-DAD-MS analysis of the flavonoids from green asparagus.

peak	t_R (min)	structure	UV (nm)	$[M-H]^-$ (m/z)	$[M-H]^+$ (m/z)	ions (ESI^+) (m/z)
1	10.9	Q-triglycoside	255, 267sh, 352	755	757	611, 465, 303
2	12.6	Q-triglycoside	256, 267sh, 354	771	773	627, 481, 303
3	12.9	IR-triglycoside	254, 268sh, 355	769	771	625, 479, 317
4	14.4	Q-3-O-rhamnoglucoside (rutin)	255, 267sh, 355	609	611	465, 303
5	15.5	IR-triglycoside	256, 268sh, 347	785	787	625, 463, 317
6	17.1	K-3-O-rhamnoglucoside (nicotiflorin)	264, 296sh, 348	593	595	449, 287
7	17.6	IR-3-O-rhamnoglucoside (narcissin)	252, 268sh, 356	623	625	479, 317
8	18.6	IR-3-O-glucoside	254, 268sh, 354	477	479	317

The MS negative ion mode spectrum of compound 1 showed a deprotonated molecular ion at m/z 755 and an ion at m/z 301 corresponding to the deprotonated aglycone, which showed that this compound is a triglycosylated quercetin. It is well established that most flavonol derivatives from plant tissues are 3-O-glycosylated and/or 7-O-glycosylated flavonoids and that they are usually linked to glucose and rhamnose residues (Ferreres *et al.*, 2004; Hvattum & Ekeberg, 2003; Ablajan *et al.*, 2006). The fact that the deprotonated ion from the aglycone was the base peak is indicative that the sugars are linked only at the 3 position (Ferreres *et al.*, 2004). The positive ion spectrum of flavonoid glycosides provided additional information about the sugars linked to the flavonol structures. The ESI spectra of the $[M + H]^+$ ion of compound 1 at m/z 757 showed two main product ions corresponding to three successive losses of sugar residues. The first loss corresponded to a rhamnose residue (146 u), yielding the major ion at m/z 611. Ion m/z 611 then decomposed into another prominent ion at m/z 465, resulting from the loss of a second unit of rhamnose (146 u). Finally, the loss of a glucose residue (162 u) generated the major ion at m/z 303,

corresponding to the protonated aglycone (quercetin). This fragmentation pattern could be compatible with a quercetin-3-rhamnosyl-rutinoside.

The MH^- spectrum of compound 2 showed a deprotonated molecular ion at m/z 771 and an ion at m/z 301 corresponding to the deprotonated aglycone, which showed that this is another quercetin triglycoside. Data from the positive ion spectrum suggested that this flavonoid derivative contained a rhamnose (146 u), the loss of which gave rise to the ion at m/z 627, and two residues of glucose (−324 u), the loss of which yielded a characteristic fragment ion of the protonated aglycone (quercetin) at m/z 303. These results suggest that compound 2 is a quercetin-3-glucosyl-rutinoside.

The MH^- spectrum of compound 3 gave a deprotonated molecular ion at m/z 769 and an ion at m/z 315 corresponding to the deprotonated aglycone (isorhamnetin). Complementary information from the positive ion spectrum revealed that the fragmentation pattern to this flavonoid derivative was similar to that from compound 1, but the aglycone was isorhamnetin instead of quercetin. Compound 3 was tentatively identified as isorhamnetin-3-rhamnosyl-rutinoside.

The MH^- spectrum of compound 5 showed a deprotonated molecular ion at m/z 785 and an ion at m/z 315 corresponding to a deprotanated aglycone. On the other hand, according to the MH^+ data, this flavonoid followed the same fragmentation pattern explained above for compound 2, but the aglycone is isorhamnetin instead of quercetin. It can be proposed that compound 5 is an isorhamnetin triglycoside with two glucoses and a rhamnose residue linked to the flavonol. This compound could be isorhamnetin 3-glucosyl-rutinoside.

On the other hand, it has been reported that the chromatographic behavior of flavonoid compounds under reversed-phase HPLC shows that, in the same conditions, a higher degree of glycosylation leads to a shorter retention time (Llorach *et al.*, 2003). Our results are in consonance with these findings, because peaks 1 (t = 10.9 min) and 2 (t_R = 12.6 min), which have been tentatively identified as quercetin triglycosides, elute earlier than rutin (t_R = 14.4 min). A similar behavior has been observed for the isorhamnetin derivatives. Therefore, peaks 3 (t_R = 12.9 min) and 5 (t_R = 15.5 min), tentatively identified as isorhamnetin-triglycosides, elute earlier than peak 7 (t_R = 17.6

min), which has been identified as isorhamnetin-rutinoside. Finally, the unique flavonoid monoglycoside detected in asparagus samples, identified as isorhamnetin-glucoside, was the last compound eluted from the column (t_R = 18.6 min).

The structures of the four new flavonol triglycosides described in this study were tentatively assigned on the basis of t_R, UV, and MS data, but further data are needed for a complete structural identification. Thus, the above four flavonoid triglycosides have been isolated and purified, and their analysis by RMN techniques will make it possible to establish the precise position of the sugars within the flavonoid molecule.

5.3.2. Quantitative analysis of flavonoids from several asparagus cultivars

Flavonoid contents of the 32 commercial hybrids of green asparagus are shown in Table 5.2. The flavonoid content of these varieties varied between 259 and 763 mg/kg of fresh weight. Rutin was the main flavonoid glycoside, and its value was ≥70% of the total flavonoid complement in all of the samples investigated. Compound 2, which has been tentatively identified as quercetin 3-O-glucosyl-rutinoside, was quantified in 31 of the 32 genotypes studied, but its content varied between 2 and 30% among the different hybrids of green asparagus. Significant quantities of nicotiflorin and narcissin were detected in most of the 32 genotypes of commercial hybrids, but these flavonoid diglycosides represented only 1−3% of the total flavonoid content, with the exception of the variety called Dulce Verde, which contained 10% of narcissin.

Table 5.2

Flavonoid content in 32 commercial hybrids of green asparagus (milligrams per kilogram of fresh weight)[a].

	Q-triglyc	Q-triglyc	IR-triglyc	Rutin	IR-triglyc	Nicoti-florin	Narcissin	IR-3-O-gluc	sum
Apollo	nd	104	nd	649	nd	tr	11	nd	763
Aragon 1978	nd	26	nd	233	nd	tr	tr	nd	259
Atlas	nd	49	nd	438	nd	7	tr	nd	494
Backlim	nd	31	nd	376	nd	tr	11	nd	418
Dulce Verde	nd	21	nd	260	nd	nd	31	nd	312
Ercole	nd	84	nd	484	nd	nd	tr	nd	568
Fileas	nd	54	nd	206	nd	tr	tr	nd	260
G Welph	nd	33	nd	328	nd	7	tr	nd	368
Grande	nd	8	nd	448	nd	8	tr	nd	465
G. Millenium	nd	55	nd	337	nd	tr	tr	nd	392
Italo	nd	79	nd	248	nd	tr	tr	nd	328
Jersey Deluxe	nd	76	nd	219	nd	tr	nd	nd	296
Jersey Giant	nd	118	nd	265	nd	8	tr	nd	391
Jersey King	nd	113	nd	345	nd	8	tr	nd	466
Jersey Night	nd	100	nd	338	nd	13	tr	nd	451
Jersey Supreme	nd	149	nd	399	nd	tr	tr	nd	547
JWC-1	nd	37	nd	544	nd	tr	tr	nd	581
NJ1016	nd	60	nd	324	nd	6	tr	nd	390
NJ953	nd	76	nd	337	nd	12	tr	nd	425
NJ956	nd	108	nd	421	nd	tr	tr	nd	529
NJ977	nd	60	nd	214	nd	tr	tr	nd	274
Pacific Purple	nd	10	nd	277	nd	tr	tr	nd	288
Purple Passion	nd	0	nd	477	nd	tr	tr	nd	477
Plaverd	nd	28	nd	407	nd	tr	12	nd	447
Rally	nd	55	nd	441	nd	7	12	nd	515
Ramada	nd	35	nd	342	nd	7	tr	nd	385
Rambo	nd	37	nd	331	nd	tr	tr	nd	369
Rapsody	nd	53	nd	706	nd	11	tr	nd	770
Ravel	nd	25	nd	273	nd	tr	tr	nd	297
Solar	nd	20	nd	542	nd	8	12	nd	582
UC115	nd	53	nd	299	nd	7	tr	nd	359
UC157	nd	44	nd	274	nd	tr	tr	nd	318

[a] *Data are the mean of three replicates. Standard deviation was <5%. nd, not detected; tr, traces.*

The detailed composition of flavonoids from 65 genotypes of triguero asparagus is shown in Table 5.3. Total flavonoid average content was 519 mg/kg of fresh weight, and rutin represented 78% of that quantity, which is comparable to the values calculated from commercial hybrids. However, significant differences were found among the flavonoid compositions from the 65 genotypes of triguero asparagus.

Table 5.3

Flavonoid content in spears from 65 genotypes of triguero asparagus (milligrams per kilogram of fresh weight)[a].

	Q-triglyc	Q-triglyc	IR-triglyc	Rutin	IR-triglyc	Nicotiflorin	Narcissin	IR-3-O-gluc	sum
HT-1	nd	nd	nd	335	9	tr	65	nd	409
HT-2	nd	nd	nd	476	19	12	65	nd	572
HT-3	nd	53	nd	331	24	38	116	nd	562
HT-4	nd	25	nd	401	35	6	108	3	578
HT-5	nd	nd	nd	382	11	108	202	nd	703
HT-6	nd	47	nd	378	33	31	203	1	693
HT-7	nd	44	nd	497	21	34	162	nd	758
HT-8	nd	28	nd	542	nd	tr	16	nd	586
HT-9	nd	16	nd	233	nd	32	159	nd	440
HT-10	41	59	nd	408	23	43	94	2	670
HT-11	nd	nd	nd	702	11	tr	tr	nd	713
HT-12	nd	16	nd	478	5	nd	tr	nd	499
HT-13	nd	19	nd	437	nd	nd	tr	nd	456
HT-14	nd	42	nd	406	16	tr	tr	nd	464
HT-15	nd	11	nd	561	3	nd	tr	nd	575
HT-16	nd	33	nd	275	nd	nd	tr	nd	308
HT-17	nd	nd	nd	583	13	nd	tr	nd	596
HT-18	nd	42	nd	220	nd	nd	tr	nd	262
HT-19	nd	17	nd	610	72	12	tr	nd	711
HT-20	nd	76	nd	716	11	tr	tr	nd	803
HT-21	nd	nd	nd	443	nd	tr	tr	nd	443
HT-22	nd	63	nd	538	10	tr	tr	nd	611
HT-23	nd	18	nd	267	nd	tr	tr	nd	285
HT-24	nd	nd	nd	490	39	tr	13	nd	542
HT-25	65	20	24	195	18	9	16	nd	347
HT-26	nd	50	nd	410	13	tr	tr	nd	473
HT-27	nd	3	nd	73	18	10	34	nd	138

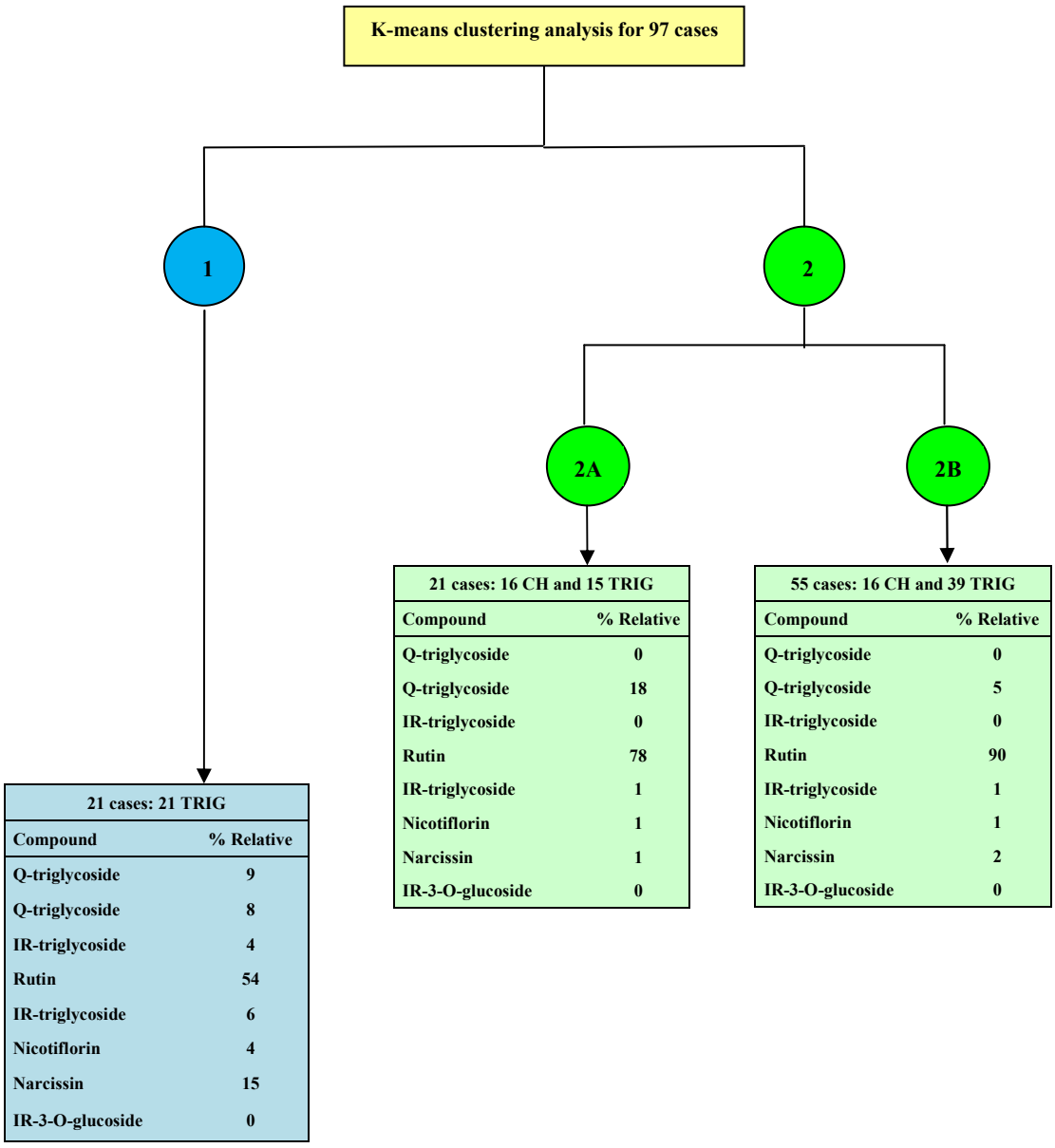
HT-28	37	32	50	366	60	tr	65	9	619
HT-29	nd	nd	nd	418	nd	tr	tr	nd	418
HT-30	nd	35	nd	384	10	nd	tr	nd	429
HT-31	nd	54	nd	478	12	tr	tr	nd	544
HT-32	nd	nd	nd	344	nd	tr	16	nd	360
HT-33	nd	57	nd	606	13	tr	tr	nd	676
HT-34	nd	3	nd	495	nd	nd	tr	nd	498
HT-35	nd	18	nd	485	nd	tr	tr	nd	503
HT-36	nd	nd	nd	462	18	tr	13	nd	493
HT-37	nd	35	nd	493	11	tr	tr	nd	539
HT-38	75	25	86	119	13	7	63	nd	388
HT-39	nd	36	nd	535	nd	6	tr	nd	577
HT-40	nd	nd	nd	717	14	tr	tr	nd	731
HT-41	nd	56	nd	390	nd	tr	nd	nd	446
HT-42	45	26	22	276	63	9	33	3	477
HT-43	nd	39	nd	458	68	18	32	nd	615
HT-44	nd	29	nd	416	42	8	26	nd	521
HT-45	43	nd	63	155	nd	14	29	nd	304
HT-46	nd	44	nd	518	nd	nd	nd	nd	562
HT-47	152	245	nd	202	20	13	116	3	751
HT-48	11	69	9	343	17	tr	14	nd	463
HT-49	nd	31	nd	328	nd	nd	nd	nd	359
HT-50	nd	23	nd	423	nd	tr	nd	nd	446
HT-51	nd	34	nd	432	8	tr	nd	nd	474
HT-52	nd	70	28	617	nd	tr	nd	nd	715
HT-53	nd	nd	nd	394	nd	tr	nd	nd	394
HT-54	80	75	35	100	21	tr	22	2	335
HT-55	nd	31	nd	377	15	tr	tr	nd	423
HT-56	38	21	24	272	39	6	30	nd	430
HT-57	36	34	nd	360	43	12	40	nd	525
HT-58	nd	96	nd	606	13	nd	tr	nd	715
HT-59	36	7	1	247	6	10	tr	nd	307
HT-60	63	82	95	371	60	9	120	4	804
HT-61	nd	86	nd	517	14	nd	nd	nd	617
HT-62	nd	55	nd	424	8	tr	tr	nd	487
HT-63	40	37	nd	172	5	8	42	5	309
HT-64	79	30	nd	310	54	tr	39	nd	512
HT-65	66	28	nd	303	47	tr	36	nd	480

[a] *Data are the mean of three replicates. Standard deviation was <5%. nd, not detected; tr, traces.*

The diverse genotypes of asparagus investigated in the present work have been classified in three distinct groups as the result of a k-means clustering analysis. This statistical test produces exactly k different clusters of greatest possible distinction. The total content of flavonoids and the relative percent of each of the eight individual flavonoids identified in green asparagus have been used as factors of classification. The distribution of the 97 genotypes of green asparagus in three clusters and the average composition of each group are shown in Figure 5.3.

Figure 5.3

Classification of genotypes of green asparagus[a] in three clusters obtained by the application of a k-means clustering analysis.



21 cases: 21 TRIG	
Compound	% Relative
Q-triglycoside	9
Q-triglycoside	8
IR-triglycoside	4
Rutin	54
IR-triglycoside	6
Nicotiflorin	4
Narcissin	15
IR-3-O-glucoside	0

21 cases: 16 CH and 15 TRIG	
Compound	% Relative
Q-triglycoside	0
Q-triglycoside	18
IR-triglycoside	0
Rutin	78
IR-triglycoside	1
Nicotiflorin	1
Narcissin	1
IR-3-O-glucoside	0

55 cases: 16 CH and 39 TRIG	
Compound	% Relative
Q-triglycoside	0
Q-triglycoside	5
IR-triglycoside	0
Rutin	90
IR-triglycoside	1
Nicotiflorin	1
Narcissin	2
IR-3-O-glucoside	0

[a] *CH: comercial hybrids of green asparagus; TRIG: triguero asparagus.*

In the first dimension, samples were divided in two clusters (1 and 2), with very different means. It is noteworthy that all 21 genotypes within the first group came from triguero asparagus, whereas the second group included both commercial hybrids and triguero genotypes. In the second dimension, the second group was divided in two clusters (2A and 2B) constituted by mixtures of commercial hybrids and triguero genotypes. Despite all of the samples within these two clusters containing a high percent of rutin, which represented up to 70% in all cases, significant differences were found between the two groups.

Cluster 1 included only genotypes from triguero asparagus, the flavonoid composition of which is significantly different from those samples within the other two clusters. These genotypes contained the greatest variety of flavonoid compounds. Rutin content was about 50% of the total flavonoids, and this was accompanied by up to seven more flavonoids, including monoglycosides (trace amounts), diglycosides, and triglycosides. The average composition for this group consisted of 54% rutin and significant quantities of other seven flavonoids, each of which represented 1−15% of total flavonoid content.

Cluster 2A comprised 16 commercial hybrids and 5 genotypes of triguero asparagus. These genotypes had an average composition consisting of 78% of rutin, which was accompanied by a significant quantity of compound 2, identified as quercetin-3-glucosyl-rutinoside, and minor quantities of other flavonoid di- and triglycosides. Cluster 2B was the greatest group, containing a mixture of 55 samples, 16 from commercial hybrids and 39 from triguero asparagus. The genotypes within this group contained almost solely rutin, which represented about 90% of the total flavonoids, accompanied by only small quantities of two or three more flavonoid glycosides.

From these results, it can be concluded that the flavonoid composition of triguero asparagus population is similar to that of commercial hybrids. This can be explained by the fact that several American hybrids of green asparagus have been included in the cultivation areas of triguero asparagus during recent years, which can influence and vary the original characteristics of the native varieties. However, the analysis of each of the 65 different genotypes of triguero asparagus revealed that several

of them (the 21 genotypes classified within cluster 1) possessed a flavonoid composition very distinct and statistically different from the majority of the samples, grouped in the two other clusters. That subgroup of triguero asparagus contained several key flavonol derivatives able to differentiate those 21 genotypes from the rest, including both commercial hybrids and triguero asparagus. Therefore, compounds 1, 3, and 8, which have been tentatively identified as quercetin-3-rhamnosyl-rutinoside, isorhamnetin-3-rhamnosyl-rutinoside, and isorhamnetin-3-O-glucoside, respectively, have been detected only in the genotypes grouped in cluster 1. On the other hand, compound 5, tentatively identified as isorhamnetin-3-glucosyl-rutinoside, is present in a greater number of genotypes of triguero asparagus but has not been detected in any of the commercial hybrids investigated in this study. This flavonol triglycoside is one of the minor flavonoid components, as can be observed in Table 5.3 and Figure 5.3. However, the fact that it is not present in any of the commercial hybrids (Table 5.2) suggests that this isorhamnetin triglycoside is key for distinguishing the triguero asparagus population from other green asparagus cultivars.

It is well established that the flavonoid profile of vegetables is influenced by genetic and environmental factors (Martínez-Sánchez *et al.*, 2008; Lachman *et al.*, 2003; Kim *et al.*, 2004; Caridi *et al.*, 2007; Harbaum *et al.*, 2007), and we have previously reported that the first determine to a greater extension both the phenolic composition and the antioxidant activity of green asparagus (Rodríguez *et al.*, 2005a). On the other hand, it has been reported that the antioxidant activity of flavonol glycosides is greatly modified by the position of the sugar group attached to the basic diphenylpropane structure (Murota & Terao, 2003). Thus, Yamamoto *et al.* (1999) demonstrated that Q40G, which has no catechol group, is greatly inferior to catechol-containing Q3G in preventing lipid peroxidation in human low-density lipoprotein (LDL). From the results of the present work it can be concluded that there are several flavonol glycosides, specific to triguero asparagus, which could be an alternative factor determining the bioactive properties of this product. Further investigations are required to determine if the characteristic flavonoid composition of triguero asparagus, reported in this work, is related to specific functional properties that may distinguish this product from other green asparagus.

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6. EFFECT OF EXTRACTION METHOD ON CHEMICAL COMPOSITION AND FUNCTIONAL CHARACTERISTICS OF HIGH DIETARY FIBRE POWDERS OBTAINED FROM ASPARAGUS BY-PRODUCTS

EFFECT OF EXTRACTION METHOD ON CHEMICAL COMPOSITION AND FUNCTIONAL CHARACTERISTICS OF HIGH DIETARY FIBRE POWDERS OBTAINED FROM ASPARAGUS BY-PRODUCTS

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Abstract

Asparagus (*Asparagus officinalis* L.) by-products, which represent around 50% of the processed vegetable, are a potential source of dietary fibre. The way that these by-products are treated affects the composition and functional properties of fibre-rich powders. Factors such as treatment intensity, solvent, and drying system were studied. Only the more soluble components (soluble sugars, uronic acids and proteins) showed significant differences. All the fibre-rich powders had high concentrations of TDF (62–77%). The IF/SF proportion decreased with the severity of treatment, in this way increasing the physiological quality of the fibre. Functional properties, namely water-holding capacity (WHC), oil-holding capacity (OHC), solubility (SOL), and glucose dialysis retardation index (GDRI), varied according to the preparation procedure. WHC and GDRI were higher in intensely extracted fibres; due to the effect of thermal processing. WHC showed values (11–20 ml water/g powder) similar to those described for other agricultural by-products, but OHC and GDRI were much higher (5–8 ml oil/g powder and 25–45%, respectively). These properties make fibre-rich powders from asparagus by-products a valuable source of dietary fibre to be included in the formulation of fibre-enriched foods.

6.1. INTRODUCTION

In developed countries, several gastrointestinal disorders (duodenal ulcer, appendicitis, constipation, hemorrhoids, colon carcinoma), diabetes mellitus, obesity, and cardiovascular diseases, have very low incidence among people consuming high amounts of fibre. Fibre benefits for the gut are widely recognized a fact that has led to its consideration as a nutrient (FDA & Human Services, 1993). The key to obtaining the level of fibre intake recommended for adults in western societies is the availability of high quality foods with a high dietary fibre content. The usual sources of dietary fibre added to commercial foods (juices, dairy products, baked goods) are cereals. Currently, however, the demand for by-products from fruits and vegetables as sources of dietary fibre has been increasing, because these sources present higher nutritional quality, higher amounts of total and soluble fibre, lower caloric content, stronger antioxidant capacity, and higher levels of fermentability and water retention (Rodríguez *et al.*, 2006).

Among the vegetables commonly consumed in the United States and Europe, asparagus has been reported as the richest in the total quality and quantity of its antioxidants (Pellegrini *et al.*, 2003 and Vinson *et al.*, 1998). Of asparagus-producing countries, Spain is in the fifth position after China, Peru, the U.S.A., and Germany. In 2005, about 50,000 tons (including both green and white asparagus) were harvested in Spain, with more than 50% coming from Andalusia (mainly green). During industrial processing, around half of the total length of each spear is discarded, which creates significant waste for producers. Assuming that the by-products have similar composition to the edible part of the spears, these represent a promising source of new value-added compounds (phytochemicals and fibre) (Nindo *et al.*, 2003). However, processing and drying may cause irreversible modifications to the fibre, affecting its original structure. Therefore, its final quality would be determined by the structural and compositional modifications that might have occurred during processing (Garau *et al.*, 2007).

The objective of this study is to prepare various fibre-rich powders from asparagus by-products, using different methods, and to evaluate their chemical composition and several functional properties by using *in vitro* tests.

6.2. MATERIALS AND METHODS

6.2.1. Asparagus by-products

Asparagus by-products were obtained from Centro Sur S.C.A. (Huétor–Tájar, Granada, Spain). Prior to canning, freshly harvested asparagus spears were cut to obtain the 15 cm length upper portion (edible part) and the rest of spear (15–18 cm) was considered a by-product. This by-product was sent to our laboratory within 24 h and held at 4 °C prior to its processing.

6.2.2. Asparagus by-product treatment

Three variables were studied (Table 6.1): extraction treatment (intense – 90 min at 60 °C, or gentle – 1 min at room temperature-), extraction solvent (water or 96% ethanol), and drying system (freeze-drying or oven treatment at 60 °C for 16 h). Two kg by-product portions were cut, homogenized and mixed using a professional homogeniser (Sirman Orione, Marsango, Italy) at top speed with each of the extraction solvents in a ratio of 1:1, solid:liquid (w/v), and at the programmed temperature (60 °C or room temperature) for 1 min. Afterwards, gently extracted samples (G) were directly processed using an industrial juicer (Tecno–Chufa, Valencia, Spain) to separate an asparagus liquid extract and a wet fibrous residue. Intensely extracted samples (I) were treated in an open reactor, designed in our laboratory, with time and temperature controls and continuous stirring. After 90 min. of extraction, samples were centrifuged as described above. Each treatment was carried out in duplicate. One batch of wet fibrous residue was freeze-dried (F-D) and the other dried in an oven (O-D). Dried fibres were ground in a hammer mill to a particle size lower than 1 mm and stored at 4 °C prior to analysis.

Table 6.1

Different conditions for obtaining a fibre-rich powder from asparagus by-product.

	DESCRIPTION
I/W O-D	Intense treatment (90 min at 60º C) with water and oven-drying
I/W F-D	Intense treatment (90 min at 60º C) with water and freeze-drying
I/E O-D	Intense treatment (90 min at 60º C) with ethanol and oven-drying
I/E F-D	Intense treatment (90 min at 60º C) with ethanol and freeze-drying
G/W O-D	Gentle treatment (1 min at room temperature) with water and oven-drying
G/W F-D	Gentle treatment (1 min at room temperature) with water and freeze-drying
G/E O-D	Gentle treatment (1 min at room temperature) with ethanol and oven-drying
G/E F-D	Gentle treatment (1 min at room temperature) with ethanol and freeze-drying

6.2.3. Proximate composition of fibres

Amounts of neutral sugars (NS), uronic acids (UA), proteins (P), and Klason lignin (KL) were determined as described previously (Fernandez-Bolaños *et al.*, 2004). For neutral sugars, sugar-free fibres (treated with 80% ethanol) were hydrolysed with trifluoroacetic acid (TFA) at 121 °C for 1 h (Ruiter & Burns, 1987) and the released sugars were quantified as alditol acetates by gas chromatography (Englyst & Cumming, 1984); uronic acids were quantified using the phenyl-phenol method after sulphuric acid hydrolysis (Ahmed & Labavitch, 1977); proteins were analysed by the Kjeldahl method, using a Büchi Digestion Unit, K-424, and a Büchi Distillation Unit, K-314, applying the factor 6.25 to convert total nitrogen into protein content; *Klason lignin* levels were determined gravimetrically as the amount of acid-insoluble material remaining after a two-stage sulphuric acid hydrolysis.

Cellulose (CEL) was quantified from the TFA-insoluble residue after 72% sulphuric acid hydrolysis, and soluble sugars (SS) from the ethanol-soluble extract of fibres, both by the anthrone method (Dische, 1962).

6.2.4. Dietary fibre content

The amount of total dietary fibre (TDF) was determined using the protocol described by Lee *et al.* (1992), with slight modifications. After digestion, insoluble fibre (IF) was recovered by filtration on a sintered glass crucible (no. 2), washed with

distilled water and dried overnight at 100 ° C (ash and protein were corrected during this step). Four volumes of hot ethanol were added to filtrates and the suspensions of soluble fibre (SF) were left overnight at 4 °C to allow SF to precipitate. Afterwards, SF was recovered by centrifugation for 20 min at 2500 g, dissolved in distilled water, and freeze-dried.

6.2.5. Functional properties

6.2.5.1. Water- and oil-holding capacities and solubility

Water-holding capacity (WHC) was determined by centrifugation as described elsewhere (Jiménez *et al.*, 2000). Samples (250 mg ×3) were suspended in 15 ml of water. After 24 h of stirring at room temperature, the suspension was centrifuged at 14000 g for 1 h. Supernatants were carefully discarded and the hydrated fibres were weighed. WHC was expressed as ml of water/g fibrous residue. Hydrated pellets were freeze-dried and solubility in water (SOL) was the weight difference between before and after the WHC assay, expressed as a percent. Oil-holding capacity (OHC) was determined under the same conditions as WHC using sunflower oil (1.0054 g/ml density), and was expressed as ml oil/g of fibrous residue.

6.2.5.2. Glucose dialysis retardation index (GDRI)

GDRI was determined as described by Lecumberri *et al.* (2007), with slight modifications. Samples of 400 mg sugar-free fibres (fibres extracted twice with 80% ethanol) were thoroughly hydrated with 15 ml of distilled water containing 30 mg glucose. After 1 h of continuous stirring, samples were transferred to 15 cm portions of previously hydrated dialysis bags (12,000 MWCO, Sigma Chemical Co.). Each bag and a control bag (with glucose, but without fibre) were put into a reservoir containing 400 ml of distilled water and held in a thermostatic water bath at 37 °C for 1 h with constant shaking. At 10 min intervals, 0.5 ml of dialysate was collected and the glucose concentration was determined spectrophotometrically by the anthrone method (Dische, 1962). GDRI was calculated as follows:

$$GDRI = 100 - \left(\frac{Total\ glucose\ diffused, sample}{Total\ glucose\ diffused, control}\ X\ 100\right)$$

6.2.6. Statistical analysis

Results were expressed as mean values ± standard deviations. To assess for differences in the composition and functional characteristics between the different treatments, a multiple sample comparison was performed using the Statgraphics Plus program version 2.1. Multivariate analysis of variance (ANOVA), followed by Duncan's multiple comparison test, was performed to contrast the groups. The level of significance used was $P < 0.05$.

6.3. RESULTS AND DISCUSSION

6.3.1. General

Asparagus by-products could be considered an interesting source of dietary fibre. By-product processing involves several factors that must be controlled in order to have a dietary fibre with optimised composition and functional characteristics. In this study, three factors have been taken into account (severity of treatment, solvent and drying system) and their effects on fibre characteristics will be considered.

6.3.2. Proximate composition

The composition of major components is presented in Table 6.2 SS and UA, the more soluble components of cell wall, varied the most – from 7–15% to 9–17%, respectively. In the case of UA, fibres from treatments with water were poorer in UA than those from treatments with ethanol. In ethanol-extracted fibres, the choice of drying system resulted in significant differences; freeze-dried fibres had higher UA levels than had oven-dried ones. In UA quantification, the three factors considered (severity of treatment, solvent and drying system) were statistically significant.

NS showed little variation among samples. Neither the drying system nor the solvent affected NS content, with only the intensity of treatment leading to significant differences. In fibres that were subjected to intense treatment, NS content was higher than in those with gentle treatment. Hemicelluloses, cell wall polysaccharides mainly composed of NS, are insoluble in both solvents, water and ethanol. Thus, during by-product processing, other components were preferentially solubilized and the resultant fibre was enriched in hemicelluloses. Considering the percentage of individual sugars (rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose) in the total content of NS (data not shown), it was remarkable that the compositions of the fibres from different treatments were very similar: xylose accounted for more than 50% of sugar content, while galactose and arabinose each contributed 10–20%. These results are in concordance with those previously reported for white and green asparagus (Rodríguez *et al*., 1999a; Rodríguez *et al*., 1999b and Waldron & Selvendran, 1992). In white spears, xylose accounted for more than 40% of all sugars while glucose, galactose

and arabinose each accounted for 15–20%. In the basal section of white asparagus (Rodríguez *et al.*, 1999a and Rodríguez *et al.*, 1999b), arabinoxylans and xyloglucans are the main hemicelluloses. This composition, above presented for by-products, suggests that these polysaccharides are also the major species in the basal section of green asparagus.

Table 6.2

Composition of fibre-rich powder obtained under different conditions from asparagus by-products (% dry matter)[a].

		SOLUBLE SUGARS[b]	NEUTRAL SUGARS	URONIC ACIDS	CELLULOSE	PROTEIN	KLASON LIGNIN
I/W	O-D	10.99±0.15ab	6.56±0.03a	9.65±0.58ab	22.10±1.16a	12.32±0.14a	16.57±0.64ab
	F-D	12.11±0.49c	6.62±0.13a	9.06±1.04a	25.25±1.46b	12.43±0.08a	18.06±1.78b
I/E	O-D	9.33±0.66d	5.87±0.15b	16.37±1.01c	19.09±2.38c	16.30±0.12b	15.23±0.36a
	F-D	13.70±0.83e	6.14±0.09ab	17.43±0.84d	21.91±0.26a	15.18±0.01c	16.61±0.10ab
G/W	O-D	10.36±0.10a	5.91±0.38b	10.22±0.45b	22.50±1.31a	13.95±0.05d	11.60±0.69c
	F-D	11.25±0.26b	5.77±0.08bc	9.89±1.11ab	22.00±1.05a	13.47±0.25e	10.97±1.52c
G/E	O-D	7.18±0.36f	5.73±0.25bc	11.99±0.58e	18.65±0.99c	16.08±0.01b	16.22±1.91ab
	F-D	15.11±0.14g	5.27±0.30c	15.11±0.52f	22.21±1.40a	15.08±0.09c	14.79±2.02a

[a] *All analyses done at least in duplicate.*
[b] *Means within a column bearing the same letter are not significantly different at 5% level as determined by the Duncan multiple range test.*

Cellulose was the main component in all samples. The intensity of treatments did not affect its amount, but the solvent and drying system did. Freeze-dried samples were richer in cellulose than were oven-dried ones. In white asparagus (Rodríguez *et al.*, 1999a and Rodríguez *et al.*, 1999b), the cellulose content was double that of NS while, in this study, it was 3–4 fold higher.

Protein content varied between 12% and 16%. The most important difference was caused by the choice of solvent, with higher protein content in ethanol-treated samples than in those treated with water. In most cases, process intensity did not cause any differences.

KL accounted for 11–18%. It is interesting to note that fibres from gentle aqueous treatment (G/W) had significantly lower lignin contents than had samples from

the other treatments. This could indicate that some additional material, or some artefacts resulting from processing, were quantified as lignin in fibres from intense treatments or treatments with ethanol.

In order to compare these fibre-rich powders with those obtained from other fruit and vegetable by-products, the composition of different fibres obtained from asparagus by-products is summarised as follows: SS 7.18–15.11%, NS 5.27–6.62%, UA 9.06–17.4%, cellulose 18.65–25.25%, protein 12.3–16.3%, and KL 11.0–18.1%. The content of SS in asparagus fibres was slightly higher than that found for other fruit or vegetable by-products. The percentage of SS was around 3% for mango peel (Larrauri *et al.*, 1996a) and red grape pomace (Llobera & Cañellas, 2007), although a content of around 10% can be found in some citrus by-products (Marín *et al.*, 2007). The content of hemicelluloses (NS), among all fruit and vegetable by-products, varies between 5% and 30%. In guava pulp (Jiménez-Escrig *et al.*, 2001), hemicelluloses accounted for about 16%, but lower levels (5–7%) were found in cocoa bean husks (Lecumberri *et al.*, 2007) and some citrus by-products (Marín *et al.*, 2007). UA contents also showed high variability, depending on the by-product studied. The lowest level was found in guava pulp (Jiménez-Escrig *et al.*, 2001), which was 2%. Lime peels had the highest level, around 25% (Ubando-Rivera *et al.*, 2005). UA levels similar to those in asparagus were found in peach pulp (Grigelmo-Miguel *et al.*, 1999). The cellulose content of fibre-rich powder from asparagus by-product was also in the range of the other products, with the lowest level, found in cocoa bean husks, being around 10% (Lecumberri *et al.*, 2007), and the highest, found in citrus, being 36–40% (Marín *et al.*, 2007). The content of protein in the fibre generated from asparagus (12.3–16.3%) was around the highest values reported for agricultural by-products, e.g. 16% for rice bran (Abdul-Hamid & Luan, 2000). The range of lignin content from other by-products was wide, varying from 6% for peach pulp (Grigelmo-Miguel *et al.*, 1999) to 32% for cocoa bean husks (Lecumberri *et al.*, 2007). The lignin content in fibres from asparagus by-product was similar to those reported by Jiménez-Escrig *et al.* (2001) for guava pulp.

6.3.3. Dietary fibre content

Table 6.3 shows the content of total dietary fibre (TDF), insoluble fibre (IF), soluble fibre (SF) and the ratio between IF and SF in fibre-rich powder from asparagus by-product. The varying extraction methods resulted in three levels of TDF content. The highest of these was found in fibres from intense treatment in water (I/W), and it was greater than 75%, probably as a consequence of the depletion of soluble components during processing, as was commented upon above. There was another group with medium content (around 69%) that included fibre from intense extraction with ethanol (I/E) and gentle extraction in water (G/W). The lowest TDF content (less than 65%) was found in fibres gently extracted in ethanol (G/E). TDF values in samples ranged from 62.1% to 77.5%, which is greater than the 49.0% previously reported by Grigelmo-Miguel and Martín-Belloso (1999) for asparagus by-product. The difference may be due to the processing that these authors used, as they only washed their samples with water and dried them in an oven. In this case, there was no depletion of soluble components at all, so the percentage of TDF decreased in the total composition.

Table 6.3

Dietary fibre fractions of different fibre-rich powder from asparagus by-products (% dry matter)[a].

		IF	SF	TDF[b]	IF/SF
I/W	O-D	66.84±2.56	10.69±0.38	77.53±2.94a	6.2
	F-D	65.15±1.12	10.27±0.35	75.42±1.47a	6.3
I/E	O-D	60.62±1.99	9.79±0.07	70.41±2.05b	6.2
	F-D	60.09±2.11	8.26±0.48	68.35±2.59b	7.2
G/W	O-D	58.22±1.89	9.90±1.69	68.11±3.58b	5.9
	F-D	58.65±1.39	10.52±0.38	69.18±1.77b	5.6
G/E	O-D	54.88±1.50	9.58±0.83	64.45±2.32c	5.7
	F-D	51.02±0.32	11.07±0.32	62.09±0.64c	4.6

[a] *Values are means of triplicate assays.*
[b] *Means bearing the same letter are not significantly different at 5% level as determined by the Duncan multiple range test.*

In the literature, there are many by-products that are valuable TDF sources, with content varying between 30% and 90%. Three different groups could be established: low-TDF sources (30–50%), such as banana (Rodríguez-Ambriz *et al.*, 2008), Marsh

grapefruit (Figuerola *et al.*, 2005), and guava (Jiménez-Escrig *et al.*, 2001); medium-TDF sources (50–70%), such as Eureka lemon (Figuerola *et al.*, 2005) and mango peel (Larrauri *et al.*, 1996b); high-TDF sources (70–90%), such as red grape pomace (Llobera & Cañellas, 2007) and lime peel (Ubando-Rivera *et al.*, 2005). Asparagus by-product fibre falls into the medium-TDF content group.

The IF/SF ratio varied from 4.6 to 7.2 (Table 6.3). The range to obtain the physiological effects associated with both the soluble and insoluble fractions was 1–2.3 (Spiller, 1986). The lowest value for the IF/SF ratio for asparagus by-product (4.6) was obtained with the G/E treatment. A decrease in this ratio was observed to be linked to the severity of the treatment and the solvent used. The decrease was due to a reduction in the percentage of IF in gently extracted samples. Most fibre from agricultural by-products also had values higher than 4, e.g. cocoa bean husks – 5 (Lecumberri *et al.*, 2007), red grape pomace – 6.4 (Llobera & Cañellas, 2007), banana – 7.6 (Rodríguez-Ambriz *et al.*, 2008), rice bran – 11 (Abdul-Hamid & Luan, 2000), and guava pulp – 27 (Jiménez-Escrig *et al.*, 2001).

6.3.4. Functional properties

6.3.4.1. Water- and oil-holding capacities and solubility

The results obtained for WHC, SOL, and OHC are presented in Table 6.4. WHC is an important property of dietary fibre from both physiological and technological points of view. WHC showed significant differences, depending on the severity of processing: fibre obtained with thermal treatment had higher WHC. Heating might modify the structural characteristics of the fibre, hence facilitating its water uptake (Figuerola *et al.*, 2005). The choice of drying system did not lead to significant differences although, in other agronomical by-products, drying at high temperatures (40–90 °C) causes a reduction in this capacity (Garau *et al.*, 2007). WHC is related to soluble dietary fibre content. As was commented upon above, fibres undergoing gentle treatment had a lower IF/SF ratio, but a higher WHC. So, the effect of heating on fibre structure seems to be greater than the influence of a higher percentage of soluble fibre. It is probably that the high solubility of asparagus fibre, 22–34% (Table 6.4), could

improve this functional property. SOL was significantly influenced by the choice of drying system, with the lowest value being in I/W samples.

Table 6.4

Functional properties of different fibre-rich powder from asparagus by-products[a].

		WHC mL water/g powder[b]	SOL %	OHC ml oil/g powder
I/W	O-D	20.34±0.54a	23.39±1.20a	7.61±0.27ab
	F-D	18.84±1.01a	22.58±0.57a	8.19±0.16c
I/E	O-D	19.65±2.08a	27.72±1.75b	7.52±0.27a
	F-D	19.69±1.54a	34.37±2.75c	8.27±0.68c
G/W	O-D	11.40±0.38b	26.53±0.53b	7.69±0.17ab
	F-D	12.58±0.77b	34.15±0.61c	8.53±0.30c
G/E	O-D	12.06±1.26b	28.91±0.96b	5.28±0.34d
	F-D	12.15±0.92b	34.73±0.76c	5.53±0.54d

[a] *Values are means of triplicate assays.*
[b] *Means within a column bearing the same letter are not significantly different at 5% level as determined by the Duncan multiple range test.*

Fibre-rich asparagus powder generated by gentle processing had a WHC similar to what was previously reported (Grigelmo-Miguel & Martín-Belloso, 1999). These results are in the range of most described fibres, e.g. 12.6 ml water/g for peach pulp fibre (Grigelmo-Miguel *et al.*, 1999) and more than 11 ml water/g for lemon fibre (Lario *et al.*, 2004). Other agricultural by-products had lower values than those commented upon above, e.g. rice bran (Abdul-Hamid & Luan, 2000) and cocoa husks (Lecumberri *et al.*, 2007), both with a WHC value of 5 ml water/g fibre. On the basis of these values, this product could be promoted as a modifier of viscosity and texture of formulated products in addition to promoting the decrease in calories that this addition could imply.

Results for OHC are presented in Table 6.4. Except for G/E samples, freeze-drying resulted in an increase of this property, ranging between 7.5 and 8.5 ml oil/g of powder. G/E samples had lower capacities. Values found in the literature were much lower than those for asparagus by-products, e.g. 0.6–1.8 ml oil/g for apple pomace and citrus peel (Figuerola *et al.*, 2005), and around 2 ml oil/g for unripe banana flour (Rodríguez-Ambriz *et al.*, 2008). The highest reported level was around 6 ml oil/g

described for carrot pulp dried at 50 °C (Garau *et al.*, 2007). Fibre-rich asparagus powder had even higher values, so the use of this fibre may be appropriate in products where emulsifying properties are required.

6.3.4.2. Glucose dialysis retardation index

In Figure 6.1 the graphs for GDRI are presented. Except for control and I/W O–D, the retardation curves of all the other samples were adjusted at *a* "square root *x*" model ($y = a+bx^{-2}$) with $R > 0.92$. Using the regression equation for each sample, the different GDRI's at 60 min. were calculated and the results are presented in Figure 6.2. Values ranged from 18% to 48%. Intense treatments led to higher GDRI than did gentle ones, although differences were stronger in samples extracted with water than in those extracted with ethanol. Fibre treated with ethanol had higher values than had fibre treated with water, and those freeze-dried were higher than those that were oven-dried. The GDRI phenomenon seems to be related to the soluble dietary fibre and uronic acid contents of insoluble fibre (Edwards *et al.*, 1987 and Wolever, 1990), although other authors have pointed to the relationship between the internal structure and surface properties of fibres and glucose diffusion (López *et al.*, 1996). Both factors could probably modulate this functional property to different degrees, depending on the studied fibre. For asparagus fibre, the surface characteristics must have some influence on GDRI because freeze-dried samples presented a longer delay of glucose dialysis. After regression analysis between chemical composition and the GDRI, uronic acids were the components that showed the highest correlation ($R = 0.72$), in agreement with above comments.

Figure 6.1

Time-related effects of the different fibre-rich powders obtained from asparagus by-products on glucose diffusion.

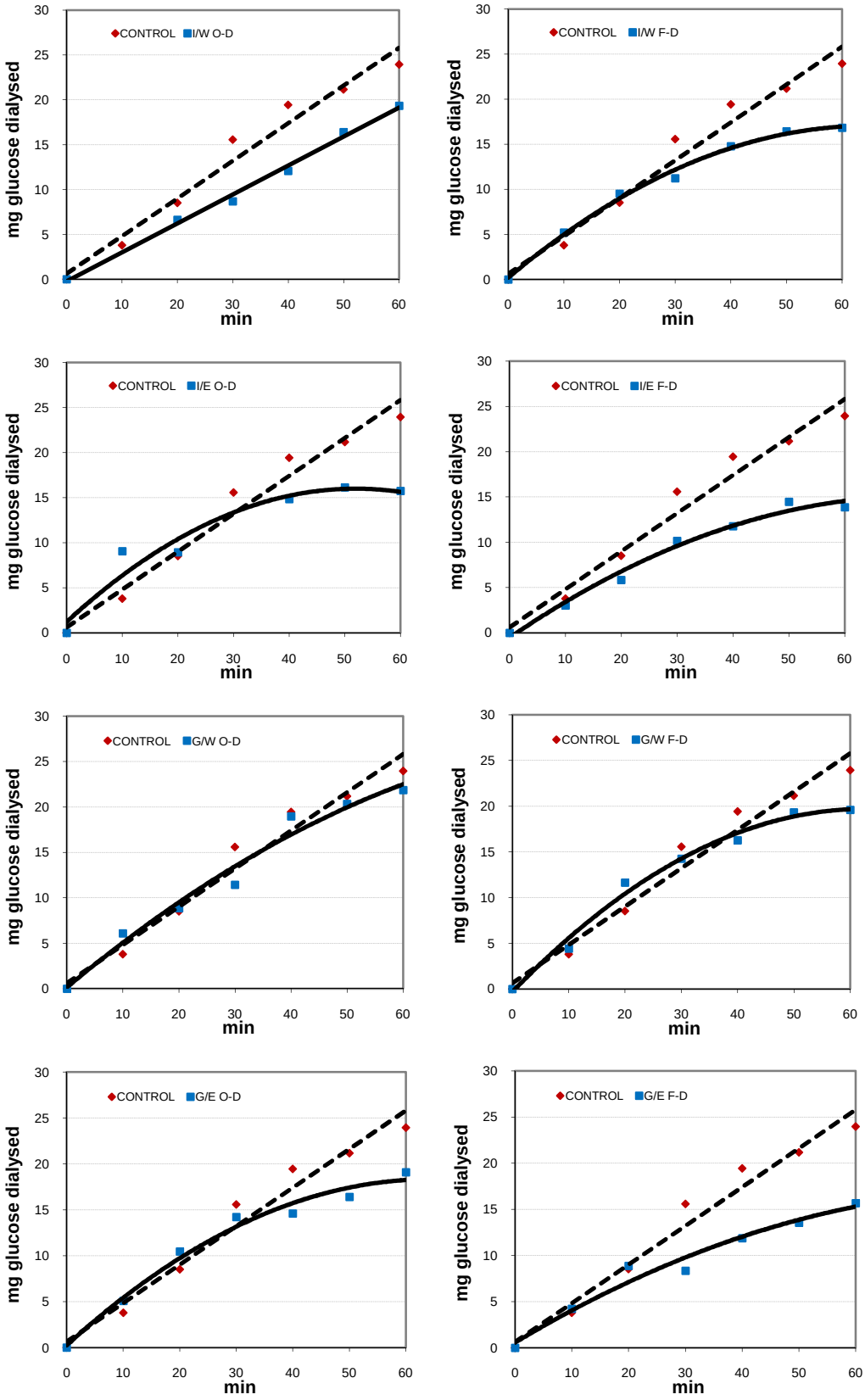
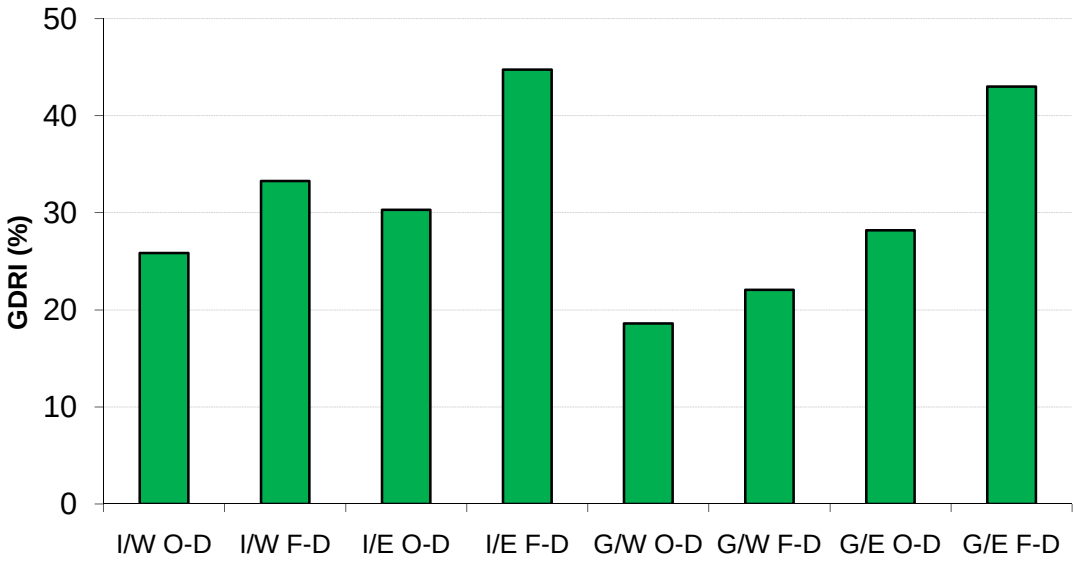


Figure 6.2

Glucose dialysis retardation index (%) of the different fibre-rich powders obtained from asparagus by-products.



The GDRI is a useful *in vitro* index to predict the effect that fibre has on the delay of glucose absorption in the gastrointestinal tract (López *et al.*, 1996). In the literature, there are a wide range of values, from wheat bran at 5.3% (Adiotomre *et al.*, 1990) to guar gum at 43% (Larrauri *et al.*, 1996b). Most of the by-products have intermediate values, e.g., artichoke fibre – 27% (López *et al.*, 1996), mango peel – 21% (Larrauri *et al.*, 1996b), and carambola pomace – 25% (Chau *et al.*, 2004). The results obtained for fibre-rich powders from asparagus by-product were similar to those reported above, with two of the treatments (I/E F–D and G/E F-D) leading to higher values.

6.4. CONCLUSIONS

The treatments applied for obtaining the fibre-rich powders from asparagus by-products caused little variability in the chemical composition of the powder. Only the components with high solubility (soluble sugars, uronic acids, and proteins) showed significant differences. With gentle treatments, especially those with ethanol, powders with lower IF/SF ratios could be obtained. Therefore, the degree of treatment severity became the key to determining the physiological quality of asparagus by-product fibre. The average composition of asparagus fibre, obtained under different conditions, was similar to those of other good sources of food fibre suggested in the literature.

The drying systems studied affected fibre surface differently. This factor had a sizeable influence on the functional properties of the fibre. Solubility and oil-holding capacity were higher in freeze-dried fibres than in oven-dried fibres. However, water-holding capacity was more influenced by thermal treatment. The functionality of thermally treated fibre makes it suitable for addition to fibre-enriched food as a modifier of viscosity and texture, and could also cause a decrease in calories. Some of the fibres examined in this study have been added to plain yoghurt and were compatible with its manufacturing process (Sanz *et al.*, 2008). Especially considering their oil-holding capacities, the high-fibre powers from asparagus by-products may be appropriate in products where emulsifying properties are required. This study also revealed that they could effectively retard the diffusion of glucose; this capacity was highly affected by the process through which it was obtained. The potential hypoglycaemic effects of these fibres suggested that they could be incorporated as low-calorie bulk ingredients in high-fibre foods to lower postprandial serum glucose levels.

Studies to identify bioactive compounds in these fibres and their *in vitro* antioxidant activities have been undertaken in order to evaluate the properties of these compounds that may be beneficial for health. These studies are almost ready to be published. Further investigations on the *in vivo* hypoglycaemic effect and other physiological effects, using animal feeding experiments, are needed to establish the possibility of the use of asparagus-derived fibres as food ingredients, and for nutritional

and technological applications, while making use of asparagus-processing waste. All these studies are thus of economic interest to producers.

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7. EFFECT OF THE EXTRACTION METHOD ON PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF HIGH DIETARY FIBRE POWDERS OBTAINED FROM ASPARAGUS BY-PRODUCTS

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Abstract

Asparagus (*Asparagus officinalis L.*) spears are highly appreciated for their composition of bioactive compounds. The method by which their by-products are treated affects the phytochemical composition and antioxidant activity of the fibre-rich powders. Factors such as the treatment intensity, the solvent used, and the drying system were studied. Among the asparagus phytochemicals, hydroxycinnamic acids (HCA), saponins, flavonoids, sterols, and fructans were quantified. HCA varied from 2.31 and 4.91 mg/g of fibre, the content being affected by the drying system and, in some cases, the solvent. Fibres from intense treatments had significantly higher amounts of saponin than samples isolated by gentle treatments. Saponin content ranged from 2.14 to 3.64 mg/g of fibre. Flavonoids were the most affected by processing conditions, being present (0.6–1.8 mg/g of fibre) only in three of the samples analysed. Continuous stirring during processing could be the main reason for this result. Sterols and fructans were present in minor amounts, 0.63–1.03 mg/g of fibre and 0.2–1.4 mg/g of fibre, respectively. Soluble and total antioxidant activities were also measured. Fibres with the highest activities corresponded to those with the highest levels of flavonoids and HCA.

7.1. INTRODUCTION

Besides their culinary quality, green asparagus spears are appreciated for their composition of bioactive compounds. Eastern civilisations have been using asparagus extracts as stimulants, laxatives, antitussives, diuretics, etc. for hundreds of years. In recent pharmacological studies (Wiboonpun *et al*., 2004 and Yu *et al.*, 1996), these extracts have been shown to have several biological activities, including antitumoral and antioxidant activities. Among all the bioactive compounds present in asparagus spears, saponins, flavonoids, and hydroxycinnamates are the main compounds responsible for the characteristics cited above. Asparagus saponins are steroidal glycosides, with protodioscin being the most abundant. Several activities of this compound have been described, with cytotoxicity against several lines of human cancerous cells (Chin, 2006; Hibasami *et al.*, 2003 and Wang *et al.*, 2003) being of special interest. Flavonoids are phenolic compounds with high antioxidant activity. Additionally, they have antitumoral and antimicrobial activities, and participate in the prevention of cardiovascular diseases (Cushine & Lamb, 2005 and Nijveldt *et al.*, 2001). Rutin is the most abundant flavonoid in asparagus spears, in addition to others that have been recently described (Fuentes-Alventosa *et al.*, 2007 and Fuentes-Alventosa *et al.*, 2008). Hydroxycinnamic acids, especially ferulic acid, are strong antioxidants. Thus, ferulic acid may be beneficial in the prevention of disorders linked to oxidative stress, including Alzheimer's disease, diabetes, cancers, hypertension, atherosclerosis, inflammatory diseases, and others (Zhao & Moghadasian, 2008). If linked to dietary fibre, ferulic acid would be desesterified in the intestinal lumen, which could offer a way to provide a slow-release form of ferulic acid that might provide a prolonged physiological effect (Plate & Gallaher, 2005).

Plant sterols (phytosterols) and fructans, mainly fructooligosaccharides, are also present in asparagus spears, but in lower amounts. The nutrition role of phytosterols is based on their cholesterol-lowering effect in human blood, based on their ability to competitively inhibit intestinal cholesterol uptake (Jiménez-Escrig *et al.*, 2006). β-Sitosterol is the most abundant compound within this group of phytochemicals. Fructooligosaccharides (FOS) have a beneficial effect on human health because they are prebiotics. FOS are not hydrolysed by digestive enzymes, but gut microbiota are able to ferment them. Several studies have found that FOS and inulin promote calcium

absorption in both the animal and human gut (Heuvel *et al.*, 1999 and Zafar *et al.*, 2004). The intestinal microflora in the lower gut can ferment FOS, which results in a reduced pH. Calcium is more soluble in acid and, therefore, more is released from the food and is available to move from the gut into the bloodstream.

Of the asparagus-producing countries, Spain ranks fifth after China, Peru, the USA, and Germany. During industrial processing, around half of the total length of each spear is discarded, which creates significant waste for producers. Assuming that the by-products have a similar composition to the edible part of the spears, their fibre-rich products could contain significant amounts of all the phytochemicals mentioned above. In addition to changes in chemical composition and functional characteristics of the fibres (Fuentes-Alventosa *et al.*, 2009), by-product processing conditions could modify the composition of bioactive compounds and, in doing so, the intrinsic antioxidant activity of these fibre-rich products. The aim of this work is to characterise phytochemicals from asparagus fibres and to study the effects that processing conditions have on their composition and antioxidant activity.

7.2. MATERIALS AND METHODS

7.2.1. Asparagus by-products

Asparagus by-products were obtained from Centro Sur S.C.A. (Huétor-Tájar, Granada, Spain). Prior to canning, freshly harvested asparagus spears were cut to obtain the 15 cm long upper portion (edible part) and the rest of spear (15–18 cm) was considered a by-product. This by-product was sent to our lab within the next 24 h and held at 4 °C until processing.

7.2.2. Asparagus by-product treatment

Three variables were studied (Table 7.1): extraction treatment (intense – 90 min at 60 °C, or gentle – 1 min at room temperature), extraction solvent (water or 96% ethanol), and drying system (freeze-drying or oven treatment at 60 °C for 16 h). Two kilograms of the by-product portions were cut, homogenised, and mixed using a professional homogeniser (Sirman Orione, Marsango, Italy). Homogenisation was performed at top speed with each of the extraction solvents in a 1:1 ratio (solid:liquid) (w/v), and at the programmed temperature (60 °C or room temperature) for 1 min. Afterwards, gently-extracted samples (G) were directly processed using a industrial juicer (Tecno-Chufa, Valencia, Spain) to separate the asparagus liquid extract from the wet fibrous residue. Intensely-extracted samples (I) were treated in an open reactor designed in our laboratory, with time and temperature controls and continuous stirring. After 90 min of extraction, samples were centrifuged as described above. Each treatment was performed in duplicate. One batch of wet fibrous residue was freeze-dried (F-D) and the other dried in an oven (O-D). Dried fibres were ground in a hammer mill to a particle size lower than 1 mm and stored at 4 °C until analysis.

Table 7.1

Different conditions for obtaining a fibre-rich powder from asparagus by-product.

	Description
I/W O-D	Intense treatment (90 min at 60º C) with water and oven-drying
I/W F-D	Intense treatment (90 min at 60º C) with water and freeze-drying
I/E O-D	Intense treatment (90 min at 60º C) with ethanol and oven-drying
I/E F-D	Intense treatment (90 min at 60º C) with ethanol and freeze-drying
G/W O-D	Gentle treatment (1 min at room temperature) with water and oven-drying
G/W F-D	Gentle treatment (1 min at room temperature) with water and freeze-drying
G/E O-D	Gentle treatment (1 min at room temperature) with ethanol and oven-drying
G/E F-D	Gentle treatment (1 min at room temperature) with ethanol and freeze-drying

7.2.3. Determination of bioactive compounds

7.2.3.1. Hydroxycinnamic acids

Total hydroxycinnamic acids (HCA) present in fibre samples were extracted and quantified as previously described (Jaramillo *et al.*, 2007). Briefly, samples (in duplicate) were treated with 2 N NaOH for 24 h, at room temperature, under nitrogen and darkness. After filtration, *trans*-cinnamic acid was added as an internal standard. Solutions were acidified and extracted three times with ethyl acetate. Ethyl acetate extracts were evaporated under nitrogen, re-dissolved in 50% methanol, and analysed by HPLC. Phenolic compounds were quantified using a Synergi 4 μm Hydro-RP80A reverse-phase column (25 cm × 4.6 mm i.d., 4 μm; Phenomenex, Macclesfield, Cheshire, UK). The gradient profile was formed using solvent A (10% aqueous acetonitrile plus 2 ml/l acetic acid) and solvent B (40% methanol, 40% acetonitrile, and 20% water plus 2 ml/l acetic acid) in the following program; the proportion of B increased from 10% to 42.5% for the first 17 min, held isocratically at 42.5% for a further 6 min, increased to 100% over the next 17 min, and finally returned to the initial conditions. The flow rate was 1 ml/min. HCA were detected using a Jasco-LC-Net II ADC liquid chromatograph system equipped with DAD and a Rheodyne injection valve (20 μl loop). Quantification was performed by integration of peak areas at 280 nm, with reference to calibrations done using known amounts of pure compounds.

7.2.3.2. Extraction of saponins and flavonoids

Two and a half grams of each fibre (in duplicate) were extracted with 100 ml of 80% ethanol. The samples were blended in a Sorvall Omnimixer, Model 17106 (Du Pont Co., Newtown, CT), at maximum speed for 1 min, and then passed through filter paper. Ethanolic extracts were stored at −20 °C until analysis.

7.2.3.3. Quantification of saponins

Five millilitre aliquots (in duplicate) of each ethanolic extract were dried under air flow and re-dissolved in 2 ml of distilled water by sonication. Water solutions were extracted twice with 2 ml of hexane, ethyl acetate, and butanol, sequentially. Butanol extracts were collected and dried under air flow, the residue was re-dissolved in 2 ml of distilled water, and then loaded onto a 1 ml Sep-Pak C_{18} cartridge (Waters Corporation, Milford, MA) preconditioned with 96% ethanol. Cartridges were washed with 5 ml of water and then with 5 ml of 96% ethanol. Ethanol fractions were assayed for saponin content.

A colorimetric method for saponin quantification was developed in our laboratory based on reactive anisaldehyde–sulphuric acid–acetic acid for TLC staining (Wang *et al.*, 2007). Two hundred microlitres of purified ethanol fractions were dispensed in quadruplicate and 400 μl of a reactive acid (sulphuric acid:acetic acid, 1:1) were added. After mixing, 20 μl of *p*-anisaldehyde were added to three of the replicates, with the same volume of water being added to the fourth to be used as a sample blank. All tubes were heated at 95–100 °C in a water bath for 2 min and then cooled with tap water. The absorbance at 630 nm was determined. In each determination, a calibration curve was done, using diosgenin as a reference standard.

7.2.3.4. Quantification of flavonoids

Flavonoids were detected and quantified by HPLC (Fuentes-Alventosa *et al.*, 2007) using a Synergi 4 μm Hydro-RP80A reverse-phase column as before. The gradient profile was formed using solvent A (10% aqueous acetonitrile plus 2 ml/l acetic acid) and solvent B (40% methanol, 40% acetonitrile, and 20% water plus 2 ml/l

acetic acid) in the following program; the proportion of B increased from 10% to 42.5% B for the first 15 min, increased to 70% over the next 6 min, remained at 70% for 3.5 min, increased again to 100% over the next 5 min, and finally returned to the initial conditions. The flow rate was 1 ml/min, and the column temperature was 30 °C. Flavonoids were detected using a Jasco-LC-Net II ADC liquid chromatograph system equipped with DAD and a Rheodyne injection valve (20 μl loop). Spectra from all peaks were recorded in the 200–600 nm range, and the chromatograms were acquired at 360 nm. Quantification was performed by integration of peak areas at 360 nm, with reference to calibrations done while using known amounts of pure compounds.

7.2.3.5. Sterols

The methods applied for hydrolysis, saponification, silylation and quantification were adapted from Jiménez-Escrig *et al.* (2006). Fibre samples (0.5 g in duplicate) were weighed in 50 ml screw-capped Erlenmeyer flasks, 4 ml of internal standard solution (20 μg cholesterol/ml ethanol) and 10 ml of 6 M HCl were added to each sample, and the flask were heated at 80 °C for 1 h in a shaking water bath. The flasks were cooled to room temperature and 20 ml of hexane:diethyl ether (1:1) mixture were added. The samples were shaken for 10 min and allowed to stand for phase separation. The upper phase was evaporated to dryness under vacuum at 50 °C. Saponification was performed as follows: 8 ml of 2 M ethanolic KOH were added to the dry extracts, the mixtures were transferred to a 50 ml screw-capped Erlenmeyer flask, and heated at 80 °C for 30 min in a shaking water bath. Then, 20 ml of cyclohexane and 12 ml of distilled water were added to each sample. The samples were shaken for 10 min and allowed to stand for phase separation. The unsaponifiable upper phase was evaporated to dryness under vacuum at 50 °C. The residue was re-dissolved in 1 ml of dichloromethane and loaded onto a 1 ml Sep-Pack C_{18} cartridge, preconditioned with methanol. Sterol fractions were eluted with 15 ml of dichloromethane:methanol (95:5) and evaporated to dryness under vacuum at 50 °C. The residue was re-dissolved in 0.5 ml of dichloromethane. Aliquots of 100 μl of the sterol fractions were placed in a pre-silanised screw-capped vial. The solvents were evaporated under nitrogen and the TMS ether derivatives of the sterols were prepared by adding 100 μl of the silylation reagent (BSTFA:TMCS, 99:1) and 100 μl of anhydrous pyridine. The samples were then heated at 60 °C for 30 min or left overnight at room temperature for silylation. The excess silylating reagent was removed

under nitrogen at 50 °C, and the residue was dissolved in 600 μl of hexane. Sterol quantification was by GC. A Hewlett–Packard 5890 Series II chromatograph, fitted with a 30 m × 0.25 mm (film thickness = 0.25 μm) cross-linked methyl siloxane capillary column (HP-1 from Agilent, Santa Clara, CA), was employed. The oven temperature program used was as follows: initial, 50 °C, 2 min; raised at 30 °C/min to 245 °C, 1 min; raised at 3 °C/min to 275 °C, and held for 28.5 min. The carrier gas was helium at a flow rate of 1 ml/min. The injector temperature was 250 °C and the FID temperature was 280 °C.

7.2.3.6. Fructans

Fructan amounts were determined using a Megazyme kit (K-FRUC), whose procedure is described in detail at http://www.megazyme.com/downloads/en/data/K-FRUC.pdf. This assay is based on AOAC method 999.03 and AACC method 32.32. Briefly, fibre samples (0.5 g in duplicate) were extracted twice with 20 ml each of hot distilled water for 15 min at 80 °C with continuous stirring. After filtering through glass filter paper, both extracts were collected and the volume made up to 50 ml. Two aliquots of 50 μl of the extracts were placed in test tubes and 50 μl of sucrase/amylase solution were added. After incubation (30 min, 40 °C), 50 μl of a 10 mg/ml sodium borohydride solution in 50 mM sodium hydroxide were added and the tubes were incubated under the same conditions. To remove excess borohydride and adjust the pH to approximately 4.5, 125 μl of 0.2 M acetic acid were added. Carefully, 50 μl of this final solution were transferred to test tubes (×3), and 25 μl of fructanase solution were added to two of the tubes and 25 μl of 0.1 M sodium acetate buffer were added to the third (sample blank). After incubation (40 °C for 20 min) to produce complete hydrolysis of fructans, 1.25 ml of *p*-hydroxybenzoic acid hydrazide (PAHBAH), reactive for reducing sugars, were dispensed into each tube. Tubes were placed in a boiling water bath for 6 min and allowed to cool in cold water. Absorbance at 410 nm was measured against a reagent blank. During each determination, a calibration curve was prepared using fructose as reference standard.

7.2.4. Antioxidant capacity

Soluble antioxidant activity was determined after fibre extraction (in duplicate) with methanol:water (50:50) and acetone:water (70:30) (Larrauri *et al.*, 1997b) by the $DPPH^{\cdot}$ method (Rodríguez *et al.*, 2005a). Total antioxidant activity was evaluated as described by Serpen *et al.* (2007). Between 3 and 20 mg of fibre was transferred to an Eppendorf tube (for weights lower than 3 mg, fibre had to be diluted with cellulose as inert material), and the reaction was started by adding 1 ml of the $DPPH^{\cdot}$ reagent (3.8 mg/50 ml methanol). After 30 min of continuous stirring, samples were centrifuged and the absorbance of the cleared supernatants was measured (in triplicate) at 480 nm. Both antioxidant activities were expressed as millimoles of Trolox equivalent antioxidant capacity (TEAC) per kilogram of sample by means of a dose–response curve for Trolox.

7.2.5. Statistical analysis

Results were expressed as mean value ± standard deviation. To assess for differences in the composition and physicochemical characteristics between the different treatments, multiple sample comparison was performed using the Statgraphics Plus program Version 2.1. (StatPoint Inc., Warrenton, VA). Multivariate analysis of variance (ANOVA), followed by Duncan's multiple comparison test, was performed to contrast the groups. The level of significance was $p < 0.05$.

7.3. RESULTS AND DISCUSSION

Asparagus by-products could be considered an interesting source of dietary fibre. By-product processing involves several factors that affect fibre composition and functionality (Fuentes-Alventosa *et al.*, 2009a). In this work, we report the composition of bioactive compounds and the antioxidant activity of a fibre-rich product obtained from asparagus by-product, which may be used as an ingredient in the preparation of additional food products. Three factors have been taken into account (severity of treatment – temperature and time –, solvent, and drying system) and their effects on fibre characteristics will be discussed.

7.3.1. Bioactive compound composition

Table 7.2 shows the composition of several bioactive compounds analysed in fibre-rich powders obtained from asparagus by-products.

Table 7.2

Bioactive components of different fibrous residues from asparagus by-products (mg/g dry matter)[a].

		HYDROXYCINNAMIC ACIDS[b]	SAPONINS	FLAVONOIDS	STEROLS	FRUCTANS
I/W	O-D	2.81±0.29a	2.50±0.12a	-	0.82±0.04a	-
	F-D	3.89±0.31b	3.18±0.33b	-	0.81±0.01a	0.21±0.03a
I/E	O-D	3.01±0.04a	3.37±0.01bc	0.64±0.03a	1.03±0.06b	1.24±.090b
	F-D	3.83±0.10b	3.64±0.33c	1.82±0.31b	1.05±0.01b	1.43±0.25c
G/W	O-D	3.07±0.23a	2.44±0.07a	1.08±0.09a	0.64±0.09c	0.33±0.04a
	F-D	4.91±0.30c	2.56±0.24a	-	0.63±0.07c	0.26±0.03a
G/E	O-D	2.31±0.06d	2.14±0.02d	-	0.83±0.06a	0.20±0.04a
	F-D	2.85±0.01a	2.74±0.01a	-	0.92±0.05a	0.77±0.19d

[a] *All analyses are done at least in duplicate.*
[b] *Means within a column bearing the same letter are not significantly different at 5% level as determined by the Duncan multiple range test.*

7.3.1.1. Hydroxycinnamic acids

The hydroxycinnamic acids present in the green spears are mainly coumaric acid, ferulic acid, and its dimers. Middle and basal portions of the spears are richer in these compounds than the upper portion, especially after a storage period (Rodríguez *et al.*, 2005b). In the fibre-rich powders assayed, the amount of HCA ranged from 2.31 to 4.91 mg/g, with ferulic acid derivatives (FAD) of 0.8 to 1.8 mg/g. In both intense and gentle treatments, significant differences were caused by the choice of drying system, with higher HCA content in the freeze-dried samples than in those dried in an oven. In the intense treatment process, the solvent did not cause any differences, but in the gentle process, fibres obtained with water had the highest HCA content.

The amounts of HCA quantified in the fibre-rich powders were higher than previously reported for green asparagus (0.6 mg HCA/g cell wall material in fresh asparagus and 1.6 mg HCA/g of cell wall material in stored spears; Rodríguez, *et al.*, 2005b), but lower than that found in the white spears, (0.7 mg FAD/g of cell wall material in fresh asparagus and 2.9 mg FAD/g of cell wall material in stored spears; Jaramillo *et al.*, 2007). It is important to take into account that these authors, when working with green asparagus, analysed only the edible portion of the spears. HCA and FAD (monomers, dimers and trimers) especially are related to asparagus hardening during storage, *via* cross-linking of plant cell wall polymers (Rodríguez-Arcos *et al.*, 2004). Asparagus by-products are harder and more fibrous than the edible portions, therefore, their HCA and FAD content must be higher than that of edible portions. One gram of these fibre-rich powders brings to the diet as much FAD as a serving of some fruits (berries, plums, and apples), vegetables (tomato, carrot, and lettuce), and beer (Zhao & Moghadasian, 2008). From a nutritional point of view, FADs have been considered to impede the degradation of polysaccharides by intestinal bacteria. Recent studies (Funk *et al.*, 2007) indicate that low to moderate amounts (1.5–15.8 mg FAD/g of cell wall material) do not interfere with hydrolysis of cell walls by human gut microbiota. Additionally, FADs have high antioxidant activity (Chen & Ho, 1997) and many other potential health benefits (Plate & Gallaher, 2005). When linked to arabinoxylan oligosaccharides, they stimulate the growth of *Bifidobacterium bifidum* (Yuan *et al.*, 2005).

7.3.1.2. Saponins

Saponins are also present in the fibre-rich powders from asparagus by-products. These bioactive compounds are present in amounts between 2.14 and 3.64 mg/g. During intense treatments, the samples treated with ethanol had higher saponin content than those treated with water, but during gentle treatments, all the samples had almost the same saponin content. In general, fibres from gentle treatments had significantly lower saponin content than samples from intense treatments, probably due to a higher solubilisation of material in the latter. In this work, we quantified saponin amounts by spectrometry, so the results expressed here could be overestimated. However, previously reported data on the basal zone of asparagus shoots (Wang *et al.*, 2003) by LC/MS (25 mg protodioscin/100 g of fresh sample) are in agreement with our results. Garlic and onion are two species of the *Allium* genus (*A. sativum* and *A. cepa*, respectively) widely used in folk medicine as hypotensive, hypoglycaemic, antimicrobial, antithrombotic, anticarcinogenic, and diuretic agents. These pharmacological activities are partially due to steroidal saponins. The content of this compound in Allium species is 2–3 mg/g of dry matter (Smoczkiewicz *et al.*, 1982), similar to the quantified amounts in the asparagus fibre-rich powders.

7.3.1.3. Flavonoids

Other bioactive compounds quantified in the asparagus by-product, fibre-rich powders are flavonoids. Asparagus flavonoids (mainly rutin) are partly responsible for the antioxidant characteristics of this vegetable (Guillén *et al.*, 2008; Makris & Rossiter, 2001 and Rodríguez *et al.*, 2005a). Only three of the analysed samples (Table 7.2) had significant amounts of flavonoids. Makris & Rossiter (2001), working with green asparagus, concluded that simply chopping and macerating caused a decrease of as much as 18.5% in rutin content without the liberation of quercetin. Therefore, rutin must be oxidatively cleaved rather than hydrolysed. However, hydrolysis might occur to some extent, but the quercetin was oxidised as soon as it was liberated from the sugar, and, thus, did not accumulate in detectable amounts. The authors in the same work quantified a 43.9% decrease in total flavonols when asparagus was boiled for 60 min. Asparagus had high levels of peroxidase activity (Rodríguez *et al.*, 1999a), with these enzymes having some specificity for flavonols (Hirota *et al.*, 1998). As observed,

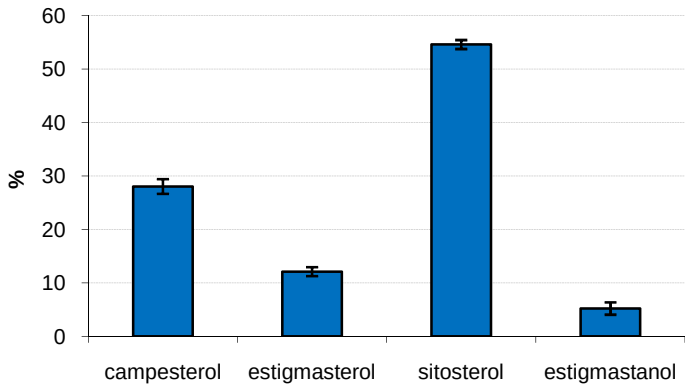
several factors could affect the stability of flavonoids during vegetable processing. Additionally, in our processing experiences, another factor must be taken into account, since continuous stirring was applied during 90 min. of processing in an open reactor. Therefore, flavonol oxidation caused by air inclusion within the asparagus by-product homogenate may occur to some extent. Presently, an optimised process (patent pending) is being applied to obtain fibre-rich powders from asparagus, in which most of the factors affecting flavonoid stability have been controlled. The relatively low content of flavonoids in these samples led to low levels of antiradical activity, which will be commented on below.

7.3.1.4. Sterols

Plant sterols are bioactive compounds present in asparagus spears and, therefore, in their by-products. In the fibre-rich powder, the amount of analysed phytosterol varied between 0.63 and 1.03 mg/g. The amount of phytosterol was affected by the solvent and intensity of treatment, but not by the drying system. Samples treated with ethanol had higher amounts of sterols than those treated with water. Samples obtained by gentle processing had lower amounts of sterols than those obtained from intense treatment. As seen in Figure 7.1, β-sitosterol was the most abundant sterol (more than 50% of total quantified sterols), as is the case in most fruits and vegetables (Jiménez-Escrig *et al.*, 2006).

Figure 7.1

Percentage contribution of each sterol to the total sterol quantified. Error bars correspond to the average value of the eight analysed samples.



7.3.1.5. Fructans

Fructans were also present in asparagus by-products and their amounts in the fibres varied with processing method, from 0.2 to 1.4 mg/g of fibre. The highest content was found in fibres obtained after intense treatment with ethanol (I/E), with the rest of the fibres having almost the same fructan content. Fructans present in asparagus were mainly fructooligosaccharides (FOS – with low polymerisation degree) instead of inulin (high molecular weight) (Shiomi *et al.*, 2007). FOS content of asparagus by-product was around 4 mg/g of dry weight (data not shown) and during the fibre obtaining process, it decreased between 25% and 95%, a reduction that was explained by the high solubility of these low molecular weight carbohydrates (3–4 polymerisation degree) in water/ethanol mixtures, even at room temperature.

7.3.2. Antioxidant activity

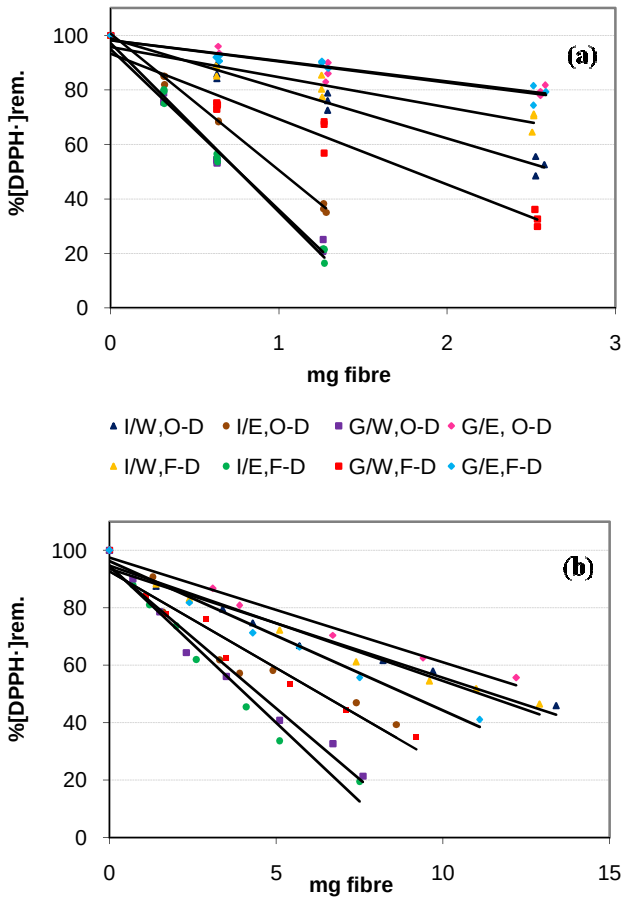
To measure antioxidant activity, the antiradical capacity (ARC) against $DPPH^{\cdot}$ was assayed in both the soluble fraction and total fibre. In Figure 7.2, the dose–response lines are presented. The correlation between dose and decrease in $DPPH^{\cdot}$ concentration was very high in all cases ($r > 0.9$). There are two fibres that have almost the same ARC; these were I/E, F-D and G/W, O-D fibres, which also had the highest content of flavonoids (Table 7.2). The I/E, O-D fibre also contained flavonoids and the ARC of its soluble extract (Subfigure (a)) was near those commented on above. Soluble extract from G/W, F-D fibre, in which flavonoids were not detected, had a lower ARC than extracts with these antioxidant compounds. However, looking at the total ARC of the fibres (Subfigure (b)) I/E, O-D and G/W, F-D fibres had the same ARC. This fact could be explained by the fact that although the latter fibre did not contain flavonoids, it had the highest amount of hydroxycinnamates (Table 7.2). These compounds, especially the ferulates, have a high antioxidant capacity and are mainly found esterified to fibre polymers in asparagus spears, therefore the ARC caused by ferulates could be measured only in the total antioxidant assay. The rest of the fibres (I/W and G/E) had similar ARC, which was much lower than those described above were.

Figure 7.2

Dose–response lines of antiradical capacity of asparagus by-product fibres. Antiradical capacity is expressed as percent DPPH$^{\bullet}$ remaining in solution (%DPPH$^{\bullet}$ rem.) after 30 min of reaction.

(a) Antiradical capacity of soluble fractions.

(b) Total antiradical capacity of fibres. Each individual point in the graphs is the average value of three replicates.



In Table 7.3, the results for antioxidant capacity expressed as Trolox equivalents/g of fibre (TEAC) are presented. The strongest antioxidant fibres (I/E, F-D, and G/W, O-D) had an equivalent of about 13 μmol Trolox/g of total fibre or 11 μmol Trolox/g of fibre (soluble extract). The second strongest ARC group (I/E, O-D and G/W, F-D) had about half of the activity listed above, and the rest at about one-third. Looking at the soluble activity only, there were differences within the last group, with G/E fibres having the lowest activity, nearly one tenth of that determined for the I/E, F-D and G/W, O-D fibres. The ARC of soluble extracts had a high correlation with the flavonoid content of fibres ($r = 0.8056$), but the total activity of fibres correlated better with ferulic acid derivatives plus flavonoids ($r = 0.8291$). The strongest

antioxidant fibres from asparagus by-products are in the same range of activity as a fibre-rich product from cocoa bean husks (Lecumberri *et al.*, 2007), and about one tenth of the ARC of fibres from different citrus by-products (Marín *et al.*, 2007), but they are much lower than fibre from guava fruit by-product (Jiménez-Escrig *et al.*, 2001) and from red grape pomace and stem (Llobera & Cañellas, 2007). The difference must be due to a higher amount of polyphenols, in addition to the presence of other antioxidant compounds (condensed tannins and ascorbic acid).

Table 7.3

Antioxidant activity (total and soluble) of the fibre-rich asparagus powders, expressed as Trolox equivalents (μmol TE/g fibre).

		TEAC (SOLUBLE)	TEAC (TOTAL)
I/W	O-D	3.30	3.80
	F-D	2.05	3.96
I/E	O-D	5.16	7.29
	F-D	11.43	13.11
G/W	O-D	11.41	12.17
	F-D	5.33	7.50
G/E	O-D	1.33	3.12
	F-D	1.40	4.55

7.4. CONCLUSIONS

Besides the chemical composition and functional characteristics (Fuentes-Alventosa *et al.*, 2009a), the bioactive profile of asparagus by-product fibres was affected by processing to obtain the powders. In general, intense treatments led to fibres with the highest content of bioactive compounds, especially those treated with ethanol, probably due to a concentration effect. The drying system had a clear effect only in the case of hydroxycinnamic acids, freeze-dried fibres having higher amounts than oven-dried ones. The most affected components were flavonoids, which are mainly responsible for the antioxidant capacity of the fibres. These compounds are oxidised very easily and were lost under most processing conditions of the fibres, decreasing the overall antioxidant capacity. Presently, we are working on an optimised process (patent pending) that maintains the maximum flavonoid content.

Although the content of bioactive compounds and the antioxidant capacity of these fibres were relatively low, it is important to remark that they are interesting sources of dietary fibres, which can be added as functional ingredients in fibre-enriched food. In comparison, other fibre sources such as cellulose, wheat bran, glucomannans, and others lack this intrinsic antioxidant activity and other bioactive compounds. These facts, together with the functional properties (water and oil holding capacities, and glucose retardation index) (Fuentes-Alventosa *et al.*, 2009a) and technical compatibility (Sanz *et al.*, 2008) possessed by fibre from asparagus by-products, increases its attractiveness as an alternative source of dietary fibre. *In vivo* studies on the antioxidant status of asparagus fibre-fed rats and other physiological parameters are under way. These results could be decisive for the use of this added-value agricultural by-product.

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8. OBTENCIÓN DE INGREDIENTES FUNCIONALES A PARTIR DE SUBPRODUCTOS DE ESPÁRRAGO

OBTENCIÓN DE INGREDIENTES FUNCIONALES A PARTIR DE SUBPRODUCTOS DE ESPÁRRAGO

RESUMEN

Muchos de los componentes bioactivos del espárrago están presentes también en las porciones del turión que se desechan durante su procesado. El objetivo de este capítulo es la puesta a punto de un método de obtención de los mismos a partir de los subproductos del espárrago. Se ha conseguido obtener extractos funcionales que contienen una riqueza en peso en flavonoides del 0,45% y en saponinas del 1,15% y extractos parcialmente purificados en flavonoides (11,08% de riqueza media en peso, que es 24 veces más concentrado que el extracto inicial) y saponinas (80,67% de riqueza media en peso, que es 70 veces más concentrado que el extracto inicial). El residuo que queda tras la obtención de los extractos está constituido por una fibra bioactiva cuya composición química, de la fibra alimentaria total, fitoquímica y capacidad antioxidante se ha optimizado con respecto al subproducto sin tratar. Tanto los extractos globales y parcialmente purificados, como la fibra bioactiva, pueden tener diferentes aplicaciones industriales como ingredientes y nutracéuticos en muy diversos campos de la industria alimentaria y farmacéutica, con lo que se conseguirá el aprovechamiento integral de estos subproductos. El proceso diseñado está pendiente de patente.

8.1. INTRODUCCIÓN

En los últimos años se han realizado numerosos estudios epidemiológicos que relacionan el consumo de frutas y verduras con un descenso en la incidencia de cáncer y mortalidad por enfermedad cardiovascular (Doll, 1990; Rimm *et al.*, 1996; Schieber *et al.*, 2001), siendo el contenido en fibra alimentaria y en fitoquímicos uno de los factores clave con los que se ha asociado este hecho.

Actualmente está bien establecido que la fibra ejerce un efecto beneficioso en el organismo, tanto por sus características físico-químicas y funcionales, como por su composición de polisacáridos y lignina (Marlett, 1990; 1997). Con el transcurso del tiempo se ha demostrado que la fibra permite un funcionamiento regular del intestino, aumenta el peso de las heces y facilita la eliminación de ácidos biliares, esteroles y grasas (Heredia Moreno *et al.*, 2002). Se recomienda el consumo de fibra alimentaria para el tratamiento y prevención de algunas enfermedades, tales como cáncer de colon, enfermedades coronarias, obesidad, diabetes y trastornos gastrointestinales (Anderson *et al.*, 1994).

Numerosos estudios clínicos apoyan el papel de los fitoquímicos como constituyentes de los alimentos que mejoran el estado de salud (Kris-Etherton *et al.*, 2002; Scalbert *et al.*, 2002). Así, por ejemplo, la función de los fitoquímicos antioxidantes en la prevención de enfermedades cardiovasculares se ha atribuido principalmente a su efecto antioxidante sobre las LDL (Rankin *et al.*, 1993; Scalbert *et al.*, 2002) por medio de la inactivación de radicales peroxilo e hidroxilo (Rankin *et al.*, 1993). Los fitoquímicos son también responsables de características de calidad como el color, sabor y aroma de frutas, verduras y productos alimenticios relacionados (vinos, zumos, etc.) (Tomás-Barberán y Espín, 2001). En las plantas son metabolitos secundarios y se les atribuyen diversas propiedades como, por ejemplo, protección contra la incidencia de rayos ultravioleta, protección contra insectos y atrayentes de animales para asegurar la polinización y la dispersión de semillas (Harbone, 1982). Por estas razones, los fitoquímicos se sintetizan preferentemente en los tejidos externos de las plantas. Estas partes externas son los principales residuos generados durante la manipulación y procesado de las frutas y verduras y constituyen una buena fuente para su extracción (Tomás-Barberán *et al.*, 2004).

El espárrago es un producto de gran interés para el sector de la población demandante de alimentos saludables, ya que contiene diversos fitoquímicos que le confieren una potencial actividad biológica importante.

Para la elaboración industrial, los turiones se cortan a una determinada longitud y, en algunos casos, se pelan, llegando a representar las porciones que se desechan más del 50% del total, lo que supone un gran problema para las industrias del sector desde el punto de vista económico y medioambiental. Hasta ahora uno de los usos de este subproducto había sido la alimentación animal. Sin embargo, actualmente existen grandes dificultades, ya que cuando se utiliza para piensos destinados al ganado bovino o caprino, se reduce considerablemente la riqueza grasa de la leche que producen estos animales.

Actualmente existe una problemática social, legislativa y económica en relación a los residuos y productos desechados de la industria de transformados vegetales. Los subproductos del espárrago se caracterizan por ser materia orgánica fácilmente degradable, carente de componentes altamente tóxicos y con un elevado grado de humedad. Muchos de los componentes bioactivos se encuentran en las porciones del turión que se desechan durante el procesado del espárrago, presentando tanto éstas como la parte comestible una composición similar en dichos compuestos, lo que resulta de gran interés científico e industrial. Así, en un trabajo previo de nuestro Grupo de Investigación, se comprobó que los extractos etanólicos de subproductos de espárragos trigueros de Huétor-Tájar poseen una alta capacidad antioxidante (Rodríguez *et al.*, 2005a).

Asumiendo, por tanto, que tienen una composición similar a la de la parte comestible de los turiones, los subproductos representan una fuente prometedora de compuestos con alto valor añadido (fitoquímicos y fibra) (Nindo *et al.*, 2003). Con una tecnología adecuada, este material residual se puede convertir en productos comerciales, así como en ingredientes funcionales.

El objetivo de este estudio es el diseño de un proceso sencillo y de fácil adaptación industrial para el aprovechamiento integral de subproductos del espárrago.

Se pretende obtener, por un lado, extractos funcionales que contengan distintos fitoquímicos y, por otro, una fibra funcional a partir del residuo que queda tras la obtención de los extractos.

8.2. MATERIALES Y MÉTODOS

8.2.1. Subproductos de espárrago

Los residuos de espárrago fueron suministrados por Centro Sur S.C.A. (Huetor-Tájar, Granada) durante las campañas de 2006 a 2008. Son la porción basal (15-20 cm) de turiones de espárrago verde destinados a su envasado comercial. Los subproductos se reciben en el laboratorio al día siguiente del procesado de los espárragos y se mantienen en cámaras frigoríficas a 4ºC hasta su tratamiento.

8.2.2. Diseño de experiencias de tratamientos de subproductos de espárrago

8.2.2.1. Reactor abierto de laboratorio con agitación continua

Se realiza un diseño experimental de cuatro variables con dos niveles cada una, según se aprecia en la Tabla 8.1. Las cuatro variables estudiadas son temperatura (25 y 60 ºC), tiempo de extracción (1 y 90 minutos), escaldado (realización o no del mismo) y disolvente (agua y etanol).

Los tratamientos a los que se someten los tallos de espárrago se describen a continuación. Se cortan 2 kg de subproductos en pequeñas porciones y se homogeneizan y mezclan con el solvente de extracción (que está a la temperatura fijada) en una homogeneizadora profesional (Sirman Orione, Marsango, Italia) a máxima velocidad, durante 1 minuto, en una proporción sólido:líquido 1:1. Las muestras que deben recibir un escaldado previo se sumergen en un baño con agua (Precisdig, J. P. Selecta, Abrera, Barcelona) durante 15 minutos a 90 ºC y a continuación se atemperan con agua corriente.

En el siguiente paso, las muestras que sólo se extraen durante 1 minuto se procesan directamente en una licuadora industrial (L-4500, Tecno-Chufa, Valencia, España), para obtener, por un lado, un extracto líquido de espárrago y, por otro, un residuo fibroso húmedo. Sin embargo, las muestras que se extraen durante 90 minutos se procesan en un reactor abierto diseñado en nuestro laboratorio, con control de tiempo

y temperatura y agitación continua, antes de su procesado en la licuadora industrial. Cada tratamiento se realiza por duplicado.

Tabla 8.1

Tratamientos realizados con tallos de espárragos.

Tratamiento	Temperatura (ºC)	Tiempo (min)	Escaldado	Disolvente
1	60	90	SI	Agua
2	60	90	SI	Etanol
3	60	90	NO	Agua
4	60	90	NO	Etanol
5	60	1	SI	Agua
6	60	1	SI	Etanol
7	60	1	NO	Agua
8	60	1	NO	Etanol
9	25	90	SI	Agua
10	25	90	SI	Etanol
11	25	90	NO	Agua
12	25	90	NO	Etanol
13	25	1	SI	Agua
14	25	1	SI	Etanol
15	25	1	NO	Agua
16	25	1	NO	Etanol

Uno de los residuos fibrosos húmedos obtenidos se liofiliza y el otro se seca en estufa con recirculación de aire (a 60 ºC durante 16 horas). Una vez secas las fibras, se pulverizan en un molino de martillo hasta obtener un tamaño de partícula inferior a 1 mm, y se almacenan a 4 ºC hasta su análisis.

8.2.2.2. Escalado a planta piloto

Este proceso, diseñado por el Grupo de Pared Celular y Componentes Bioactivos de Alimentos del Instituto de la Grasa (CSIC), no se describe en la presente memoria ya que está pendiente de obtención de patente.

8.2.3. Análisis de flavonoides y saponinas en los extractos

Los flavonoides y saponinas se determinan según se describe en Fuentes-Alventosa *et al.* (2009b), artículo que forma parte de esta memoria de Tesis. Para cuantificar el contenido total de flavonoides y saponinas, los subproductos se extrajeron con etanol 80%, en proporción 1:4 (peso de muestra fresca: volumen de etanol). Las muestras se mezclan en el homogeneizador Sorvall Omnimixer, modelo 17106 (Du Pont Co., Newtown, Estados Unidos), durante 1 minuto a velocidad máxima. Se filtran con papel de filtro (Anoia, Barcelona, España) y se llevan a volumen con etanol al 80%. Los extractos etanólicos se almacenan a -20 ºC hasta su posterior análisis. Las extracciones se hacen por duplicado. Éstos son los valores de referencia para calcular los rendimientos de extracción obtenidos en las distintas experiencias.

8.2.4. Análisis de la fibra bioactiva

Los análisis realizados en las fracciones de fibra en cuanto a composición (azúcares solubles, fibra y proteína), fibra total (azúcares neutros, ácidos urónicos y celulosa) y fitoquímicos (flavonoides, saponinas, esteroles y ácidos hidroxicinámicos) se realizan según se describen en Fuentes-Alventosa *et al.* (2009a y 2009b).

Para la determinación de la actividad antioxidante de la fracción soluble (AAO-FS) de la fibra se realizan tres ensayos diferentes: capacidad antirradical, poder reductor y capacidad de inhibición de la oxidación primaria.

Para todos los ensayos de AAO-FS se parte de un extracto antioxidante de la fibra obtenido según se describe en Lecumberri *et al.* (2007). Se pesa por duplicado 1 g de fibra en un tubo de centrífuga y se extrae secuencialmente (1 hora a temperatura ambiente, en agitación continua) con 30 mL de HCl 16 mM en metanol:agua (50:50) y 30 mL de acetona:agua (70:30). Después de centrifugar (15 minutos, 3000 g), se unen los sobrenadantes de cada extracción, se concentran y se llevan a volumen.

La capacidad antirradical (CAR) se determina por el método del DPPH· (2,2-difenil-1-picrilhidracilo) [Rodríguez *et al.*, 2005a]. Se dosifican en una placa multipocillo 5 µL de extracto y 195 µL del reactivo DPPH· (3,8 mg/50 mL de metanol).

Transcurridos 30 minutos se mide la absorbancia a 490 nm en un lector de placas (Microplate Reader Model 550, Bio-Rad, California, Estados Unidos).

Para el estudio del poder reductor (PR) se usa una modificación del método de Psarra *et al.* (2002). Se emplea como oxidante $FeCl_3$. El ion Fe^{2+} producido en la reacción redox forma un producto coloreado con 2,2'-dipiridilo. Se dosifican 10 µL de cada extracto y 10 µL de $FeCl_3$ 6 mM en ácido cítrico 5 mM en los pocillos de la microplaca por cuadruplicado. Para cada muestra, se utiliza un blanco sin $FeCl_3$. Después de la dosificación, la microplaca se incuba durante 20 minutos a 50 ºC en una estufa Binder (Tuttlingen, Alemania). Posteriormente se añaden 180 µL de solución de dipiridilo (5 g/L) en ácido tricloroacético al 1,2% a cada pocillo. Se mantiene a temperatura ambiente durante 30 minutos y se lee la absorbancia a 490 nm.

El método que se utiliza para calcular la capacidad de inhibición de la oxidación primaria (CIOP) está basado en la determinación de peróxidos formados a partir de dienos conjugados del ácido linoleico en presencia o ausencia de un antioxidante potencial (Ruberto *et al.*, 2000). Se prepara una solución 0,1 M de lauril sulfato sódico en Na_2HPO_4 acuoso 0,01 M y se ajusta a pH 7,4 con H_3PO_4. Se añade ácido linoleico hasta una concentración de 2,6 mM y se agita hasta su completa emulsión. Como iniciador de radicales libres se utiliza una solución 0,07 M de 2,2'-azobis(2-amidinopropano) (ABAP) en agua. Se añaden en un tubo de ensayo 5 µL de solución de ABAP, 25 µL de cada extracto y 1 mL de la solución de ácido linoleico. Cada extracto se dosifica por cuadruplicado. Se emplea un blanco sin ácido linoleico para cada muestra. Los tubos de ensayo se incuban a 50ºC en estufa durante 1 hora, y después de este período de tiempo se mide la absorbancia a 232 nm. La absorbancia del ácido linoleico sin la solución de antioxidante se considera como el 100% de oxidación.

Los residuos de fibra tras la obtención del extracto antioxidante se secan al aire y sobre ellos se determina la actividad antioxidante de la fracción insoluble (AAO-FI) por el método del DPPH· descrito por Serpen *et al.* (2007). En los ensayos en los que hay que pesar menos de 3 mg las fibras se diluyen con celulosa, como material inerte. En tubos eppendorf se añaden las fibras y 1 mL de reactivo DPPH· (3,8 mg/50 mL de metanol). Las muestras se agitan durante 30 minutos, se centrifugan y se mide, por triplicado, la absorbancia de los sobrenadantes a 480 nm.

Los resultados de todos los ensayos de actividad antioxidante se expresan como equivalentes Trolox (mmoles equivalentes Trolox/kg de fibra).

8.3. RESULTADOS Y DISCUSIÓN

8.3.1. Puesta a punto del proceso

8.3.1.1. Reactor abierto de laboratorio con agitación continua

- **Contenido de flavonoides y saponinas en los extractos**

La Tabla 8.2 muestra la concentración y porcentaje de recuperación de flavonoides en los diferentes tratamientos. El valor de referencia para calcular este porcentaje fue 282,88 mg/kg.

La mayor concentración de flavonoides en los extractos fue de 131,14 mg/kg de subproductos, obtenida con el tratamiento 4. Hay que indicar que, en general, se obtuvieron valores muy bajos y una alta variabilidad entre replicados del mismo tratamiento. La ausencia de flavonoides en los extractos no se corresponde con una mayor presencia de estos compuestos en las fibras bioactivas que se obtuvieron en estos tratamientos (Fuentes-Alventosa *et al.*, 2009b). La pérdida de flavonoides puede deberse, posiblemente, a procesos de oxidación enzimática, por acción de la peroxidasa (Hirota *et al.*, 1998), o química, debido a la aireación de la muestra en las distintas etapas de los tratamientos (homogeneización, extracción y centrifugación).

La realización de análisis estadístico mediante un ANOVA factorial y Tabla de Medias (Tablas 8.3 y 8.4) para los tratamientos con etanol (cuyos resultados fueron más homogéneos que los obtenidos con las extracciones acuosas) indica que de los tres factores, un mayor tiempo, una mayor temperatura y la combinación de ambos influyeron positivamente en la extracción de flavonoides, mientras que el escaldado y la combinación del mismo con los dos niveles de tiempo o temperatura produjeron efectos negativos.

Tabla 8.2

Concentración y porcentaje de recuperación de flavonoides en los diferentes tratamientos.

Tratamiento	Características	mg flavonoides/ kg subproducto			% Recuperación
1	60ºC-90'-agua-escaldado	0,57	±	1,14	0,20
2	60ºC-90'-etanol-escaldado	50,48	±	6,42	17,85
3	60ºC-90'-agua-no escaldado	13,96	±	16,28	4,93
4	60ºC-90'-etanol-no escaldado	131,14	±	9,48	46,36
5	60ºC-1'-agua-escaldado	44,68	±	55,66	15,79
6	60ºC-1'-etanol-escaldado	14,05	±	5,33	4,97
7	60ºC-1'-agua-no escaldado	10,45	±	15,09	3,69
8	60ºC-1'-etanol-no escaldado	10,90	±	2,92	3,85
9	25ºC-90'-agua-escaldado	0,99	±	1,98	0,35
10	25ºC-90'-etanol-escaldado	14,57	±	4,18	5,15
11	25ºC-90'-agua-no escaldado	0			0
12	25ºC-90'-etanol-no escaldado	25,04	±	9,00	8,85
13	25ºC-1'-agua-escaldado	24,75	±	31,25	8,75
14	25ºC-1'-etanol-escaldado	10,59	±	5,73	3,74
15	25ºC-1'-agua-no escaldado	76,04	±	1,10	26,88
16	25ºC-1'-etanol-no escaldado	14,14	±	5,99	5,00

Tabla 8.3

Tabla ANOVA para la extracción de flavonoides con etanol[a] (fuente: Statgraphics Plus 5.1).

Análisis de la Varianza paraCol 1 - Sumas de Cuadrados de Tipo III

Fuente	Suma de cuadrados	GL	Cuadrado Medic	Cociente-F	P-Valor
EFECTOS PRINCIPALES					
A:Col 2	10115,4	1	10115,4	241,48	0,0000
B:Col 3	14716,4	1	14716,4	351,31	0,0000
C:Col_4	4188,41	1	4188,41	99,99	0,0000
INTERACCIONES					
AB	10051,5	1	10051,5	239,95	0,0000
AC	2015,49	1	2015,49	48,11	0,0000
BC	4116,42	1	4116,42	98,27	0,0000
ABC	2956,8	1	2956,8	70,59	0,0000
RESIDUOS	1005,35	24	41,8896		
TOTAL (CORREGIDO)	49165,8	31			

Los cocientes F están basados en el error cuadrático medic residual.

[a] *Col 1: concentración de flavonoides; A:Col 2: Temperatura; B:Col 3: Tiempo; C:Col_4: Escaldado.*

Tabla 8.4

Tabla de Medias para la extracción de flavonoides con etanol[a] (fuente: Statgraphics Plus 5.1).

Tabla de Medias por mínimos cuadrados para Col_1
con 95,0 Intervalos de confianza

Nivel	Frecuencia	Media	Error Estándar	Límite Inferior	Límite Superior
Media Total	32	33,8638			
Col 2					
1	16	51,6431	1,61805	48,3036	54,9826
2	16	16,0844	1,61805	12,7449	19,4239
Col 3					
1	16	55,3088	1,61805	51,9692	58,6483
2	16	12,4188	1,61805	9,07924	15,7583
Col 4					
1	16	22,4231	1,61805	19,0836	25,7626
2	16	45,3044	1,61805	41,9649	48,6439
Col 2 según Col 3					
1 1	8	90,8113	2,28827	86,0985	95,534
1 2	8	12,475	2,28827	7,75223	17,1978
2 1	8	19,8062	2,28827	15,0935	24,529
2 2	8	12,3625	2,28827	7,63973	17,0853
Col 2 según Col 4					
1 1	8	32,2663	2,28827	27,5435	36,989
1 2	8	71,02	2,28827	66,2972	75,7428
2 1	8	12,58	2,28827	7,85723	17,3028
2 2	8	19,5887	2,28827	14,866	24,3115
Col 3 según Col 4					
1 1	8	32,5263	2,28827	27,8035	37,249
1 2	8	78,0913	2,28827	73,3685	82,814
2 1	8	12,32	2,28827	7,59723	17,0428
2 2	8	12,5175	2,28827	7,79473	17,2403

[a] *Col_1: concentración de flavonoides; Col 2: Temperatura (1: 60 ºC; 2: 25 ºC); Col 3: Tiempo (1: 90'; 2: 1'); Col 4: Escaldado (1: Sí; 2: No).*

En la Tabla 8.5 se muestra la concentración y porcentaje de recuperación de saponinas en los diferentes tratamientos. El valor de referencia para calcular este porcentaje fue 645,56 mg/kg.

Tabla 8.5

Concentración y porcentaje de recuperación de saponinas en los diferentes tratamientos.

Tratamiento	Características	mg diosgenina/ kg subproducto	% Recuperación
1	60ºC-90'-agua-escaldado	244,78 ± 0,99	37,95
2	60ºC-90'-etanol-escaldado	198,33 ± 23,88	30,75
3	60ºC-90'-agua-no escaldado	352,54 ± 39,24	54,66
4	60ºC-90'-etanol-no escaldado	204,71 ± 23,68	31,74
5	60ºC-1'-agua-escaldado	273,49 ± 18,91	42,40
6	60ºC-1'-etanol-escaldado	401,07 ± 28,40	62,18
7	60ºC-1'-agua-no escaldado	282,50 ± 29,06	43,80
8	60ºC-1'-etanol-no escaldado	307,29 ± 40,55	47,64
9	25ºC-90'-agua-escaldado	173,96 ± 34,68	26,97
10	25ºC-90'-etanol-escaldado	346,74 ± 45,69	53,76
11	25ºC-90'-agua-no escaldado	282,96 ± 31,30	43,87
12	25ºC-90'-etanol-no escaldado	406,26 ± 23,07	62,99
13	25ºC-1'-agua-escaldado	179,72 ± 16,86	27,86
14	25ºC-1'-etanol-escaldado	354,05 ± 27,76	54,89
15	25ºC-1'-agua-no escaldado	335,01 ± 24,62	51,94
16	25ºC-1'-etanol-no escaldado	443,26 ± 51,63	68,72

En general, las saponinas son más estables al calentamiento y la agitación que los flavonoides. La mayor concentración y, por tanto, mayor porcentaje de recuperación se obtuvo con el tratamiento 16 (443,26 mg de diosgenina / kg de residuo; 68,72% de recuperación). También se superó el 60% de recuperación con el tratamiento 12 (62,99%) y 6 (62,18%). Los menores porcentajes de recuperación se obtuvieron con el tratamiento 9 (26,97%) y el tratamiento 13 (27,86%). De los resultados obtenidos parece deducirse que los tratamientos con etanol son más efectivos en la extracción (cinco de los ocho superan el 50% de recuperación) que los acuosos (sólo dos superan el 50%).

También se realizó, en el caso de las saponinas, un ANOVA factorial y Tabla de Medias (Tablas 8.6 a 8.9) para los tratamientos con agua y etanol.

En el caso de las extracciones acuosas, una mayor temperatura y la combinación de la misma con un mayor tiempo influyeron positivamente, mientras que el escaldado y la combinación del mismo con los dos niveles de temperatura produjeron efectos negativos. El factor tiempo no tuvo influencia significativa.

Tabla 8.6

Tabla ANOVA para la extracción de saponinas con agua[a] (fuente: Statgraphics Plus 5.1).

Análisis de la Varianza paraCol 1 - Sunas de Cuadrados de Tipo III

Fuente	Suma de cuadrados	GL	Cuadrado Medio	Cociente-F	P-Valor
EFECTOS PRINCIPALES					
A:Col 2	14142,2	1	14142,2	20,67	0,0002
B:Col 3	116,325	1	116,325	0,17	0,6842
C:Col_4	62226,6	1	62226,6	90,97	0,0000
INTERACCIONES					
AB	4211,47	1	4211,47	6,16	0,0216
AC	9326,64	1	9326,64	13,63	0,0014
BC	1179,45	1	1179,45	1,72	0,2033
ABC	9015,06	1	9015,06	13,18	0,0016
RESIDUOS	14365,5	21	684,07		
TOTAL (CORREGIDO)	114936,0	28			

Los cocientes F están basados en el error cuadrático medio residual.

[a] *Col 1: concentración de saponinas; A:Col 2: Temperatura; B:Col 3: Tiempo; C:Col_4: Escaldado.*

En las extracciones etanólicas, una mayor temperatura y tiempo influyeron negativamente, mientras que el escaldado no tuvo influencia significativa.

Según estos resultados, y los obtenidos en el caso de los flavonoides, se descartó el escaldado para sucesivas experiencias.

Tabla 8.7

Tabla de Medias para la extracción de saponinas con agua[a] (fuente: Statgraphics Plus 5.1).

Tabla de Medias por mínimos cuadrados para Col_1
con 95,0 Intervalos de confianza

Nivel	Frecuencia	Media	Error Estándar	Límite Inferior	Límite Superior
Media Total	29	265,619			
Col 2					
1	13	288,326	7,55022	272,624	304,027
2	16	242,912	6,53868	229,314	256,51
Col 3					
1	13	263,559	7,55022	247,858	279,261
2	16	267,678	6,53868	254,08	281,276
Col 4					
1	15	217,988	6,80568	203,835	232,141
2	14	313,249	7,31047	298,046	328,452
Col 2 según Col 3					
1 1	5	298,658	11,9379	273,831	323,484
1 2	8	277,994	9,24709	258,763	297,224
2 1	8	228,461	9,24709	209,231	247,692
2 2	8	257,362	9,24709	238,132	276,593
Col 2 según Col 4					
1 1	7	259,135	9,988	238,364	279,906
1 2	6	317,516	11,3253	293,964	341,069
2 1	8	176,841	9,24709	157,611	196,072
2 2	8	308,982	9,24709	289,752	328,213
Col 3 según Col 4					
1 1	7	209,371	9,988	188,6	230,142
1 2	6	317,748	11,3253	294,195	341,3
2 1	8	226,605	9,24709	207,375	245,835
2 2	8	308,751	9,24709	289,521	327,982

[a] *Col_1: concentración de saponinas; Col 2: Temperatura (1: 60 ºC; 2: 25 ºC); Col 3: Tiempo (1: 90'; 2: 1'); Col 4: Escaldado (1: Sí; 2: No).*

Tabla 8.8

Tabla ANOVA para la extracción de saponinas con etanol[a] (fuente: Statgraphics Plus 5.1).

Análisis de la Varianza paraCol 1 - Sumas de Cuadrados de Tipo III

Fuente	Suma de cuadrados	GL	Cuadrado Medio	Cociente-F	P-Valor
EFECTOS PRINCIPALES					
A:Col 2	96215,8	1	96216,8	80,03	0,0000
B:Col 3	61201,9	1	61201,9	50,90	0,0000
C:Col_4	1875,02	1	1875,02	1,56	0,2238
INTERACCIONES					
AB	34123,0	1	34123,0	28,38	0,0000
AC	27895,8	1	27895,8	23,20	0,0001
BC	2487,77	1	2487,77	2,07	0,1632
ABC	8439,28	1	8439,28	7,02	0,0140
RESIDUOS	28855,3	24	1202,31		
TOTAL (CORREGIDO)	261095,0	31			

Los cocientes F están basados en el error cuadrático medio residual.

[a] *Col 1: concentración de saponinas; A:Col 2: Temperatura; B:Col 3: Tiempo; C:Col_4: Escaldado.*

Tabla 8.9

Tabla de Medias para la extracción de saponinas con etanol[a] (fuente: Statgraphics Plus 5.1).

Tabla de Medias por mínimos cuadrados para Col_1
con 95,0 Intervalos de confianza

Nivel	Frecuencia	Media	Error Estándar	Límite Inferior	Límite Superior
Media Total	32	332,741			
Col 2					
1	16	277,907	8,66357	260,016	295,798
2	16	387,575	8,66357	369,684	405,466
Col 3					
1	16	289,008	8,66357	271,117	306,899
2	16	376,474	8,66357	358,583	394,365
Col 4					
1	16	325,086	8,66357	307,195	342,977
2	16	340,396	8,66357	322,505	358,287
Col 2 según Col 3					
1 1	8	201,519	12,2592	176,217	226,821
1 2	8	354,295	12,2592	329,093	379,597
2 1	8	376,497	12,2592	351,196	401,799
2 2	8	398,653	12,2592	373,351	423,954
Col 2 según Col 4					
1 1	8	299,777	12,2592	274,476	325,079
1 2	8	256,036	12,2592	230,734	281,338
2 1	8	350,395	12,2592	325,093	375,697
2 2	8	424,755	12,2592	399,453	450,057
Col 3 según Col 4					
1 1	8	272,536	12,2592	247,234	297,838
1 2	8	305,48	12,2592	280,178	330,782
2 1	8	377,636	12,2592	352,334	402,938
2 2	8	375,311	12,2592	350,009	400,613

[a] *Col_1: concentración de saponinas; Col 2: Temperatura (1: 60 ºC; 2: 25 ºC); Col 3: Tiempo (1: 90'; 2: 1'); Col 4: Escaldado (1: Sí; 2: No).*

De estas primeras experiencias de extracción de flavonoides y saponinas con el reactor abierto de laboratorio podríamos concluir que:

- La agitación y la exposición al aire durante el tratamiento son factores que afectan negativamente a los flavonoides, mientras que las saponinas parecen ser mucho más estables.

- El etanol extrae mayor cantidad de ambos compuestos.

- Un mayor tiempo de extracción y temperatura producen efectos dispares según el solvente, fitoquímico y combinación de las variables estudiadas.

- El escaldado tiene, en general, un efecto negativo en la extracción de flavonoides y saponinas.

- **Fibras bioactivas**.

Las fibras obtenidas de los tratamientos en condiciones extremas, es decir, los tratamientos 3 y 4 (60 ºC, 90 minutos, sin escaldado y en agua o etanol respectivamente), y 15 y 16 (temperatura ambiente, 1 minuto, sin escaldado y en agua o etanol respectivamente), se secaron por dos métodos, liofilización y secado en estufa de recirculación de aire, según lo expuesto en el apartado 8.2.2.1.

Se decidió escoger estas muestras porque eran en las que previsiblemente mayores diferencias se podrían encontrar. Así se determinaría mejor el efecto que los distintos factores (temperatura, tiempo de extracción, disolvente y proceso de secado) tienen sobre las características estudiadas.

Estas muestras se han caracterizado desde el punto de vista de composición química, características funcionales y componentes bioactivos. Los resultados han sido recogidos en dos publicaciones incluidas en esta memoria de Tesis.

8.3.1.2. Escalado a planta piloto

Tras la realización de los ensayos preliminares en el reactor abierto de laboratorio con agitación continua, se diseñó una experiencia a nivel de planta piloto.

Se obtuvo un extracto de 205,5 L, que contenía 4,04 g de flavonoides y 10,36 g de saponinas, lo cual supuso un 37,34% y un 52,59% de recuperación de ambos compuestos, respectivamente. Estos resultados pueden considerarse satisfactorios, teniendo en cuenta, además, que en la fibra que queda tras la obtención de los extractos, se recupera gran parte del resto de flavonoides y saponinas.

A continuación este extracto se purificó parcialmente en estos fitoquímicos y se logró concentrar el 99% (3,99 g) de los flavonoides en 20 L y el 98% (10,19 g) de las saponinas en 8 L.

Con estos extractos parcialmente purificados y enriquecidos en flavonoides y saponinas se están realizando actualmente experiencias con animales de laboratorio,

para determinar su potencial actividad biológica *"in vivo"*. Además, los extractos liofilizados constituyen productos entregables.

8.3.2. Caracterización de la fibra bioactiva

El residuo procedente de la etapa de extracción, tras su liofilización o secado en estufa con recirculación de aire y molido en molino de martillos, constituye la fibra bioactiva del espárrago (FBS: fibra bioactiva seca; FBL: fibra bioactiva liofilizada). Ésta está constituida por polisacáridos estructurales de la pared celular (pectinas, hemicelulosas y celulosa) y compuestos fenólicos, incluyendo ácidos hidroxicinámicos, como el ácido p-cumárico y ferúlico, además de proteínas y de un porcentaje variable de los componentes funcionales solubles (Fuentes-Alventosa *et al.*, 2009a y 2009b). Este tipo de fibra se podría utilizar como un nuevo ingrediente de alimentos funcionales, ya que además de los efectos fisiológicos propios de la fibra, posee la capacidad antioxidante propia de los compuestos fenólicos unidos a ella (Saura-Calixto, 1998) y actividades biológicas que proporcionan otros fitoquímicos que contiene, como saponinas y esteroles.

A continuación se muestran los resultados obtenidos en las campañas de 2007 y 2008 a partir de subproductos tratados con el proceso pendiente de patente. Estas fibras bioactivas se han comparado con los subproductos secos (SS) y liofilizados (SL) sin tratamiento extractivo previo, para valorar si estos productos se podrían considerar también como productos entregables. Las experiencias se realizaron a escala piloto y se han determinado composición, fibra total, fitoquímicos y capacidad antioxidante.

8.3.2.1. Composición química

En la Tabla 8.10 se presenta la composición de los subproductos y fibras bioactivas expresada en g/100 g de materia seca. La realización de análisis estadístico mediante un ANOVA simple (datos no mostrados) nos indica que, en general, no existen diferencias significativas entre campañas, salvo para los azúcares solubles, pero los niveles de éstos no son muy diferentes en los distintos productos. Por tanto, se puede decir que los productos son bastante homogéneos entre campañas, presentando un bajo contenido en azúcares solubles (entre 3-6%), alto en fibra (superior al 50%) y moderado

en proteínas (15-23%). Con respecto al proceso de secado de la fibra (en estufa o por liofilización), no se afectó ningún componente de forma significativa. En cuanto al tratamiento, sólo existen diferencias significativas en el contenido de fibra total. Esto último es debido a la extracción de los componentes más solubles del espárrago durante el tratamiento, enriqueciéndose las fibras bioactivas en fibra total (porción más insoluble).

Tabla 8.10

Composición química de los subproductos y fibra bioactiva expresada en g/100 g de materia seca.

		AZÚCARES SOLUBLES	FIBRA TOTAL	PROTEÍNA
2007	**SS**	6,20±0,39	49,13±0,50	21,47±0,74
	SL	4,25±0,29	50,57±0,47	19,68±0,15
	FBS	5,07±0,47	64,58±0,53	16,92±0,22
	FBL	4,17±0,21	52,14±0,33	17,84±0,07
2008	**SS**	3,36±0,24	51,44±0,27	23,56±0,10
	SL	5,88±0,35	56,40±0,41	15,55±0,28
	FBS	4,05±0,27	61,28±0,73	20,22±0,20
	FBL	3,37±0,28	56,38±0,41	21,19±0,91

Si comparamos los resultados de este tratamiento con los obtenidos en las experiencias a escala de laboratorio (Fuentes-Alventosa *et al.*, 2009a), observamos la ventaja de que disminuyen los azúcares solubles en la fibra (3-5% frente a 7-15%). Estos resultados son comparables a los encontrados en otros productos vegetales considerados una buena fuente de fibra, tales como la cáscara del mango (Larrauri *et al.*, 1996a) y el bagazo de la uva tinta (Llobera y Cañellas, 2007). Además, con el tratamiento propuesto en esta Memoria ha aumentado el porcentaje de proteínas (17-21% frente a 12-16%), encontrándose dentro del grupo de los valores más altos indicados para los subproductos agrícolas, como, por ejemplo, el 16% del salvado de arroz (Abdul-Hamid y Luan, 2000), y ha disminuido la fibra total cuantificada, estando ésta en un porcentaje superior al 50% y situándose en el grupo de contenido intermedio en la misma indicado en Fuentes-Alventosa *et al.* (2009a), a nivel de la cáscara del limón Eureka (Figuerola *et al.*, 2005) y del mango (Larrauri *et al.*, 1996a).

8.3.2.2. Fibra alimentaria total

En la Tabla 8.11 se presenta la composición de la fibra alimentaria total de los subproductos y fibras bioactivas, expresada en g/100 g de fibra alimentaria total. Un ANOVA simple entre campañas (datos no mostrados) nos indica que no existen diferencias estadísticamente significativas en los tres componentes analizados. Tampoco se detectaron diferencias según el sistema de secado de la fibra. Sin embargo, el tratamiento afectó de forma significativa al contenido de ácidos urónicos. Se observa que éste redujo el porcentaje de ácidos urónicos entre un 5-10% aproximadamente con respecto a los subproductos sin tratar, ya que son muy solubles y gran parte pasaron a los extractos. Sin embargo, no hubo diferencias significativas en cuanto al contenido de celulosa y hemicelulosas, ya que son más insolubles y se afectan menos con las extracciones.

Tabla 8.11

Composición en azúcares neutros, ácidos urónicos y celulosa de la fibra alimentaria total expresada en g/100 g de fibra alimentaria total.

		AZÚCARES NEUTROS	ÁCIDOS URÓNICOS	CELULOSA
2007	**SS**	9,00±0,32	25,88±1,83	34,95±2,09
	SL	10,16±0,46	26,72±0,48	36,05±3,12
	FBS	9,82±0,81	14,71±0,57	43,51±2,88
	FBL	9,20±0,32	17,46±1,80	39,24±2,84
2008	**SS**	9,08±0,03	23,71±0,63	35,45±2,18
	SL	8,89±0,00	22,07±0,52	42,46±3,79
	FBS	8,73±1,05	16,34±0,54	34,08±2,96
	FBL	9,65±0,50	18,68±1,73	36,71±2,91

En la Tabla 8.12 se presenta la composición glicosídica porcentual de los azúcares neutros. Es de resaltar en todos los casos la cuantificación en más de un 60% de xilosa, lo que indica una alta presencia de xilanas en la fibra del espárrago. Si unimos este porcentaje al de glucosa, ya que las xiloglucanas son también hemicelulosas abundantes en la pared de este producto vegetal, se observa que más del 70% de los azúcares neutros cuantificados proceden de polisacáridos difícilmente extraíbles de la pared, que formarán parte necesariamente de la fracción insoluble de la fibra total. En este componente insoluble de la fibra también hay que tener en cuenta el alto porcentaje

de celulosa que presenta la fibra (Tabla 8.11), por lo que se puede concluir que entre un 60-75% de los azúcares cuantificados forman parte de la fibra insoluble siendo ésta por tanto la mayoritaria, lo que ya se puso de manifiesto en las experiencias a nivel de laboratorio (Fuentes-Alventosa *et al.*, 2009a).

Tabla 8.12

Composición glicosídica porcentual de los azúcares neutros de la fibra alimentaria total de los subproductos y fibras bioactivas.

	2007				2008			
	SS	**SL**	**FBS**	**FBL**	**SS**	**SL**	**FBS**	**FBL**
Ramnosa	4,61	4,64	2,63	3,32	3,58	3,01	3,06	3,41
Fucosa	1,78	1,68	1,54	1,78	1,56	0,84	1,66	1,60
Arabinosa	6,85	5,76	4,95	6,49	7,21	4,16	7,81	7,07
Xilosa	62,15	65,79	69,52	65,10	65,20	75,56	64,25	65,22
Manosa	5,91	5,74	4,35	4,98	3,93	3,78	4,56	4,82
Galactosa	11,55	10,32	10,42	12,19	12,34	7,67	13,00	12,37
Glucosa	7,14	6,06	6,58	6,14	6,18	4,97	5,65	5,51

8.3.2.3. Composición en fitoquímicos

Tanto los subproductos originales como las fibras obtenidas tras el tratamiento objeto de patente, contienen distintos fitoquímicos, por lo que se pueden considerar fibras funcionales, según lo presentado en la Tabla 8.13. Un ANOVA simple entre campañas (datos no mostrados) nos indica que, en general, no existen diferencias estadísticamente significativas en los componentes analizados, exceptuando los esteroles, pero los niveles de éstos en los distintos productos no son muy diferentes. El tratamiento afectó de forma significativa al contenido de saponinas y esteroles, si bien, de forma global se puede concluir que tras éste, la fibra queda enriquecida en componentes bioactivos, ya que el contenido de éstos (referido a materia seca) es superior en las fibras que en los subproductos originales. El sistema de secado de la fibra afectó de forma significativa a los flavonoides y saponinas y, en general, la cantidad de fitoquímicos es mayor en los productos liofilizados que en los correspondientes secados en estufa.

Tabla 8.13

Componentes funcionales de la fibra expresados como mg/g de materia seca.

		FLAVONOIDES	SAPONINAS	ESTEROLES	HIDROXICINAMATOS
2007	**SS**	0,57±0,05	1,18±0,08	1,56±0,04	1,57±0,03
	SL	2,31±0,05	3,47±0,72	1,74±0,01	2,88±0,16
	FBS	1,06±0,15	4,48±0,59	1,69±0,17	2,08±0,10
	FBL	1,91±0,16	5,51±0,05	1,90±0,12	2,46±0,02
2008	**SS**	0,22±0,04	2,54±0,14	0,95±0,01	3,87±0,10
	SL	1,80±0,04	5,00±0,37	1,18±0,01	1,74±0,15
	FBS	1,33±0,04	4,39±0,13	1,50±0,03	2,62±0,13
	FBL	1,94±0,03	6,09±0,26	1,77±0,05	3,42±0,25

Si comparamos estos resultados con los expresados en Fuentes-Alventosa *et al.* (2009b), observamos que se ha corregido el problema de oxidación de los flavonoides, ya que el contenido de los mismos fue muy similar, e incluso superior al máximo cuantificado en las experiencias de laboratorio (1,06-1,94 mg/g de fibra bioactiva frente a 0,64-1,82 mg/g de fibra en el artículo mencionado). La fibra bioactiva es más rica en saponinas (4,39-6,09 frente a 2,14-3,64 mg/g fibra) y esteroles (1,50-1,90 frente a 0,63-1,05 mg/g fibra). El contenido en hidroxicinamatos es algo menor (2,08-3,42 frente a 2,31-4,91 mg/g fibra), aunque este hecho puede venir determinado por la duración del tiempo de almacenamiento de los subproductos antes de ser caracterizados y/o tratados, o incluso por las distintas campañas.

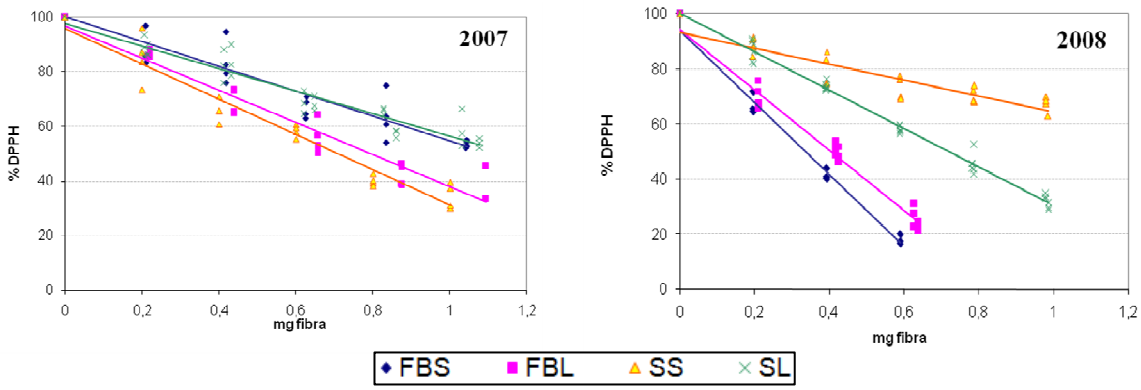
8.3.2.4. Capacidad antioxidante

- **Actividad antioxidante de las fracciones soluble (AAO-FS) e insoluble (AAO-FI)**

La Figura 8.1 muestra las curvas dosis-respuesta de la capacidad antirradical (CAR) de la fracción soluble de fibras de subproductos de espárrago (campañas 2007 y 2008). Se observa que en la campaña de 2008 las fibras bioactivas poseen una mayor CAR que los subproductos. Las fibras bioactivas son similares pero existen diferencias en los subproductos, teniendo los liofilizados más actividad que los secos. En la campaña de 2007 los resultados han sido diferentes, presentando tanto las fibras como los subproductos una actividad similar. Además hay un claro descenso de actividad en la fibra bioactiva. Esta diferencia en los resultados de 2007 podría estar relacionada con la estabilidad de las fibras durante su almacenamiento, por lo que se ha diseñado una experiencia de conservación en distintas condiciones de atmósferas y temperaturas que se llevará a cabo con muestras de la campaña 2009. Los resultados de esta experiencia serán de gran interés en vista a la consecución y explotación de la patente.

Figura 8.1

Curvas dosis-respuesta de CAR[a] de la fracción soluble de fibras de subproductos de espárrago (campañas 2007 y 2008).



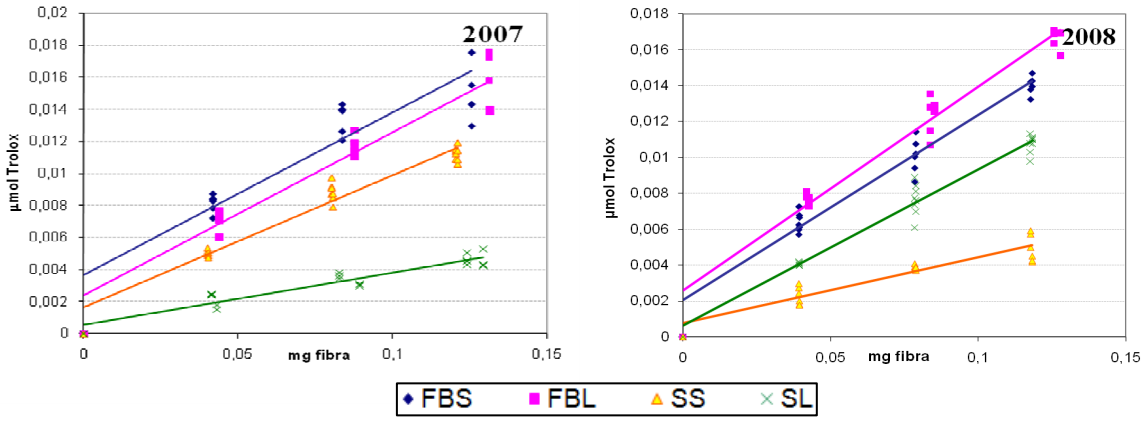
[a] *La CAR está expresada como porcentaje de DPPH* $^{\cdot}$ *que permanece en la solución después de 30 minutos de reacción. Cada punto de la gráfica es el valor medio de tres replicados.*

En la Figura 8.2 se representan las curvas dosis-respuesta del poder reductor (PR) de la fracción soluble de fibras de subproductos de espárrago (campañas 2007 y

2008). Las fibras bioactivas poseen un mayor PR que los subproductos sin tratar. Además se observa el mismo patrón que en la CAR: las dos fibras bioactivas tienen similar capacidad, teniendo los subproductos liofilizados mayor actividad que los secos. En la campaña 2007 se obtienen resultados similares, aunque se observa que el subproducto liofilizado tiene una actividad muy inferior al seco.

Figura 8.2

Curvas dosis-respuesta del PR[a] de la fracción soluble de fibras de subproductos de espárrago (campañas 2007 y 2008).



[a] *El PR está expresado como µmoles de Trolox por miligramo de fibra. Cada punto de la gráfica es el valor medio de cuatro replicados.*

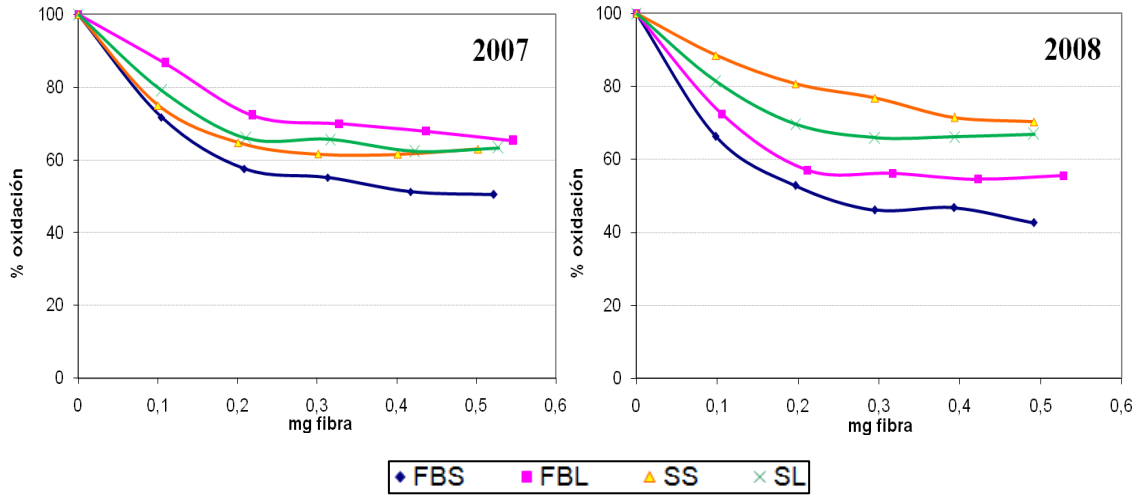
En la Figura 8.3 se representa la capacidad de inhibición de la oxidación primaria (CIOP) de la fracción soluble de fibras de subproductos de espárrago (campañas 2007 y 2008). Aquí se vuelven a obtener unos mejores resultados en las fibras bioactivas de la campaña 2008, además con la misma relación entre las distintas fibras (la fibra bioactiva seca y liofilizada presentan actividades similares pero mayor que el subproducto liofilizado y éste a su vez mayor que el subproducto seco). En la campaña de 2007 se observa de nuevo un comportamiento diferente: el mayor porcentaje de oxidación ocurrió en la fibra bioactiva liofilizada y a continuación en los subproductos liofilizados, posiblemente por el problema anteriormente mencionado en la CAR de la fracción soluble.

A la vista de los resultados en estos tres ensayos de AAO-FS podemos concluir que el proceso propuesto en esta Memoria aumenta esta actividad. Pero se hace necesario un estudio de estabilidad de las fibras durante el almacenamiento, ya que en

las fibras y subproductos que tardaron más tiempo en analizarse (campaña de 2007) los resultados fueron inferiores en algunos ensayos.

Figura 8.3

Curvas dosis-respuesta de la CIOP[a] de la fracción soluble de fibras de subproductos de espárrago (campañas 2007 y 2008).

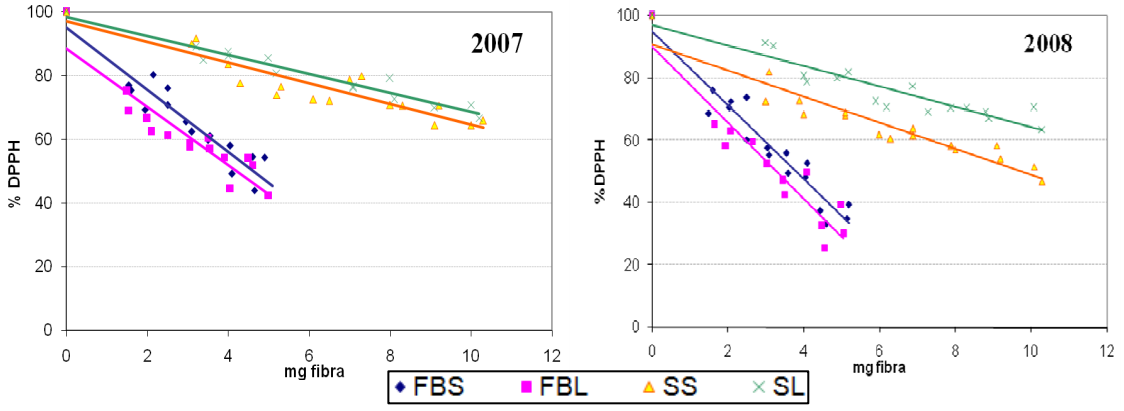


[a] *La oxidación primaria está expresada como porcentaje de oxidación de ácido linoleico por miligramo de fibra. Cada punto de la gráfica es el valor medio de cuatro replicados.*

En la Figura 8.4 se presentan los resultados obtenidos para la CAR de la fracción insoluble de las fibras. En este caso no existen grandes diferencias entre las dos campañas, teniendo las fibras bioactivas mayor actividad que los subproductos sin tratar.

Figura 8.4

Curvas dosis-respuesta de CAR[a] de la fracción insoluble de subproductos de espárrago (campañas 2007 y 2008).



[a] *La CAR está expresada como porcentaje de DPPH* $^{\cdot}$ *que permanece en la solución después de 30 minutos de reacción. Cada punto de la gráfica es el valor medio de tres replicados.*

Para obtener unos valores que puedan servir de comparación entre las distintas muestras así como con otras fibras comerciales, hay que expresar estos resultados en función de un patrón. Para ello, a partir de las ecuaciones de las rectas de regresión calculadas para la CAR se calcula la EC_{50} para cada fibra, es decir, la cantidad de fibra necesaria para disminuir un 50% la concentración inicial de DPPH. En el caso de la CIOP no se puede calcular la EC_{50} porque hay fibras que no llegan al 50% de inhibición, por ello se determina la EC_{25}. Estos valores de EC_{50} ó EC_{25} se comparan con la sustancia patrón (Trolox) y se halla la equivalencia entre las fibras en estudio y el patrón. Para el ensayo del PR al estar los resultados ya expresados como µmoles Trolox, se calcula la equivalencia directamente de las ecuaciones de regresión de las rectas.

En la Tabla 8.14 se recogen los valores de AAO-FS y AAO-FI, expresados como equivalentes Trolox (TE).

Si se comparan estos resultados con los expresados en Fuentes-Alventosa *et al.* (2009b), se observa que se obtienen mayores valores de CAR de la fracción soluble en la campaña de 2008. Así, las fibras bioactivas secas y liofilizadas presentaron respectivamente 25,74 y 21,32 mmol TE/Kg fibra, mientras que las fibras de las experiencias a nivel de laboratorio presentaron como máximo alrededor de 11 mmol TE/kg fibra (Fuentes-Alventosa *et al.*, 2009b).

Tabla 8.14

AAO-FS y AAO-FI, expresados como equivalentes Trolox (mmol TE/kg de fibra).

		AAO-FS			AAO-FI
		CAR	**PR**	**CIOP**	**CAR**
2007	**SS**	12,27	84,00	35,61	3,08
	SL	7,51	31,00	27,67	2,75
	FBS	7,90	104,90	44,21	9,61
	FBL	10,91	104,10	17,12	10,60
2008	**SS**	5,81	37,50	12,80	4,56
	SL	12,06	87,50	26,05	3,11
	FBS	25,74	105,10	52,11	11,73
	FBL	21,32	116,10	43,34	13,51

Las fibras bioactivas obtenidas y analizadas en 2008 tienen una capacidad antirradical total superior a otra fibras de subproductos vegetales propuestos como posibles ingredientes de alimentos enriquecidos en fibra, como es el caso de los subproductos del cacao (7,73 μmol TE/g peso seco; Lecumberri *et al.*, 2007). Sin embargo, siguen teniendo valores muy inferiores a otros propuestos, como los subproductos de cítricos (70-240 μmol TE/g fibra; Marín *et al.*, 2007) y el hollejo de uva tinta (427 μmol TE/g fibra; Llobera y Cañellas, 2007).

- **Relación entre la actividad antioxidante y fitoquímicos**

Como ya se ha descrito anteriormente, los flavonoides son compuestos altamente antioxidantes (Makris y Rossiter, 2001; Rodríguez *et al.*, 2005; Guillén *et al.*, 2008). Los hidroxicinamatos, especialmente el ácido ferúlico, también poseen una gran actividad antioxidante (Chen y Ho, 1997). Ambos compuestos están presentes como fitoquímicos en las fibras estudiadas. Los flavonoides son fácilmente extraíbles, mientras que los hidroxicinamatos permanecen unidos por enlaces éster a la estructura de la fibra.

A continuación se va a discutir si ambos grupos de compuestos están relacionados con la AAO-FS y la AAO-FI, respectivamente. Para ello, se ha realizado un análisis de regresión entre la actividad antioxidante de las fibras y su contenido en los citados fitoquímicos. Dado que los resultados de AAO-FS de la campaña 2007 han resultado algo anómalos, se van a excluir de este estudio.

Como muestra la Figura 8.5 las curvas se han ajustado a un modelo de raíz cuadrada-X. Para los tres ensayos (Tabla 8.15) se han obtenido valores del coeficiente de correlación (R) entre 0,8 y 0,9, lo cual indica cierta correlación entre el contenido de flavonoides de las fibras y su AAO-FS.

Figura 8.5

Ajuste al modelo raíz cuadrada-X para describir la relación entre la concentración de flavonoides y la AAO-FS (capacidad antirradical, poder reductor e inhibición de la oxidación primaria) en las fibras bioactivas y subproductos de espárrago (campaña 2008).

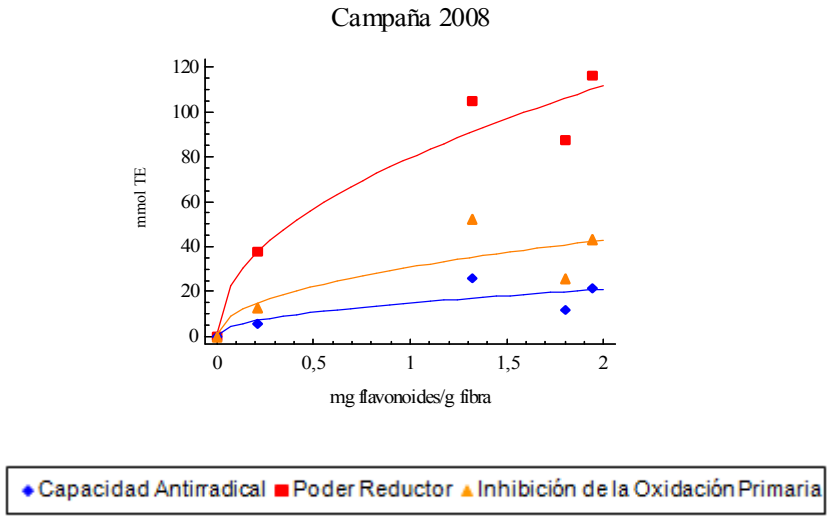


Tabla 8.15

Coeficiente de correlación (R) entre la concentración de flavonoides y la AAO-FS en las fibras bioactivas y subproductos de espárrago (campaña 2008).

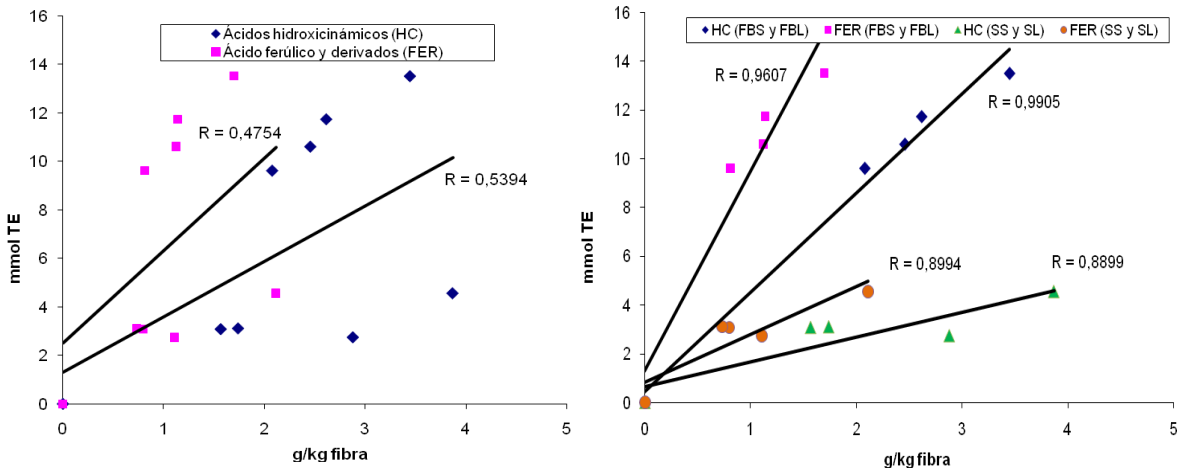
	R
CAR	0,8335
PR	0,9693
CIOP	0,8483

Aunque parece haber cierta relación entre contenido en flavonoides y capacidad antioxidante de la fracción soluble de las fibras, no se puede establecer que sean los únicos responsables de dicha actividad. De hecho, en el espárrago existen otros compuestos antioxidantes solubles (glutatión, ácido ascórbico, etc.) cuyo estudio aún no se ha abordado en los subproductos. La presencia de estos compuestos bioactivos en las fibras obtenidas y su implicación en la actividad antioxidante son estudios de gran interés que se llevarán a cabo con vistas a la consecución y explotación de la patente.

En la Figura 8.6 se representan los datos del contenido de hidroxicinamatos y de ácido ferúlico y derivados frente a la AAO-FI de las muestras de las campañas 2007 y 2008. Como se puede observar en la subfigura izquierda, no existe correlación entre ambas variables, encontrándose valores de R cercanos a 0,5. En la subfigura derecha se representa el estudio de regresión realizado para los datos de fibra tratada (FBS y FBL) con el proceso descrito en la presente Memoria y los de fibra sin tratar (SS y SL) de forma independiente. Se observa que ahora sí se encuentra correlación entre CAR y cantidad de hidroxicinamatos y ferúlico en las fibras, siendo la correlación mejor en las fibras tratadas (R>0,9) que sin tratar (R=0,8). Las fibras que han sido tratadas presentan mucha más actividad que las que no lo han sido, lo que se comprueba por la mayor pendiente de las rectas de regresión.

Figura 8.6

Correlación entre la concentración de ácidos hidroxicinámicos (y dentro de éstos, el ácido ferúlico y sus derivados) y la AAO-FI en las fibras bioactivas y subproductos de espárrago (campañas 2007 y 2008).



Este hecho parece indicar que es preciso un tratamiento que cambie la estructura de la fibra para que los hidroxicinamatos, unidos por fuertes enlaces a ella, puedan actuar como antioxidantes. Es un hecho ampliamente reconocido en la bibliografía que los tratamientos térmicos provocan cambios en las características superficiales de las fibras obtenidas, como por ejemplo, en la porosidad, capacidad de hinchado, etc. (Figuerola *et al.*, 2005). En el caso de los subproductos de espárrago, las fibras obtenidas con el proceso desarrollado en esta Memoria deben tener sus características superficiales mejoradas, de forma que, una vez rehidratadas, los hidroxicinamatos unidos a ella puedan mostrar un mayor potencial antioxidante.

8.4. PRODUCTOS DEL PROCESO Y CARACTERÍSTICAS

Con el proceso desarrollado se consigue el aprovechamiento integral de los subproductos del espárrago. Mediante procedimientos simples y de fácil adaptación industrial se obtienen los siguientes ingredientes funcionales a partir de estos residuos de la industria alimentaria:

- **Subproductos secos y liofilizados de espárrago, en polvo**. Se mantienen prácticamente todos los componentes químicos presentes en los tallos frescos, pero más concentrados. La Tabla 8.16 muestra la composición expresada en g/100 g de materia seca de los subproductos secos y liofilizados obtenidos en las campañas de 2007 y 2008.

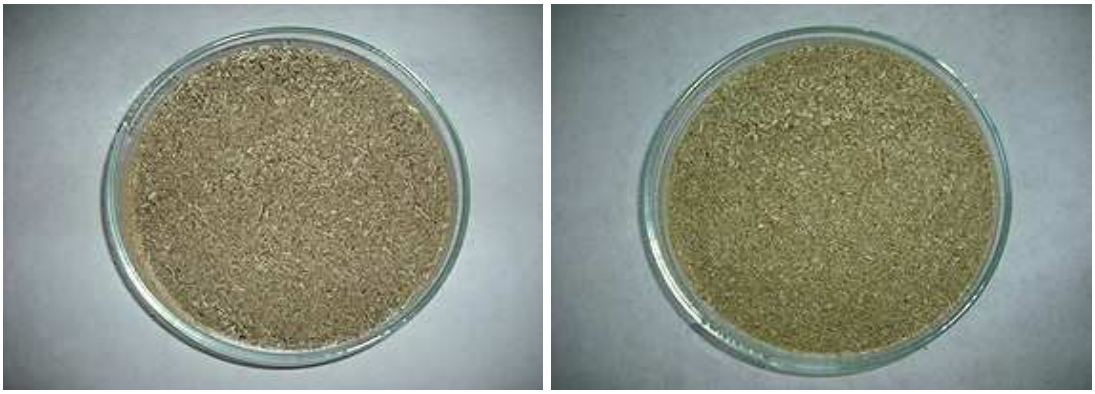
Tabla 8.16

Composición de los subproductos secos y liofilizados obtenidos en las campañas de 2007 y 2008 expresada en g/100 g de materia seca.

	2007		2008	
	SS	**SL**	**SS**	**SL**
Fibra total	49,13	50,57	51,54	56,40
Proteínas	21,47	19,68	23,56	15,55
Azúcares solubles	6,20	4,25	3,36	5,88
Saponinas	0,12	0,35	0,25	0,50
Flavonoides	0,06	0,23	0,02	0,18
Hidroxicinamatos	0,16	0,29	0,39	0,17
Esteroles	0,16	0,17	0,10	0,12

Imagen 8.1

Subproductos secos (izquierda) y liofilizados (derecha) de espárrago, en polvo.



- **Extracto funcional de espárrago**. Contiene la mayor parte de los compuestos solubles bioactivos. La riqueza en peso de este extracto global es de un 0,45% de flavonoides y un 1,15% de saponinas. Este producto, que se puede presentar en forma de solución o extracto seco, una vez realizados los correspondientes ensayos de toxicidad y bioactividad, podrá utilizarse como ingrediente funcional en distintos productos alimenticios. Pero además, esta mezcla de compuestos saludables podría tener aplicación en dietética y parafarmacia, en presentaciones similares a las numerosas cápsulas y preparados de plantas que existen actualmente en el mercado.

Imagen 8.2

Extracto funcional de subproductos de espárrago obtenido tras el tratamiento.

- **Extractos parcialmente purificados y enriquecidos**. Se han obtenido extractos parcialmente purificados en flavonoides (11,08% de riqueza media en peso, que es 24 veces más concentrado que el extracto inicial) y saponinas (80,67% de riqueza media en peso, que es 70 veces más concentrado que el extracto inicial) que, una vez liofilizados, se pueden presentar en forma de polvo.

Imagen 8.3

Concentrado de flavonoides (izquierda) y saponinas (derecha).

- **Fibra bioactiva.** La Tabla 8.17 muestra la composición expresada en g/100 g de materia seca de las fibras bioactivas obtenidas en las campañas de 2007 y 2008. La fibra bioactiva de espárrago que se obtiene tiene un amplio rango de aplicación en la formulación de alimentos funcionales de distinta naturaleza como el yogur (Sanz *et al.*, 2008).

Tabla 8.17

Composición de la fibra bioactiva obtenida en las campañas de 2007 y 2008 expresada en g/100 g de materia seca.

	2007		2008	
	FBS	**FBL**	**FBS**	**FBL**
Fibra total	64,58	52,14	61,28	56,38
Proteínas	16,92	17,84	20,22	21,19
Azúcares solubles	5,07	4,17	4,05	3,30
Saponinas	0,45	0,55	0,44	0,61
Flavonoides	0,11	0,19	0,13	0,19
Hidroxicinamatos	0,21	0,25	0,26	0,34
Esteroles	0,17	0,19	0,15	0,18

Imagen 8.4

Fibra bioactiva obtenida con el tratamiento.

8.5. CONCLUSIONES

Se ha diseñado un proceso (pendiente de patente) de fácil aplicación industrial, cuyo fin es el de obtener extractos funcionales y fibra bioactiva.

El uso de un reactor abierto, donde los subproductos eran agitados y estaban expuestos al aire durante el tratamiento, afectó negativamente a los flavonoides. Sin embargo, el método desarrollado para la obtención de extractos bioactivos controla la oxidación y permite obtener mayores cantidades de estos fitoquímicos.

Con el proceso se consigue una purificación parcial de los extractos ricos en flavonoides y saponinas, concentrándose los mismos hasta 25 y 70 veces, respectivamente. Se obtiene, por tanto, unos extractos con una mayor riqueza en cada uno de estos compuestos bioactivos.

El otro ingrediente funcional obtenido con el proceso es una fibra bioactiva de mayor calidad que la de los subproductos sin tratar, ya que se le han retirado gran parte de los compuestos solubles, por lo que aumenta el porcentaje de fibra en la composición de la misma. También aumenta su contenido en componentes bioactivos y, debido a esta mayor concentración de fitoquímicos, también es mayor su capacidad antioxidante. Incluso se ha comprobado que el tratamiento aquí propuesto es necesario para que algunos de ellos muestren su actividad. Además se trata de una fibra optimizada con respecto a las que se obtuvieron con los diferentes tratamientos realizados en el reactor abierto de laboratorio (mostrados en Fuentes-Alventosa *et al.*, 2009a y 2009b).

Ya que el secado por liofilización puede ser un proceso que encarezca el coste de la aplicación industrial, se presenta la opción del secado de la fibra en estufa, que aunque disminuye levemente el contenido de algunos fitoquímicos mantiene una composición y actividad antioxidante similar a la de la fibra liofilizada y muy superior a los subproductos secos o liofilizados sin tratar.

Con la fibra bioactiva y los extractos obtenidos, parcialmente purificados y enriquecidos en flavonoides y saponinas, se están realizando actualmente experiencias con animales de laboratorio, para determinar su potencial actividad biológica *"in vivo"*.

En función de los resultados que se obtengan se podrá establecer la posibilidad de uso de ambos productos como ingredientes alimentarios y para aplicaciones nutricionales y tecnológicas.

Se concluye, finalmente, que en los residuos de espárrago tenemos una variedad de ingredientes funcionales que presentan una gran flexibilidad para su inclusión en la formulación de diferentes alimentos. Por tanto, podremos encontrar aplicaciones específicas para estos ingredientes y nutracéuticos en muy diversos campos de la industria alimentaria y farmacéutica, recurriendo a la preparación que resulte más adecuada para cada caso concreto: extractos globales, parcialmente purificados, fibra bioactiva, etc.

9. CONCLUSIONES

1. Se ha puesto a punto un método de extracción de flavonoides en espárrago, optimizado en términos de solvente de extracción, tamaño de muestra, volumen y concentración de etanol, tiempo y forma de extracción.

2. Se ha desarrollado y validado un método de HPLC-MS que permite una buena separación de ocho flavonoides distintos, lo que ha permitido su identificación, cuantificación y aislamiento. La validación se realizó empleando patrones de los flavonoides para el estudio de respuesta lineal, límite de detección y cuantificación, precisión y exactitud del método. Cuando no se disponía de patrón para algún compuesto, la cuantificación se basó en el valor medio para esa clase de compuesto (según la aglicona), debido a que las respuestas son similares entre clases.

3. Siete de los flavonoides detectados no habían sido previamente descritos en el espárrago verde: kaempferol-3-O-rutinósido (nicotiflorina), isoramnetina-3-O-rutinósido (narcisina), isoramnetina-3-O-glucósido, dos triglicósidos de quercetina y dos triglicósidos de isoramnetina. Se ha de resaltar que aunque en la bibliografía prácticamente sólo se cita a la rutina como flavonoide en el espárrago verde, algunos de los indicados se han detectado en la mayoría de las variedades comerciales, si bien de forma minoritaria con respecto a aquella.

4. La composición de flavonoides de la población triguero de Huétor Tájar es, en general, similar a la de las variedades comerciales de espárrago verde analizadas. Sin embargo, el análisis por separado de los 65 genotipos distintos que componen la población triguero de Huétor Tájar reveló que un grupo de 21 poseen una composición en flavonoides distinta y estadísticamente diferente al resto de las muestras. Así, isoramnetina-3-O-glucósido y los flavonoides identificados tentativamente como quercetina-3-ramnosil-rutinósido e isoramnetina-3-ramnosil-rutinósido sólo se han detectado en genotipos de dicho grupo.

5. Se ha detectado un flavonoide que puede ser clave para distinguir a la población de espárrago triguero de otros cultivares de espárrago verde. Se trata del flavonoide identificado tentativamente como isoramnetina-3-glucosil-rutinósido, el cual se ha detectado en la mayoría de los genotipos de espárrago triguero de Huétor Tájar y en ninguna de las variedades comerciales de espárrago verde.

6. Estos resultados evidencian que el espárrago triguero de Huétor Tájar presenta una mayor variedad en este fitoquímico que las variedades comerciales. Por tanto, el distinto perfil de este compuesto bioactivo en los dos grupos de muestras estudiadas, hace que éste sea un factor a tener en cuenta en futuros estudios de mejora o selección de cultivares de mayor valor añadido, para la revalorización de las variedades denominadas como "triguero", así como la autenticación de las mismas frente a las comerciales.

7. Con el fin de diseñar un proceso para el aprovechamiento de subproductos de espárrago se realizaron experiencias a escala de laboratorio para estudiar la influencia de distintos factores. El amplio estudio realizado, tanto sobre los extractos obtenidos como sobre los residuos fibrosos, permite concluir que:

a) Los flavonoides se afectan negativamente por la agitación y la exposición al aire durante el tratamiento.

b) El etanol extrae mayor cantidad de flavonoides y saponinas que el agua.

c) Con los tratamientos intensos, especialmente los realizados con etanol, se obtienen fibras con mayor contenido en compuestos bioactivos, probablemente debido a un efecto de concentración por la pérdida de componentes solubles de la misma.

8. Se ha diseñado un proceso a escala de planta piloto (pendiente de patente) que consigue el aprovechamiento integral de los subproductos del espárrago, mediante procedimientos simples y de fácil adaptación industrial. Gracias a este proceso se obtienen inicialmente, un extracto total de compuestos bioactivos de espárrago y un residuo fibroso.

9. Se ha conseguido separar del extracto total los dos grupos de compuestos bioactivos mayoritarios presentes, flavonoides y saponinas, obteniendo extractos parcialmente purificados y enriquecidos en cada uno de estos fitoquímicos.

10. Con el proceso propuesto, la fibra resultante es de mejor calidad (composición química, fibra total, fitoquímicos y capacidad antioxidante) que la de los subproductos sin tratar. Además, la composición de esta fibra bioactiva de espárrago y su actividad antioxidante es similar a otras fibras estudiadas en la bibliografía, lo que hace que los subproductos de espárrago se puedan considerar como una fuente de fibra de interés alimentario.

11. Con el proceso diseñado se han obtenido una serie de productos entregables cuya utilización se pretende patentar, y que son de interesante aplicación en la industria alimentaria y farmacéutica, entre los que destacamos:

a) Liofilizado de extracto funcional de espárrago, que contiene una riqueza en peso en flavonoides de un 0,45% y en saponinas de un 1,15%, además de otros compuestos solubles bioactivos.

b) Liofilizado de extractos parcialmente purificados en flavonoides, con una riqueza del 11%.

c) Liofilizado de extractos parcialmente purificados en saponinas, con una riqueza del 81%.

d) Fibra bioactiva seca o liofilizada de espárrago, en polvo, con una interesante composición en fibra total, proteínas y fitoquímicos, lo que la cualifica para su aplicación en la formulación de alimentos funcionales.

12. Se hacen necesarios la realización de experiencias para estudiar la estabilidad de los productos durante el almacenamiento y para determinar la potencial actividad biológica *"in vivo"* de estos ingredientes funcionales, antes de encontrar aplicaciones específicas en diversos campos de la industria alimentaria y farmacéutica.

10. BIBLIOGRAFÍA

Aaby K., Skrede G., Wrolstad R.E. 2005. Phenolic composition and antioxidant activities in flesh and achenes of strawberries (Fragaria ananassa). *J. Agric. Food Chem.* 53, 4032-4040.

Abdul-Hamid A., Luan Y.S. 2000. Functional properties of dietary fibre prepared from deffated rice bran. *Food Chem.* 68, 15–19.

Ablajan K., Abliz Z., Shang X.-Y., He J.-M., Zhang R.-P., Shi J.-G. 2006. Structural characterization of flavonol 3,7-di-O-glycosides and determination of the glycosylation position by using negative ion electrospray ionization tandem mass spectrometry. *J. Mass Spectrom.* 41, 352-360.

Adiotomre J., Eastwood M.A., Edwards C.A., Brydon W.G. 1990. Dietary fiber: *In vitro* methods that anticipate nutrition and metabolic activity in humans. *Am. J. Clin. Nutr.* 52, 128–134.

Ahmed A.R., Labavitch J.M. 1977. A simplified method for accurate determination of cell wall uronide content. *J. Food Biochem.* 1, 361–365.

Alcázar M.J., Jiménez M.J. 1988. Flavonoids as anti-inflammatory agents. *Fitoterapia* 59, 25-38.

Al-Habori M., Raman A. 1998. Antidiabetic and hypocholesterolaemic Effects of fenugreek. *Phytother. Res.* 12, 233-242.

Amaro M.A., Moreno R., Zurera G., Sánchez P.J. 1999. Nutricional changes in the essential trace elements content of asparagus during industrial processing. *Food Res. Int.* 32, 479-486.

Amaro, M.A., Moreno, R., Zurera, G., García, R.M. 1995. Influence of vegetative cycle of asparagus (*Asparagus officinalis*, L.) on cooper, iron, zinc and manganese content. *Plant Foods Hum. Nutr*. 47, 349-355.

American Dietetic Association. 2008. Position of the American Dietetic Association: health implications of dietary fiber. *J. Am. Diet. Assoc.* 108, 1716-1731.

Anderson J.W., Smith B.M., Gustafson N.J. 1994. Health benefits and practical aspects of high-fibre diets. *Am. J. Clin. Nutr.* 59 (supplement), 1242S-1247S.

Arai, S. 1996. Studies on functional foods in Japan-state of the art. *Biosci. Biotechnol. Biochem.* 60, 9–15.

Asp N.G.; Johansson C.G. 1984. Dietary fibre analysis. *Nutr. Abstr. Rew. Clin. Nutr. Series A*, 54, 735-752.

Assmann G., Wahrburg U. 2004. Beneficios de los componentes secundarios del aceite de oliva. Asajanet: el portal de la Asociación Agraria Jóvenes Agricultores.

Balasundram N., Sundram K., Samman S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem.* 99, 191-203.

Bast A. 2005. Functional food and nutraceuticals. En: *Proceedings of the XI International Asparagus Symposium in Acta Horticulturae*.

Bazzano L.A., He J., Ogden L.G., Loria C.M., Whelton P.K. 2003. Dietary fiber intake and reduced risk of coronary heart disease in US men and women: The National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study. *Arch. Intern. Med*. 163, 1897-1904.

Behr H.-C. 2006. International asparagus production and markets. En: *Proceedings of the XI International Asparagus Symposium in Acta Horticulturae.*

Benages, S. 1990. El espárrago. *Ediciones Mundi-Prensa, Bilbao* 179-212.

Benson B.L. 2008. 2005 Update of the world's asparagus production areas, spear utilization and production periods. Proc. of 11th Int. Asparagus Symp. *Acta Hort.* 776, 495-507.

Bergquist S.A., Gertsson U.E., Knuthsen P., Olsson M.E. 2005. Flavonoids in baby spinach (Spinacia oleracea L.): Changes during plant growth and storage. *J. Agric. Food Chem.* 53, 9459-9464.

Bingham S.A., Day N.E., Luben R., Ferrari P., Slimani N., Norat T., Clavel-Chapelon F., Kesse E, Nieters A., Boeing H., Tjonneland A., Overvad K., Martinez C., Dorronsoro M., Gonzalez C.A., Key T.J., Trichopoulou A., Naska A., Vineis P., Tumino R., Krogh V., Bueno-de-Masquita H., Peeters P.H.M., Berglund G., Hallmans G., Lund E., Skele G., Kaaks R., Riboll E. 2003. Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): An observational study. *Lancet* 361, 1496-1501.

Birt D.F., Hendrich S., Wang W. 2001. Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacol. Ther*. 90, 157-177.

Bors W., Heller W., Christa M. y cols. 1990. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. *Methods Enzymol.* 186, 343-355.

Bramlett K.S., Houck K.A., Borchert K.M., Dowless M.S., Kulanthaivel P., Zhang Y., Beyer T.P., Schmidt R., Thomas J.S., Michael L.F., Barr R., Montrose C., Eacho P.I., Cao G., Burris T.P.. 2003. A natural product ligand of the oxysterol receptor, liver X receptor. *J. Pharmacol. Exp. Therapeutics.* 307, 291-296.

Brasseur T. 1989. Propriétés anti-inflamatoires de flavonoides. *J. Pharmacol. Bel.* 44, 235-241.

Browning B.L. 1967. Carboxy methyl cellulose. *Methods World Chem.* 2, 490-492.

Bruneton J. 1995. Pharmacognosy, phytochem., medicinal plants. *Lavoisier Publishing, Paris,* pp. 538–544 (ISBN 2-4730-0028-7).

Cantos E., Espín J.C., Tomás-Barberán F.A. 2002. Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC–DAD–MS–MS. *J. Agric. Food Chem.* 50, 5691-5696.

Caridi D., Trenerry V.C., Rochfort S., Duong S., Laugher D., Jones R. 2007. Profiling and quantifying quercetin glucosides in onion (Allium cepa L.) varieties using capillary zone electrophoresis and high performance liquid chromatography. *Food Chem.* 105, 691-699.

Chau C.-F., Chen C.-H., Lin. C.-Y. 2004. Insoluble fiber-rich fractions derived from *Averrhoa carambola*: Hypoglycemic effects determined by *in vitro* methods. *Lebensm.-Wiss.u.-Technol.* 37, 331–335.

Chen J.H., Ho C.-T. 1997. Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *J. Agric. Food Chem.* 45, 2374–2378.

Chin, C.K. 2006. Functional elements from asparagus for human health. In *Proceedings of the XI international asparagus symposium in acta horticulturae*.

Cody V., Middleton E., Harborne J.B. y cols. 1998. Plant flavonoids in biology and medicine. Biochemical, pharmacological and structure-activity relatioships. *Alan R. Liss, New York.*

Colic M., Pavelic K. 2000. Molecular mechanisms of anticancer activity of natural dietetic products. *J. Mol. Med.* 78 (6), 333-336.

Cummings J.H. 1996. *Metabolic and Physiological Aspects of Dietary Fibre.* Commission of the European Communities. Bruselas.

Cushine T, Lamb A.J. 2005. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* 26, 343–356.

Da Silva J., Herrmann S.M., Peres W., Possa Marroni N., Gonzalez Gallego J., Erdtmann B. 2002. Evaluation of the genotoxic effect of rutin and quercetin by comet assay and micronucleus test. *Food Chem. Toxicol.* 40, 941-947.

De Jong, N., Ocke, M. C., Branderhorst, H. A., Friele, R. 2003. Demographic and lifestyle characteristics of functional food consumers and dietary supplement users. *Br. J. Nutr.* 89, 273–281.

Dini I., Schettino O., Simioli T., Dini A. 2001. Studies on the constituents of *Chenopodium quinoa* seeds: isolation and characterization of new triterpene saponins. *J. Agric. Food Chem.* 49, 741–746.

Dische Z. 1962. Color reactions of carbohydrates. In: R.L. Whistler and M.L. Wolfram, Editors, *Methods in Carbohydrate Chemistry*, Academic Press, New York, pp. 477–512.

Doll R. 1990. An overview of the epidemiological evidence linking dite and cancer. *Proceedings of the National Academy of Sciences of the United States of America* 49, 119-131.

Dopico-García M.S., Valentão P., Guerra L., Andrade P.B., Seabra R.M. 2007. Experimental design for extraction and quantification of phenolic compounds and organic acids in white "Vinho Verde" grapes. *Anal. Chim. Acta* 583, 15-22.

Dunaif G., Schneeman B.O. 1981. The effect of dietary fibre on human pancreatic enzyme activity in vitro. *Am. J. Clin. Nutr.* 34, 1034-1035.

Edwards C.A., Blackburn N.A., Craigen L., Davinson P., Tomlin J., Sugden K., Johnson I.T., Read N.W. 1987. Viscosity of food gums determined *in vitro* related to their hypoglycaemic actions. *Am. J. Clin. Nutr.* 46, 72–77.

Englyst H.N., Cumming J.H. 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-chromatography of constituent sugars as alditol acetates. *Analyst* 109, 937–942.

Escarpa, A.; Pérez-Cabrera, C.; González, M. C. 2000. Optimization and validation of a fast liquid gradient for determination of prominent flavan-3-ols and flavonols in fresh vegetables. *J. High Resolut. Chromatogr.* 23, 637-643.

Espejo J.A., González-Fernández J.L. 2000. En: El arte culinario del espárrago de Huétor-Tájar. *Consejo Regulador de la Denominación Específica "Espárrago de Huétor-Tájar"*.

Espín J.C., García-Conesa M.T., Tomás-Barberán F.A. 2007. Nutraceuticals: Facts and fiction. *Phytochem.* 68, 2986–3008.

FDA, Department of Health and Human Services. 1993. Health Claims: fruits and vegetables and cancer (Revised as of 1 April 2005). In: *FDA, Code of Federal Regulations* (vol. 2, Title 21. Chapter I. Subchapter B. Part 101. Subpart E. section 101.78). Rockville, MD: GPO.

Fengel D., Wegener G. 1984. *Wood chemistry, ultraestructure and reactions*. Ed. Walter de Gruyter. Berlin-New York.

Fernández-Bolaños J., Felizón B., Brenes M., Guillén R., Heredia A. 1998. Hydroxytyrosol and tyrosol as the main compounds found in the phenolic fraction of steam-exploted olive stones. *J. Agric. Food Chem.* 75, 1643-1649.

Fernandez-Bolaños J., Rodríguez G., Gómez E., Guillén R., Jiménez A., Heredia A. 2004. Total recovery of the waste of two-phase olive oil processing: isolation of added-value compounds. *J. Agric. Food Chem.* 52, 5849–5855.

Ferreres F., Llorach R., Gil-Izquierdo A. 2004. Characterization of the interglycosidic linkage in di-, tri-, tetra- and pentaglycosylated flavonoids and differentiation of positional isomers by liquid chromatography/electrospray ionization tandem mass spectrometry. *J. Mass Spectrom.* 39, 312–321.

Figuerola F., Hurtado M.L., Estévez A.M., Chiffelle, I., Asenjo, F. 2005. Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. *Food Chem.* 91 (3) 395-401.

Fincher G.B., Stone B.A., Clarke A.E. 1983. Arabinogalactan-proteins: structure, biosynthesis and function. *Ann. Rev. Plant Physiol.* 34, 47-70.

Formica J.V., Regelson W. 1995. Review of the biology of quercetin and related bioflavonoids. *Food Chem. Toxicol.* 33, 1061-1080.

Frydoonfar H.R, McGrath DR, Spigelman AD. 2003. The variable effect on proliferation of a colon cancer cell line by the citrus fruit flavonoid naringenin. *Colorectal Dis.* 5, 149– 152.

Fuentes-Alventosa J.M., Jaramillo S., Rodríguez-Gutiérrez G., Cermeño P., Espejo J.A., Jiménez-Araujo A. 2008. Flavonoid profile of green asparagus genotypes. *J. Agric. Food Chem.* 56, 6977–6984.

Fuentes-Alventosa J.M., Jaramillo-Carmona S., Rodríguez-Gutiérrez G., Espejo-Calvo J.A., Rodríguez-Arcos R., Fernández-Bolaños J., Guillén-Bejarano R., Jiménez-Araujo A. 2009b. Effect of the extraction method on phytochemical composition and antioxidant activity of high dietary fibre powders obtained from asparagus by-products. *Food Chem.* 116, 484-490.

Fuentes-Alventosa J.M., Rodríguez G., Cermeño P., Jiménez A., Guillén R., Fernandez-Bolaños J., Rodríguez-Arcos R. 2007. Identification of flavonoid diglycosides in several genotypes of asparagus from the Huétor-Tájar population variety. *J. Agric. Food Chem.* 55, 10028–10035.

Fuentes-Alventosa J.M., Rodríguez-Gutiérrez G., Jaramillo-Carmona S., Espejo-Calvo J.A., Rodríguez-Arcos R., Fernández-Bolaños J., Guillén-Bejarano R.,

Jiménez-Araujo A. 2009a. Effect of extraction method on chemical composition and functional characteristics of high dietary fibre powders obtained from asparagus by-products. *Food Chem.* 113, 665–671.

Fuentes-Alventosa, J.M. 2007. Caracterización de flavonoides y saponinas en el espárrago triguero de Huétor Tájar (Granada). Trabajo de Investigación correspondiente al *Máster en Agroalimentación 2006/2007*. Universidad de Córdoba.

Fuleki, T. 1999. Rutin, the main component of surface deposits on pickled green asparagus. *J. Food Sci.* 64, 252-254.

Funk C., Braune A., Grabber J.H., Steinhart H., Bunzel M. 2007. Moderate ferulate and diferulate levels do not impede maize cell wall degradation by human intestinal microbiota. *J. Agric. Food Chem.* 55, 2418–2423.

Gallaher D.D., Locket P.L., Gallaher C.M. 1992. Bile acid metabolism in rats fed two levels of corn oil and brans of oat, rye and barley and sugar beet fiber. *J. Nutr.* 122, 473-481.

Garau M.C., Simal S., Rosselló C., Femenia A. 2007. Effect of air drying temperature on physico-chemical properties of dietary fibre and antioxidant capacity of orange (*Citrus aurantium* v, *Canoneta)* by-products. *Food Chem.* 104, 1014–1024.

Gardner P.T., White T.A.C., McPhail D.B., Duthie G.G. 2000. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chem.* 68, 471-474.

Geleijnse J.M., Launer L.J., Van der Kuip D.A., Hofman A., Witteman J.C. 2002. Inverse association of tea and flavonoid intakes with incident myocardial infarction: the Rotterdam study. *Am. J. Clin. Nutr.* 75, 880-886.

Gil M.I., Tomás-Barberán F.A., Hess-Pierce B., Holcroft D.M., Kader A. 2000. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J. Agric. Food Chem.* 48, 4581-4589.

González A.G., Hernández J.C., León F., Padrón J.I., Estévez F., Quintana J., Bermejo J. 2003. Steroidal saponins from the bark of *Dracaena draco* and their cytotoxic activities. *J. Nat. Prod*. 66 (6), 793-798.

Gorinstein S., Zachwieja Z., Katrich E., Pawelzik E., Haruenkit R., Trakhtenberg S., Martin-Belloso O. 2004. Comparison of the contents of the main antioxidant compounds and the antioxidant activity of white grapefruit and its new hybrid. *Lebensm.-Wiss.-Technol.* 37, 337-343.

Gregory J., Foster K., Tyler H., Wiseman M. 1990. The dietary and nutritional survey of british adults. HMSO, London, UK.

Grigelmo-Miguel N., Gorinstein S., Martín-Belloso O. 1999. Characterisation of peach dietary fibre concentrate as a food ingredient. *Food Chem.* 65, 175-181.

Grigelmo-Miguel N., Martín-Belloso O. 1999. Comparison of dietary fibre from by-products of processing fruits and greens and from cereals. *Lebensm.-Wiss.u.-Technol.* 32, 503–508.

Gryglewki R.J., Korbut R., Robak J., Swies J. 1987. On the mechanism of antithrombotic action of flavonoids. *Biochem. Pharmacol.* 36, 317-322.

Guillén R., Rodríguez R., Jaramillo S., Rodríguez G., Espejo J.A., Fernandez-Bolaños J., Heredia A., Jiménez A. 2008. Antioxidants from asparagus spears: Phenolics. *Acta Hort.* 776, 247–254.

Han L.K., Zheng Y.N., Xu B.J., Okuda H., Kimura Y. 2002. Saponins from Platycodi Radix Ameliorate high fat diet-induced obesity in mice. *J. Nutr.* 132, 2241-2245.

Haralampidis K., Trojanowska M., Osbourn A.E. 2002. Biosynthesis of triterpenoid saponins in plants. *Adv. Biochem. Eng. Biotechnol.* 75, 31–49.

Harbaum B., Hubbermann E.M., Wolff C., Herges R., Zhu Z., Schwarz K. 2007. Identification of flavonoids and hydroxycinnamic acids in pak choi varieties (Brassica campestris L. ssp.chichensis var. communis) by HPLC-ESI-MS and NMR and their quantification by HPLC-DAD. *J. Agric. Food Chem.* 55, 8251-8260.

Harbone, J.B. 1982. Introduction to ecological biochemistry. *Academic Press*, London, ISBN 0-12-324680-6.

Harborne J.B., Williams C.A. 2000. Advance in flavonoid research since 1992. *Phytochem.* 55, 481-504.

Hardigree A.A., Epler J.L. 1978 Comparative mutagenesis of plants flavonoids in microbial system. *Mutation Res.* 58, 231-239.

Harland B.F., Narula G. 2001. Dietary fiber and mineral interaction. En: *Handbook of Dietary Fiber*, Cho S. y Dreher M. (eds.). Marcell Dekker, Inc. Nueva York, pp. 219-225.

Harris P.J. 1990. Plant cell wall structure and development. En: *Microbial and Plant Opportunities to Improve Lignocellulose Utilization by Rumiants.* Alkin, E.D. (ed.). Elsevier Sci. Publ. Co. Nueva York, pp. 71-90.

Harwood H.J., Chandler C.E., Pellarin L.D., Bangerter F.W., Wilkins R.W., Long C.A., Cosgrove P.G., Malinow M.R., Marzetta C.A., Pettini J.L. 1993. Pharmacologic consequences of cholesterol absorption inhibition: alteration in cholesterol metabolism and reduction in plasma cholesterol concentration induced by

the synthetic saponin beta-tigogenin cellobioside (CP-88818; tiqueside). *J. Lipid. Res*. 34, 377-395.

Heaton K.W., Pomare E.W. 1974. Effect of bran on blood lipids and calcium. *Lancet* 1, 49-50.

Heredia A., Fernández M.J. 1979. Composición de fibra. II. Lignina y celulosa. *Grasas y aceites* 30 (3) 141-143.

Heredia A., Guillén R. 1986. Valor de la fibra vegetal. *Alimentación, Equipos y Tecnología* 4, 149-154.

Heredia A., Guillén R., Jiménez A, Fernández-Bolaños J. 1993. Plant cell wall structure. *Rev. Esp. Cienc. Tecnol. Ali.* 33, 113-131.

Heredia A., Jiménez A., Guillén R. 1995. Composition of plant cell wall. *Z Lebensm. Unter Forsch.* 200, 24-31.

Heredia Moreno A., Jiménez Araujo A.J., Fernández-Bolaños Guzmán J., Guillén Bejarano R., Rodríguez Arcos R. 2002. Fibra alimentaria. *Biblioteca de Ciencias*, 4. Consejo Superior de Investigaciones Científicas. ISBN: 84-00-08108-0.

Hertog M.G.L., Hollman P.C.H., Putte van de B. 1996. Content of potentially anticarcinogenic flavonoids of tea, infusions, wines, and fruit juices. *J. Agric. Food Chem.* 41, 1242-1246.

Hibasami H., Moteki H., Ishikawa K., Katsuzaki H., Imai K., Yoshioka K., Ishii Y., Komiya T. 2003. Protodioscin isolated from fenugreek (*Trigonella foenumgraecum* L.) induces cell death and morphological change indicative of apoptosis in leukemic cell line H-60, but not in gastric cancer cell line KATO III. *Intl. J. Mol. Med.* 11, 23–26.

Hirano R., Sasamoto W., Matsumoto A., Itakura H., Igarashi O., Kondo K. 2001. Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. Internal Medicine I, National Defense Medical College, Tokorozawa, Saitama, Japan. *J. Nutr. Sci. Vitaminol.* (Tokyo) 47, 357-362.

Hirota S., Shimoda T., Takahama U. 1998. Tissue and spatial distribution of flavonol and peroxidase in onion bulbs and stability of flavonol glucosides during boiling of the scales. *J. Agric. Food Chem.* 46, 3497–3502.

Hollman P.C.H. 2004. Absorption, Bioavailability, and Metabolism of Flavonoids. *Pharmaceutical Biol.* 42, 74-83.

Hollman P.C.H., Katan M.B. 1999. Dietary flavonoids: Intake, health effects and bioavailability. *Food Chem. Toxicol.* 37, 937-942 .

Hollman P.C.H., Katan M.B. 1998. Bioavailability and health effects od dietary flavonols in man. *Archives of Toxicology*. *Supplement*. *Arch. Fur Toxicol.* 20, 237-248.

Hu F.B., Willett W.C. 2002. Optimal diets for prevention of coronary heart disease. *JAMA* 288, 2569-78.

Huggett, A.C., Schliter, B. 1996. Research needs for establishing the safety of functional foods. *Nutr. Rev.* 54, S143– S148.

Hvattum E., Ekeberg D. 2003. Study of the collision-induced radical cleavage of flavonoid glycosides using negative electrospray ionization tandem quadrupole mass spectrometry. *J. Mass Spectrom.* 38, 43-49.

Iiyama K, Lam T.B., Meikle P.J. Rhodes D.I., Stone B.A. 1993. Cell wall biosynthesis and its regulation. En: *Forage cell wall structure and digestibility*. Jung H.G., Buxton D.R., Hatfield R.D., Ralph J. (eds). Madison, Winscosin. pp. 621-683.

Iiyama K, Lam T.B., Stone B.A. 1990. Phenolic acid bridges between polysaccharides and lignin in wheat internodes. *Phytochem.* 29, 733-737.

Jaramillo S., Rodríguez R., Jiménez A., Guillén R., Fernandez-Bolaños J., Heredia A. Effects of storage conditions on the accumulation of ferulic acid derivatives in white asparagus cell wall. *J. Sci. Food Agric.* 87, 286–296.

Jensen M.K., Koh-Banerjee P., Hu F.B., Franz M., Sampson L., Gronbaek M., Rimm E.B. 2004. Intakes of whole grains, bran, and germ and the risk of coronary heart disease in men. *Am. J. Clin. Nutr*. 80, 1492-1499.

Jiménez A. 1993. Modificación de la pared cellular de aceitunas durante su elaboración. *Tesis Doctoral*.

Jiménez A., Rodríguez R., Fernández-Caro I., Guillén R., Fernandez-Bolaños J., Heredia A. 2000. Dietary fibre content of tables olives processed under different European styles: study of physicochemical characteristics. *J. Sci. Food Agric.* 80, 1–6.

Jiménez-Escrig A., Rincón M., Pulido R., Saura-Calixto F. 2001. Guava fruit (*Psidium guajava* L) as a new source of antioxidant dietary fiber. *J. Agric. Food Chem.* 49, 5489–5493.

Jiménez-Escrig A., Santos-Hidalgo A.B., Saura-Calixto F. 2006. Common sources and estimated intake of plant sterols in the Spanish diet. *J. Agric. Food Chem.* 54, 3462–3471.

Johnson I.T., Southgate D.A.T. 1994. Dietary fibre and related substances. *Food Safety Series nº 3.* Chapman y Hall (eds). Londres-Nueva York.

Kaline K., Bornstein S.R., Bergmann A., Hauner H., Schwarz P.E.H. 2007. The importance and effect of dietary fiber in diabetes prevention with particular consideration of whole grain products. *Horm. Metab. Res*. 39, 687-693.

Kamat J., Boloor K., Devasagayam P.A., Venkatachalam S.R. 2000. Antioxidant properties of *Asparagus racemosus* against damage induced by irradiation in rat liver mitochondria. *J. Ethnopharmacol.* 71, 425–435.

Kaur N, Gupta AK. 2002. Applications of inulin and oligofructose in health and nutrition. *J. Biosci.* 27 (7), 703-714.

Kim D.-O., Padilla-Zakour O.I., Griffiths P.D. 2004. Flavonoids and antioxidant capacity of various cabbage genotypes at juvenile stage. *J. Food Sci.* 69, 685-689.

Kim G.-S., Kim H.-T., Seong J.-D., Oh S.-R., Lee C.-O., Bang J.-K., Seong N.-S., Song K.S. 2005. Cytotoxic steroidal saponins from the rhizomes of Asparagus oligoclonos. *J. Nat. Prod.* 68 (5), 766-768.

Kite G.C., Porter E.A., Simmonds M.S.J. 2007. Chromatographic behaviour of steroidal saponins studied by high-performance liquid chromatography–mass spectrometry. *J. Chromatogr. A* 1148, 177–183.

Konishi M., Hano Y., Takayama M., Nomura T., Hamzah A.S., Ahmad R.B., Jamani H. 1998. Triterpenoid saponins from *Hedyotis nudicaulis*. *Phytochem.* 48, 525–528.

Koo H.N., Jeong H.J., Choi J.Y., Choi S.D., Choi T.J., Cheon Y.S., Kim K.S., Kang B.K., Park S.T., Chang C.H., Kim C.H., Lee Y.M., Kim H.M., An N.H., Kim J.J. 2000. Inhibition of tumor necrosis factor-α-induced apoptosis by *Asparagus cochinchinensis* in Hep G2 cells. *J. Ethnopharmacol.* 73 (1-2), 137-43.

Kris-Etherton P.M., Hecker K.D., Bonanome A., Coval S.M., Binkoski A.E., Hilpert K.F. Griel A.E., Etherton T.D. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am. J. Med.* 113, 71S-88S.

Kritchevsky D. 1988. Dietary fiber. *Ann. Rew. Nutr.* 8, 301-328.

Kühnau J. 1976. The Flavonoids: a class of semi-essential food components: their role in human nutrition. *World Rev. Nutr. Diet* 24, 117-190.

Kuti J.O., Konuru H.B. 2004. Antioxidant capacity and phenolic content in leaf extracts of tree spinach (Cnidoscolus spp.). *J. Agric. Food Chem.* 52, 117-121.

Lachman J., Pronek D., Hejtmánková A., Dudjak J., Pivec V., Faitová K. 2003. Total poyphenol and main flavonoid antioxidants in different onion (Allim cepa L.) varieties. *Hortic. Sci.* 30, 142-147.

Lako J., Trenerry V.C., Wahlqvist M., Wattanapenpaiboon N., Sotheeswaran S., Premier R. 2007. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chem.* 10, 1727-1741.

Lapointe A., Couillard C., Lemieux S. 2006. Effects of dietary factors on oxidation of low-density lipoprotein particles. *J. Nutr. Biochem.*, Volume 17, Issue 10, pp. 645-658.

Lario Y., Sendra E., García-Pérez J., Fuentes C., Sayas-Barberá E., Fernández-López J., Pérez-Alvarez J.A. 2004. Preparation of high dietary fiber powder from lemon juice by-products. *Innov. Food Sci. Emerg. Technol.* 5 (1) 113-117.

Larrauri J.A., Goñi I., Martín-Carrón N., Rupérez P., Saura-Calixto F. 1996b. Measurement of health-promoting properties in fruit dietary fibres: antioxidant capacity, fermentability and glucose retardation index. *J. Sci. Food Agric.* 71, 515–519.

Larrauri J.A., Rupérez P., Borroto B., Saura-Calixto F. 1996a. Mango peels as a new tropical fibre: preparation and characterization. *Lebensm.-Wiss.u.-Technol* 29, 729–733.

Larrauri J.A., Rupérez P., Saura-Calixto F. 1997b. Mango peel fibres with antioxidant activity. *Z. Lebensm. Unters. Forsch. A* 205, 39–42.

Larrauri J.A., Rupérez P., Saura-Calixto F. 1997a. Pineapple shell as a source of dietary fiber with associated polyphenols. *J. Agric. Food Chem.* 45, 4028-4031.

Laughton M.J., Evans P.J., Moroney M.A. y cols. 1991. Inhibition of mammalian 5-lipoxygenase and cyclo-oxigenase by flavonoids and phenolic dietary additives. Relationship to antioxidant activity and to iron ion-reducing ability. *Biochem. Pharmacol.* 42, 1673-1681.

Laughton M.J., Halliwell B., Evans P.J. y cols. 1989. Antioxidant and prooxidant actions of the plant phenolic quercitin, gossypol and myricetin. Effect on lipid peroxidation, hydroxyl radical generation, and bleomycin-dependent damage to DNA. *Biochem. Pharmacol.* 38, 2859-286.

Lecumberri E., Mateos R., Izquierdo-Pulido M., Rupérez P., Goya L, Bravo L. 2007. Dietary fibre composition, antioxidant capacity and physico-chemical properties of a fibre-rich product from cocoa (*Theobroma cacao* L.). *Food Chem.* 104, 948-954.

Lee S.C., Prosky L., De Vries J.W. 1992. Determination of total, soluble and insoluble dietary fiber in food Enzymatic, gravimetric method, MES-TRIS buffer: collaborative study. *J. Assoc. Off. Anal. Chem.* 75, 395–416.

Lee S.-O., Simons A.L., Murphy P.A., Hendrich S. 2005. Soyasaponins lowered plasma cholesterol and increased fecal bile acids in female golden syrian hamsters. *Exp. Biol. Med.* 230, 472-478.

Lefebvre A.C., Thébaudin J.Y. 2002. Fibras extraídas de las hortalizas. En: *Tecnología de las Hortalizas*. Tirilly Y. y Bourgeois C. (eds). Acribia, S.A., Zaragoza, pp. 459-481.

Letan A. 1966. The relation of structure to antioxidant activity of quercitin and some of its derivates. *J. Food Sci.* 31, 518-523.

Lineback D., Drener M., Devries J. 1995. Complex carbohydrates: the science and the label. *Nutr. Rev.* 53, 186-193.

Llobera A., Cañellas J. 2007. Dietary fibre content and antioxidant activity of Manto Negro red grape (*Vitis vinifera*): Pomace and stem. *Food Chem.* 101, 659–666.

Llorach R., Espín J.C., Tomás-Barberán F.A., Ferreres F. 2003. Aprovechamiento y gestión de subproductos de la manipulación e industrialización de hortalizas. Posible uso como fuente de compuestos de interés para la salud. *CTC alimentación* 16, 6-12.

Llorach R., Gil-Izquierdo A., Ferreres F., Tomás-Barberán F.A. 2003. HPLC-DAD-MS/MS ESI characterization of inusual highly glycosylated acylated flavonoids from cauliflower (Brassica oleracea L. var. botrytis) agroindustrial byproducts. *J. Agric. Food Chem.* 51, 3895-3899.

López G., Ros G., Rincón F., Periago M.J., Martínez M.C., Ortuño. J. 1996. Relationship between physical and hydration properties of soluble and insoluble fiber of artichoke. *J. Agric. Food Chem.* 44, 2773–2778.

Lu, Y.-H.; Li, Z.-H.; Wang, Z.-T.; Wei, D.-Z. 2006. Quality evaluation of Platycladus orientalis (L.) Franco through simultaneous determination of four bioactive flavonoids by high-performance liquid chromatography. *J. Pharm. Biomed. Anal.* 41, 1186-1190.

Madar Z., Odes H.S. 1990. Dietary fibre in metabolic diseases. En: *Dietary Fibre Research*, Paoletti (ed), pp. 1-65.

Maeda T., Kakuta H., Sonoda S., Ueno R., Suzuki T., Oosawa K. 2005. Antioxidant capacities of extracts from green, purple, and white asparagus spears related to polyphenol concentration. *Hortic. Sci.* 40, 1221-1224.

Mahato S.B., Ganguly A.N., Sahu N.P. 1982. Steroid saponins. *Phytochem.*, Vol. 21 (5), 959-978.

Makris D.P., Rossiter J.T. 2001. Domestic processing of onion bulbs (*Allium cepa*) and asparagus spears (*Asparagus officinalis*): Effect on flavonol content and antioxidant status. *J. Agric. Food Chem.* 49, 3216–3222.

Manach C., Morand C., Crespy V., Demigné C., Texier O., Régérat F., Rémésy C. 1998. Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. *F.E.B.S. Letters* 426, 331-336.

Mandalari G., Bennett R.N., Bisignano G., Saija A., Dugo G., Lo Curto R.B. Faulds C.B. Waldron K.W. 2006. Characterization of flavonoids and pectins from bergamot (*Citrus bergamia* Risso) peel, a major byproduct of essential oil extraction. *J. Agric. Food Chem.* 54, 197-203.

Mandel S., Packer L., Youdim M.B.H., Weinreb O. 2005. Proceedings from the Third International Conference on Mechanism of Action of Nutraceuticals. *J. Nutr. Biochem.* 16, 513–520.

Mandel S., Weinreb O., Amit T., Youdim M.B.H. 2004. Cell signalling pathways in the neuroprotective actions of the green tea polyphenol (-)-epigallocatechin-3-gallate: implications for neurodegenerative diseases. *J. Neurochem.* 88, 1555–1569.

Mann J.I., Cummings J.H. 2009. Possible implications for health of the different definitions of dietary fibre. *Nutr. Metab. Cardiovasc. Dis.* 19, 226-229.

Marín F.R., Soler-Rivas C., Benavente-García O., Castillo J., Pérez-Álvarez J.A. 2007. By-products from different citrus processes as a source of customized functional fibres. *Food Chem.* 100, 736–741.

Marlett J.A. 1990. Analysis of dietary fiber in foods. En: *Dietary Fiber Research*. Kritchevsky D., Bonfield Ch. (eds), Plenum Press, Nueva York, pp 31-48.

Marlett J.A. 1997. Soluble dietary fiber workshop. En: *Dietary Fiber in Health and Disease*. Kritchevsky D., Bonfield Ch. (eds), Plenum Press, Nueva York, pp. 311-313.

Marquina S., Maldonado N., Garduño-Ramírez M.L., Aranda E., Villarreal M.L., Navarro V., Bye R., Delgado G., Alvarez L. 2001. Bioactive oleanolic acid saponins and other constituents from the roots of *Viguiera decurrens*. *Phytochem.* 56, 93–97.

Martín-Belloso O., Llanos-Barriobero E. 2001. Proximate composition, minerals and vitamins in selected canned vegetables. *Eur. Food Res. Technol.* 212, 182-187.

Martin-Cabrejas M.A., Esteban R., López-Andreu F.J., Waldron K., Selvendran R.R. 1995. Dietary fiber content of pear and kiwi pomaces. *J. Agric. Food Chem.* 43, 662-666.

Martínez-Flores S., González-Gallego J., Culebras J.M. y Muñón Mª.J. 2002. Los flavonoides: popiedades y acciones antioxidantes. *Nutr. Hosp.* XVII (6) 271-278.

Martínez-Sánchez A., Gil-Izquierdo A., Gil M.I., Ferreres F. 2008. A comparative study of flavonoid compounds, vitamin C, and antioxidant properties of baby leaf Brassicaceae species. *J. Agric. Food Chem.* 56, 2330-2340.

Martínez-Sánchez A., Llorach A., Gil M.I., Ferreres F. 2007. Identification of new flavonoid glycosides and flavonoid profiles to characterize rocket leafy salads (Eruca vesicaria and Diplotaxis tenuifolia). *J. Agric. Food Chem.* 55, 1356-1363.

Matsuura H. 2001. Saponins in garlic as modifiers of the risk of cardiovascular disease. *J. Nutr.* 131, 1000S–1005S.

Matysik G., Wójciak-Koiseor M., Paduch R. 2005. The influence of Calendulae officinalis flos extracts on cell cultures, and the chromatographic analysis of extracts. *J. Pharm. Biomed. Anal.* 38, 285-292.

McDougall G.J., Morrison I.M., Stewart D, Hillman J.R. 1996. Plant cell walls as a dietary fibre: range, structure, processing and function. *J. Sci. Food Agric.* 70, 133-150.

Mendez M.A., Pera G., Agudo A., Bueno-de-Mesquita H., Palli D., Boeing H., Carneiro F., Berrino F., Sacerdote C., Tumino R., Panico S., Berglund G., Manjer J., Johansson I., Stenling R., Martinez C., Dorronsoro M., Barricarte A., Tormo M.J., Quiros J.R., Allen N., Key T.J., Bingham S., Linseisen J., Kaaks R., Overvad K., Jensen J., Olsen A., Tjonneland A., Peeters P.H.M., Numans M.E., Ocke M.C., Clavel-Chapelon F., Boutron-Ruault M., Trichopoulou A., Lund E., Slimani N., Jenab M., Perrair P., Riboli E., Gonzalez C.A. 2007. Cereal fiber intake may reduce risk of gastric adenocarcinomas: The EPIC-EURGAST study. *Int. J. Cancer* 121, 1618-1623.

Merck, S.A. Industrias Químicas. 2000. Bioflavonoides: quercetina y rutina. *Informe a Profesionales*.

Mertz C., Cheynier V., Günata, Z., Brat P. 2007. Analysis of phenolic compounds in two blackberry species (Rubus glaucus and Rubus adenotrichus) by high-performance liquid chromatography with diode array detection and electrospray ion trap mass spectrometry. *J. Agric. Food Chem.* 55, 8616-8624.

Milbury P.E., Chen C.Y., Dolnikowski G.G., Blumberg J.B. 2006. Determination of flavonoids and phenolics and their distribution in almonds. *J. Agric. Food Chem.* 54, 5027-5033.

Mimaki Y., Kuroda M., Kameyama A., Yosuka A., Sashida Y. 1998. Steroidal saponins from the rhizomes of Hosta sieboldii and their cytostatic activity on HL-60 cells. *Phytochem*. 48 (8), 1361-1369.

Mimaki Y., Watanabe K., Ando Y., Sakuma C., Sashida Y., Furuya S. Sakagami H., 2001b. Flavonol glycosides and steroidal saponins from the leaves of *Cestrum nocturnum* and their cytotoxicity. *J. Nat. Prod.* 64, 17–22.

Mimaki Y., Yokosuka A., Kuroda M., Sashida Y. 2001a. Cytotoxic activities and structure-cytotoxic relationships of steroidal saponins. *Biol. Pharm. Bull.* 24, 1286–1289.

Miyakoshi M., Tamura Y., Masuda H., Mizutani K., Tanaka O., Ikeda T., Ohtani K., Kasai R., Yamasaki K. 2000. Antiyeast steroidal saponins from *Yucca schidigera* (*Mohave yucca*), a new anti-food-deteriorating agent. *J. Nat. Prod.* 63, 332–338.

Moira, H. 1998. The market for functional foods. *Int. Dairy J.* 8, 349 –353.

Monties B. 1980. Les polymères vegetaux. Polymères pariétaux et alimentaires non azotés. *Collection Biochimie Appliqué*. Ed. Gauthier-Villars, París.

Moon Y.J., Wang X., Morris M.E. 2006. Dietary flavonoids: effects on xenobiotic and carcinogen metabolism. *Toxicology in vitro* 20, 187-200.

Moreau R.A., Whitaker B.D., Hicks K.B. 2002. Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. *Progress in Lipid Res*. 41, 457-500.

Morrissey J.P., Osbourn A.E. 1999. Fungal resistance to plant antibiotics as a mechanism of pathogenesis. *Microbiol. Mol. Biol. Revs* 63, 708–724.

Murota K., Terao J. 2003. Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. *Arch. Biochem. Biophys.* 417, 12-17.

Murray T.M. 1996. Prevention and management of osteoporosis: consensus statements from the Scientific Advisory Board of the Osteoporosis Society of Canada: 4. Calcium nutrition and osteoporosis. *CMAJ* 155, 935–939.

Myhrstad M.C., Carlsen H., Nordstrom O., Blomhoff R., Moskaug J.J.O. 2002. Flavonoids increase the intracellular glutathione level by transactivation of the gamma-glutamylcysteine synthetase catalytical subunit promoter. *Free Radic. Biol. Med.* 32, 386-393.

Nijveldt R.J., VanNood E., VanHoorn D.E., Boelens P., VanNorren K., VanLeeuwen P. 2001. Flavonoids: A review of probable mechanisms of action and potential applications. *Am. J. Clin. Nutr.* 74, 418–425.

Nindo C.I., Sun T., Wang S.W., Tang J., Powers J.R. 2003. Evaluation of drying technologies for retention of physical quality and antioxidants in asparagus. *Eur. Food Res. Technol.* 36, 507–516.

Niness K.R. 1999. Inulin and oligofructose: What Are They? *J. Nutr.* 129 (7), 1402S-1406S.

Nkondjock A., Ghadirian P. 2004. Dietary carotenoids and risk of colon cancer: case-control study. *Int. J. Cancer* 110, 110–116.

Oakenfull D. 1981. Saponins in food – A review. *Food Chem.* 6 (1981) 19-40.

Oakenfull D. 1986. Aggregation of saponins and bile acids in aqueous solution. *Aust. J. Chem. 39*, 1671–1683.

Oakenfull D., Sidhu G.S. 1990. Could saponins be a useful treatment for hypercholesterolaemia? *Eur. J. Clin. Nutr.* 44 (1), 79-88.

Okoli C.O., Akah P.A. 2004. Mechanisms of the anti-inflamatory activity of the leaf extracts of Culcasia scadens P. Beauv (*Araceae*). *Pharmacol. Biochem. Behav*. 79, 473-481.

Oleszek W., Bialy Z. 2006. Chromatographic determination of plant saponins. An update (2002–2005). *J. Chromatogr. A*, 1112, 78–91.

Osbourn A.E. 2003. Molecules of interest, saponins in cereals. *Phytochem.* 62, 1–4.

Pace-Asciak C.R., Hahn S., Diamandis E.P., Soleas G., Goldberg D.M. 1995. The red wine phenolics trans-resveratrol and quercitin block human platelet aggregation in eicosanoid synthesis: implication for protection against coronary heart disease. *Clin. Chim. Acta* 235, 207-219.

Parr A.J., NG A., Waldron K.W. 1997. Ester-linked phenolic components of carrots cell walls. *J. Agric. Food Chem.* 45, 2468-2471.

Pascal, G. 1996. Functional food in the European Union. *Nutr. Rev.* 54, S29–S32.

Pellegrini N., Serafini M., Colombi B., Del Rio D., Salvatore S., Bianchi M., Brighenti F. 2003. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different *in vitro* assays. *J. Nutr.* 33, 2812–2819.

Peschel W., Sánchez-Rabaneda F., Diekmann W., Plescher A., Gartzia I., Jiménez D., Lamuela-Raventós R., Buxaderas S., Codina C. 2006. An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. *Food Chem.* 97, 137-150.

Pierron-Darbonne A. 2006. Past, present and future of the asparagus cultivation around the mediterranean sea. En: *Proceedings of the XI International Asparagus Symposium in Acta Horticulturae.*

Pinelo M, Del Fabbro P., Manzocco L., Núñez M.J., Nicoli M.C. 2005. Optimization of continuous phenol extraction from *Vitis vinífera* byproducts. *Food Chem.* 92, 109-117.

Plate A.Y.A., Gallaher D.D. 2005. The potential health benefits of corn components and products. *Cereal Foods World* 50, 305–314.

Pool-Zobel B.L. 2005. Inulin-type fructans and reduction in colon cancer risk: review of experimental and human data. *Br. J. Nutr.* 93, S73-S90.

Potter J.D. 1997. Food nutrition and the prevention of cancer. A global perspective. Potter J.D. (ed.). *World Cancer Research Fund/American Institute for Cancer Research.* Washington DC.

Proestos C., Boziaris I.S., Nychas G.-J.E., Komaitis M. 2006. Analysis of flavonoids and phenolic acids in Greek aromatic plants: investigation of their antioxidant capacity and antimicrobial activity. *Food Chem.* 95, 664-671.

Psarra E., Makris D.P., Kallithraka S., Kefalas P. 2002. Evaluation of the antiradical and reducing properties of selected Greek white wines: correlation with polyphenolic composition. *J. Sci. Food Agric.* 82, *1014−1020.*

Qian Z.-H., Li H.-J., Li P., Ren M.-T., Tang D. 2007. Simultaneous qualitation and quantification of thirteen bioactive compounds in Flos Lonicerae by high-performace liquid chromatography with diode array detector and mass spectrometry. *Chem. Pharm. Bull.* 55, 1073-1076.

Rankin S., de Whaley C., Hoult J., Jessup W., Wilkins G., Gollard J., Leake D. (1993). The modification of low density lipoprotein by the flavonoids myricetin and gossypetin. *Biochem. Pharmacol.* 45, 67-75.

Rechner A.R., Kuhnle G., Bremner P., Hubbard G.P., Moore K.P., Rice-Evans C.A. 2002.The metabolic fate of dietary polyphenols in humans. *Free Radical Biol. Med.* 33, 220-235.

Reglamento (CE) Nº 1924. 2006. Diario Oficial de la Unión Europea L 12/3-18.

Reinhold J.G., Faraji B., Abadi P., Ismail-Beigi F. 1976. Decreased absorption of calcium, magnesium, zinc and phosphorous by humans due to increased fiber and phosphorous consumption as wheat bread. *J. Nutr.* 106, 493-503.

Reinhold J.G., Nasr K., Lahimgarzadeh A, Hedayati H. 1973. Effects of purified phylate and phytate-rich bread upon metabolism of zinc, calcium, phosphorous and nitrogen in man. *Lancet* 1, 283-288.

Rice-Evans C.A., Miller N.J., Paganga J. 1996. Structure antioxidant ctivity relationships of flavonoids and phenolic acids. *Free Rad. Biol. Med.* 20, 933-956.

Rice-Evans C.A., Packer L. 1998. En: *Flavonoids in health and disease*. Ed. Marcel Dekker, INC. New York 20, 447-467.

Rimm E.B., Katan M.B., Ascherio A., Stampfer M.J., Willett W.C. 1996. Relation between intake and risk of coronary heart disease in male health professionals. *Ann. Intern. Med.* 125, 384-389.

Roberfroid M.B. 2005. Introducing inulin-type fructans. *Br. J. Nutr.* 93 (Suppl 1), S13-25.

Rochfort S.J., Imsic M., Jones R., Trenerry V.C., Tomkins, B. 2006. Characterization of flavonol conjugates in immature leaves of Pak Choi [Brassica rapa L. ssp. chinensis L. (Hanelt.)] by HPLC–DAD and LC–MS/MS. *J. Agric. Food Chem.* 54, 4855-4860.

Rodríguez R., Guillén R., Heredia A., Fernandez-Bolaños J., Jiménez A. 1999. Postharvest changes on cell wall and peroxidases of white asparagus (*Asparagus officinalis* L.). *Acta Hort.* 479, 477–482.

Rodríguez R., Jaramillo S., Guillén R., Jiménez A., Fernandez-Bolaños J., Heredia A. 2005b. Cell wall phenolics of white and green asparagus. *J. Sci. Food Agri*c. 85, 971–978.

Rodríguez R., Jaramillo S., Rodríguez G., Espejo J.A., Guillén R., Fernández-Bolaños J., Heredia A., Jiménez A. 2005a. Antioxidant activity of ethanolic extracts from several asparagus cultivars. *J. Agric. Food Chem.* 53, 5212-5217.

Rodríguez R., Jiménez A., Fernandez-Bolaños J., Guillén R., Heredia A. 2006. Dietary fibre from vegetable products as source of functional ingredients. *Trends Food Sci. Technol.* 17, 3–15.

Rodríguez R., Jiménez A., Guillén R., Heredia A., Fernandez-Bolaños J. 1999a. Postharvest changes in white asparagus cell wall during refrigerated storage. *J. Agric. Food Chem.* 47, 3551–3557.

Rodríguez R., Jiménez A., Guillén R., Heredia A., Fernandez-Bolaños J. 1999b. Turnover of white asparagus cell wall polysaccharides during postharvest storage. *J. Agric. Food Chem.* 47, 4525–4531.

Rodríguez R., Smith A.C., Waldron K.W. 2004. Ferulic acid cross-links in asparagus cell walls in relation to texture. *J. Agric. Food Chem.* 52, 4740-4750.

Rodríguez-Ambriz S.L., Islas-Hernández J.J., Agama-Acevedo E., Tovar J., Bello-Pérez L.A. 2008. Characterization of a fibre-rich powder prepared by liquefaction of unripe banana flour. *Food Chem.* 107, 1515–1521.

Rodríguez-Arcos R., Smith A.C., Waldron K.W. 2004. Ferulic acid cross-links in asparagus cell wall in relation to texture. *J. Agric. Food Chem.* 52, 4740–4750.

Romani A., Vignolini P., Isolani L., Ieri F., Heimler D. 2006. HPLC–DAD/MS Characterization of flavonoids and hydroxycinnamic derivatives in turnip tops (Brassica rapa L. subsp. sylvestris L.). *J. Agric. Food Chem.* 54, 1342-1346.

Ross J.A., Kasum C.M. 2002. Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annu Rev. Nutr.* 22, 19-34.

Ross J.K., Pusateri D.J., Shultz T.D. 1990. Dietary and hormonal evaluation of men at different risks for prostate cancer: fiber intake, excretion, and composition, with *in vitro* evidence for an association between steroids hormones and specific fiber components. *Am. J. Clin. Nutr.* 51, 365-370.

Ruberto G., Baratta M.T. 2000. Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem.* 69, 167−174.

Ruiter J.M., Burns J.C. 1987. Characterization of trifluoroacetic acid hydrolized of subtropical forage grass cell walls. *J. Agric. Food Chem.* 35, 308–316.

Salovaara H., Gates F., Tenkanen M. 2007. Dietary fibre-components and functions. *Wageningen Academic Publishers*, The Netherlands (344 pp., ISBN: 90-8686-019-7).

Salvatore S., Pellegrini N., Brenna O.V., Del Rio D., Frasca G., Brighenti F., Tumino R. 2005. Antioxidant characterization of some Sicilian edible wild greens. *J. Agric. Food Chem.* 53, 9465-9471.

Salvi M.J., Rajput C. 1995. Pineapple. En: *Handbook of food science and technology. production, composition, storage and processing.* Salunke, Kadam, Dekker (eds). New York, pp. 171-182.

Sanz T., Salvador A., Jiménez A., Fiszman S.M. 2008. Yogurt enrichment with functional asparagus fibre. Effect of extraction method on rheological properties, colour, and sensory acceptance. *Eur. Food Res. Technol.* 227, 1515–1521.

Saskia A.B.E., van Accker, Bast A.A.L.T. 1998. Structural aspects of antioxidant activity of flavonoids. En: *Flavonoids in health and disease*. Ed Marcel Dekker, INC. New York 9:221-251.

Saura-Calixto F. 1998. Antioxidant dietary fiber product: a new concept and a potential food ingredient. *J. Agric. Food Chem.* 46, 4303-4306.

Scalbert A., Morand C., Manach C., Rémésy C. 2002. Absorption and metabolism of polyphenols in the gut and impact on health. *Biomed. Pharmacother.* 56, 276-282.

Scheneeman B.O. 1987. Soluble vs insoluble fibre-different physiological responses. *Food Technol.* 41, 81-82.

Schieber A., Stintzing F.C., Carle R. 2001. By-products of plant food processing as a source of functional compounds – recent developments. *Trends Food Sci. Technol.* 12, 401-413.

Schwarzbach A., Schreiner M., Knorr D. 2006. Effect of cultivars and deep freeze storage on saponin content of white asparagus spears (*Asparagus officinalis L.)*. *Eur. Food Res. Technol.* 222, 32-35.

Selvendran R.R., Robertson J.A. 1994. Dietary fibre in foods: amount and type. En: *Physico-chemical properties of dietary fibre and effect of processing on micronutrients availability*. Amado R., Bary J.J., Frolich W. (eds), Luxemburgo, pp. 11-19.

Serpen A., Capuano E., Fogliano V., Gokmen V. 2007. A new procedure to measure the antioxidant activity of insoluble food components. *J. Agric. Food Chem.* 55, 7676–7681.

Seyoum, A.; Asres, K.; El-Fiky, F. K. 2006. Structure–radical scavenging activity relationships of flavonoids. *Phytochem.* 67, 2058-2070.

Shiomi N., Benkeblia N., Onodera S., Omori T., Takahashi N., Fujishima M. Taiki Yoshihira T., Kosaka S. 2007. Saccharide and fructooligosaccharide contents, and invertase, 1-KHE, 1-SST, 1-FFT and 6G-FFT activities in green asparagus spears during storage: Effects of temperature and spear portion. *J. Appl. Glycosci.* 54, 187–194.

Showalter A.M. 1993. Structure and function of plant cell wall proteins. *Plant Cell* 5, 9-23.

Sidhu K.S. 2003. Health benefits and potential risks related to consumption of fish or fish oil. *Regul. Toxicol. Pharmacol.* 38, 336– 44.

Silva B.M., Andrade P.B., Ferreres F., Domingues A.L., Seabra R.M., Ferreira M.A. 2002. Phenolic profile of quince fruit (Cydonia oblonga Miller) (pulp and peel). *J. Agric. Food Chem.* 50, 4615-4618.

Silvis N. 1992. Nutritional recommendations for individuals with diabetes mellitus. *S. Afr. Med. J.* 81, 162– 6.

Sims I.M. 2004. Structural diversity of fructans from members of the order Asparagales in New Zeland. *Phytochem*. 63, 351-359.

Sjösttröm E. 1981. *Wood Chemistry. Fundamentals and Applications*. Academic Press. London. pp. 15-50.

Slavin J.L. 2001. Dietary fiber and colon cancer. En: *Handbook of Dietary Fiber.* Cho S. y Dreher M. (eds), Marcel Dekker, Inc., New York, pp. 31-45.

Slimestad R., Fosen T., Vågen I.M. 2007. Onions: as source of unique dietary flavonoids. *J. Agric. Food Chem.* 55, 10067-10080.

Smoczkiewicz M.A., Nitschke D., Wieladek H. 1982. Microdetermination of steroid and triterpene saponin glycosides in various plant materials. I. Allium species. *Mikrochim. Acta* II, pp. 43–53.

Southgate D.A.T. 1990. *Determination of Food Carbohydrates*. Elsevier Applied Science. Londres.

Southon S., Price K.R., Johnson I.T., Gee J.M. 1988. The effect of Gypshophila saponins in the diet on mineral status and plasma cholesterol concentration in the rat. *Br. J. Nutr.* 59 (1), 49-55.

Sparg S.G., Light M.E., van Staden J. 2004. Biological activities and distribution of plant saponins. *J. Ethnopharmacol.* 94, 219–243.

Spencer J.P.E. 1999. Metabolism of tea flavonoids in the gastrointestinal tract. *J. Nutr.* 133, 3255-3261S

Spiller G.A. 1986. Suggestion for a basis on which to determine a desirable intake of dietary fibre. In: G.A. Spiller, Editor, *CRC Handbook of Dietary Fibre in Human Nutrition*, CRC Press, Florida, pp. 281–283.

Stavric B. 1994. Quercitin in our diet: from potent mutagen to probable anticarcinogen. *Clin. Biochem.* 27, 245-248.

Stahl W., Ale-Agha N., Polidori M.C. 2002. Non-antioxidant properties of carotenoids. *Biol. Chem.* 383, 553-558.

Steinmetz K.A., Potter J.D. 1991. Vegetables, fruit, and cancer. I. Epidemiology. *Cancer Causes and Control* 2, 325-357.

Stobiecki M. 2000. Application of mass spectrometry for identification and structural studies of flavonoid glycosides. *Phytochem.* 54, 237-256.

Stone J.E., Scallaham A.M. 1967. The effect of component and their constituent non-cellulosic polymers. *Ind. Crops Prod.* 6, 17-26.

Sudhakar D.V., Maini S.B. 2000. Isolation and characterization of mango peel pectins. *J. Food Proc. Preserv.* 24, 209-227.

Sun, T.; Tang, J.; Powers, J. R. 2007. Antioxidant activity and quality of asparagus affected by microwave-circulated water combination and conventional sterilization. *Food Chem.* 100, 813-819.

Swies J., Robak J., Dabrowski L., Duniec Z., Michalska Z., Gryglewski R.J. 1984. Antiaggregatory effects of flavonoids in vivo and their influence on lipoxygenase and cyclooxygenase in vitro. *Pol. J. Pharmacol. Pharm.* 36, 455-463.

Temple N.J. 2002. Nutrition and disease: challenges of research design. *Nutrition* 18, 343–347.

Terao J., Yamaguchi S., Shirai M. y cols. 2001. Protection by quercetin and quercetin 3-O-beta-D-glucuronide of peroxynitrite-induced antioxidant consumption in human plasma low-density lipoprotein. *Free Radic. Res.* 35, 925-931.

Tomás-Barberán F.A., Llorach R., Espín J.C., Ferreres F. 2004. Agri-food residues as a source of phytochemicals. *Total Food Proceedings* 42-48. Institute of Food Research (Norwich, Reino Unido). ISBN 0-7084-0644-5.

Tomás-Barberán F.A., Espín J.C. 2001. Phenolic compounds and related encimes as determinants of quality in fruit and vegetables. *J. Sci. Food Agric.* 81, 853-876.

Tran Q.L., Tezuka Y., Banskota A.H., Tran Q.K., Saiki I., Kadota S. 2001. New spirostanol steroids and steroidal saponins from roots and rhizomes of Dracaena angustifolia and their antiproliferative activity. *J. Nat. Prod.* 64 (9), 1127-32.

Trowell H.C. 1976. Definition of dietary fiber and hyphoteses that it is a protective factor in certain diseases. *Am. J. Clin. Nutr.* 29, 417-427.

Ubando-Rivera J., Navarro-Ocaña A., Valdivia-López M.A. 2005. Mexican lime peel: comparative study on contents of dietary fibre and associated antioxidant activity. *Food Chem.* 89, 57–61.

Ursini F., Maiorino M., Morazzoni P. y cols. 1994. A novel antioxidant flavonoid (IdB 1031) affecting molecular mechanisms of cellular activation. *Free Rad. Biol. Med.* 16, 547-553.

Vallejo F., Gil-Izquierdo A., Pérez-Vicente A., García-Viguera C. 2004. In vitro gastrointestinal digestion study of broccoli inflorescence phenolic compounds, glucosinolates, and vitamin C. *J. Agric. Food Chem.* 52, 135-138.

Van Buren J.P. 1979. The chemistry of texture in fruits and vegetables. *J. Texture Studies* 10, 1-23.

Van den Heuvel E.G.H.M., Muys T., Dokkum W.v., Schaasfma G. 1999. Oligofructose stimulates calcium absorption in adolescents. *Am. J. Clin. Nutr.* 69, 544–548.

Vasconcellos J.A. 2000. Alimentos funcionales. Conceptos y beneficios para la salud. The World of Food Science. IFT y IUFoST. www.worldfoodscience.org.

Vincken J.P., Heng L., de Groot A., Gruppen H. 2007. Saponins, classification and occurrence in the plant kingdom. *Phytochem.* 68, 275–297.

Vinson J.A., Hao Y., Su X., Zubik L. 1998. Phenol antioxidant quantity and quality in foods: vegetables. *J. Agric. Food Chem.* 46, 3630–3634.

Vrijsen R., Everaert L., Boeté A. 1988. Antiviral activity of flavones and potentiation by ascorbate. *J. Gen. Virol.* 69, 1749-1751.

Waldron K.W., Selvendran R.R. 1992. Cell wall changes in immature asparagus stem tissue after excision. *Phytochem.* 31, 1931–1940.

Wang H.X., Ng T.B. 1999. Natural products with hypoglycaemic, hypotensive, hypocholesterolemic, antiatherosclerotic and antithrombotic activities. *Life Sci.* 65, 2663–2677.

Wang M., Tadmor Y., Wu Q.L., Chin C.K., Garrison S.A., Simon J.E. 2003. Quantification of protodioscin and rutin in asparagus shoots by LC/MS and HPLC methods. *J. Agric. Food Chem.* 51, 6132-6136.

Wang S.Y., Lin H.-S. 2000. Antioxidant activitiy in fruits and leaves of blackberry, raspberry and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem.* 48, 140–146.

Wang T.S., Lii C.K., Chang J.Y., Kuo C.I., Chen H.P. 2007. Anti-Fenton reaction activity of three taxa of water yam (*Dioscorea alata* L.). *Int J. Food Sci. Tech.* 42, 1107–1113.

WHO/FAO. 2008. Codex Alimentarius Commission (CNFSDU). 30th Session. ALINORM 09/32/26, Apéndice 2, pp. 49-50.

Wiboonpun N., Phuwapraisirisan P., Tip-pyang S. 2004. Identification of antioxidant compound from *Asparagus racemosus*. *Phytother. Res.* 18, 771-773.

Widdowson E.M., McCance R.A. 1942. Iron exchanges of adults on white and brown bread. *Lancet* 1, 588-591.

Wildman R.E.C. 2001. En: Handbook of nutraceuticals and functional foods. *CRC Series In Modern Nutrition*, Chapter 1, 1-12.

Willis M.S., Wians F.H. 2003. The role of nutrition in preventing prostate cancer: a review of the proposed mechanism of action of various dietary substances. *Clin. Chim. Acta* 330, 57– 83.

Wilson J.R. 1993. Organization of forage plant tissues. En: *Forage Cell Wall Structure and Digestibility*. Jung H.G., Buxton D.R., Hatfield R.D., Ralph J. (eds). Madison, Wiscosin, pp. 1-32.

Wilson W.D., Jarvis M.C., Duncan H.J. 1989. In-vitro digestibility of kale (*Brassica oleracea)* secondary xylem and parenchyma cell walls and their polysaccharide components. *J. Sci. Food Agric.* 48, 9-14.

Wolever T.M.S. 1990. Relationship between dietary fibre content and composition in foods and the glycemic index. *Am. J. Clin. Nutr.* 51, 72–75.

Yamamoto N., Moon J.-H., Tsushida T., Nagao A., Terao J. 1999. Inhibitory effect of quercetin metabolites and their related derivativeson copper ion-induced lipid peroxidation in human low-density lipoprotein. *Arch. Biochem. Biophys.* 372, 347-354.

Yokosuka A., Mimaki Y., Sashida Y. 2002. Spirostanol saponins from the rhizomes of *Tacca chantrieri* and their cytotoxic activity. *Phytochem.* 61, 73–78.

Yu S., Chee-Kok C., Chi-Tang H., Wei M., Garrison S.A., Ho C. Huang M.-T. 1996. Anti-tumor activity of the crude saponins obtained from asparagus. *Cancer Lett.* 104, 31–36.

Yuan X., Wang J., Yao H. 2005. Feruloyl oligosaccharides stimulate the growth of *Bifidobacterium bifidum*. *Anaerobe* 11, 225–229.

Zafar T.A., Weaver C.M., Zhao Y., Martin B.R., Wasttney M.E. 2004. Nondigestible oligosaccharides increase calcium absorption and supress bone resorption in ovariectomized rats. *J. Nutr.* 134, 399–402.

Zhao Z., Moghadasian M.H. 2008. Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review. *Food Chem.* 109, 691–702.

ABREVIATURAS

AAO-FI: actividad antioxidante de la fracción insoluble de la fibra

AAO-FS: actividad antioxidante de la fracción soluble de la fibra

ABAP: 2,2'-azobis(2-amidinopropano)

ANOVA: análisis de la varianza

ARC: antiradical capacity

BSTFA: N,O-bis-trimethylsilyl-trifluoroacetamide

CAR: capacidad antirradical

CEL: cellulose

CIOP: capacidad de inhibición de la oxidación primaria

DAD: diode-array detection

DPPH: 2,2-difenil-1-picrilhidracilo

ESI: electrospray ionization

FAD: ferulic acid derivatives

FBL: fibra bioactiva liofilizada

FBS: fibra bioactiva seca

FID: flame ionization detector

FOS: fructooligosaccharides

G/E F-D: gentle treatment (1 min at room temperature) with ethanol and freeze-drying

G/E O-D: gentle treatment (1 min at room temperature) with ethanol and oven-drying

G/W F-D: gentle treatment (1 min at room temperature) with water and freeze-drying

G/W O-D: gentle treatment (1 min at room temperature) with water and oven-drying

GC: gas chromatography

GDRI: glucose dialysis retardation index

HCA: hydroxycinnamic acids

HPLC: high-performance liquid chromatography

i.d.: internal diameter

I/E F-D: intense treatment (90 min at 60º C) with ethanol and freeze-drying

I/E O-D: intense treatment (90 min at 60º C) with ethanol and oven-drying

I/W F-D: intense treatment (90 min at 60º C) with water and freeze-drying

I/W O-D: intense treatment (90 min at 60º C) with water and oven-drying

IF: insoluble fibre

IR: isorhamnetin

K: kaempferol

KL: Klason lignin

LC: liquid chromatography

LOD: limits of detection

LOQ: limits of quantification

LSD: least-square differences

MS: mass spectrometry

NS: neutral sugars

OHC: oil-holding capacity

P: proteins

PR: poder reductor

Q: quercetin

R: coeficiente de correlación de Pearson

RSD: relative standard deviation

SF: soluble fibre

SL: subproductos liofilizados

SOL: solubility

SS: subproductos secos

TDF: total dietary fibre

TE: equivalentes Trolox

TEAC: Trolox equivalent antioxidant capacity

TFA: trifluoroacetic acid

TMCS: trimethylchlorosilane

t_R: retention times

UA: uronic acids

UV: ultraviolet

WHC: water-holding capacity

ANEXO

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Identification of Flavonoid Diglycosides in Several Genotypes of Asparagus from the Huétor-Tájar Population Variety

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The qualitative and quantitative composition of flavonoids from the Huétor-Tájar population variety of asparagus (commonly known as "*triguero*") was investigated. Flavonoids were analyzed by reversed-phase high-performance liquid chromatography–diode array detection (HPLC–DAD). Liquid chromatography–mass spectrometry (LC–MS) under identical HPLC conditions was used to verify the identities of the flavonoid glycosides from *triguero* asparagus. The quantities of asparagus flavonoids were calculated according to concentration curves constructed with authentic standards. Total flavonoid contents, calculated as the sum of individual compounds, were determined and ranged from 400 to 700 mg/kg fresh weight. The most abundant was rutin, which represented 55–98% of the total flavonoid complement. *Triguero* asparagus were revealed to be an important source of not only quercetin derivatives but also kaempferol and isorhamnetin glycosides. Significant differences ($p < 0.05$) in the content and relative composition of flavonoids were found among the spears of the distinct asparagus genotypes from the Huétor-Tájar population variety.

KEYWORDS: ***Triguero* asparagus; flavonoid diglycosides; HPLC–DAD; MS**

INTRODUCTION

Flavonoids represent the most common and widely distributed group of plant-food phenolics, and their contents and compositions have been related to the antioxidant properties of different fruits and vegetables (*1, 2*). The beneficial effects of the consumption of vegetables, such as broccoli (*3*), spinach (*4*), onion, and asparagus (*5*), on human health can, at least partly, be explained by their flavonoid content.

Asparagus is a vegetable that has traditionally been very appreciated for its organoleptic and nutritional characteristics, but this product is also a good source of bioactive compounds that may contribute to enhancing its cultivation and consumption. It has been established that, among the most commonly consumed vegetables, asparagus has the highest antioxidant capacity (*6, 7*), and this property is associated to a great extent to its total phenolic content (*8, 9*).

Scarce information is available regarding asparagus spear phenolic characterization, but we have recently investigated the phenolic profile of both white and green asparagus, and the results revealed that, whereas white spears mainly contained hydroxycinnamic acid derivatives, flavonoids were the major phenolics in green asparagus (*10*). In agreement with Maeda et al. (*11*), who reported that rutin represented 60–80% of the total phenolic content of purple and green asparagus extracts, we have found that rutin constituted more than 70% of the total phenolic content of asparagus from commercial hybrids. This flavonoid glycoside has been shown to act as a strong free-radical scavenger and may have a protective role in carcinogenesis and cardiovascular diseases (*2*). The high content of rutin of green asparagus could be directly related to its antioxidant properties. However, other flavonoids that are much more abundant in *triguero* asparagus than in commercial hybrids (*10*) may contribute to that activity and have not been characterized yet.

Wang et al. (*12*) developed a liquid chromatography–mass spectrometry (LC–MS) method for the characterization of the main bioactive compounds, including saponins and flavonoids, in asparagus spears. These authors detected only two flavonoid compounds in asparagus extracts; the major compound was identified as rutin, and the other peak appeared to be a rutin-type flavonoid that should have an extra sugar molecule on its structure.

Several recent studies have been conducted with a focus on the influence of genetics and cultivation area on the phenolic profile of plant foods. Significant differences have been found not only in the total content but also in the flavonoid profile from different varieties of several plant foods, such as strawberries (*13*), grapes (*14*), spinach (*15*), and *Brassica* species (*16, 17*).

We have previously reported that the flavonoid profile of green asparagus is determined by sample origin and variety (*10*).

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The main objective of the present study was the quantitative determination of the different flavonoid glycosides detected and identified from several genotypes of asparagus from the Huétor-Tájar population variety. Those flavonoids, which are not found in most commercial varieties of green asparagus, were identified and quantified by high-performance liquid chromatography–diode array detection (HPLC–DAD), and LC–MS was used to confirm their structures.

MATERIALS AND METHODS

Plant Material. The samples investigated consisted of spears from 10 native lines of *triguero* asparagus from the Huétor-Tájar population variety and a sample of commercial green asparagus. *Triguero* asparagus are tetraploid subspecies that come from wild asparagus, autochthonous to the Huétor-Tájar area. Asparagus spears were harvested from 10 lines of *triguero* asparagus (HT-1, HT-2, HT-3, HT-4, HT-5, HT-6, HT-7, HT-8, HT-9, and HT-10) from Huétor-Tájar, Granada, Spain, in the spring of 2005 and 2006. These asparagus have been developed and cloned in Las Torres Agricultural Research Center, Alcalá del Río, Sevilla, Spain, during the last year, and they have been classified and selected by their agronomic characteristics. Their chemical characterization, on the basis of flavonoid profiles, may help to establish new criteria for the selection of these *triguero* asparagus.

The spears investigated in this work were harvested from experimental fields of Huétor-Tájar (Granada, Spain). On harvest day, asparagus spears were transported to the laboratory and then weighed, frozen at −20 °C, and freeze-dried. This plant tissue was ground into a fine powder and stored at −20 °C for further analysis.

Phenolic Extraction. Each sample, consisting of 2.5 g of freeze-dried material, was extracted with 100 mL of 80% ethanol (EtOH). The samples were blended in a Sorvall Omnimixer, Model 17106 (Du Pont Co., Newtown, CT), at maximum speed for 1 min, and then filtered through filter paper. Ethanolic extracts were stored at −20 °C until analysis by HPLC. The method was optimized in terms of the extraction of the solvent, sample size, volume, and concentration of ethanol for asparagus spears. All extractions were made in duplicate.

Acid Hydrolysis. The free flavonoid aglycones were released by acidic hydrolysis as follows: 2.5 g of freeze-dried material were extracted with 80 mL of 80% EtOH as described above. A total of 20 mL of 6 M HCl was added, and the solution was incubated for 2 h, with constant mixing, at 90 °C. The extract was filtered through filter paper and made to 100 mL with 80% ethanol. The extracts were stored at −20 °C until analysis.

Qualitative Analysis of Flavonoids by HPLC–DAD. Phenolic compounds were detected and quantified by HPLC using a SYNERGI 4 μHYDRO-RP80A reverse-phase column (25 cm × 4.6 mm i.d., 4 μm; Phenomenex, Macclesfield, Cheshire, U.K.). The gradient profile for the separation of flavonoids was formed using solvent A [10% (v/v) aqueous acetonitrile plus 2 mL/L acetic acid] and solvent B (40% methanol, 40% acetonitrile, and 20% water plus 2 mL/L acetic acid) in the following program: the proportion of B was increased from 10 to 42.5% B for the first 17 min, then to 70% B over the next 6 min, maintained at 70% B for 3.5 min, then to 100% B over the next 5 min, maintained at 100% B for 5 min, and finally returned to the initial conditions. The flow rate was 1 mL/min, and the column temperature was 30 °C. Phenolic compounds were detected using a Jasco-LC-Net II ADC liquid chromatograph system equipped with DAD and a Rheodyne injection valve (20 μL loop). Spectra from all peaks were recorded in the 200–600 nm range, and the chromatograms were acquired at 360 nm.

Isolation of the New Flavonoids Identified in *Triguero* Asparagus. A HPLC method similar to that described above but using a semi-preparative SYNERGI 4 μHYDRO-RP80A reverse-phase column (25 cm × 46 mm i.d., 4 μm; Phenomenex, Macclesfield, Cheshire, U.K.) was developed for the isolation of the new flavonoids. The flow rate was maintained at 10 mL/min, and the injection volume was 400 μL. Elution was monitored by UV at 360 nm, and the flavonoids were manually collected after the UV detector. The two fractions containing each individual compound were then reinjected onto the analytical column, to purify the two isolated flavonoids. Those were concentrated under nitrogen prior to lyophilization.

Table 1. Effect of the Solvent Type and Solvent/Solid Ratio on the Flavonoid Contents Extracted from *Triguero* Asparagus[a]

	mg/kg fresh weight			
	ratio of 1:1	ratio of 1:2	ratio of 1:4	ratio of 1:8
water	214 ± 15	227 ± 9	252 ± 6	250 ± 9
80% ethanol	233 ± 4	545 ± 8	591 ± 6	594 ± 15
80% methanol	443 ± 6	514 ± 17	583 ± 13	594 ± 9

[a] Data are the means of three replicates.

Characterization of Flavonoids by HPLC–DAD–MS. Rutin and the "new flavonoids" detected in asparagus were separated by HPLC, as described above, and identified by their electron impact mass data collected on a quadrupole mass analyzer (ZMD4, Micromass, Waters, Inc., Manchester, U.K.). Electrospray ionization (ESI) mass spectra were obtained at ionization energies of 50 and 100 eV (negative mode) and 50 eV (positive mode), with MS scans from *m*/*z* 100 to 1000. Capillary voltage was 3 kV; desolvation temperature was 200 °C; source temperature was 100 °C; and extractor voltage was 12 V. The flow was maintained at 1 mL min^{-1}.

HPLC–DAD–MS System for Quantitative Analysis. Flavonoid compound quantification was achieved by integration of peak areas, with reference to calibrations made using known amounts of pure compounds.

Results were calculated from the mean of three replicates. Comparisons among samples were done by the analysis of variation (ANOVA) test and the least-square deconvolution (LSD) method at a 95% confidence level.

Validation of the Method of HPLC–DAD–MS for Quantitative Analysis. Calibration curves were established on 8 data points that covered a concentration range of 5–250 μg/mL for each flavonoid glycoside. The linearity response of rutin, kaempferol-3-*O*-rutinoside, and isorhamnetion-3-*O*-rutinoside was determined using standards purchased from Megazyme. Eight concentrations of the mixed standard 80% ethanol solution were injected in duplicate. The calibration curves were constructed by plotting the mean peak area versus the concentration of standards. The limits of detection (LOD) and quantification (LOQ) were determined at a signal-to-noise ratio (S/N) of 3 and 10, respectively.

The precision of the method was evaluated from the measurement of intra- and interday variability. For this purpose, the same mixed standard 80% ethanol solution was analyzed 3 times within the same day and then for 3 consecutive days. The assays were realized by triplicate, and the relative standard deviation (RSD) was taken as a measure of precision.

A recovery test was used to evaluate the accuracy of the method. Accurate amounts of the three standards were added to 2.5 g of freeze-dried asparagus (plant material) and then extracted and analyzed as described above. The average percentage recoveries were calculated as the ratio of detected amount versus added amount. The recovery experiment was performed with three replicates at two concentration levels.

RESULTS AND DISCUSSION

Extraction Procedure. Prior to achieving the detailed analysis of the phenolic profile of green asparagus, the influence of different process conditions (raw material, solvent type, solvent/solid ratio, simple or sequential extraction, time of extraction) on the phenolics extraction efficiency was studied. The results (**Tables 1**–**3**) showed the following: (1) The yield of total soluble phenolics was equivalent from fresh asparagus and freeze-dried material. The water content of the spears was 90%, and the values of the distinct samples investigated were not significantly different ($p < 0.05$). (2) The yield of phenolics was also equivalent when using methanol or ethanol aqueous

Table 2. Effect of Sequential Extraction on the Flavonoid Contents Extracted from *Triguero* Asparagus[a]

	mg/kg fresh weight			
	1st extraction	2nd extraction	3rd extraction	4th extraction
1 × 25 mL of 80% EtOH	233 ± 4			
1 × 100 mL of 80% EtOH	591 ± 6			
4 × 25 mL of 80% EtOH	233 ± 4	155 ± 21	71 ± 5	0
4 × 100 mL of 80% EtOH	591 ± 6	9 ± 1	0	0

[a] Data are the means of three replicates.

Table 3. Effect of the Extraction Time on the Flavonoid Contents Extracted from *Triguero* Asparagus[a]

	mg/kg fresh weight			
	1 min	1 h	2 h	16 h
1 × 25 mL of 80% EtOH	233 ± 4	237 ± 6	230 ± 4	235 ± 9
1 × 100 mL of 80% EtOH	591 ± 6	588 ± 11	605 ± 8	593 ± 8

[a] Data are the means of three replicates.

solutions and higher than that reached using water as the extraction solvent. It is noteworthy that the highest results were attained when the alcohol concentration was ≥70%. (3) The solvent/solid ratio greatly influenced the phenolic yield. Preliminary assays were conducted by extracting 25 g of fresh sample and 2.5 g of freeze-dried material with 25–50 and 100 mL of 80% ethanol or water, respectively, and the results revealed that the higher the solvent/solid ratio, the higher the total amount of phenolics solubilized. Extraction volumes higher than 100 mL did not increase the amount of phenolics released, which revealed that the optimal solvent/solid ratio was 1:4 (g of fresh sample/mL of ethanol). (4) Sequential extraction of asparagus samples, using 4 × 25 or 4 × 100 mL aliquots of extraction solvent, did not result in a higher yield of phenolics compared to simple extraction with 100 mL of 80% ethanol. (5) In the same way, increasing the time of extraction did not have a positive effect on extraction efficiency, because extraction for 1, 2, and 24 h with constant mixing did not make a significant difference in the amount of solubilized phenolics compared to that obtained by extracting the samples for 1–2 min.

Characterization of Flavonoid Glycosides from *Triguero* Asparagus. A representative chromatogram of the ethanolic extract from *triguero* asparagus is shown in **Figure 1**. From the HPLC–DAD data, the major peak was identified as rutin, because its retention time and UV spectrum were identical to those of the rutin standard purchased from Sigma. In addition to rutin, there were two significant peaks on the chromatogram of the ethanolic extract from *triguero* asparagus (**Figure 1A**). From the HPLC–DAD data, those peaks were tentatively identified as flavonoid glycosides, because the UV spectra from peak 1 (264, 280sh, and 348) and peak 2 (252, 280, and 356) were similar to that from rutin (255, 279sh, and 355). The chromatogram from the ethanolic extract of commercial green asparagus was also recorded (inset of **Figure 1A**), and it consisted of a very prominent peak of rutin, which was only accompanied by one or two other minor peaks of flavonoids that, in many cases, were only detected in trace amounts.

After acid hydrolysis of the *triguero* and commercial green asparagus ethanolic extracts, three different flavonol aglycones were detected for the former (**Figure 1B**), while a unique peak was found for the latter (inset of **Figure 1B**). The aglycones were identified by a comparison of retention times, DAD information, and co-injection with standards. The results revealed that commercial asparagus only yielded quercetin. Analysis of the hydrolyzate from *triguero* asparagus revealed that quercetin was the most prominent aglycone as expected, because rutin represented more than 50% of the total flavonoid complement in all samples investigated in this study. However, it has been revealed that *triguero* asparagus is also a good source of glycosides from kaempferol and isorhamnetin, flavonols that are not found in most varieties of green asparagus. By a comparison of the UV spectra from the new flavonoid glycosides with those from the aglycones, it can be proposed that peak 1 must be a kaempferol (264, 280sh, and 364) derivative, while peak 2 could derive from quercetin (252, 284sh, and 372) or isorhamnetin (252, 284sh, and 368).

Because UV spectra were not enough to identify the flavonoids, a HPLC–MS method for recording the MS spectra from both aglycones and flavonoid glycosides was developed. The ESI–HPLC–MS analyses allowed the first structure hypotheses to be established. The use of alternating positive/negative ionization modes during recording was preferred to ensure the assignment of the molecular weights.

Figure 2A shows the negative-ion MS spectrum of rutin (*m*/*z* 609). It can be observed that, apart from the molecular ion, the main product ions were at *m*/*z* 301, 300, 271, 255, and 151. As reported previously (*18*), deprotonated flavonoid-*O*-glycosides, such as rutin (quercetin-3-*O*-rhamnoglucoside), provide both a radical aglycone anion $(Y_0 - H)^{-\bullet}$ at *m*/*z* 301 and an aglycone product ion (Y_0^-) at *m*/*z* 300.

The $[M - H]^-$ product ion spectra of rutin also reveals an abundant $[Y_0 - H - CO - H]^-$ ion at *m*/*z* 271 and a $[Y_0 - H - CO_2 - H]^-$ ion at *m*/*z* 255. These ions provide structural information for isomeric differentiation and determination of the glycosylation position (*19*), because their presence is indicative of 3-O-glycosylation, while 7-O-glycosylated flavonoids would provide an abundant $[Y_0 - CO]^-$ ion at *m*/*z* 273, which was not detected in this case.

The relative abundances of the Y_0^- and $[Y_0 - H]^{-\bullet}$ ions have been proposed to be related to the flavonoid glycosylation position. According to Ferreres et al. (*20*), the Y_0^- ion was the base peak for the flavonoid *O*-diglycosides, whereas it was represented about 30% relative abundance for the flavonoid di-*O*-glycosides. In agreement with these findings, **Figure 2A** shows that rutin, with the two sugars linked in position 3, yielded Y_0^- as the main ion in the negative mode.

The positive-ion spectrum of flavonoid glycosides provides additional and complementary information about structural characteristics, mainly on those aspects related to the position of the sugars. As observed in **Figure 2B**, the ESI spectra of the $[M - H]^+$ ion of rutin, at *m*/*z* 611, showed two main product ions corresponding to two successive losses of sugar residues. The first loss corresponded to a rhamnose residue (146 units), yielding the Y_1^+ ion at *m*/*z* 465, and this was followed by the elimination of glucose (162 units), giving the Y_0^+ ion at *m*/*z* 303.

On the basis of the hypothesis illustrated above by the characterization of rutin with the application of ESI–MS techniques in positive- and negative-ion modes, it has been possible to determine the tentative structures of the two new flavonoid diglycosides detected in *triguero* asparagus. The MS analysis of compound 1 showed a molecular ion at *m*/*z* 593 (**Figure 3**). Its $[M - H]^-$ product ion spectrum gave rise to the Y_0^- [M – H – 308] at *m*/*z* 285 as the base peak and also revealed an abundant $[Y_0 - H - CO - H]$ ion at *m*/*z* 255. This fragmentation pattern indicates that compound 1 is a kaempferol

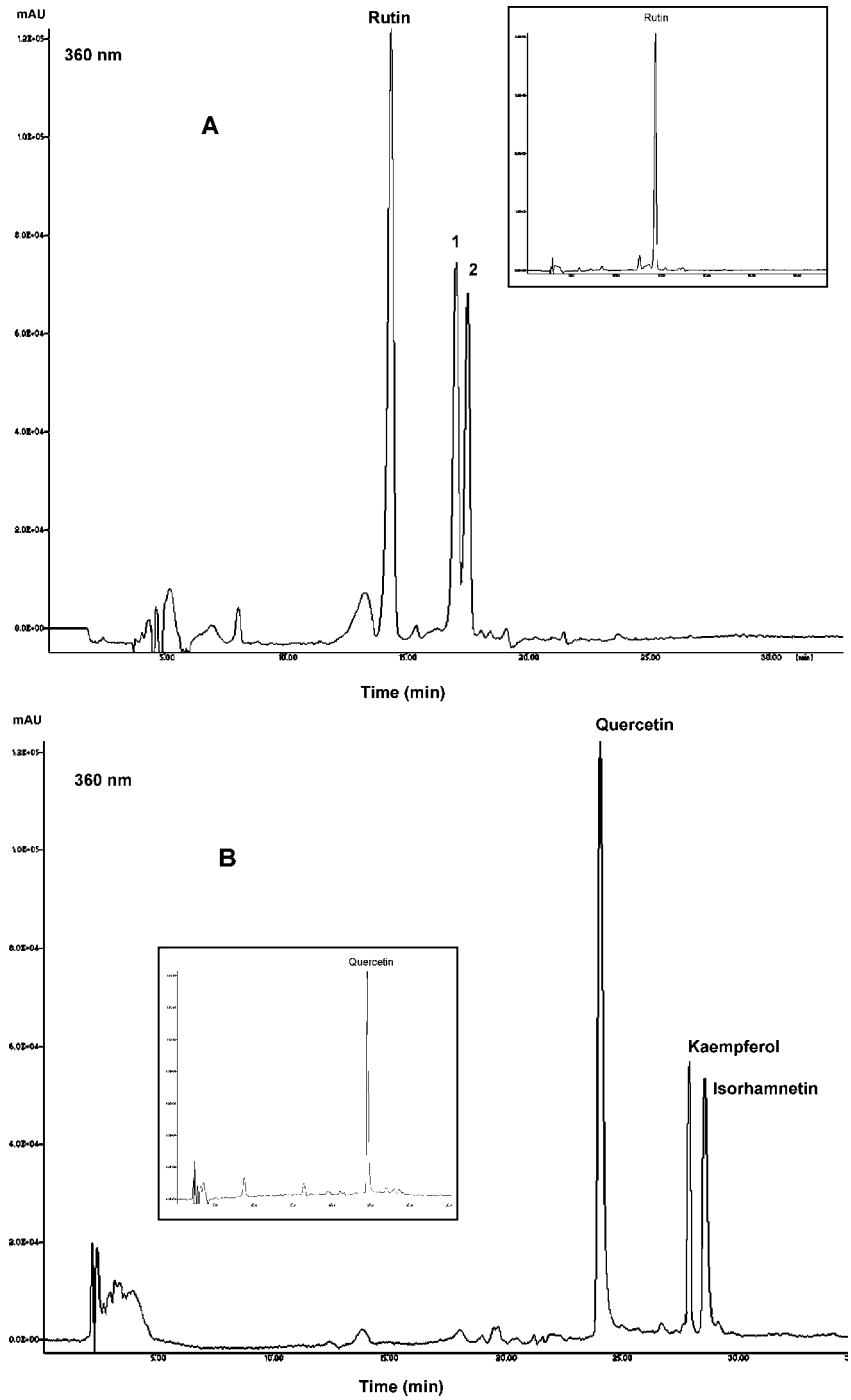

Figure 1. Chromatographic profiles acquired by HPLC–DAD (360 nm) of the 80% ethanolic extract of *triguero* asparagus (A) and its acid hydrolyzate (B). (Insets) Chromatograpahic profiles acquired by HPLC–DAD (360 nm) of the 80% ethanolic extract of commercial asparagus (A) and its acid hydrolyzate (B).

glycosylated with two sugar residues, consisting of a hexose, likely glucose (162 units), and a deoxyhexose, likely rhamnose (146 units). According to Ferreres et al. (*20*), the flavonoid diglycosides with sugar moieties linked to different phenolic positions of the flavonoid nucleus provided a Y_1^- [M – H – 162] ion, which is formed by a loss of a glucosyl from the [M − H]$^-$ as the base peak, and the Y_0^- represented around 30% relative abundance, whereas the flavonoid diglycosides with two sugar moieties linked to the same phenolic position yielded the aglycone ion product (Y_0^-) as the base peak. As described above for rutin, an aglycone product ion is the base peak for compound 1, because it can be observed in its negative-ion spectrum (**Figure 3A**). It has also been established that the glycosylation position significantly influences the fragmentation behavior of flavonoid *O*-glycosides and has been shown to affect the relative abundances of radical aglycone ions, which are most pronounced for flavonol 3-*O*-glycosides. The presence of an abundant $[Y_0 - H]^{-\bullet}$ ion at *m*/*z* 284 and a significant $[Y_0 - H - CO - H]$ ion at *m*/*z* 255 supports the fact that the rhamnose and glucose residues are located at the 3-O positions. In addition to these findings, the fact that the characteristic $[Y_0 - CO]^-$ ion of the 7-*O*-glycosides was not detected is consistent with the two sugar residues being located at the 3-O position. Detailed analysis of the positive-ion MS spectrum allowed for the confirmation of the nature and position of the sugars linked to this kaempferol derivative. The ESI spectrum of the $[M - H]^+$ ion at *m*/*z* 595

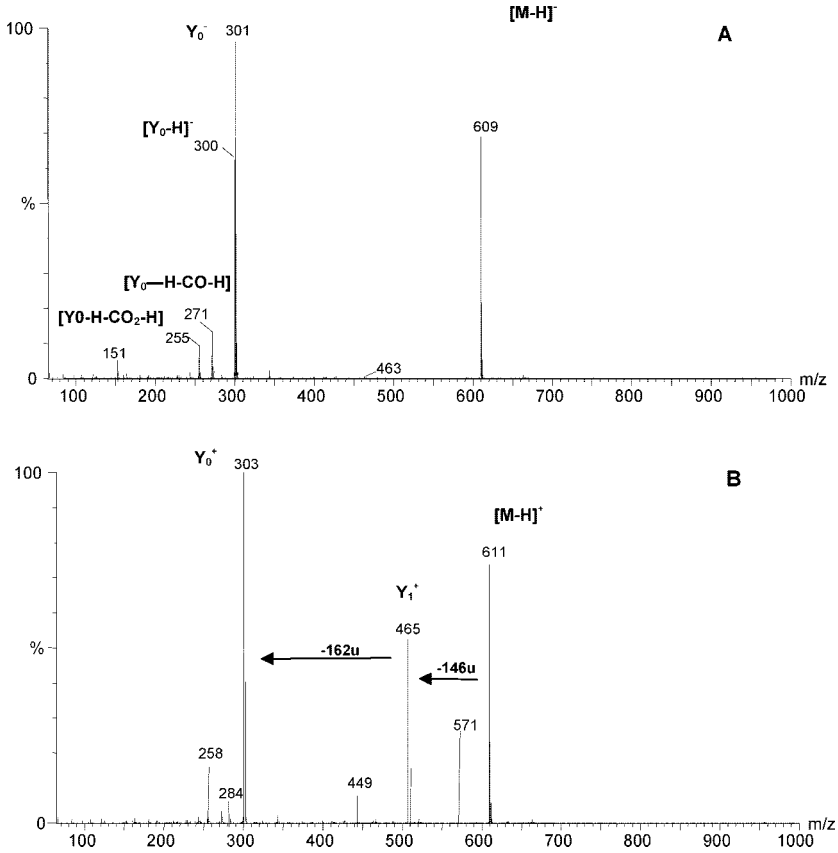

Figure 2. ESI spectra of rutin in negative (A) and positive (B) modes.

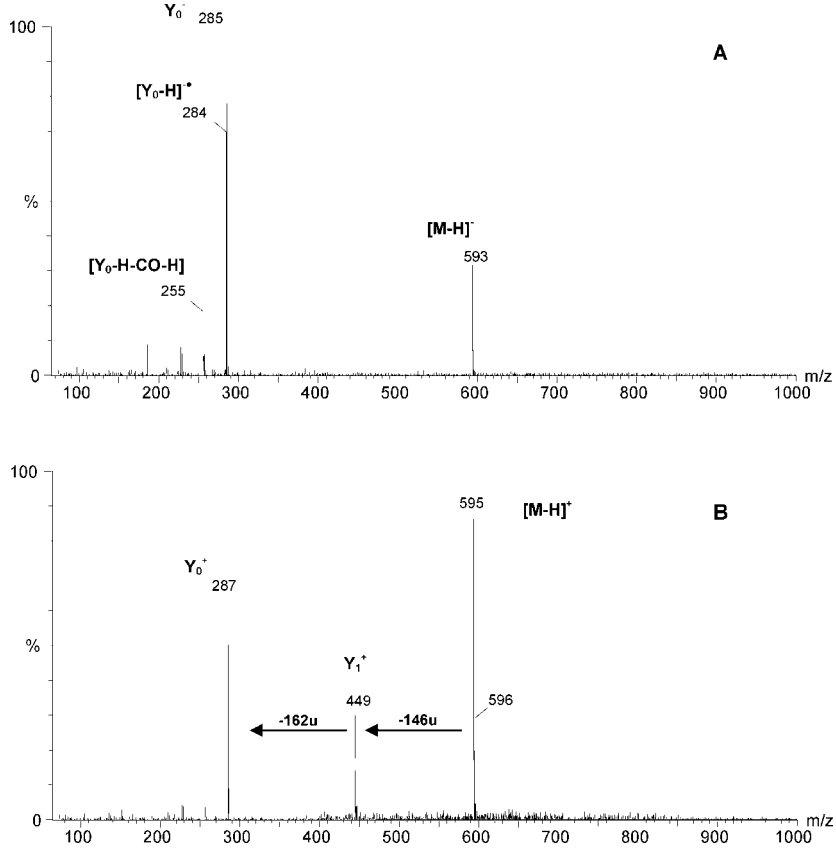

Figure 3. ESI spectra of kaempferol diglycoside in negative (A) and positive (B) mode.

from compound 1 (**Figure 3B**) showed two main product ions, indicating two losses of sugar residues. The first loss corresponded to a rhamnose (146 units), giving Y_1^+ at *m/z* 449, and then the loss of glucose (162 units) yielded the Y_0^+ ion at *m/z* 287, which was assigned as protonated kaempferol. These results are in agreement with the fragmentation behavior of a

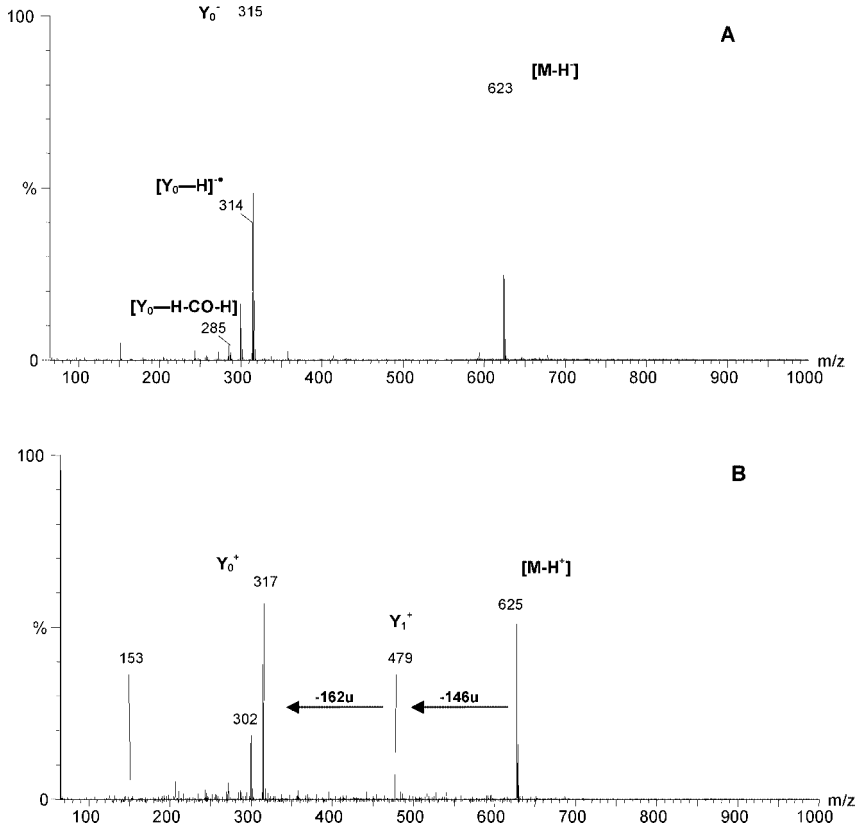

Figure 4. ESI spectra of isorhamnetin diglycoside in negative (A) and positive (B) mode.

3-*O*-rutinoside flavonol, which, in this case, would be the kaempferol-3-*O*-rutinoside. This compound, known as nicotiflorin, has not been previously detected in asparagus, but its presence has been reported in other plant food, such as quince fruit (*21*).

The mass spectra of compound 2 revealed that this was also a flavonoid diglycoside, whose fragmentation pattern was similar to that of rutin and compound 1. **Figure 4** shows the negative and positive spectra of compound 2. After ensuring the assignment of its molecular weight (623), as well as that of the corresponding aglycone (315), by analyzing the fragments issued from the $[MH]^-$ ion at *m*/*z* 623 (**Figure 4A**), the nature and position of the two sugar residues present in this compound were determined from the information generated by the fragmentation of the $[M - H]^+$ ion at *m*/*z* 625 (**Figure 4B**). Following the premises established for the characterization of compound 1, the other flavonoid detected in *triguero* asparagus has been tentatively assigned as isorhamnetin-3-*O*-rutinoside. This compound, which is also present in significant quantities in the *triguero* cultivars investigated in this work, had not been previously described in asparagus, because it is not present in the green spears from commercial hybrids. To our knowledge, almonds are the unique plant food that contain isorhamnetin-3-*O*-rutinoside as a predominant flavonoid (*22*).

The identities of kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-rutinoside were confirmed by injection of authentic standards purchased from Extrasynthese. Authentic compounds were injected alone and with their corresponding flavonoids isolated from *triguero* asparagus extracts. Retention times and UV and MS profiles were the same for pure kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-rutinoside as for those of peaks 1 and 2.

Validation of the Method for Quantitative Analysis of Flavonoids from *Triguero* Asparagus. To ensure the accurate assessment of the contents of the three flavonoid glycosides found in *triguero* asparagus, the HPLC–DAD–MS method was validated prior to its application for the quantitative analysis of different asparagus cultivars. The calibration curves of rutin, nicotiflorin, and narcissin showed good linear regression within test ranges, as observed in **Table 4**. The limit of detection, defined as the lowest sample concentration that can be detected (signal-to-noise-ratio = 3), was 4.61 μg/mL for rutin, 1.89 μg/mL for nicotiflorin, and 2.90 μg/mL for narcissin, and the limit of quantification, defined as the lowesr sample concentration that can be quantitatively determined with suitable precision and accuracy (signal-to-noise ratio = 10), was 15.39 μg/mL for rutin, 6.31 μg/mL for nicotiflorin, and μg/mL for narcissin. As shown in **Table 5**, the developed analytical method provided good precision and stability, because the overall intra- and interday variations were less than 4.1% for all flavonoids. To test the recovery of the method, one asparagus sample was added to known quantities of each of the reference flavonoids. The samples were analyzed before and after the additions in triplicate. Results showed that 105% of rutin, 93% of kaempferol-3-*O*-rutinoside, and 95% of isorhamnetin-3-*O*-rutinoside were recovered (**Table 5**).

Analytical characteristics of the calibration graphs as well as the precision and accuracy of the method were satisfactory and comparable to those reported by other authors that have

Table 4. Calibration Curves, LODs, and LOQs of the Three Flavonoid Diglycosides[a]

flavonoid diglycoside	calibration curve	r^2	test range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
rutin	$y = 25\,074x + 126\,136$	0.9978	25–250	4.61	15.39
k-3-*O*-rutinoside	$y = 27\,162x - 55\,839$	0.9996	5–250	1.89	6.31
i-3-*O*-rutinoside	$y = 29\,329x + 32\,576$	0.9991	5–250	2.90	9.67

[a] Data are the means of three replicates.

Table 5. Precisions and Recoveries of the Three Flavonoid Diglycosides[a]

flavonoid diglycoside	precision		recovery ($n = 3$)	
	intraday RSD (%)	interday RSD (%)	recovery (%)	RSD (%)
rutin	0.45	0.46	104.93	2.80
k-3-*O*-rutinoside	0.42	1.33	92.97	4.09
i-3-*O*-rutinoside	0.43	0.54	95.94	1.67

[a] Data are the means of three replicates.

Table 6. *Triguero* Asparagus Flavonoids Identified by HPLC–DAD and HPLC–MS[a]

asparagus line	mg/kg fresh weight						
	rutin	(%)	k-3-*O*-rutinoside	(%)	i-3-*O*-rutinoside	(%)	total flavonoids
HT-1	336 ± 15	(83)	5 ± 0	(1)	66 ± 0	(16)	407 ± 13
HT-2	476 ± 30	(86)	13 ± 1	(2)	65 ± 1	(12)	553 ± 44
HT-3	332 ± 16	(69)	39 ± 4	(8)	111 ± 18	(23)	481 ± 37
HT-4	401 ± 13	(78)	6 ± 0	(1)	108 ± 3	(21)	515 ± 16
HT-5	382 ± 28	(55)	108 ± 2	(16)	203 ± 16	(29)	692 ± 47
HT-6	368 ± 15	(67)	35 ± 1	(6)	147 ± 2	(27)	549 ± 14
HT-7	498 ± 12	(72)	34 ± 1	(5)	162 ± 4	(23)	694 ± 18
HT-8	548 ± 8	(97)	5 ± 0	(1)	9 ± 4	(2)	562 ± 5
HT-9	230 ± 5	(55)	31 ± 1	(7)	157 ± 3	(38)	418 ± 9
HT-10	411 ± 4	(75)	42 ± 2	(8)	97 ± 4	(18)	549 ± 6

[a] Data are the means of three replicates. Data in parentheses represent the relative percent.

developed HPLC–DAD–MS analytical methods for the determination of flavonoid compounds from several plant materials (*23–25*). It can be concluded that the recommended method is reliable and accurate for the qualitative and quantitative determination of flavonoid diglycosides from asparagus.

Quantification of Flavonoids from 10 Varieties of *Triguero* Asparagus. The main flavonoid glycosides found in all of the asparagus varieties were rutin, kaempferol-3-*O*-rutinoside, and isorhamnetin-3-*O*-rutinoside, but significant differences were found in the total quantities and the relative compositions of the distinct asparagus varieties (**Table 6**). The flavonoid content of the 10 varieties investigated varied between 400 and 700 mg/kg fresh weight, which was within the range of the values reported for green asparagus from commercial varieties (*12, 26*). Rutin has been reported as the main phenolic compound in green asparagus, and it represents more than 80% of the total phenolic complement of commercial hybrids (*10, 11*). Therefore, this has been described as the main flavonoid related to the antioxidant activity of ethanolic extracts from different varieties of green asparagus (*11, 26, 27*), and although the presence of other related flavonoids accompanying rutin has recently been reported (*12*), they have not been characterized yet. As observed in **Table 6**, there are several varieties of *triguero* asparagus, such as HT-1, HT-2, and HT-8, whose flavonoid profiles are similar to that found in commercial hybrids (**Figure 1A**). However, there is another group of *triguero* varieties, such as HT-5, HT-6, and HT-9, in which kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-rutinoside represent nearly 50% of the total flavonoid content. Fuleki (*27*) has reported that rutin is the only flavonoid present in green asparagus spears, and this author suggested that other minor peaks detected in the chromatogram from asparagus methanolic extracts were impurities, because most of them were even detected in commercial rutin used as a standard. From the results of the present paper, it can be proposed that some of those peaks did not correspond to impurities but to the kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-rutinoside, which are present in significantly greater quantities in *triguero* asparagus than other green varieties. These findings may explain why these compounds had not been previously described in green asparagus, because of the fact they are not found or are present in very low quantities in most commercial varieties.

A recent study about the characterization of antioxidant components of some Italian edible wild greens (*8*) showed that wild asparagus species, such as *Asparagus acutifolius*, have a considerable antioxidant capacity that seems to be related to their flavonoid content. The detailed analysis of their flavonoid profiles revealed that, apart from rutin and other quercetin glycosides, those asparagus contain significant amounts of kaempferol and isorhamnetin derivatives. Because the samples were analyzed after acidic hydrolysis, just the aglycones were determined and flavonoid content was quantified as quercetin equivalents, because that was the most abundant aglycone. These data are in agreement with our results and support the fact that *triguero* asparagus may come from wild species distinct from *Asparagus officinalis*, which gives them a characteristic phytochemical profile that can be used for differentiating and revalorizating native asparagus cultivars. It has been established that the bioactive properties of flavonoid compounds are dependent upon their structure and that minor differences in the number and position of –OH and sugar residues linked to the flavonol skeleton may lead to great differences in the bioactive properties of the individual compounds (*28*). Thus, deeper studies on the isolation and structural characterization of flavonoids and other bioactive compounds from *triguero* asparagus will establish relationships between individual components and specific beneficial actions associated with asparagus, mainly derived from its antioxidant capacity.

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LITERATURE CITED

(1) Hollman, P. C. H.; Katan, M. B. Dietary flavonoids: Intake, health effects and bioavailability. *Food Chem. Toxicol.* **1999**, *37*, 937–942.

(2) Harborne, J. B.; Williams, C. A. Advance in flavonoid research since 1992. *Phytochemistry* **2000**, *55*, 481–504.

(3) Vallejo, F.; Gil-Izquierdo, A.; Pérez-Vicente, A.; García-Viguera, C. In vitro gastrointestinal digestion study of broccoli inflorescence phenolic compounds, glucosinolates, and vitamin C. *J. Agric. Food Chem.* **2004**, *52*, 135–138.

(4) Kuti, J. O.; Konuru, H. B. Antioxidant capacity and phenolic content in leaf extracts of tree spinach (*Cnidoscolus* spp.). *J. Agric. Food Chem.* **2004**, *52*, 17–121.

(5) Makris, D. P.; Rossiter, J. T. Domestic processing of onion bulbs and asparagus spears: Effect of flavonol content and antioxidant status. *J. Agric. Food Chem.* **2001**, *49*, 3216–3222.

(6) Vinson, J. A.; Hao, Y.; Su, X.; Zubik, L. Phenol antioxidant quantity and quality in foods: Vegetables. *J. Agric. Food Chem.* **1998**, *46*, 3630–3634.

(7) Pellegrini, N.; Serafini, M.; Colombi, B.; Del Rio, D.; Salvatore, S.; Bianchi, M.; Brighenti, F. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *J. Nutr.* **2003**, *133*, 2812–2819.

(8) Salvatore, S.; Pellegrini, N.; Brenna, O. V.; Del Rio, D.; Frasca, G.; Brighenti, F.; Tumino, R. Antioxidant characterization of some Sicilian edible wild greens. *J. Agric. Food Chem.* **2005**, *53*, 9465–9471.

(9) Rodríguez, R.; Jaramillo, S.; Rodríguez, G.; Espejo, J. A.; Guillén, R.; Fernández-Bolaños, J.; Heredia, A.; Jiménez, A. Antioxidant activity of ethanolic extracts from several asparagus cultivars. *J. Agric. Food Chem.* **2005**, *53*, 5212–5217.

(10) Guillén, R.; Rodríguez, R.; Jaramillo, S.; Rodríguez, G.; Espejo, J. A.; Fernández-Bolaños, J.; Heredia, A.; Jiménez, A. Antioxidants from asparagus spears: Flavonoids. *Acta Hortic.* 2007, in press.

(11) Maeda, T.; Kakuta, H.; Sonoda, S.; Ueno, R.; Suzuki, T.; Oosawa, K. Antioxidant capacities of extracts from green, purple, and white asparagus spears related to polyphenol concentration. *Hortic. Sci.* **2005**, *40*, 1221–1224.

(12) Wang, M.; Tadmor, Y.; Wu, Q. L.; Chin, C. K.; Garrison, S. A.; Simon, J. E. Quantification of protodioscin and rutin in asparagus shoots by LC/MS and HPLC methods. *J. Agric. Food Chem.* **2003**, *51*, 6132–6136.

(13) Aaby, K.; Skrede, G.; Wrolstad, R. E. Phenolic composition and antioxidant activities in flesh and achenes of strawberries (*Fragaria ananassa*). *J. Agric. Food Chem.* **2005**, *53*, 4032–4040.

(14) Cantos, E.; Espín, J. C.; Tomás-Barberán, F. A. Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC–DAD–MS–MS. *J. Agric. Food Chem.* **2002**, *50*, 5691–5696.

(15) Bergquist, S. A.; Gertsson, U. E.; Knuthsen, P.; Olsson, M. E. Flavonoids in baby spinach (*Spinacia oleracea* L.): Changes during plant growth and storage. *J. Agric. Food Chem.* **2005**, *53*, 9459–9464.

(16) Romani, A.; Vignolini, P.; Isolani, L.; Ieri, F.; Heimler, D. HPLC–DAD/MS Characterization of flavonoids and hydroxycinnamic derivatives in turnip tops (*Brassica rapa* L. subsp. *sylvestris* L.). *J. Agric. Food Chem.* **2006**, *54*, 1342–1346.

(17) Rochfort, S. J.; Imsic, M.; Jones, R.; Trenerry, V. C.; Tomkins, B. Characterization of flavonol conjugates in immature leaves of Pak Choi [*Brassica rapa* L. ssp. *chinensis* L. (Hanelt.)] by HPLC–DAD and LC–MS/MS. *J. Agric. Food Chem.* **2006**, *54*, 4855–4860.

(18) Hvattum, E.; Ekeberg, D. Study of the collision-induced radical cleavage of flavonoid glycosides using negative electrospray ionization tandem quadrupole mass spectrometry. *J. Mass Spectrom.* **2003**, *38*, 43–49.

(19) Ablajan, K.; Abliz, Z.; Shang, X.-Y.; He, J.-M.; Zhang, R.-P.; Shi, J.-G. Structural characterization of flavonol 3,7-di-*O*-glycosides and determination of the glycosylation position by using negative ion electrospray ionization tandem mass spectrometry. *J. Mass Spectrom.* **2006**, *41*, 352–360.

(20) Ferreres, F.; Llorach, R.; Gil-Izquierdo, A. Characterization of the interglycosidic linkage in di-, tri-, tetra- and pentaglycosylated flavonoids and differentiation of positional isomers by liquid chromatography/electrospray ionization tandem mass spectrometry. *J. Mass Spectrom.* **2004**, *39*, 312–321.

(21) Silva, B. M.; Andrade, P. B.; Ferreres, F.; Domingues, A. L.; Seabra, R. M.; Ferreira, M. A. Phenolic profile of quince fruit (*Cydonia oblonga* Miller) (pulp and peel). *J. Agric. Food Chem.* **2002**, *50*, 4615–4618.

(22) Milbury, P. E.; Chen, C. Y.; Dolnikowski, G. G.; Blumberg, J. B. Determination of flavonoids and phenolics and their distribution in almonds. *J. Agric. Food Chem.* **2006**, *54*, 5027–5033.

(23) Qian, Z.-H.; Li, H.-J.; Li, P.; Ren, M.-T.; Tang, D. Simultaneous qualitation and quantification of thirteen bioactive compounds in Flos Lonicerae by high-performace liquid chromatography with diode array detector and mass spectrometry. *Chem. Pharm. Bull.* **2007**, *55*, 1073–1076.

(24) Lu, Y.-H.; Li, Z.-H.; Wang, Z.-T.; Wei, D.-Z. Quality evaluation of *Platycladus orientalis* (L.) Franco through simultaneous determination of four bioactive flavonoids by high-performance liquid chromatography. *J. Pharm. Biomed. Anal.* **2006**, *41*, 1186–1190.

(25) Escarpa, A.; Pérez-Cabrera, C.; González, M. C. Optimization and validation of a fast liquid gradient for determination of prominent flavan-3-ols and flavonols in fresh vegetables. *J. High Resolut. Chromatogr.* **2000**, *23*, 637–643.

(26) Sun, T.; Tang, J.; Powers, J. R. Antioxidant activity and quality of asparagus affected by microwave-circulated water combination and conventional sterilization. *Food Chem.* **2007**, *100*, 813–819.

(27) Fuleki, T. Rutin, the main component of surface deposits on pickled green asparagus. *J. Food Sci.* **1999**, *64*, 252–254.

(28) Seyoum, A.; Asres, K.; El-Fiky, F. K. Structure–radical scavenging activity relationships of flavonoids. *Phytochemistry* **2006**, *67*, 2058–2070.

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Flavonoid Profile of Green Asparagus Genotypes

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The determination of flavonoid profiles from different genotypes of *triguero* asparagus and their comparison to those from green asparagus commercial hybrids was the main goal of this study. The samples consisted of 32 commercial hybrids and 65 genotypes from the Huétor-Tájar population variety (*triguero*). The analysis of individual flavonoids by HPLC-DAD-MS has allowed the determination of eight naturally occurring flavonol derivatives in several genotypes of *triguero* asparagus. Those compounds included mono-, di-, and triglycosides of three flavonols, that is, quercetin, isorhamnetin, and kaempferol. The detailed analysis of the flavonoid profiles revealed significant differences among the distinct genotypes. These have been classified in three distinct groups as the result of a *k*-means clustering analysis, two of them containing both commercial hybrids and *triguero* asparagus and another cluster constituted by 21 genotypes of *triguero* asparagus, which contain several key flavonol derivatives able to differentiate them. Hence, the triglycosides tentatively identified as quercetin-3-rhamnosyl-rutinoside, isorhamnetin-3-rhamnosyl-rutinoside, and isorhamnetin-3-*O*-glucoside have been detected only in the genotypes grouped in the above-mentioned cluster. On the other hand, the compound tentatively identified as isorhamnetin-3-glucosyl-rutinoside was present in most genotypes of triguero *asparagus*, whereas it has not been detected in any of the commercial hybrids.

KEYWORDS: Asparagus; genotypes; cluster analysis; flavonoid glycosides; HPLC-DAD-MS

INTRODUCTION

Asparagus known as *triguero* are tetraploid subspecies from Huétor-Tájar, Granada, which proceed from wild species that have traditionally been collected and consumed throughout southern Spain. These green-purple asparagus are very appreciated for their organoleptic and nutritional properties, but their cultivation is being replaced by commercial hybrids, which yield more homogeneous production and spears of greater caliber.

Because health-promoting characteristics in food are increasingly demanded and included in the purchase decision by the discriminating consumer, the investigation of the bioactive compounds responsible of the beneficial effects associated with a specific product is of great interest for its revalorization.

Experimental evidence has demonstrated that each plant species is characterized by a limited number of phytochemicals; and within the same species, the nature of those compounds can vary from organ to organ but is constant enough toward several other factors (*1, 2*). Therefore, the characterization of fruits and vegetables can be made from their phytochemical profile, and it may be used, for example, to differentiate varieties of a plant food (*3–5*).

We have previously reported that there is high correlation between antioxidant capacity and total phenol content in *triguero* asparagus (*6*), which suggests that phenols could be mainly responsible for that activity as happens for other plant-derived products (*7–10*). It has also been established that flavonoids are the most abundant phenolics in green asparagus and that their profile is significantly different from that found in green spears from commercial hybrids (*11, 12*). The composition of flavonoids in plants is influenced by both genetic factors and environmental conditions. The former seem to be the most determinant factor because significant differences were found between *triguero* native spears and commercial hybrids both cultivated in Huétor-Tájar (*12*). The determination of the flavonoid profiles from different genotypes of *triguero* asparagus and their comparison to those from green asparagus hybrids developed in recent years by major international asparagus

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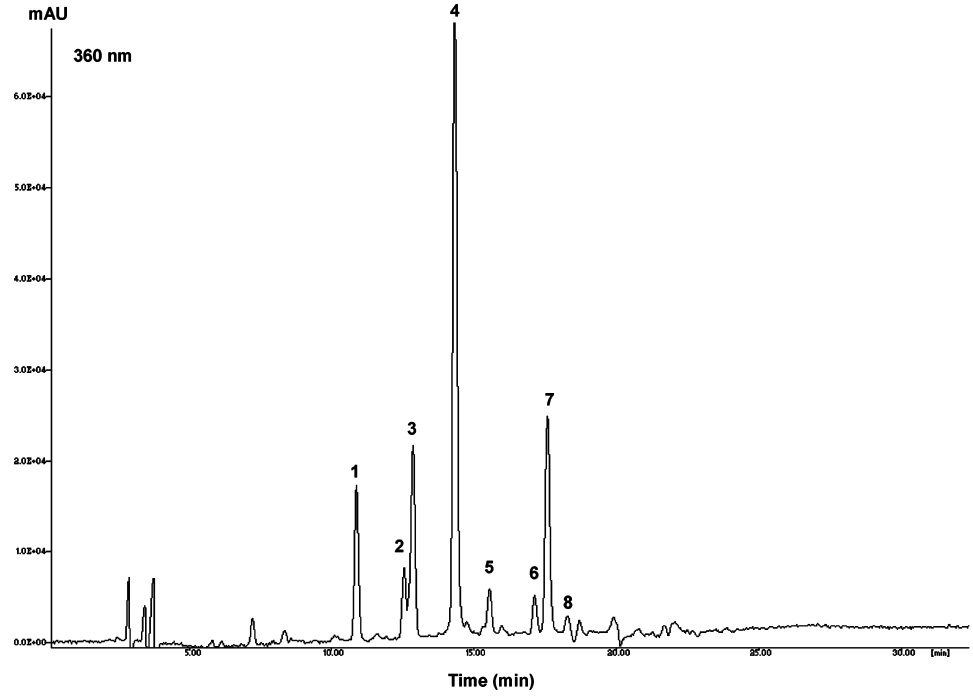

Figure 1. Chromatograpahic profile acquired by HPLC-DAD (360 nm) of a *triguero* asparagus ethanolic extract.

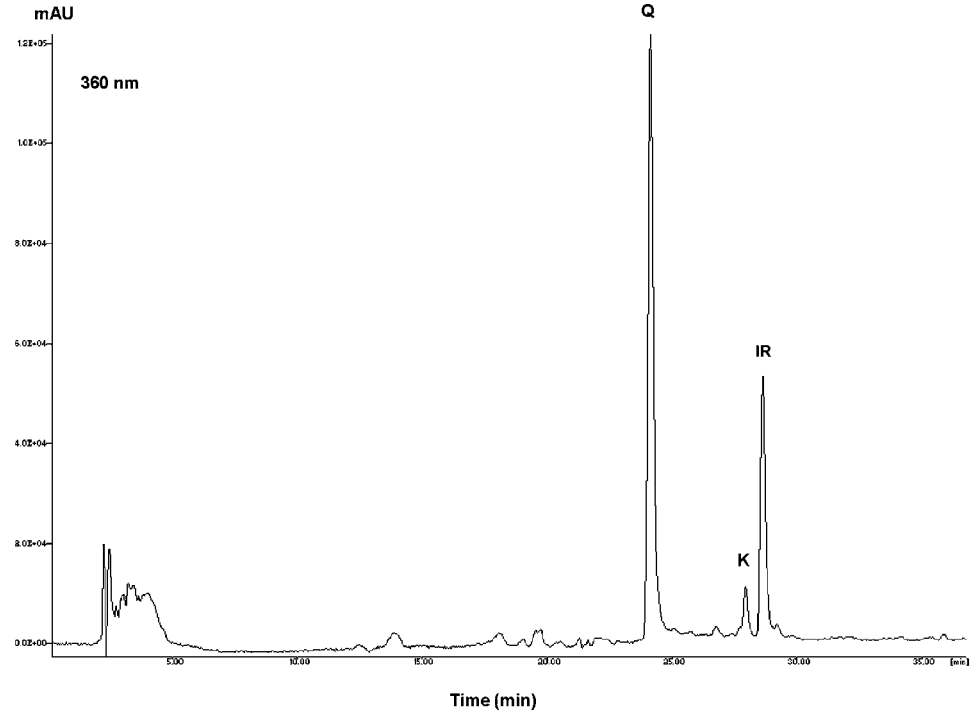

Figure 2. HPLC-DAD chromatogram at 370 nm of the hydrolysate extract of *triguero* asparagus.

programs was the main goal of this study. The chemical characterization of those native spears may allow new criteria to be established for selection and contribute to the promotion of the cultivation and consumption of a very high quality product that is still poorly known.

MATERIALS AND METHODS

Plant Material. The samples investigated consisted of spears from 32 green asparagus hybrids developed in recent years by major international asparagus programs and 65 different native lines of *triguero* asparagus from the Huétor-Tájar population variety. The first were cultivated in Las Torres Agricultural Research Center, Alcalá del Río, Sevilla, Spain, and the *triguero* asparagus samples were collected from Huétor-Tájar, Granada, Spain.

The asparagus samples were collected over a 2 year period and from experimental fields under controlled conditions. The spears were harvested at the same point of the harvest period (April−May 2005 and 2006), cut to the same length, and kept under refrigerated conditions ($T^a = 4$ °C) from the field to the laboratory to minimize the influence of environmental and storage conditions on the flavonoid profile.

Prior to being analyzed, the spears were washed with sodium hypochlorite solution (50 ppm of active Cl_2) and cut to a distance of 20 cm from the tip. Asparagus samples were weighed, frozen at −20 °C, and freeze-dried. This plant tissue was ground into a fine powder and stored at −20 °C for further analysis.

Table 1. Peak Numbers, Retention Times (t_R), Assigned Structures, UV, and Main Ion Species Observed during HPLC-DAD-MS Analysis of the Flavonoids from Green Asparagus

peak	t_R (min)	structure	UV (nm)	$[M - H]^-$ (*m*/*z*)	$[M - H]^+$ (*m*/*z*)	ions (ESI^+) (*m*/*z*)
1	10.9	Q-triglycoside	255, 267sh, 352	755	757	611, 465, 303
2	12.6	Q-triglycoside	256, 267sh, 354	771	773	627, 481, 303
3	12.9	IR-triglycoside	254, 268sh, 355	769	771	625, 479, 317
4	14.4	Q-3-*O*-rhamnoglucoside (rutin)	255, 267sh, 355	609	611	465, 303
5	15.5	IR-triglycoside	256, 268sh, 347	785	787	625, 463, 317
6	17.1	K-3-*O*-rhamnoglucoside (nicotiflorin)	264, 296sh, 348	593	595	449, 287
7	17.6	IR-3-*O*-rhamnoglucoside (narcissin)	252, 268sh, 356	623	625	479, 317
8	18.6	IR-3-*O*-glucoside	254, 268sh, 354	477	479	317

Table 2. Flavonoid Content in 32 Commercial Hybrids of Green Asparagus (Milligrams per Kilogram of Fresh Weight)[a]

	Q-triglyc	Q-triglyc	IR-triglyc	rutin	IR-triglyc	nicotiflorin	narcissin	IR-3-*O*-gluc	sum
Apollo	nd	104	nd	649	nd	tr	11	nd	763
Aragon 1978	nd	26	nd	233	nd	tr	tr	nd	259
Atlas	nd	49	nd	438	nd	7	tr	nd	494
Backlim	nd	31	nd	376	nd	tr	11	nd	418
Dulce Verde	nd	21	nd	260	nd	nd	31	nd	312
Ercole	nd	84	nd	484	nd	nd	tr	nd	568
Fileas	nd	54	nd	206	nd	tr	tr	nd	260
G Welph	nd	33	nd	328	nd	7	tr	nd	368
Grande	nd	8	nd	448	nd	8	tr	nd	465
G. Millenium	nd	55	nd	337	nd	tr	tr	nd	392
Italo	nd	79	nd	248	nd	tr	tr	nd	328
Jersey Deluxe	nd	76	nd	219	nd	tr	nd	nd	296
Jersey Giant	nd	118	nd	265	nd	8	tr	nd	391
Jersey King	nd	113	nd	345	nd	8	tr	nd	466
Jersey Night	nd	100	nd	338	nd	13	tr	nd	451
Jersey Supreme	nd	149	nd	399	nd	tr	tr	nd	547
JWC-1	nd	37	nd	544	nd	tr	tr	nd	581
NJ1016	nd	60	nd	324	nd	6	tr	nd	390
NJ953	nd	76	nd	337	nd	12	tr	nd	425
NJ956	nd	108	nd	421	nd	tr	tr	nd	529
NJ977	nd	60	nd	214	nd	tr	tr	nd	274
Pacific Purple	nd	10	nd	277	nd	tr	tr	nd	288
Purple Passion	nd	0	nd	477	nd	tr	tr	nd	477
Plaverd	nd	28	nd	407	nd	tr	12	nd	447
Rally	nd	55	nd	441	nd	7	12	nd	515
Ramada	nd	35	nd	342	nd	7	tr	nd	385
Rambo	nd	37	nd	331	nd	tr	tr	nd	369
Rapsody	nd	53	nd	706	nd	11	tr	nd	770
Ravel	nd	25	nd	273	nd	tr	tr	nd	297
Solar	nd	20	nd	542	nd	8	12	nd	582
UC115	nd	53	nd	299	nd	7	tr	nd	359
UC157	nd	44	nd	274	nd	tr	tr	nd	318

[a] Data are the mean of three replicates. Standard deviation was <5%. nd, not detected; tr, traces.

Chemicals and Reagents. Authentic standards of quercetin (Q), kaempferol (K), isorhamnetin (IR), and rutin (quercetin 3-*O*-rutinoside) were purchased from Sigma-Aldrich Quimica (Madrid, Spain); kaempferol-3-*O*-rutinoside (nicotiflorin), isorhamnetin 3-*O*-rutinoside (narcissin), and isorhamnetin 3-*O*-glucoside were purchased from Extrasynthese (Genay, France).

All solvents were of HPLC grade purity (Romyl, Teknokroma, Barcelona, Spain). All sample solutions were prepared using Milli-Q water.

Flavonoids Extraction. Phenolic compounds, mainly flavonoids, were extracted as described in Fuentes-Alventosa et al. (*12*). Each sample, consisting of 2.5 g of freeze-dried material, was extracted with 100 mL of 80% ethanol (EtOH). The samples were blended in a Sorvall Omnimixer, model 17106 (DuPont Co., Newtown, CT), at maximum speed for 1 min and then filtered through filter paper. Ethanolic extracts were stored at −20 °C until analysis by HPLC. All extractions were made in duplicate.

Acid Hydrolysis. The free flavonoid aglycones were released by acidic hydrolysis as follows: 2.5 g of freeze-dried material was extracted with 80 mL of 80% EtOH as described above. Twenty milliliters of 6 M HCl was added, and the solution was incubated for 2 h, with constant mixing, at 90 °C. The extract was filtered through filter paper and made up to 100 mL with 80% ethanol. The extracts were stored at −20 °C until analysis.

HPLC-DAD Analysis. Analyses of flavonoids were carried out using a Jasco-LC-Net II ADC liquid chromatograph system equipped with a diode array detector (DAD). Flavonoid compounds were separated by using a SYNERGI 4 μHYDRO-RP80A reverse phase column (25 cm × 4.6 mm i.d., 4 μm particle size; Phenomenex, Macclesfield, Cheshire, U.K.). The gradient profile for the separation of flavonoids was formed using solvent A [10% (v/v) aqueous acetonitrile plus 2 mL/L acetic acid] and solvent B (40% methanol, 40% acetonitrile, 20% water plus 2 mL/L acetic acid) in the following program: the proportion of B was increased from 10 to 42.5% B for the first 17 min, then to 70% B over the next 6 min, maintained at 70% B for 3.5 min, then to 100% B over the next 5 min, maintained at 100% B for 5 min, and finally returned to the initial conditions. The flow rate was 1 mL/min, and the column temperature was set at 30 °C. Spectra from all peaks were recorded in the 200−600 nm range, and the chromatograms were acquired at 360 nm for flavonoid glycosides and at 370 nm for their aglycones.

Table 3. Flavonoid Content in Spears from 65 Genotypes of *Triguero* Asparagus (Milligrams per Kilogram of Fresh Weight)

	Q-triglyc	Q-triglyc	IR-triglyc	rutin	IR-triglyc	nicotiflorin	narcisin	IR-3-*O*-gluc	sum
HT-1	nd	nd	nd	335	9	tr	65	nd	409
HT-2	nd	nd	nd	476	19	12	65	nd	572
HT-3	nd	53	nd	331	24	38	116	nd	562
HT-4	nd	25	nd	401	35	6	108	3	578
HT-5	nd	nd	nd	382	11	108	202	nd	703
HT-6	nd	47	nd	378	33	31	203	1	693
HT-7	nd	44	nd	497	21	34	162	nd	758
HT-8	nd	28	nd	542	nd	tr	16	nd	586
HT-9	nd	16	nd	233	nd	32	159	nd	440
HT-10	41	59	nd	408	23	43	94	2	670
HT-11	nd	nd	nd	702	11	tr	tr	nd	713
HT-12	nd	16	nd	478	5	nd	tr	nd	499
HT-13	nd	19	nd	437	nd	nd	tr	nd	456
HT-14	nd	42	nd	406	16	tr	tr	nd	464
HT-15	nd	11	nd	561	3	nd	tr	nd	575
HT-16	nd	33	nd	275	nd	nd	tr	nd	308
HT-17	nd	nd	nd	583	13	nd	tr	nd	596
HT-18	nd	42	nd	220	nd	nd	tr	nd	262
HT-19	nd	17	nd	610	72	12	tr	nd	711
HT-20	nd	76	nd	716	11	tr	tr	nd	803
HT-21	nd	nd	nd	443	nd	tr	tr	nd	443
HT-22	nd	63	nd	538	10	tr	tr	nd	611
HT-23	nd	18	nd	267	nd	tr	tr	nd	285
HT-24	nd	nd	nd	490	39	tr	13	nd	542
HT-25	65	20	24	195	18	9	16	nd	347
HT-26	nd	50	nd	410	13	tr	tr	nd	473
HT-27	nd	3	nd	73	18	10	34	nd	138
HT-28	37	32	50	366	60	tr	65	9	619
HT-29	nd	nd	nd	418	nd	tr	tr	nd	418
HT-30	nd	35	nd	384	10	nd	tr	nd	429
HT-31	nd	54	nd	478	12	tr	tr	nd	544
HT-32	nd	nd	nd	344	nd	tr	16	nd	360
HT-33	nd	57	nd	606	13	tr	tr	nd	676
HT-34	nd	3	nd	495	nd	nd	tr	nd	498
HT-35	nd	18	nd	485	nd	tr	tr	nd	503
HT-36	nd	nd	nd	462	18	tr	13	nd	493
HT-37	nd	35	nd	493	11	tr	tr	nd	539
HT-38	75	25	86	119	13	7	63	nd	388
HT-39	nd	36	nd	535	nd	6	tr	nd	577
HT-40	nd	nd	nd	717	14	tr	tr	nd	731
HT-41	nd	56	nd	390	nd	tr	nd	nd	446
HT-42	45	26	22	276	63	9	33	3	477
HT-43	nd	39	nd	458	68	18	32	nd	615
HT-44	nd	29	nd	416	42	8	26	nd	521
HT-45	43	nd	63	155	nd	14	29	nd	304
HT-46	nd	44	nd	518	nd	nd	nd	nd	562
HT-47	152	245	nd	202	20	13	116	3	751
HT-48	11	69	9	343	17	tr	14	nd	463
HT-49	nd	31	nd	328	nd	nd	nd	nd	359
HT-50	nd	23	nd	423	nd	tr	nd	nd	446
HT-51	nd	34	nd	432	8	tr	nd	nd	474
HT-52	nd	70	28	617	nd	tr	nd	nd	715
HT-53	nd	nd	nd	394	nd	tr	nd	nd	394
HT-54	80	75	35	100	21	tr	22	2	335
HT-55	nd	31	nd	377	15	tr	tr	nd	423
HT-56	38	21	24	272	39	6	30	nd	430
HT-57	36	34	nd	360	43	12	40	nd	525
HT-58	nd	96	nd	606	13	nd	tr	nd	715
HT-59	36	7	1	247	6	10	tr	nd	307
HT-60	63	82	95	371	60	9	120	4	804
HT-61	nd	86	nd	517	14	nd	nd	nd	617
HT-62	nd	55	nd	424	8	tr	tr	nd	487
HT-63	40	37	nd	172	5	8	42	5	309
HT-64	79	30	nd	310	54	tr	39	nd	512
HT-65	66	28	nd	303	47	tr	36	nd	480

Isolation of the New Flavonoids Identified in Green Asparagus. A HPLC method similar to that described above, but using a semipreparative SYNERGI 4 HYDRO-RP80A reverse phase column (25 cm × 46 mm i.d., 4 μm; Phenomenex), was developed for the isolation of the new flavonoids. The flow rate was maintained at 10 mL/min, and the injection volume was 400 μL. Elution was monitored by UV at 360 nm, and the flavonoids were manually collected after the UV detector. The four fractions containing each individual compound were then re-injected onto the analytical column to purify the four isolated flavonoids. Those were concentrated under nitrogen prior to lyophilization.

Characterization of Flavonoids by HPLC-DAD-MS. Flavonoid glycosides detected in green asparagus were separated by HPLC as described above and identified by their electron impact mass data

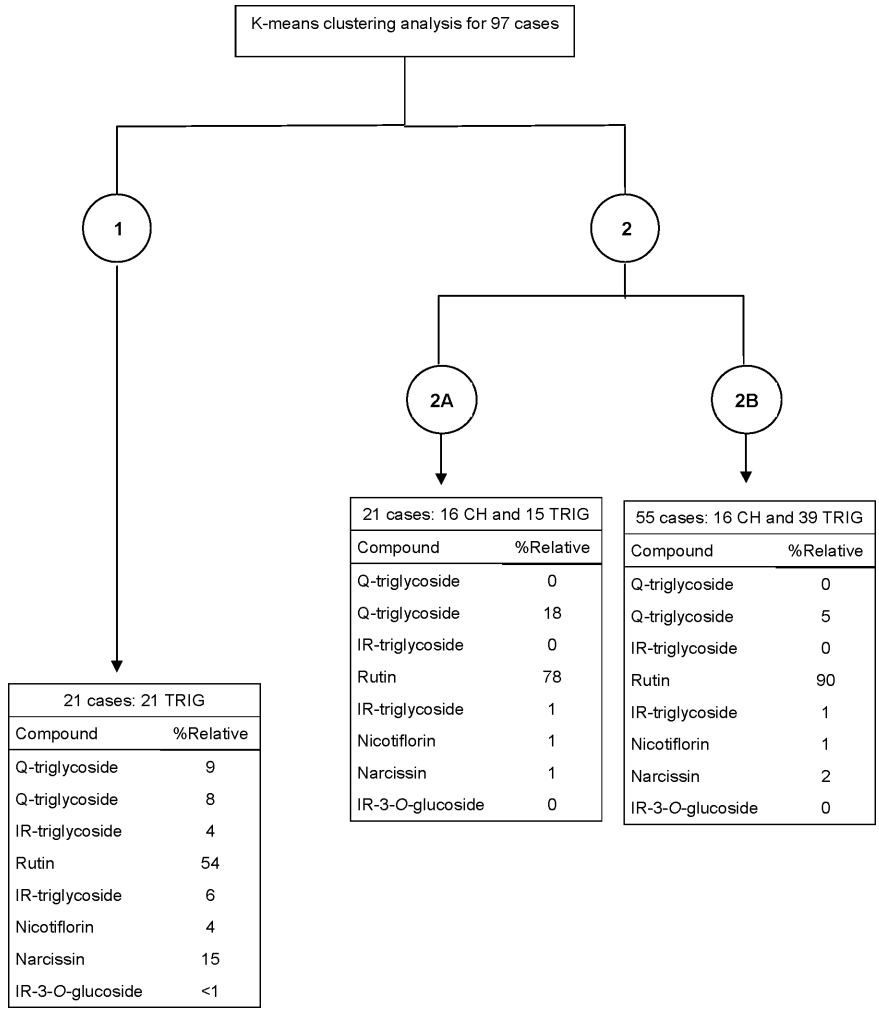

Figure 3. Classification of genotypes of green asparagus in three clusters obtained by the application of a *k*-means clustering analysis.

collected on a quadrupole mass analyzer (ZMD4, Micromass, Waters Inc., Manchester, U.K.). Electrospray ionization (ESI) mass spectra were obtained at ionization energies of 50 and 100 eV (negative mode) and 50 eV (positive mode), with MS scans from *m*/*z* 100 to 1000. Capillary voltage was 3 kV, desolvation temperature was 200 °C, source temperature was 100 °C, and extractor voltage was 12 V. The flow was maintained at 1 mL min^{-1}.

Identification and Quantification of Individual Flavonoids. Quantitative evaluation of flavonoid content was carried out as described by Fuentes-Alventosa et al. (*12*). Identification of individual flavonoid glycosides was carried out using their retention times and both spectroscopic and mass spectrometric data. Quantification of individual flavonoid monoglycosides and flavonoid diglycosides was directly performed by HPLC-DAD using an eight-point regression curve in the range of 0−250 μg on the basis of standards. When standards were not available, as in the case of the new flavonid triglycosides described in the present work, quantification was based on an average value for that class of compound, because responses were essentially similar within classes. Results were calculated from the mean of three replicates. Comparisons among samples were done by the ANOVA test and the LSD method at 95% confidence level.

RESULTS AND DISCUSSION

Determination of Flavonoids from Green Asparagus. The separation of flavonol glycosides in a *triguero* asparagus ethanolic extract is presented in **Figure 1**. As can be seen, the analytical method allowed the determination of eight flavonol glycosides. From HPLC-DAD data, all flavonoids are glycosylated derivatives of three flavonols, that is, quercetin (252, 267sh, 372), kaempferol (264, 296sh, 364), and isorhamnetin (252, 268sh, 368). No free aglycones were detected in the ethanolic extracts from triguero asparagus, but acid hydrolysis of the samples confirmed that *triguero* asparagus flavonoids are derivatives of three different aglycones, quercetin (Q) being the major flavonol, followed by isorhamnetin (IR) and kaempferol (K) (**Figure 2**). The identities of these aglycones were confirmed by the comparison of the HPLC-DAD-MS data of the hydrolysate to those of commercial standards.

Peaks 4, 6, 7, and 8 were respectively identified as rutin (quercetin-3-*O*-rutinoside), nicotiflorin (kaempferol-3-*O*-rutinoside), narcissin (isorhamnetin-3-*O*-rutinoside), and isorhamnetin-3-*O*-glucoside, on the basis of their spectral characteristics and comparison to standards. The above three flavonol diglycosides had been previously described in *triguero* asparagus (*12*), and the isorhamnetin-3-*O*-glucoside is the first flavonol monoglycoside detected in green asparagus. This compound has been described in other plant tissues, such as grapes (*13*), calendula (*14*), and turnip tops (*15*).

The four flavonol triglycosides, which had not been previously described in asparagus, were tentatively identified by means of a combination of the retention times (t_R), UV, and mass spectra obtained by HPLC-DAD-MS. Peaks 1 and 2 were tentatively identified as quercetin derivatives and peaks 3 and 5 as isorhamnetin derivatives.

As the UV spectra from all of the flavonol glycosides are very similar, they were not very useful for identification. MS fragmentation patterns of these compounds were used to obtain more information about their molecular masses and structural

characteristics. **Table 1** shows the values obtained for each of the detected compounds (numbered from 1 to 8 following their retention times).

The MS negative ion mode spectrum of compound **1** showed a deprotonated molecular ion at *m/z* 755 and an ion at *m/z* 301 corresponding to the deprotonated aglycone, which showed that this compound is a triglycosylated quercetin. It is well established that most flavonol derivatives from plant tissues are 3-*O*-glycosylated and/or 7-*O*-glycosylated flavonoids and that they are usually linked to glucose and rhamnose residues (*16–18*). The fact that the deprotonated ion from the aglycone was the base peak is indicative that the sugars are linked only at the 3 position (*16*). The positive ion spectrum of flavonoid glycosides provided additional information about the sugars linked to the flavonol structures. The ESI spectra of the $[M + H]^+$ ion of compound **1** at *m/z* 757 showed two main product ions corresponding to three successive losses of sugar residues. The first loss corresponded to a rhamnose residue (146 u), yielding the major ion at *m/z* 611. Ion *m/z* 611 then decomposed into another prominent ion at *m/z* 465, resulting from the loss of a second unit of rhamnose (146 u). Finally, the loss of a glucose residue (162 u) generated the major ion at *m/z* 303, corresponding to the protonated aglycone (quercetin). This fragmentation pattern could be compatible with a quercetin-3-rhamnosyl-rutinoside.

The MH^- spectrum of compound **2** showed a deprotonated molecular ion at *m/z* 771 and an ion at *m/z* 301 corresponding to the deprotonated aglycone, which showed that this is another quercetin triglycoside. Data from the positive ion spectrum suggested that this flavonoid derivative contained a rhamnose (146 u), the loss of which gave rise to the ion at *m/z* 627, and two residues of glucose (−324 u), the loss of which yielded a characteristic fragment ion of the protonated aglycone (quercetin) at *m/z* 303. These results suggest that compound **2** is a quercetin-3-glucosyl-rutinoside.

The MH^- spectrum of compound **3** gave a deprotonated molecular ion at *m/z* 769 and an ion at *m/z* 315 corresponding to the deprotonated aglycone (isorhamnetin). Complementary information from the positive ion spectrum revealed that the fragmentation pattern to this flavonoid derivative was similar to that from compound **1**, but the aglycone was isorhamnetin instead of quercetin. Compound **3** was tentatively identified as isorhamnetin-3-rhamnosyl-rutinoside.

The MH^- spectrum of compound **5** showed a deprotonated molecular ion at *m/z* 785 and an ion at *m/z* 315 corresponding to a deprotanated aglycone. On the other hand, according to the MH^+ data, this flavonoid followed the same fragmentation pattern explained above for compound **2**, but the aglycone is isorhamnetin instead of quercetin. It can be proposed that compound **5** is an isorhamnetin triglycoside with two glucoses and a rhamnose residue linked to the flavonol. This compound could be isorhamnetin 3-glucosyl-rutinoside.

On the other hand, it has been reported that the chromatographic behavior of flavonoid compounds under reversed-phase HPLC shows that, in the same conditions, a higher degree of glycosylation leads to a shorter retention time (*19*). Our results are in consonance with these findings, because peaks 1 (t = 10.9 min) and 2 (t_R = 12.6 min), which have been tentatively identified as quercetin triglycosides, elute earlier than rutin (t_R = 14.4 min). A similar behavior has been observed for the isorhamnetin derivatives. Therefore, peaks 3 (t_R = 12.9 min) and 5 (t_R = 15.5 min), tentatively identified as isorhamnetin-triglycosides, elute earlier than peak 7 (t_R = 17.6 min), which has been identified as isorhamnetin-rutinoside. Finally, the unique flavonoid monoglycoside detected in asparagus samples, identified as isorhamnetin-glucoside, was the last compound eluted from the column (t_R = 18.6 min).

The structures of the four new flavonol triglycosides described in this study were tentatively assigned on the basis of t_R, UV, and MS data, but further data are needed for a complete structural identification. Thus, the above four flavonoid triglycosides have been isolated and purified, and their analysis by RMN techniques will make it possible to establish the precise position of the sugars within the flavonoid molecule.

Quantitative Analysis of Flavonoids from Several Asparagus Cultivars. Flavonoid contents of the 32 commercial hybrids of green asparagus are shown in **Table 2**. The flavonoid content of these varieties varied between 259 and 763 mg/kg of fresh weight. Rutin was the main flavonoid glycoside, and its value was ≥70% of the total flavonoid complement in all of the samples investigated. Compound **2**, which has been tentatively identified as quercetin 3-*O*-glucosyl-rutinoside, was quantified in 31 of the 32 genotypes studied, but its content varied between 2 and 30% among the different hybrids of green asparagus. Significant quantities of nicotiflorin and narcissin were detected in most of the 32 genotypes of commercial hybrids, but these flavonoid diglycosides represented only 1−3% of the total flavonoid content, with the exception of the variety called Dulce Verde, which contained 10% of narcissin.

The detailed composition of flavonoids from 65 genotypes of *triguero* asparagus is shown in **Table 3**. Total flavonoid average content was 519 mg/kg of fresh weight, and rutin represented 78% of that quantity, which is comparable to the values calculated from commercial hybrids. However, significant differences were found among the flavonoid compositions from the 65 genotypes of *triguero* asparagus.

The diverse genotypes of asparagus investigated in the present work have been classified in three distinct groups as the result of a *k*-means clustering analysis. This statistical test produces exactly *k* different clusters of greatest possible distinction. The total content of flavonoids and the relative percent of each of the eight individual flavonoids identified in green asparagus have been used as factors of classification. The distribution of the 97 genotypes of green asparagus in three clusters and the average composition of each group are shown in **Figure 3**.

In the first dimension, samples were divided in two clusters (1 and 2), with very different means. It is noteworthy that all 21 genotypes within the first group came from *triguero* asparagus, whereas the second group included both commercial hybrids and *triguero* genotypes. In the second dimension, the second group was divided in two clusters (2A and 2B) constituted by mixtures of commercial hybrids and *triguero* genotypes. Despite all of the samples within these two clusters containing a high percent of rutin, which represented up to 70% in all cases, significant differences were found between the two groups.

Cluster 1 included only genotypes from *triguero* asparagus, the flavonoid composition of which is significantly different from those samples within the other two clusters. These genotypes contained the greatest variety of flavonoid compounds. Rutin content was about 50% of the total flavonoids, and this was accompanied by up to seven more flavonoids, including monoglycosides (trace amounts), diglycosides, and triglycosides. The average composition for this group consisted of 54% rutin and significant quantities of other seven flavonoids, each of which represented 1−15% of total flavonoid content.

Cluster 2A comprised 16 commercial hybrids and 5 genotypes of *triguero* asparagus. These genotypes had an average com-

position consisting of 78% of rutin, which was accompanied by a significant quantity of compound **2**, identified as quercetin-3-glucosyl-rutinoside, and minor quantities of other flavonoid di- and triglycosides. Cluster 2B was the greatest group, containing a mixture of 55 samples, 16 from commercial hybrids and 39 from *triguero* asparagus. The genotypes within this group contained almost solely rutin, which represented about 90% of the total flavonoids, accompanied by only small quantities of two or three more flavonoid glycosides.

From these results, it can be concluded that the flavonoid composition of *triguero* asparagus population is similar to that of commercial hybrids. This can be explained by the fact that several American hybrids of green asparagus have been included in the cultivation areas of *triguero* asparagus during recent years, which can influence and vary the original characteristics of the native varieties. However, the analysis of each of the 65 different genotypes of *triguero* asparagus revealed that several of them (the 21 genotypes classified within cluster 1) possessed a flavonoid composition very distinct and statistically different from the majority of the samples, grouped in the two other clusters. That subgroup of *triguero* asparagus contained several key flavonol derivatives able to differentiate those 21 genotypes from the rest, including both commercial hybrids and *triguero* asparagus. Therefore, compounds **1**, **3**, and **8**, which have been tentatively identified as quercetin-3-rhamnosyl-rutinoside, isorhamnetin-3-rhamnosyl-rutinoside, and isorhamnetin-3-*O*-glucoside, respectively, have been detected only in the genotypes grouped in cluster 1. On the other hand, compound **5**, tentatively identified as isorhamnetin-3-glucosyl-rutinoside, is present in a greater number of genotypes of *triguero* asparagus but has not been detected in any of the commercial hybrids investigated in this study. This flavonol triglycoside is one of the minor flavonoid components, as can be observed in **Table 3** and **Figure 3**. However, the fact that it is not present in any of the commercial hybrids (**Table 2**) suggests that this isorhamnetin triglycoside is key for distinguishing the *triguero* asparagus population from other green asparagus cultivars.

It is well established that the flavonoid profile of vegetables is influenced by genetic and environmental factors (*20–24*), and we have previously reported that the first determine to a greater extension both the phenolic composition and the antioxidant activity of green asparagus (*6*). On the other hand, it has been reported that the antioxidant activity of flavonol glycosides is greatly modified by the position of the sugar group attached to the basic diphenylpropane structure (*25*). Thus, Yamamoto et al. (*26*) demonstrated that Q40G, which has no catechol group, is greatly inferior to catechol-containing Q3G in preventing lipid peroxidation in human low-density lipoprotein (LDL). From the results of the present work it can be concluded that there are several flavonol glycosides, specific to *triguero* asparagus, which could be an alternative factor determining the bioactive properties of this product. Further investigations are required to determine if the characteristic flavonoid composition of *triguero* asparagus, reported in this work, is related to specific functional properties that may distinguish this product from other green asparagus.

LITERATURE CITED

(1) Proestos, C.; Boziaris, I. S.; Nychas, G.-J. E.; Komaitis, M. Analysis of flavonoids and phenolic acids in Greek aromatic plants: investigation of their antioxidant capacity and antimicrobial activity. *Food Chem.* **2006**, *95*, 664–671.

(2) Lako, J.; Trenerry, V. C.; Wahlqvist, M.; Wattanapenpaiboon, N.; Sotheeswaran, S.; Premier, R. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chem.* **2007**, *10*, 1727–1741.

(3) Martínez-Sánchez, A.; Llorach, A.; Gil, M. I.; Ferreres, F. Identification of new flavonoid glycosides and flavonoid profiles to characterize rocket leafy salads (*Eruca vesicaria* and *Diplotaxis tenuifolia*). *J. Agric. Food Chem.* **2007**, *55*, 1356–1363.

(4) Mertz, C.; Cheynier, V.; Günata, Z.; Brat, P. Analysis of phenolic compounds in two blackberry species (*Rubus glaucus* and *Rubus adenotrichus*) by high-performance liquid chromatography with diode array detection and electrospray ion trap mass spectrometry. *J. Agric. Food Chem.* **2007**, *55*, 8616–8624.

(5) Slimestad, R.; Fosen, T.; Vågen, I. M. Onions: as source of unique dietary flavonoids. *J. Agric. Food Chem.* **2007**, *55*, 10067–10080.

(6) Rodríguez, R.; Jaramillo, S.; Rodríguez, G.; Espejo, J. A.; Guillén, R.; Fernández-Bolaños, J.; Heredia, A.; Jiménez, A. Antioxidant activity of ethanolic extracts from several asparagus cultivars. *J. Agric. Food Chem.* **2005**, *53*, 5212–5217.

(7) Gardner, P. T.; White, T. A. C.; McPhail, D. B.; Duthie, G. G. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chem.* **2000**, *68*, 471–474.

(8) Gil, M. I.; Tomás-Barberán, F. A.; Hess-Pierce, B.; Holcroft, D. M.; Kader, A. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J. Agric. Food Chem.* **2000**, *48*, 4581–4589.

(9) Wang, S. Y.; Lin, H.-S. Antioxidant activitiy in fruits and leaves of blackberry, raspberry and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem.* **2000**, *48*, 140–146.

(10) Gorinstein, S.; Zachwieja, Z.; Katrich, E.; Pawelzik, E.; Haruenkit, R.; Trakhtenberg, S.; Martin-Belloso, O. Comparison of the contents of the main antioxidant compounds and the antioxidant activity of white grapefruit and its new hybrid. *Lebensm.-Wiss.-Technol.* **2004**, *37*, 337–343.

(11) Guillén, R.; Rodríguez, R.; Jaramillo, S.; Rodríguez, G.; Espejo, J. A.; Fernández-Bolaños, J.; Heredia, A; Jiménez, A. Antioxidants from asparagus spears: flavonoids. *Acta Hortic.* **2008**, *776*, 247–254.

(12) Fuentes-Alventosa, J. M.; Rodríguez, G.; Cermeño, P.; Jiménez, A.; Guillén, R.; Fernández-Bolaños, J.; Rodríguez-Arcos, R. Identification of flavonoid diglycosides in several genotypes of asparagus from the Huétor-Tájar population variety. *J. Agric. Food Chem.* **2007**, *55*, 10028–10035.

(13) Dopico-García, M. S.; Valentão, P.; Guerra, L.; Andrade, P. B.; Seabra, R. M. Experimental design for extraction and quantification of phenolic compounds and organic acids in white "Vinho Verde" grapes. *Anal. Chim. Acta* **2007**, *583*, 15–22.

(14) Matysik, G.; Wójciak-Koiseor, M.; Paduch, R. The influence of *Calendulae officinalis flos* extracts on cell cultures, and the chromatographic analysis of extracts. *J. Pharm. Biomed. Anal.* **2005**, *38*, 285–292.

(15) Romani, A.; Vignolini, P.; Isolani, L.; Ieri, F.; Heimler, D. HPLC-DAD/MS characterization of flavonoids and hydroxycinnamic derivatives in turnip tops (*Brassica rapa* L. subsp. *sylvestris* L.). *J. Agric. Food Chem.* **2006**, *54*, 1342–1346.

(16) Ferreres, F.; Llorach, R; Gil-Izquierdo, A. Characterization of the interglycosidic linkage in di-, tri-, tetra- and pentaglycosylated flavonoids and differentiation of positional isomers by liquid chromatography/electrospray ionization tandem mass spectrometry. *J. Mass Spectrom.* **2004**, *39*, 312–321.

(17) Hvattum, E.; Ekeberg, D. Study of the collision-induced radical cleavage of flavonoid glycosides using negative electrospray ionization tandem quadrupole mass spectrometry. *J. Mass Spectrom.* **2003**, *38*, 43–49.

(18) Ablajan, K.; Abliz, Z.; Shang, X.-Y.; He, J.-M.; Zhang, R.-P.; Shi, J.-G. Structural characterization of flavonol 3,7-di-*O*-glycosides and determination of the glycosylation position by using negative ion electrospray ionization tandem mass spectrometry. *J. Mass Spectrom.* **2006**, *41*, 352–360.

(19) Llorach, R.; Gil-Izquierdo, A.; Ferreres, F.; Tomás-Barberán, F. A. HPLC-DAD-MS/MS ESI characterization of inusual highly glycosylated acylated flavonoids from cauliflower (*Brassica oleracea* L. var. *botrytis*) agroindustrial byproducts. *J. Agric. Food Chem.* **2003**, *51*, 3895–3899.

(20) Martínez-Sánchez, A.; Gil-Izquierdo, A.; Gil, M. I.; Ferreres, F. A comparative study of flavonoid compounds, vitamin C, and antioxidant properties of baby leaf Brassicaceae species. *J. Agric. Food Chem.* 2008*56*, 2330–2340.

(21) Lachman, J.; Pronek, D.; Hejtmánková, A.; Dudjak, J.; Pivec, V.; Faitová, K. Total poyphenol and main flavonoid antioxidants in different onion (*Allim cepa* L.) varieties. *Hortic. Sci.* **2003**, *30*, 142–147.

(22) Kim, D.-O.; Padilla-Zakour, O. I.; Griffiths, P. D. Flavonoids and antioxidant capacity of various cabbage genotypes at juvenile stage. *J. Food Sci.* **2004**, *69*, 685–689.

(23) Caridi, D.; Trenerry, V. C.; Rochfort, S.; Duong, S.; Laugher, D.; Jones, R. Profiling and quantifying quercetin glucosides in onion (*Allium cepa* L.) varieties using capillary zone electrophoresis and high performance liquid chromatography. *Food Chem.* **2007**, *105*, 691–699.

(24) Harbaum, B.; Hubbermann, E. M.; Wolff, C.; Herges, R.; Zhu, Z.; Schwarz, K. Identification of flavonoids and hydroxycinnamic acids in pak choi varieties (*Brassica campestris* L. ssp. *chichensis* var. *communis*) by HPLC-ESI-MS and NMR and their quantification by HPLC-DAD. *J. Agric. Food Chem.* **2007**, *55*, 8251–8260.

(25) Murota, K.; Tera, J. Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. *Arch. Biochem. Biophys.* **2003**, *417*, 12–17.

(26) Yamamoto, N.; Moon, J.-H.; Tsushida, T.; Nagao, A.; Terao, J. Inhibitory effect of quercetin metabolites and their related derivatives on copper ion-induced lipid peroxidation in human low-density lipoprotein. *Arch. Biochem. Biophys.* **1999**, *372*, 347–354.

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Analytical Methods

Effect of extraction method on chemical composition and functional characteristics of high dietary fibre powders obtained from asparagus by-products

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ABSTRACT

Asparagus (*Asparagus officinalis L.*) by-products, which represent around 50% of the processed vegetable, are a potential source of dietary fibre. The way that these by-products are treated affects the composition and functional properties of fibre-rich powders. Factors such as treatment intensity, solvent, and drying system were studied. Only the more soluble components (soluble sugars, uronic acids and proteins) showed significant differences. All the fibre-rich powders had high concentrations of TDF (62–77%). The IF/SF proportion decreased with the severity of treatment, in this way increasing the physiological quality of the fibre. Functional properties, namely water-holding capacity (WHC), oil-holding capacity (OHC), solubility (SOL), and glucose dialysis retardation index (GDRI), varied according to the preparation procedure. WHC and GDRI were higher in intensely extracted fibres; due to the effect of thermal processing. WHC showed values (11–20 ml water/g powder) similar to those described for other agricultural by-products, but OHC and GDRI were much higher (5–8 ml oil/g powder and 25–45%, respectively). These properties make fibre-rich powders from asparagus by-products a valuable source of dietary fibre to be included in the formulation of fibre-enriched foods.

1. Introduction

In developed countries, several gastrointestinal disorders (duodenal ulcer, appendicitis, constipation, hemorrhoids, colon carcinoma), diabetes mellitus, obesity, and cardiovascular diseases, have very low incidence among people consuming high amounts of fibre. Fibre benefits for the gut are widely recognized a fact that has led to its consideration as a nutrient (FDA & Human Services, 1993). The key to obtaining the level of fibre intake recommended for adults in western societies is the availability of high quality foods with a high dietary fibre content. The usual sources of dietary fibre added to commercial foods (juices, dairy products, baked goods) are cereals. Currently, however, the demand for by-products from fruits and vegetables as sources of dietary fibre has been increasing, because these sources present higher nutritional quality, higher amounts of total and soluble fibre, lower caloric content, stronger antioxidant capacity, and higher levels of fermentability and water retention (Rodríguez, Jiménez, Fernandez-Bolaños, Guillén, & Heredia, 2006).

Among the vegetables commonly consumed in the United States and Europe, asparagus has been reported as the richest in the total quality and quantity of its antioxidants (Pellegrini et al., 2003; Vinson, Hao, Su, & Zubik, 1998). Of asparagus-producing countries, Spain is in the fifth position after China, Peru, the U.S.A., and Germany. In 2005, about 50,000 tons (including both green and white asparagus) were harvested in Spain, with more than 50% coming from Andalusia (mainly green). During industrial processing, around half of the total length of each spear is discarded, which creates significant waste for producers. Assuming that the by-products have similar composition to the edible part of the spears, these represent a promising source of new value-added compounds (phytochemicals and fibre) (Nindo, Sun, Wang, Tang, & Powers, 2003). However, processing and drying may cause irreversible modifications to the fibre, affecting its original structure. Therefore, its final quality would be determined by the structural and compositional modifications that might have occurred during processing (Garau, Simal, Rosselló, & Femenia, 2007).

The objective of this study is to prepare various fibre-rich powders from asparagus by-products, using different methods, and to evaluate their chemical composition and several functional properties by using *in vitro* tests.

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2. Materials and methods

2.1. Asparagus by-products

Asparagus by-products were obtained from Centro Sur S.C.A. (Huétor–Tajar, Granada, Spain). Prior to canning, freshly harvested asparagus spears were cut to obtain the 15 cm length upper portion (edible part) and the rest of spear (15–18 cm) was considered a by-product. This by-product was sent to our laboratory within 24 h and held at 4 °C prior to its processing.

2.2. Asparagus by-product treatment

Three variables were studied (Table 1): extraction treatment (intense – 90 min at 60 °C, or gentle – 1 min at room temperature-), extraction solvent (water or 96% ethanol), and drying system (freeze-drying or oven treatment at 60 °C for 16 h). Two kg by-product portions were cut, homogenized and mixed using a professional homogeniser (Sirman Orione, Marsango, Italy) at top speed with each of the extraction solvents in a ratio of 1:1, solid:liquid (w/v), and at the programmed temperature (60 °C or room temperature) for 1 min. Afterwards, gently extracted samples (G) were directly processed using an industrial juicer (Tecno–Chufa, Valencia, Spain) to separate an asparagus liquid extract and a wet fibrous residue. Intensely extracted samples (I) were treated in an open reactor, designed in our laboratory, with time and temperature controls and continuous stirring. After 90 min. of extraction, samples were centrifuged as described above. Each treatment was carried out in duplicate. One batch of wet fibrous residue was freeze-dried (F-D) and the other dried in an oven (O-D). Dried fibres were ground in a hammer mill to a particle size lower than 1 mm and stored at 4 °C prior to analysis.

2.3. Proximate composition of fibres

Amounts of neutral sugars (NS), uronic acids (UA), proteins (P), and Klason lignin (KL) were determined as described previously (Fernandez-Bolaños et al., 2004). For neutral sugars, sugar-free fibres (treated with 80% ethanol) were hydrolysed with trifluoroacetic acid (TFA) at 121 °C for 1 h (Ruiter & Burns, 1987) and the released sugars were quantified as alditol acetates by gas chromatography (Englyst & Cumming, 1984); uronic acids were quantified using the phenyl-phenol method after sulphuric acid hydrolysis (Ahmed & Labavitch, 1977); proteins were analysed by the Kjeldahl method, using a Büchi Digestion Unit, K-424, and a Büchi Distillation Unit, K-314, applying the factor 6.25 to convert total nitrogen into protein content; *Klason lignin* levels were determined gravimetrically as the amount of acid-insoluble material remaining after a two-stage sulphuric acid hydrolysis.

Cellulose (CEL) was quantified from the TFA-insoluble residue after 72% sulphuric acid hydrolysis, and soluble sugars (SS) from the ethanol-soluble extract of fibres, both by the anthrone method (Dische, 1962).

Table 1
Different conditions for obtaining a fibre-rich powder from asparagus by-product

	Description
I/W O-D	Intense treatment (90 min at 60 °C) with water and oven-drying
I/W F-D	Intense treatment (90 min at 60 °C) with water and freeze-drying
I/E O-D	Intense treatment (90 min at 60 °C) with ethanol and oven-drying
I/E F-D	Intense treatment (90 min at 60 °C) with ethanol and freeze-drying
G/W O-D	Gentle treatment (1 min at room temperature) with water and oven-drying
G/W F-D	Gentle treatment (1 min at room temperature) with water and freeze-drying
G/E O-D	Gentle treatment (1 min at room temperature) with ethanol and oven-drying
G/E F-D	Gentle treatment (1 min at room temperature) with ethanol and freeze-drying

2.4. Dietary fibre content

The amount of total dietary fibre (TDF) was determined using the protocol described by Lee, Prosky, and De Vries (1992), with slight modifications. After digestion, insoluble fibre (IF) was recovered by filtration on a sintered glass crucible (no. 2), washed with distilled water and dried overnight at 100 ° C (ash and protein were corrected during this step). Four volumes of hot ethanol were added to filtrates and the suspensions of soluble fibre (SF) were left overnight at 4 °C to allow SF to precipitate. Afterwards, SF was recovered by centrifugation for 20 min at 2500 g, dissolved in distilled water, and freeze-dried.

2.5. Functional properties

2.5.1. Water- and oil-holding capacities and solubility

Water-holding capacity (WHC) was determined by centrifugation as described elsewhere (Jiménez et al., 2000). Samples (250 mg ×3) were suspended in 15 ml of water. After 24 h of stirring at room temperature, the suspension was centrifuged at 14000 g for 1 h. Supernatants were carefully discarded and the hydrated fibres were weighed. WHC was expressed as ml of water/g fibrous residue. Hydrated pellets were freeze-dried and solubility in water (SOL) was the weight difference between before and after the WHC assay, expressed as a percent. Oil-holding capacity (OHC) was determined under the same conditions as WHC using sunflower oil (1.0054 g/ml density), and was expressed as ml oil/g of fibrous residue.

2.5.2. Glucose dialysis retardation index (GDRI)

GDRI was determined as described by Lecumberri et al. (2007), with slight modifications. Samples of 400 mg sugar-free fibres (fibres extracted twice with 80% ethanol) were thoroughly hydrated with 15 ml of distilled water containing 30 mg glucose. After 1 h of continuous stirring, samples were transferred to 15 cm portions of previously hydrated dialysis bags (12,000 MWCO, Sigma Chemical Co.). Each bag and a control bag (with glucose, but without fibre) were put into a reservoir containing 400 ml of distilled water and held in a thermostatic water bath at 37 °C for 1 h with constant shaking. At 10 min intervals, 0.5 ml of dialysate was collected and the glucose concentration was determined spectrophotometrically by the anthrone method (Dische, 1962). GDRI was calculated as follows:

$$\mathrm{GDRI} = 100 - \left(\frac{\text{Total glucose diffused, sample}}{\text{Total glucose diffused, control}} \times 100\right) \quad (1)$$

2.6. Statistical analysis

Results were expressed as mean values ± standard deviations. To assess for differences in the composition and functional characteristics between the different treatments, a multiple sample comparison was performed using the Statgraphics Plus program version 2.1. Multivariate analysis of variance (ANOVA), followed by Duncan's multiple comparison test, was performed to contrast the groups. The level of significance used was $P < 0.05$.

3. Results and discussion

3.1. General

Asparagus by-products could be considered an interesting source of dietary fibre. By-product processing involves several fac-

tors that must be controlled in order to have a dietary fibre with optimised composition and functional characteristics. In this study, three factors have been taken into account (severity of treatment, solvent and drying system) and their effects on fibre characteristics will be considered.

3.2. Proximate composition

The composition of major components is presented in Table 2. SS and UA, the more soluble components of cell wall, varied the most – from 7–15% to 9–17%, respectively. In the case of UA, fibres from treatments with water were poorer in UA than those from treatments with ethanol. In ethanol-extracted fibres, the choice of drying system resulted in significant differences; freeze-dried fibres had higher UA levels than had oven-dried ones. In UA quantification, the three factors considered (severity of treatment, solvent and drying system) were statistically significant.

NS showed little variation among samples. Neither the drying system nor the solvent affected NS content, with only the intensity of treatment leading to significant differences. In fibres that were subjected to intense treatment, NS content was higher than in those with gentle treatment. Hemicelluloses, cell wall polysaccharides mainly composed of NS, are insoluble in both solvents, water and ethanol. Thus, during by-product processing, other components were preferentially solubilized and the resultant fibre was enriched in hemicelluloses. Considering the percentage of individual sugars (rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose) in the total content of NS (data not shown), it was remarkable that the compositions of the fibres from different treatments were very similar: xylose accounted for more than 50% of sugar content, while galactose and arabinose each contributed 10–20%. These results are in concordance with those previously reported for white and green asparagus (Rodríguez, Jiménez, Guillén, Heredia, & Fernandez-Bolaños, 1999a, 1999b; Waldron & Selvendran, 1992). In white spears, xylose accounted for more than 40% of all sugars while glucose, galactose and arabinose each accounted for 15–20%. In the basal section of white asparagus Rodríguez et al., 1999a, 1999b), arabinoxylans and xyloglucans are the main hemicelluloses. This composition, above presented for by-products, suggests that these polysaccharides are also the major species in the basal section of green asparagus.

Cellulose was the main component in all samples. The intensity of treatments did not affect its amount, but the solvent and drying system did. Freeze-dried samples were richer in cellulose than were oven-dried ones. In white asparagus (Rodríguez et al., 1999a, 1999b), the cellulose content was double that of NS while, in this study, it was 3–4 fold higher.

Protein content varied between 12% and 16%. The most important difference was caused by the choice of solvent, with higher protein content in ethanol-treated samples than in those treated with water. In most cases, process intensity did not cause any differences.

KL accounted for 11–18%. It is interesting to note that fibres from gentle aqueous treatment (G/W) had significantly lower lignin contents than had samples from the other treatments. This could indicate that some additional material, or some artefacts resulting from processing, were quantified as lignin in fibres from intense treatments or treatments with ethanol.

In order to compare these fibre-rich powders with those obtained from other fruit and vegetable by-products, the composition of different fibres obtained from asparagus by-products is summarised as follows: SS 7.18–15.11%, NS 5.27–6.62%, UA 9.06–17.4%, cellulose 18.65–25.25%, protein 12.3–16.3%, and KL 11.0–18.1%. The content of SS in asparagus fibres was slightly higher than that found for other fruit or vegetable by-products. The percentage of SS was around 3% for mango peel (Larrauri, Rupérez, Borroto, & Saura-Calixto, 1996) and red grape pomace (Llobera & Cañellas, 2007), although a content of around 10% can be found in some citrus by-products (Marín, Soler-Rivas, Benavente-García, Castillo, & Pérez-Álvarez, 2007). The content of hemicelluloses (NS), among all fruit and vegetable by-products, varies between 5% and 30%. In guava pulp (Jiménez-Escrig, Rincón, Pulido, & Saura-Calixto, 2001), hemicelluloses accounted for about 16%, but lower levels (5–7%) were found in cocoa bean husks (Lecumberri et al., 2007) and some citrus by-products (Marín et al., 2007). UA contents also showed high variability, depending on the by-product studied. The lowest level was found in guava pulp (Jiménez-Escrig et al., 2001), which was 2%. Lime peels had the highest level, around 25% (Ubando-Rivera, Navarro-Ocaña, & Valdivia-López, 2005). UA levels similar to those in asparagus were found in peach pulp (Grigelmo-Miguel, Gorinstein, & Martín-Belloso, 1999). The cellulose content of fibre-rich powder from asparagus by-product was also in the range of the other products, with the lowest level, found in cocoa bean husks, being around 10% (Lecumberri et al., 2007), and the highest, found in citrus, being 36–40% (Marín et al., 2007). The content of protein in the fibre generated from asparagus (12.3–16.3%) was around the highest values reported for agricultural by-products, e.g. 16% for rice bran (Abdul-Hamid & Luan, 2000). The range of lignin content from other by-products was wide, varying from 6% for peach pulp (Grigelmo-Miguel et al., 1999) to 32% for cocoa bean husks (Lecumberri et al., 2007). The lignin content in fibres from asparagus by-product was similar to those reported by Jiménez-Escrig et al. (2001) for guava pulp.

3.3. Dietary fibre content

Table 3 shows the content of total dietary fibre (TDF), insoluble fibre (IF), soluble fibre (SF) and the ratio between IF and SF in fibre-rich powder from asparagus by-product. The varying extraction methods resulted in three levels of TDF content. The highest of these was found in fibres from intense treatment in water (I/W), and it was

Table 2
Composition of fibre-rich powder obtained under different conditions from asparagus by-products (% dry matter)[a]

		Soluble sugars[b]	Neutral sugars	Uronic acids	Cellulose	Protein	Klason lignin
I/W	O-D	11.0 ± 0.15ab	6.56 ± 0.03a	9.65 ± 0.58ab	22.1 ± 1.16a	12.3 ± 0.14a	16.6 ± 0.64ab
	F-D	12.1 ± 0.49c	6.62 ± 0.13a	9.06 ± 1.04a	25.3 ± 1.46b	12.4 ± 0.08a	18.1 ± 1.78b
I/E	O-D	9.33 ± 0.66d	5.87 ± 0.15b	16.4 ± 1.01c	19.1 ± 2.38c	16.3 ± 0.12b	15.2 ± 0.36a
	F-D	13.7 ± 0.83e	6.14 ± 0.09ab	17.4 ± 0.84d	21.9 ± 0.26a	15.2 ± 0.01c	16.6 ± 0.10ab
G/W	O-D	10.4 ± 0.10a	5.91 ± 0.38b	10.2 ± 0.45b	22.5 ± 1.31a	14.0 ± 0.05d	11.6 ± 0.69c
	F-D	11.3 ± 0.26b	5.77 ± 0.08bc	9.89 ± 1.11ab	22.0 ± 1.05a	13.5 ± 0.25e	11.0 ± 1.52c
G/E	O-D	7.18 ± 0.36f	5.73 ± 0.25bc	12.0 ± 0.58e	18.7 ± 0.99c	16.1 ± 0.01b	16.2 ± 1.91ab
	F-D	15.1 ± 0.14g	5.27 ± 0.30c	15.1 ± 0.52f	22.2 ± 140a	15.1 ± 0.09c	14.8±2.02a

[a] All analyses done at least in duplicate.
[b] Means within a column bearing the same letter are not significantly different at 5% level as determined by the Duncan multiple range test.

greater than 75%, probably as a consequence of the depletion of soluble components during processing, as was commented upon above. There was another group with medium content (around 69%) that included fibre from intense extraction with ethanol (I/E) and gentle extraction in water (G/W). The lowest TDF content (less than 65%) was found in fibres gently extracted in ethanol (G/E). TDF values in samples ranged from 62.1% to 77.5%, which is greater than the 49.0% previously reported by Grigelmo-Miguel and Martín-Belloso (1999) for asparagus by-product. The difference may be due to the processing that these authors used, as they only washed their samples with water and dried them in an oven. In this case, there was no depletion of soluble components at all, so the percentage of TDF decreased in the total composition.

In the literature, there are many by-products that are valuable TDF sources, with content varying between 30% and 90%. Three different groups could be established: low-TDF sources (30–50%), such as banana (Rodríguez-Ambriz, Islas-Hernández, Agama-Acevedo, Tovar, & Bello-Pérez, 2008), Marsh grapefruit (Figuerola, Hurtado, Estévez, Chiffelle, & Asenjo, 2005), and guava (Jiménez-Escrig et al., 2001); medium-TDF sources (50–70%), such as Eureka lemon (Figuerola et al., 2005) and mango peel (Larrauri, Goñi, Martín-Carrón, Rupérez, & Saura-Calixto, 1996); high-TDF sources (70–90%), such as red grape pomace (Llobera & Cañellas, 2007) and lime peel (Ubando-Rivera et al., 2005). Asparagus by-product fibre falls into the medium-TDF content group.

The IF/SF ratio varied from 4.6 to 7.2 (Table 3). The range to obtain the physiological effects associated with both the soluble and insoluble fractions was 1–2.3 (Spiller, 1986). The lowest value for the IF/SF ratio for asparagus by-product (4.6) was obtained with the G/E treatment. A decrease in this ratio was observed to be linked to the severity of the treatment and the solvent used. The decrease was due to a reduction in the percentage of IF in gently extracted samples. Most fibre from agricultural by-products also had values higher than 4, e.g. cocoa bean husks – 5 (Lecumberri et al., 2007), red grape pomace – 6.4 (Llobera & Cañellas, 2007), banana – 7.6 (Rodríguez-Ambriz et al., 2008), rice bran – 11 (Abdul-Hamid & Luan, 2000), and guava pulp – 27 (Jiménez-Escrig et al., 2001).

3.4. Functional properties

3.4.1. Water- and oil-holding capacities and solubility

The results obtained for WHC, SOL, and OHC are presented in Table 4. WHC is an important property of dietary fibre from both physiological and technological points of view. WHC showed significant differences, depending on the severity of processing: fibre obtained with thermal treatment had higher WHC. Heating might modify the structural characteristics of the fibre, hence facilitating its water uptake (Figuerola et al., 2005). The choice of drying system did not lead to significant differences although, in other agronomical by-products, drying at high temperatures (40–90 °C) causes a reduction in this capacity (Garau et al., 2007). WHC is related to soluble dietary fibre content. As was commented upon above, fibres undergoing gentle treatment had a lower IF/SF ratio, but a higher WHC. So, the effect of heating on fibre structure seems to be greater than the influence of a higher percentage of soluble fibre. It is probably that the high solubility of asparagus fibre, 22–34% (Table 4), could improve this functional property. SOL was significantly influenced by the choice of drying system, with the lowest value being in I/W samples.

Fibre-rich asparagus powder generated by gentle processing had a WHC similar to what was previously reported (Grigelmo-Miguel & Martín-Belloso, 1999). These results are in the range of most described fibres, e.g. 12.6 ml water/g for peach pulp fibre (Grigelmo-Miguel et al., 1999) and more than 11 ml water/g for lemon fibre (Lario et al., 2004). Other agricultural by-products had lower values than those commented upon above, e.g. rice bran (Abdul-Hamid & Luan, 2000) and cocoa husks (Lecumberri et al., 2007), both with a WHC value of 5 ml water/g fibre. On the basis of these values, this product could be promoted as a modifier of viscosity and texture of formulated products in addition to promoting the decrease in calories that this addition could imply.

Results for OHC are presented in Table 4. Except for G/E samples, freeze-drying resulted in an increase of this property, ranging between 7.5 and 8.5 ml oil/g of powder. G/E samples had lower capacities. Values found in the literature were much lower than those for asparagus by-products, e.g. 0.6–1.8 ml oil/g for apple pomace and citrus peel (Figuerola et al., 2005), and around 2 ml oil/g for unripe banana flour (Rodríguez-Ambriz et al., 2008). The highest reported level was around 6 ml oil/g described for carrot pulp dried at 50 °C (Garau et al., 2007). Fibre-rich asparagus powder had even higher values, so the use of this fibre may be appropriate in products where emulsifying properties are required.

3.4.2. Glucose dialysis retardation index

In Fig. 1 the graphs for GDRI are presented. Except for control and I/W O–D, the retardation curves of all the other samples were adjusted at *a* "square root *x*" model ($y = a + bx^{-2}$) with $R > 0.92$. Using the regression equation for each sample, the different GDRI's at 60 min. were calculated and the results are presented in Fig. 2. Values ranged from 18% to 48%. Intense treatments led to higher GDRI than did gentle ones, although differences were stronger in samples extracted with water than in those extracted with ethanol. Fibre treated with ethanol had higher values than had fibre treated with water, and those freeze-dried were higher than those that were oven-dried. The GDRI phenomenon seems to be related to the soluble dietary fibre and uronic acid contents of insoluble fibre (Edwards et al., 1987; Wolever, 1990), although other authors have pointed to the relationship between the internal structure and surface properties of fibres and glucose diffusion (López et al., 1996).

Table 3
Dietary fibre fractions of different fibre-rich powder from asparagus by-products (% dry matter)[a]

		IF	SF	TDF[b]	IF/SF
I/W	O-D	66.8 ± 2.56	10.7 ± 0.38	77.5 ± 2.94a	6.2
	F-D	65.2 ± 1.12	10.3 ± 0.35	75.4 ± 1.47a	6.3
I/E	O-D	60.6 ± 1.99	9.79 ± 0.07	70.4 ± 2.05b	6.2
	F-D	60.1 ± 2.11	8.26 ± 0.48	68.4 ± 2.59b	7.2
G/W	O-D	58.2 ± 1.89	9.90 ± 1.69	68.1 ± 3.58b	5.9
	F-D	58.7 ± 1.39	10.5 ± 0.38	69.2 ± 1.77b	5.6
G/E	O-D	54.9 ± 1.50	9.58 ± 0.83	64.5 ± 2.32c	5.7
	F-D	51.0 ± 0.32	11.1 ± 0.32	62.1 ± 0.64c	4.6

[a] Values are means of triplicate assays.
[b] Means bearing the same letter are not significantly different at 5% level as determined by the Duncan multiple range test.

Table 4
Functional properties of different fibre-rich powder from asparagus by-products[a]

		WHC[b] ml water/g powder	SOL %	OHC ml oil/g powder
I/W	O-D	20.3 ± 0.54a	23.4 ± 1.20a	7.61 ± 0.27ab
	F-D	18.8 ± 1.01a	22.6 ± 0.57a	8.19 ± 0.16c
I/E	O-D	19.7 ± 2.08a	27.7 ± 1.75b	7.52 ± 0.27a
	F-D	19.7 ± 1.54a	34.4 ± 2.75c	8.27 ± 0.68c
G/W	O-D	11.4 ± 0.38b	26.5 ± 0.53b	7.69 ± 0.17ab
	F-D	12.6 ± 0.77b	34.2 ± 0.61c	8.53 ± 0.30c
G/E	O-D	12.1 ± 1.26b	28.9 ± 0.96b	5.28 ± 0.34d
	F-D	12.2 ± 0.92b	34.7 ± 0.76c	5.53 ± 0.54d

[a] Values are means of triplicate assays.
[b] Means within a column bearing the same letter are not significantly different at 5% level as determined by the Duncan multiple range test.

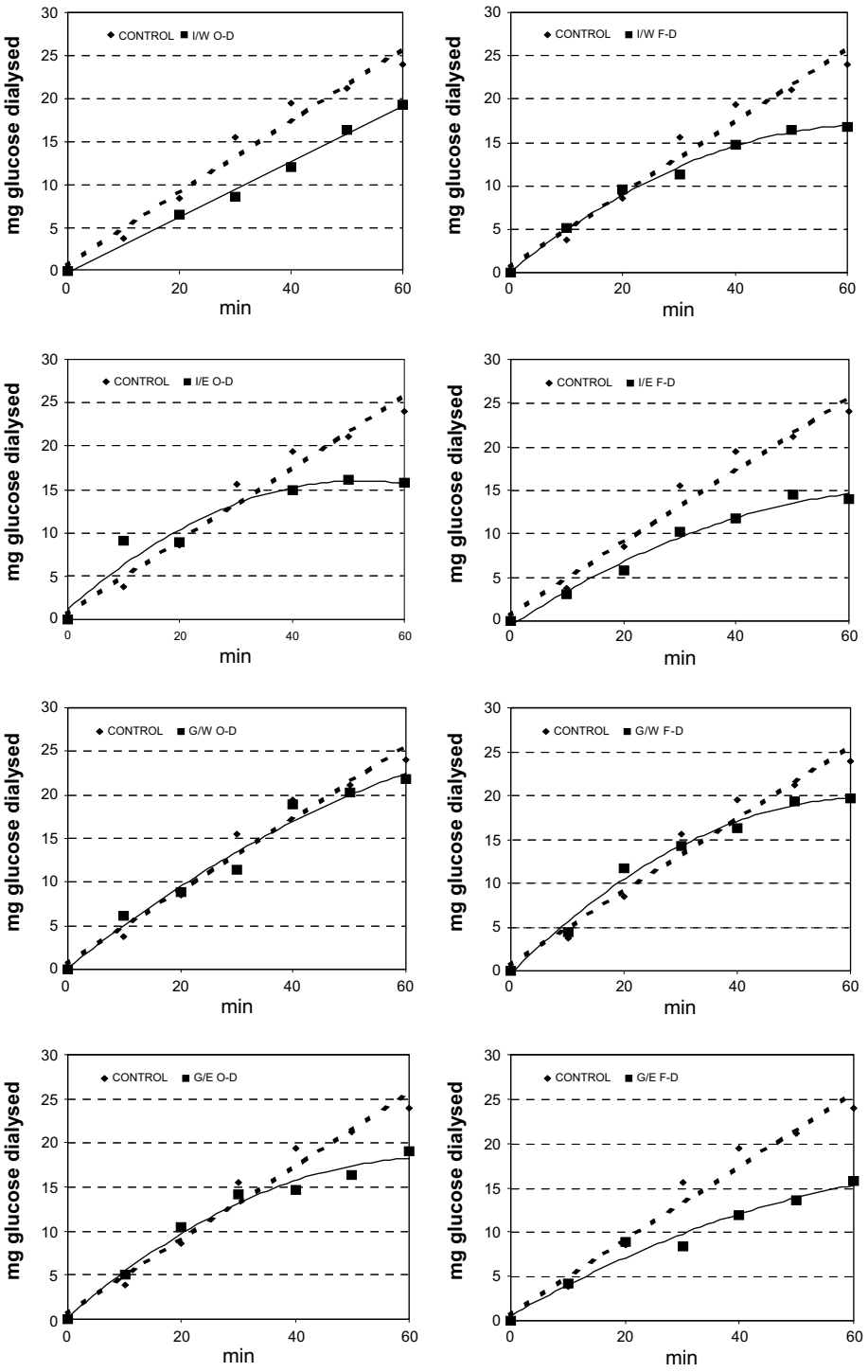

Fig. 1. Time-related effects of the different fibre-rich powders obtained from asparagus by-products on glucose diffusion.

Both factors could probably modulate this functional property to different degrees, depending on the studied fibre. For asparagus fibre, the surface characteristics must have some influence on GDRI because freeze-dried samples presented a longer delay of glucose dialysis. After regression analysis between chemical composition and the GDRI, uronic acids were the components that showed the highest correlation ($R = 0.72$), in agreement with above comments.

The GDRI is a useful *in vitro* index to predict the effect that fibre has on the delay of glucose absorption in the gastrointestinal tract (López et al., 1996). In the literature, there are a wide range of values, from wheat bran at 5.3% (Adiotomre, Eastwood, Edwards, & Brydon, 1990) to guar gum at 43% (Larrauri et al., 1996). Most of the by-products have intermediate values, e.g., artichoke fibre – 27% (López et al., 1996), mango peel – 21% (Larrauri et al., 1996), and carambola pomace – 25% (Chau, Chen, & Lin, 2004). The results obtained for fibre-rich powders from asparagus by-product were similar to those reported above, with two of the treatments (I/E F–D and G/E F-D) leading to higher values.

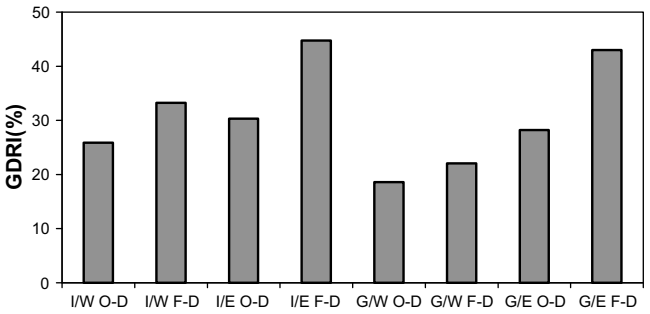

Fig. 2. Glucose dialysis retardation index (%) of the different fibre-rich powders obtained from asparagus by-products.

4. Conclusions

The treatments applied for obtaining the fibre-rich powders from asparagus by-products caused little variability in the chemical composition of the powder. Only the components with high solubility (soluble sugars, uronic acids, and proteins) showed significant differences. With gentle treatments, especially those with ethanol, powders with lower IF/SF ratios could be obtained. Therefore, the degree of treatment severity became the key to determining the physiological quality of asparagus by-product fibre. The average composition of asparagus fibre, obtained under different conditions, was similar to those of other good sources of food fibre suggested in the literature.

The drying systems studied affected fibre surface differently. This factor had a sizeable influence on the functional properties of the fibre. Solubility and oil-holding capacity were higher in freeze-dried fibres than in oven-dried fibres. However, water-holding capacity was more influenced by thermal treatment. The functionality of thermally treated fibre makes it suitable for addition to fibre-enriched food as a modifier of viscosity and texture, and could also cause a decrease in calories. Some of the fibres examined in this study have been added to plain yoghurt and were compatible with its manufacturing process (Sanz, Salvador, Jiménez, & Fiszman, 2008). Especially considering their oil-holding capacities, the high-fibre powers from asparagus by-products may be appropriate in products where emulsifying properties are required. This study also revealed that they could effectively retard the diffusion of glucose; this capacity was highly affected by the process through which it was obtained. The potential hypoglycaemic effects of these fibres suggested that they could be incorporated as low-calorie bulk ingredients in high-fibre foods to lower postprandial serum glucose levels.

Studies to identify bioactive compounds in these fibres and their *in vitro* antioxidant activities have been undertaken in order to evaluate the properties of these compounds that may be beneficial for health. These studies are almost ready to be published. Further investigations on the *in vivo* hypoglycaemic effect and other physiological effects, using animal feeding experiments, are needed to establish the possibility of the use of asparagus-derived fibres as food ingredients, and for nutritional and technological applications, while making use of asparagus-processing waste. All these studies are thus of economic interest to producers.

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References

Abdul-Hamid, A., & Luan, Y. S. (2000). Functional properties of dietary fibre prepared from deffated rice bran. *Food Chemistry, 68*, 15–19.

Adiotomre, J., Eastwood, M. A., Edwards, C. A., & Brydon, W. G. (1990). Dietary fiber: *In vitro* methods that anticipate nutrition and metabolic activity in humans. *American Journal of Clinical Nutrition, 52*, 128–134.

Ahmed, A. R., & Labavitch, J. M. (1977). A simplified method for accurate determination of cell wall uronide content. *Journal of Food Biochemistry, 1*, 361–365.

Chau, C.-F., Chen, C.-H., & Lin, C.-Y. (2004). Insoluble fiber-rich fractions derived from *Averrhoa carambola*: Hypoglycemic effects determined by *in vitro* methods. *Lebensm.-Wiss.u.-Technol., 37*, 331–335.

Dische, Z. (1962). Color reactions of carbohydrates. In R. L. Whistler & M. L. Wolfram (Eds.), *Methods in Carbohydrate Chemistry* (pp. 477–512). New York: Academic Press.

Edwards, C. A., Blackburn, N. A., Craigen, L., Davinson, P., Tomlin, J., Sugden, K., et al. (1987). Viscosity of food gums determined *in vitro* related to their hypoglycaemic actions. *American Journal of Clinical Nutrition, 46*, 72–77.

Englyst, H. N., & Cumming, J. H. (1984). Simplified method for the measurement of total non-starch polysaccharides by gas-chromatography of constituent sugars as alditol acetates. *Analyst, 109*, 937–942.

FDA, Department of Health and Human Services (1993). Health Claims: fruits and vegetables and cancer (Revised as of 1 April 2005). In: FDA, Code of Federal Regulations (vol. 2, Title 21. Chapter I. Subchapter B. Part 101. Subpart E. section 101.78). Rockville, MD: GPO.

Fernandez-Bolaños, J., Rodríguez, G., Gómez, E., Guillén, R., Jiménez, A., Heredia, A., et al. (2004). Total recovery of the waste of two-phase olive oil processing: isolation of added-value compounds. *Journal of Agricultural and Food Chemistry, 52*, 5849–5855.

Figuerola, F., Hurtado, M. L., Estévez, A. M., Chiffelle, I., & Asenjo, F. (2005). Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. *Food Chemistry, 91*, 395–401.

Garau, M. C., Simal, S., Rosselló, C., & Femenia, A. (2007). Effect of air drying temperature on physico-chemical properties of dietary fibre and antioxidant capacity of orange (*Citrus aurantium* v. *Canoneta) by-products. Food Chemistry, 104*, 1014–1024.

Grigelmo-Miguel, N., & Martín-Belloso, O. (1999). Comparison of dietary fibre from by-products of processing fruits and greens and from cereals. *Lebensm.-Wiss.u.-Technol, 32*, 503–508.

Grigelmo-Miguel, N., Gorinstein, S., & Martín-Belloso, O. (1999). Characterisation of peach dietary fibre concentrate as a food ingredient. *Food Chemistry, 65*, 175–181.

Jiménez, A., Rodríguez, R., Fernández-Caro, I., Guillén, R., Fernandez-Bolaños, J., & Heredia, A. (2000). Dietary fibre content of tables olives processed under different European styles: study of physicochemical characteristics. *Journal of the Science of Food and Agriculture, 80*, 1–6.

Jiménez-Escrig, A., Rincón, M., Pulido, R., & Saura-Calixto, F. (2001). Guava fruit (*Psidium guajava* L) as a new source of antioxidant dietary fiber. *Journal of Agricultural and Food Chemistry, 49*, 5489–5493.

Lario, Y., Sendra, E., García-Pérez, C., Fuentes, E., Sayas-Barberá, E., Fernández-López, J., & Pérez-Álvarez, J. A. (2004). Preparation of high dietary fiber powder from lemon juice by-product. *Innovative Food Science and Emerging Technologies, 5*, 113–117.

Larrauri, J. A., Goñi, I., Martín-Carrón, N., Rupérez, P., & Saura-Calixto, F. (1996). Measurement of health-promoting properties in fruit dietary fibres: antioxidant capacity, fermentability and glucose retardation index. *Journal of the Science of Food and Agriculture, 71*, 515–519.

Larrauri, J. A., Rupérez, P., Borroto, B., & Saura-Calixto, F. (1996). Mango peels as a new tropical fibre: preparation and characterization. *Lebensm.-Wiss.u.-Technol, 29*, 729–733.

Lecumberri, E., Mateos, R., Izquierdo-Pulido, M., Rupérez, P., Goya, L., & Bravo, L. (2007). Dietary fibre composition, antioxidant capacity and physico-chemical properties of a fibre-rich product fron cocoa (*Theobroma cacao* L). *Food Chemistry, 104*, 948–954.

Lee, S. C., Prosky, L., & De Vries, J. W. (1992). Determination of total, soluble and insoluble dietary fiber in food Enzymatic, gravimetric method, MES-TRIS buffer: collaborative study. *Journal of the Association of Official Analytical Chemistry, 75*, 395–416.

Llobera, A., & Cañellas, J. (2007). Dietary fibre content and antioxidant activity of Manto Negro red grape (*Vitis vinifera*): pomace and stem. *Food Chemistry, 101*, 659–666.

López, G., Ros, G., Rincón, F., Periago, M. J., Martínez, M. C., & Ortuño, J. (1996). Relationship between physical and hydration properties of soluble and insoluble fiber of artichoke. *Journal of Agricultural and Food Chemistry, 44*, 2773–2778.

Marín, F. R., Soler-Rivas, C., Benavente-García, O., Castillo, J., & Pérez-Álvarez, J. A. (2007). By-products from different citrus processes as a source of customized functional fibres. *Food Chemistry, 100*, 736–741.

Nindo, C. I., Sun, T., Wang, S. W., Tang, J., & Powers, J. R. (2003). Evaluation of drying technologies for retention of physical quality and antioxidants in asparagus. *European Food Research and Technology, 36*, 507–516.

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M., & Brighenti, F. (2003). Total antioxidant capacity of plant foods, beverages and oils

consumed in Italy assessed by three different *in vitro* assays. *Journal of Nutrition, 133*, 2812–2819.

Rodríguez, R., Jiménez, A., Fernandez-Bolaños, J., Guillén, R., & Heredia, A. (2006). Dietary fibre from vegetable products as source of functional ingredients. *Trends in Food Science and Technology, 17*, 3–15.

Rodríguez, R., Jiménez, A., Guillén, R., Heredia, A., & Fernandez-Bolaños, J. (1999a). Postharvest changes in white asparagus cell wall during refrigerated storage. *Journal of Agricultural and Food Chemistry, 47*, 3551–3557.

Rodríguez, R., Jiménez, A., Guillén, R., Heredia, A., & Fernandez-Bolaños, J. (1999b). Turnover of white asparagus cell wall polysaccharides during postharvest storage. *Journal of Agricultural and Food Chemistry, 47*, 4525–4531.

Rodríguez-Ambriz, S. L., Islas-Hernández, J. J., Agama-Acevedo, E., Tovar, J., & Bello-Pérez, L. A. (2008). Characterization of a fibre-rich powder prepared by liquefaction of unripe banana flour. *Food Chemistry, 107*, 1515–1521.

Ruiter, J. M., & Burns, J. C. (1987). Characterization of trifluoroacetic acid hydrolized of subtropical forage grass cell walls. *Journal of Agricultural and Food Chemistry, 35*, 308–316.

Sanz, T., Salvador, A., Jiménez, A., & Fiszman, S. M. (2008). Yogurt enrichment with functional asparagus fibre Effect of extraction method on rheological properties, colour, and sensory acceptance. *European Food Research and Technology, 227*, 1515–1521.

Spiller, G. A. (1986). Suggestion for a basis on which to determine a desirable intake of dietary fibre. In G. A. Spiller (Ed.), *CRC Handbook of Dietary Fibre in Human Nutrition* (pp. 281–283). Florida: CRC Press.

Ubando-Rivera, J., Navarro-Ocaña, A., & Valdivia-López, M. A. (2005). Mexican lime peel: comparative study on contents of dietary fibre and associated antioxidant activity. *Food Chemistry, 89*, 57–61.

Vinson, J. A., Hao, Y., Su, X., & Zubik, L. (1998). Phenol antioxidant quantity and quality in foods: vegetables. *Journal of Agricultural and Food Chemistry, 46*, 3630–3634.

Waldron, K. W., & Selvendran, R. R. (1992). Cell wall changes in immature asparagus stem tissue after excision. *Phytochemistry, 31*, 1931–1940.

Wolever, T. M. S. (1990). Relationship between dietary fibre content and composition in foods and the glycemic index. *American Journal of Clinical Nutrition, 51*, 72–75.

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Effect of the extraction method on phytochemical composition and antioxidant activity of high dietary fibre powders obtained from asparagus by-products

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ABSTRACT

Asparagus (*Asparagus officinalis L.*) spears are highly appreciated for their composition of bioactive compounds. The method by which their by-products are treated affects the phytochemical composition and antioxidant activity of the fibre-rich powders. Factors such as the treatment intensity, the solvent used, and the drying system were studied. Among the asparagus phytochemicals, hydroxycinnamic acids (HCA), saponins, flavonoids, sterols, and fructans were quantified. HCA varied from 2.31 and 4.91 mg/g of fibre, the content being affected by the drying system and, in some cases, the solvent. Fibres from intense treatments had significantly higher amounts of saponin than samples isolated by gentle treatments. Saponin content ranged from 2.14 to 3.64 mg/g of fibre. Flavonoids were the most affected by processing conditions, being present (0.6–1.8 mg/g of fibre) only in three of the samples analysed. Continuous stirring during processing could be the main reason for this result. Sterols and fructans were present in minor amounts, 0.63–1.03 mg/g of fibre and 0.2–1.4 mg/g of fibre, respectively. Soluble and total antioxidant activities were also measured. Fibres with the highest activities corresponded to those with the highest levels of flavonoids and HCA.

1. Introduction

Besides their culinary quality, green asparagus spears are appreciated for their composition of bioactive compounds. Eastern civilisations have been using asparagus extracts as stimulants, laxatives, antitussives, diuretics, etc. for hundreds of years. In recent pharmacological studies (Kamat, Boloor, Devasagayam, & Venkatachalam, 2000; Wiboonpun, Phuwapraisirisan, & Tip-pyang, 2004; Yu et al., 1996), these extracts have been shown to have several biological activities, including antitumoral and antioxidant activities. Among all the bioactive compounds present in asparagus spears, saponins, flavonoids, and hydroxycinnamates are the main compounds responsible for the characteristics cited above. Asparagus saponins are steroidal glycosides, with protodioscin being the most abundant. Several activities of this compound have been described, with cytotoxicity against several lines of human cancerous cells (Chin, 2006; Hibasami et al., 2003; Wang et al., 2003) being of special interest. Flavonoids are phenolic compounds with high antioxidant activity. Additionally, they have antitumoral and antimicrobial activities, and participate in the prevention of cardiovascular diseases (Cushine & Lamb, 2005; Nijveldt et al., 2001). Rutin is the most abundant flavonoid in asparagus spears, in addition to others that have been recently described (Fuentes-Alventosa et al., 2007, 2008). Hydroxycinnamic acids, especially ferulic acid, are strong antioxidants. Thus, ferulic acid may be beneficial in the prevention of disorders linked to oxidative stress, including Alzheimer's disease, diabetes, cancers, hypertension, atherosclerosis, inflammatory diseases, and others (Zhao & Moghadasian, 2008). If linked to dietary fibre, ferulic acid would be desesterified in the intestinal lumen, which could offer a way to provide a slow-release form of ferulic acid that might provide a prolonged physiological effect (Plate & Gallaher, 2005).

Plant sterols (phytosterols) and fructans, mainly fructooligosaccharides, are also present in asparagus spears, but in lower amounts. The nutrition role of phytosterols is based on their cholesterol-lowering effect in human blood, based on their ability to competitively inhibit intestinal cholesterol uptake (Jiménez-Escrig, Santos-Hidalgo, & Saura-Calixto, 2006). β-Sitosterol is the most abundant compound within this group of phytochemicals. Fructooligosaccharides (FOS) have a beneficial effect on human health because they are prebiotics. FOS are not hydrolysed by digestive

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enzymes, but gut microbiota are able to ferment them. Several studies have found that FOS and inulin promote calcium absorption in both the animal and human gut (Heuvel, Muys, Dokkum, & Schaasfma, 1999; Zafar, Weaver, Zhao, Martin, & Wasttney, 2004). The intestinal microflora in the lower gut can ferment FOS, which results in a reduced pH. Calcium is more soluble in acid and, therefore, more is released from the food and is available to move from the gut into the bloodstream.

Of the asparagus-producing countries, Spain ranks fifth after China, Peru, the USA, and Germany. During industrial processing, around half of the total length of each spear is discarded, which creates significant waste for producers. Assuming that the by-products have a similar composition to the edible part of the spears, their fibre-rich products could contain significant amounts of all the phytochemicals mentioned above. In addition to changes in chemical composition and functional characteristics of the fibres (Fuentes-Alventosa et al., 2009), by-product processing conditions could modify the composition of bioactive compounds and, in doing so, the intrinsic antioxidant activity of these fibre-rich products. The aim of this work is to characterise phytochemicals from asparagus fibres and to study the effects that processing conditions have on their composition and antioxidant activity.

2. Materials and methods

2.1. Asparagus by-products

Asparagus by-products were obtained from Centro Sur S.C.A. (Huétor-Tajar, Granada, Spain). Prior to canning, freshly harvested asparagus spears were cut to obtain the 15 cm long upper portion (edible part) and the rest of spear (15–18 cm) was considered a by-product. This by-product was sent to our lab within the next 24 h and held at 4 °C until processing.

2.2. Asparagus by-product treatment

Three variables were studied (Table 1): extraction treatment (intense – 90 min at 60 °C, or gentle – 1 min at room temperature), extraction solvent (water or 96% ethanol), and drying system (freeze-drying or oven treatment at 60 °C for 16 h). Two kilograms of the by-product portions were cut, homogenised, and mixed using a professional homogeniser (Sirman Orione, Marsango, Italy). Homogenisation was performed at top speed with each of the extraction solvents in a 1:1 ratio (solid:liquid) (w/v), and at the programmed temperature (60 °C or room temperature) for 1 min. Afterwards, gently-extracted samples (G) were directly processed using a industrial juicer (Tecno-Chufa, Valencia, Spain) to separate the asparagus liquid extract from the wet fibrous residue. Intensely-extracted samples (I) were treated in an open reactor designed in our laboratory, with time and temperature controls and continuous stirring. After 90 min of extraction, samples were centrifuged as described above. Each treatment was performed in duplicate. One batch of wet fibrous residue was freeze-dried (F-D) and the other dried in an oven (O-D). Dried fibres were ground in a hammer mill to a particle size lower than 1 mm and stored at 4 °C until analysis.

2.3. Determination of bioactive compounds

2.3.1. Hydroxycinnamic acids

Total hydroxycinnamic acids (HCA) present in fibre samples were extracted and quantified as previously described (Jaramillo et al., 2007). Briefly, samples (in duplicate) were treated with 2 N NaOH for 24 h, at room temperature, under nitrogen and darkness. After filtration, *trans*-cinnamic acid was added as an internal standard. Solutions were acidified and extracted three times with ethyl acetate. Ethyl acetate extracts were evaporated under nitrogen, re-dissolved in 50% methanol, and analysed by HPLC. Phenolic compounds were quantified using a Synergy 4μ Hydro-RP80A reverse-phase column (25 cm × 4.6 mm i.d., 4 μm; Phenomenex, Macclesfield, Cheshire, UK). The gradient profile was formed using solvent **A** (10% aqueous acetonitrile plus 2 ml/l acetic acid) and solvent **B** (40% methanol, 40% acetonitrile, and 20% water plus 2 ml/l acetic acid) in the following program; the proportion of **B** increased from 10% to 42.5% for the first 17 min, held isocratically at 42.5% for a further 6 min, increased to 100% over the next 17 min, and finally returned to the initial conditions. The flow rate was 1 ml/min. HCA were detected using a Jasco-LC-Net II ADC liquid chromatograph system equipped with DAD and a Rheodyne injection valve (20 μl loop). Quantification was performed by integration of peak areas at 280 nm, with reference to calibrations done using known amounts of pure compounds.

2.3.2. Extraction of saponins and flavonoids

Two and a half grams of each fibre (in duplicate) were extracted with 100 ml of 80% ethanol. The samples were blended in a Sorvall Omnimixer, Model 17106 (Du Pont Co., Newtown, CT), at maximum speed for 1 min, and then passed through filter paper. Ethanolic extracts were stored at −20 °C until analysis.

2.3.3. Quantification of saponins

Five millilitre aliquots (in duplicate) of each ethanolic extract were dried under air flow and re-dissolved in 2 ml of distilled water by sonication. Water solutions were extracted twice with 2 ml of hexane, ethyl acetate, and butanol, sequentially. Butanol extracts were collected and dried under air flow, the residue was re-dissolved in 2 ml of distilled water, and then loaded onto a 1 ml Sep-Pak C_{18} cartridge (Waters Corporation, Milford, MA) preconditioned with 96% ethanol. Cartridges were washed with 5 ml of water and then with 5 ml of 96% ethanol. Ethanol fractions were assayed for saponin content.

A colorimetric method for saponin quantification was developed in our laboratory based on reactive anisaldehyde–sulphuric acid–acetic acid for TLC staining (Wang, Lii, Chang, Kuo, & Chen, 2007). Two hundred microlitres of purified ethanol fractions were dispensed in quadruplicate and 400 μl of a reactive acid (sulphuric

Table 1
Different conditions for obtaining a fibre-rich powder from asparagus by-product.

	Description
I/W O-D	Intense treatment (90 min at 60 °C) with water and oven-drying
I/W F-D	Intense treatment (90 min at 60 °C) with water and freeze-drying
I/E O-D	Intense treatment (90 min at 60 °C) with ethanol and oven-drying
I/E F-D	Intense treatment (90 min at 60 °C) with ethanol and freeze-drying
G/W O-D	Gentle treatment (1 min at room temperature) with water and oven-drying
G/W F-D	Gentle treatment (1 min at room temperature) with water and freeze-drying
G/E O-D	Gentle treatment (1 min at room temperature) with ethanol and oven-drying
G/E F-D	Gentle treatment (1 min at room temperature) with ethanol and freeze-drying

acid:acetic acid, 1:1) were added. After mixing, 20 μl of *p*-anisaldehyde were added to three of the replicates, with the same volume of water being added to the fourth to be used as a sample blank. All tubes were heated at 95–100 °C in a water bath for 2 min and then cooled with tap water. The absorbance at 630 nm was determined. In each determination, a calibration curve was done, using diosgenin as a reference standard.

2.3.4. Quantification of flavonoids

Flavonoids were detected and quantified by HPLC (Fuentes-Alventosa et al., 2007) using a Synergy 4μ Hydro-RP80A reverse-phase column as before. The gradient profile was formed using solvent **A** (10% aqueous acetonitrile plus 2 ml/l acetic acid) and solvent **B** (40% methanol, 40% acetonitrile, and 20% water plus 2 ml/l acetic acid) in the following program; the proportion of **B** increased from 10% to 42.5% **B** for the first 15 min, increased to 70% over the next 6 min, remained at 70% for 3.5 min, increased again to 100% over the next 5 min, and finally returned to the initial conditions. The flow rate was 1 ml/min, and the column temperature was 30 °C. Flavonoids were detected using a Jasco-LC-Net II ADC liquid chromatograph system equipped with DAD and a Rheodyne injection valve (20 μl loop). Spectra from all peaks were recorded in the 200–600 nm range, and the chromatograms were acquired at 360 nm. Quantification was performed by integration of peak areas at 360 nm, with reference to calibrations done while using known amounts of pure compounds.

2.3.5. Sterols

The methods applied for hydrolysis, saponification, silylation and quantification were adapted from Jiménez-Escrig et al. (2006). Fibre samples (0.5 g in duplicate) were weighed in 50 ml screw-capped Erlenmeyer flasks, 4 ml of internal standard solution (20 μg cholesterol/ml ethanol) and 10 ml of 6 M HCl were added to each sample, and the flask were heated at 80 °C for 1 h in a shaking water bath. The flasks were cooled to room temperature and 20 ml of hexane:diethyl ether (1:1) mixture were added. The samples were shaken for 10 min and allowed to stand for phase separation. The upper phase was evaporated to dryness under vacuum at 50 °C. Saponification was performed as follows: 8 ml of 2 M ethanolic KOH were added to the dry extracts, the mixtures were transferred to a 50 ml screw-capped Erlenmeyer flask, and heated at 80 °C for 30 min in a shaking water bath. Then, 20 ml of cyclohexane and 12 ml of distilled water were added to each sample. The samples were shaken for 10 min and allowed to stand for phase separation. The unsaponifiable upper phase was evaporated to dryness under vacuum at 50 °C. The residue was re-dissolved in 1 ml of dichloromethane and loaded onto a 1 ml Sep-Pack C_{18} cartridge, preconditioned with methanol. Sterol fractions were eluted with 15 ml of dichloromethane:methanol (95:5) and evaporated to dryness under vacuum at 50 °C. The residue was re-dissolved in 0.5 ml of dichloromethane. Aliquots of 100 μl of the sterol fractions were placed in a pre-silanised screw-capped vial. The solvents were evaporated under nitrogen and the TMS ether derivatives of the sterols were prepared by adding 100 μl of the silylation reagent (BSTFA:TMCS, 99:1) and 100 μl of anhydrous pyridine. The samples were then heated at 60 °C for 30 min or left overnight at room temperature for silylation. The excess silylating reagent was removed under nitrogen at 50 °C, and the residue was dissolved in 600 μl of hexane. Sterol quantification was by GC. A Hewlett–Packard 5890 Series II chromatograph, fitted with a 30 m × 0.25 mm (film thickness = 0.25 μm) cross-linked methyl siloxane capillary column (HP-1 from Agilent, Santa Clara, CA), was employed. The oven temperature program used was as follows: initial, 50 °C, 2 min; raised at 30 °C/min to 245 °C, 1 min; raised at 3 °C/min to 275 °C, and held for 28.5 min. The carrier gas was helium at a flow rate of 1 ml/min. The injector temperature was 250 °C and the FID temperature was 280 °C.

2.3.6. Fructans

Fructan amounts were determined using a Megazyme kit (K-FRUC), whose procedure is described in detail at http://www.megazyme.com/downloads/en/data/K-FRUC.pdf. This assay is based on AOAC method 999.03 and AACC method 32.32. Briefly, fibre samples (0.5 g in duplicate) were extracted twice with 20 ml each of hot distilled water for 15 min at 80 °C with continuous stirring. After filtering through glass filter paper, both extracts were collected and the volume made up to 50 ml. Two aliquots of 50 μl of the extracts were placed in test tubes and 50 μl of sucrase/amylase solution were added. After incubation (30 min, 40 °C), 50 μl of a 10 mg/ml sodium borohydride solution in 50 mM sodium hydroxide were added and the tubes were incubated under the same conditions. To remove excess borohydride and adjust the pH to approximately 4.5, 125 μl of 0.2 M acetic acid were added. Carefully, 50 μl of this final solution were transferred to test tubes (×3), and 25 μl of fructanase solution were added to two of the tubes and 25 μl of 0.1 M sodium acetate buffer were added to the third (sample blank). After incubation (40 °C for 20 min) to produce complete hydrolysis of fructans, 1.25 ml of *p*-hydroxybenzoic acid hydrazide (PAHBAH), reactive for reducing sugars, were dispensed into each tube. Tubes were placed in a boiling water bath for 6 min and allowed to cool in cold water. Absorbance at 410 nm was measured against a reagent blank. During each determination, a calibration curve was prepared using fructose as reference standard.

2.4. Antioxidant capacity

Soluble antioxidant activity was determined after fibre extraction (in duplicate) with methanol:water (50:50) and acetone:water (70:30) (Larrauri, Rupérez, & Saura-Calixto, 1997) by the DPPH· method (Rodríguez, Jaramillo, Rodríguez, et al., 2005). Total antioxidant activity was evaluated as described by Serpen, Capuano, Fogliano, and Gokmen (2007). Between 3 and 20 mg of fibre was transferred to an Eppendorf tube (for weights lower than 3 mg, fibre had to be diluted with cellulose as inert material), and the reaction was started by adding 1 ml of the DPPH· reagent (3.8 mg/50 ml methanol). After 30 min of continuous stirring, samples were centrifuged and the absorbance of the cleared supernatants was measured (in triplicate) at 480 nm. Both antioxidant activities were expressed as millimoles of Trolox equivalent antioxidant capacity (TEAC) per kilogram of sample by means of a dose–response curve for Trolox.

2.5. Statistical analysis

Results were expressed as mean value ± standard deviation. To assess for differences in the composition and physicochemical characteristics between the different treatments, multiple sample comparison was performed using the Statgraphics Plus program Version 2.1. (StatPoint Inc., Warrenton, VA). Multivariate analysis of variance (ANOVA), followed by Duncan's multiple comparison test, was performed to contrast the groups. The level of significance was $p < 0.05$.

3. Results and discussion

Asparagus by-products could be considered an interesting source of dietary fibre. By-product processing involves several factors that affect fibre composition and functionality (Fuentes-Alventosa et al., 2009). In this work, we report the composition of bioactive compounds and the antioxidant activity of a fibre-rich

product obtained from asparagus by-product, which may be used as an ingredient in the preparation of additional food products. Three factors have been taken into account (severity of treatment – temperature and time, solvent, and drying system) and their effects on fibre characteristics will be discussed.

3.1. Bioactive compound composition

Table 2 shows the composition of several bioactive compounds analysed in fibre-rich powders obtained from asparagus by-products.

3.1.1. Hydroxycinnamic acids

The hydroxycinnamic acids present in the green spears are mainly coumaric acid, ferulic acid, and its dimers. Middle and basal portions of the spears are richer in these compounds than the upper portion, especially after a storage period (Rodríguez, Jaramillo, Guillén, et al., 2005). In the fibre-rich powders assayed, the amount of HCA ranged from 2.31 to 4.91 mg/g, with ferulic acid derivatives (FAD) of 0.8 to 1.8 mg/g. In both intense and gentle treatments, significant differences were caused by the choice of drying system, with higher HCA content in the freeze-dried samples than in those dried in an oven. In the intense treatment process, the solvent did not cause any differences, but in the gentle process, fibres obtained with water had the highest HCA content.

The amounts of HCA quantified in the fibre-rich powders were higher than previously reported for green asparagus (0.6 mg HCA/g cell wall material in fresh asparagus and 1.6 mg HCA/g of cell wall material in stored spears; Rodríguez, Jaramillo, Guillén, et al., 2005), but lower than that found in the white spears, (0.7 mg FAD/g of cell wall material in fresh asparagus and 2.9 mg FAD/g of cell wall material in stored spears; Jaramillo et al., 2007). It is important to take into account that these authors, when working with green asparagus, analysed only the edible portion of the spears. HCA and FAD (monomers, dimers and trimers) especially are related to asparagus hardening during storage, *via* cross-linking of plant cell wall polymers (Rodríguez-Arcos, Smith, & Waldron, 2004). Asparagus by-products are harder and more fibrous than the edible portions, therefore, their HCA and FAD content must be higher than that of edible portions. One gram of these fibre-rich powders brings to the diet as much FAD as a serving of some fruits (berries, plums, and apples), vegetables (tomato, carrot, and lettuce), and beer (Zhao & Moghadasian, 2008). From a nutritional point of view, FADs have been considered to impede the degradation of polysaccharides by intestinal bacteria. Recent studies (Funk, Braune, Grabber, Steinhart, & Bunzel, 2007) indicate that low to moderate amounts (1.5–15.8 mg FAD/g of cell wall material) do not interfere with hydrolysis of cell walls by human gut microbiota. Additionally, FADs have high antioxidant activity (Chen & Ho, 1997) and many other potential health benefits (Plate & Gallaher, 2005). When linked to arabinoxylan oligosaccharides, they stimulate the growth of *Bifidobacterium bifidum* (Yuan, Wang, & Yao, 2005).

3.1.2. Saponins

Saponins are also present in the fibre-rich powders from asparagus by-products. These bioactive compounds are present in amounts between 2.14 and 3.64 mg/g. During intense treatments, the samples treated with ethanol had higher saponin content than those treated with water, but during gentle treatments, all the samples had almost the same saponin content. In general, fibres from gentle treatments had significantly lower saponin content than samples from intense treatments, probably due to a higher solubilisation of material in the latter. In this work, we quantified saponin amounts by spectrometry, so the results expressed here could be overestimated. However, previously reported data on the basal zone of asparagus shoots (Wang et al., 2003) by LC/MS (25 mg protodioscin/100 g of fresh sample) are in agreement with our results. Garlic and onion are two species of the *Allium* genus (*A. sativum* and *A. cepa*, respectively) widely used in folk medicine as hypotensive, hypoglycaemic, antimicrobial, antithrombotic, anticarcinogenic, and diuretic agents. These pharmacological activities are partially due to steroidal saponins. The content of this compound in Allium species is 2–3 mg/g of dry matter (Smoczkiewicz, Nitschke, & Wieladek, 1982), similar to the quantified amounts in the asparagus fibre-rich powders.

3.1.3. Flavonoids

Other bioactive compounds quantified in the asparagus by-product, fibre-rich powders are flavonoids. Asparagus flavonoids (mainly rutin) are partly responsible for the antioxidant characteristics of this vegetable (Guillén et al., 2008; Makris & Rossiter, 2001; Rodríguez, Jaramillo, Rodríguez, et al., 2005). Only three of the analysed samples (Table 2) had significant amounts of flavonoids. Makris and Rossiter (2001), working with green asparagus, concluded that simply chopping and macerating caused a decrease of as much as 18.5% in rutin content without the liberation of quercetin. Therefore, rutin must be oxidatively cleaved rather than hydrolysed. However, hydrolysis might occur to some extent, but the quercetin was oxidised as soon as it was liberated from the sugar, and, thus, did not accumulate in detectable amounts. The authors in the same work quantified a 43.9% decrease in total flavonols when asparagus was boiled for 60 min. Asparagus had high levels of peroxidase activity (Rodríguez, Guillén, Heredia, Fernandez-Bolaños, & Jiménez, 1999), with these enzymes having some specificity for flavonols (Hirota, Shimoda, & Takahama, 1998). As observed, several factors could affect the stability of flavonoids during vegetable processing. Additionally, in our processing experiences, another factor must be taken into account, since continuous stirring was applied during 90 min. of processing in an open reactor. Therefore, flavonol oxidation caused by air inclusion within the asparagus by-product homogenate may occur

Table 2
Bioactive components of different fibrous residues from asparagus by-products (mg/g dry matter).[a]

		Hydroxycinnamic acids[b]	Saponins	Flavonoids	Sterols	Fructans
I/W	O-D	2.81 ± 0.29a	2.50 ± 0.12a	–	0.82 ± 0.04a	–
	F-D	3.89 ± 0.31b	3.18 ± 0.33b	–	0.81 ± 0.01a	0.21 ± 0.03a
I/E	O-D	3.01 ± 0.04a	3.37 ± 0.01bc	0.64 ± 0.03a	1.03 ± 0.06b	1.24 ± .09b
	F-D	3.83 ± 0.10b	3.64 ± 0.33c	1.82 ± 0.31b	1.05 ± 0.01b	1.43 ± 0.25c
G/W	O-D	3.07 ± 0.23a	2.44 ± 0.07a	1.08 ± 0.09a	0.64 ± ± 0.09c	0.33 ± 0.04a
	F-D	4.91 ± 0.30c	2.56 ± 0.24a	–	0.63 ± ± 0.07c	0.26 ± 0.03a
G/E	O-D	2.31 ± 0.06d	2.14 ± 0.02d	–	0.83 ± 0.06a	0.20 ± 0.04a
	F-D	2.85 ± 0.01a	2.74 ± 0.01a	–	0.92 ± 0.05a	0.77 ± 0.19d

[a] All analyses are done in quadruplicate at least.
[b] Means within a column bearing the same letter are not significantly different at 5% level, as determined by the Duncan multiple range test.

to some extent. Presently, an optimised process (patent pending) is being applied to obtain fibre-rich powders from asparagus, in which most of the factors affecting flavonoid stability have been controlled. The relatively low content of flavonoids in these samples led to low levels of antiradical activity, which will be commented on below.

3.1.4. Sterols

Plant sterols are bioactive compounds present in asparagus spears and, therefore, in their by-products. In the fibre-rich powder, the amount of analysed phytosterol varied between 0.63 and 1.03 mg/g. The amount of phytosterol was affected by the solvent and intensity of treatment, but not by the drying system. Samples treated with ethanol had higher amounts of sterols than those treated with water. Samples obtained by gentle processing had lower amounts of sterols than those obtained from intense treatment. As seen in Fig. 1, β-sitosterol was the most abundant sterol (more than 50% of total quantified sterols), as is the case in most fruits and vegetables (Jiménez-Escrig et al., 2006).

3.1.5. Fructans

Fructans were also present in asparagus by-products and their amounts in the fibres varied with processing method, from 0.2 to 1.4 mg/g of fibre. The highest content was found in fibres obtained after intense treatment with ethanol (I/E), with the rest of the fibres having almost the same fructan content. Fructans present in asparagus were mainly fructooligosaccharides (FOS – with low polymerisation degree) instead of inulin (high molecular weight) (Shiomi et al., 2007). FOS content of asparagus by-product was around 4 mg/g of dry weight (data not shown) and during the fibre obtaining process, it decreased between 25% and 95%, a reduction that was explained by the high solubility of these low molecular weight carbohydrates (3–4 polymerisation degree) in water/ethanol mixtures, even at room temperature.

3.2. Antioxidant activity

To measure antioxidant activity, the antiradical capacity (ARC) against DPPH· was assayed in both the soluble fraction and total fibre. In Fig. 2, the dose–response lines are presented. The correlation between dose and decrease in DPPH· concentration was very high in all cases ($r > 0.9$). There are two fibres that have almost the same ARC; these were I/E, F-D and G/W, O-D fibres, which also had the highest content of flavonoids (Table 2). The I/E, O-D fibre also contained flavonoids and the ARC of its soluble extract (Subfigure (a)) was near those commented on above. Soluble extract from G/W, F-D fibre, in which flavonoids were not detected, had a lower ARC than extracts with these antioxidant compounds. However, looking at the total ARC of the fibres (Subfigure (b)) I/E, O-D and G/W, F-D fibres had the same ARC. This fact could be explained by the fact that although the latter fibre did not contain flavonoids, it had the highest amount of hydroxycinnamates (Table 2). These compounds, especially the ferulates, have a high antioxidant capacity and are mainly found esterified to fibre polymers in asparagus spears, therefore the ARC caused by ferulates could be measured only in the total antioxidant assay. The rest of the fibres (I/W and G/E) had similar ARC, which was much lower than those described above were.

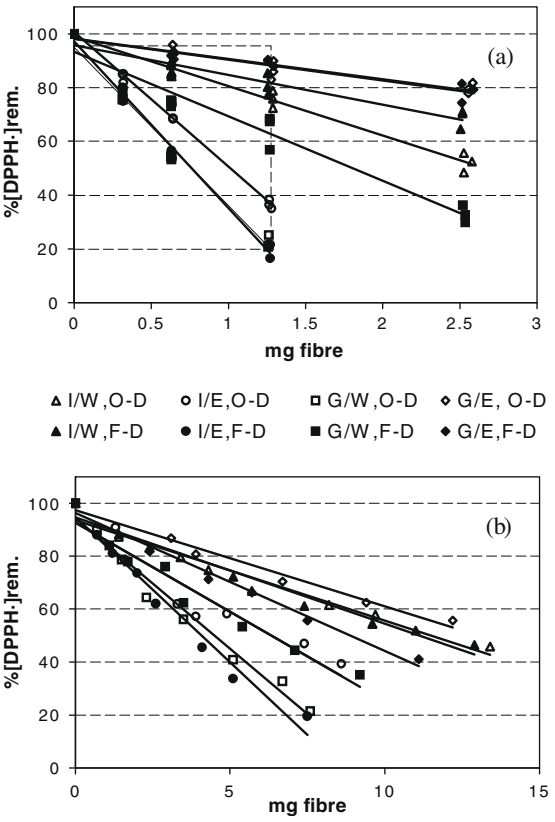

Fig. 2. Dose–response lines of antiradical capacity of asparagus by-product fibres. Antiradical capacity is expressed as percent DPPH· remaining in solution (%DPPH· rem.) after 30 min of reaction. (a) Antiradical capacity of soluble fractions. (b) Total antiradical capacity of fibres. Each individual point in the graphs is the average value of three replicates.

In Table 3, the results for antioxidant capacity expressed as Trolox equivalents/g of fibre (TEAC) are presented. The strongest antioxidant fibres (I/E, F-D, and G/W, O-D) had an equivalent of about 13 μmol Trolox/g of total fibre or 11 μmol Trolox/g of fibre (soluble extract). The second strongest ARC group (I/E, O-D and G/W, F-D) had about half of the activity listed above, and the rest at about one-third. Looking at the soluble activity only, there were differences within the last group, with G/E fibres having the lowest activity, nearly one tenth of that determined for the I/E, F-D and G/

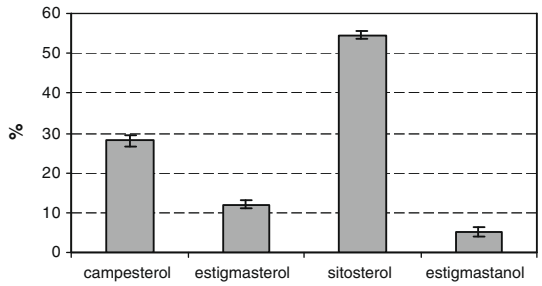

Fig. 1. Percentage contribution of each sterol to the total sterol quantified. Error bars correspond to the average value of the eight analysed samples.

Table 3
Antioxidant activity (total and soluble) of the fibre-rich asparagus powders, expressed as Trolox equivalents (μmol TE/g fibre).

		TEAC (soluble)	TEAC (total)
I/W	O-D	3.30	3.80
	F-D	2.05	3.96
I/E	O-D	5.16	7.29
	F-D	11.43	13.11
G/W	O-D	11.41	12.17
	F-D	5.33	7.50
G/E	O-D	1.33	3.12
	F-D	1.40	4.55

W, O-D fibres. The ARC of soluble extracts had a high correlation with the flavonoid content of fibres ($r = 0.8056$), but the total activity of fibres correlated better with ferulic acid derivatives plus flavonoids ($r = 0.8291$). The strongest antioxidant fibres from asparagus by-products are in the same range of activity as a fibre-rich product from cocoa bean husks (Lecumberri et al., 2007), and about one tenth of the ARC of fibres from different citrus by-products (Marín, Soler-Rivas, Benavente-García, Castillo, & Pérez-Álvarez, 2007), but they are much lower than fibre from guava fruit by-product (Jiménez-Escrig, Rincón, Pulido, & Saura-Calixto, 2001) and from red grape pomace and stem (Llobera & Cañellas, 2007). The difference must be due to a higher amount of polyphenols, in addition to the presence of other antioxidant compounds (condensed tannins and ascorbic acid).

4. Conclusions

Besides the chemical composition and functional characteristics (Fuentes-Alventosa et al., 2009), the bioactive profile of asparagus by-product fibres was affected by processing to obtain the powders. In general, intense treatments led to fibres with the highest content of bioactive compounds, especially those treated with ethanol, probably due to a concentration effect. The drying system had a clear effect only in the case of hydroxycinnamic acids, freeze-dried fibres having higher amounts than oven-dried ones. The most affected components were flavonoids, which are mainly responsible for the antioxidant capacity of the fibres. These compounds are oxidised very easily and were lost under most processing conditions of the fibres, decreasing the overall antioxidant capacity. Presently, we are working on an optimised process (patent pending) that maintains the maximum flavonoid content.

Although the content of bioactive compounds and the antioxidant capacity of these fibres were relatively low, it is important to remark that they are interesting sources of dietary fibres, which can be added as functional ingredients in fibre-enriched food. In comparison, other fibre sources such as cellulose, wheat bran, glucomannans, and others lack this intrinsic antioxidant activity and other bioactive compounds. These facts, together with the functional properties (water and oil holding capacities, and glucose retardation index); (Fuentes-Alventosa et al., 2009) and technical compatibility (Sanz, Salvador, Jiménez, & Fiszman, 2008) possessed by fibre from asparagus by-products, increases its attractiveness as an alternative source of dietary fibre. *In vivo* studies on the antioxidant status of asparagus fibre-fed rats and other physiological parameters are under way. These results could be decisive for the use of this added-value agricultural by-product.

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References

Chen, J. H., & Ho, C.-T. (1997). Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *Journal of Agricultural and Food Chemistry, 45*, 2374–2378.

Chin, C. K. (2006). Functional elements from asparagus for human health. In *Proceedings of the XI international asparagus symposium in acta horticulturae*.

Cushine, T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents, 26*, 343–356.

Fuentes-Alventosa, J. M., Jaramillo, S., Rodríguez-Gutiérrez, G., Cermeño, P., Espejo, J. A., Jiménez-Araujo, A., et al. (2008). Flavonoid profile of green asparagus genotypes. *Journal of Agricultural and Food Chemistry, 56*, 6977–6984.

Fuentes-Alventosa, J. M., Rodríguez, G., Cermeño, P., Jiménez, A., Guillén, R., Fernandez-Bolaños, J., et al. (2007). Identification of flavonoid diglycosides in several genotypes of asparagus from the Huétor-Tájar population variety. *Journal of Agricultural and Food Chemistry, 55*, 10028–10035.

Fuentes-Alventosa, J. M., Rodríguez-Gutiérrez, G., Jaramillo-Carmona, S., Espejo-Calvo, J. A., Rodríguez-Arcos, R., Fernández-Bolaños, J., et al. (2009). Effect of extraction method on chemical composition and functional characteristics of high dietary fibre powders obtained from asparagus by-products. *Food Chemistry, 113*, 665–671.

Funk, C., Braune, A., Grabber, J. H., Steinhart, H., & Bunzel, M. (2007). Moderate ferulate and diferulate levels do not impede maize cell wall degradation by human intestinal microbiota. *Journal of Agricultural and Food Chemistry, 55*, 2418–2423.

Guillén, R., Rodríguez, R., Jaramillo, S., Rodríguez, G., Espejo, J. A., Fernandez-Bolaños, J., et al. (2008). Antioxidant from asparagus spear: Phenolics. *Acta Horticulturae, 776*, 247–254.

Hibasami, H., Moteki, H., Ishikawa, K., Katsuzaki, H., Imai, K., Yoshioka, K., et al. (2003). Protodioscin isolated from fenugreek (*Trigonella foenumgraecum* L.) induces cell death and morphological change indicative of apoptosis in leukemic cell line H-60, but not in gastric cancer cell line KATO III. *International Journal of Molecular Medicine, 11*, 23–26.

Hirota, S., Shimoda, T., & Takahama, U. (1998). Tissue and spatial distribution of flavonol and peroxidase in onion bulbs and stability of flavonol glucosides during boiling of the scales. *Journal of Agricultural and Food Chemistry, 46*, 3497–3502.

Jaramillo, S., Rodríguez, R., Jiménez, A., Guillén, R., Fernandez-Bolaños, J., & Heredia, A. (2007). Effects of storage conditions on the accumulation of ferulic acid derivatives in white asparagus cell wall. *Journal of the Science of Food and Agriculture, 87*, 286–296.

Jiménez-Escrig, A., Rincón, M., Pulido, R., & Saura-Calixto, F. (2001). Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. *Journal of Agricultural and Food Chemistry, 49*, 5489–5493.

Jiménez-Escrig, A., Santos-Hidalgo, A. B., & Saura-Calixto, F. (2006). Common sources and estimated intake of plant sterols in the Spanish diet. *Journal of Agricultural and Food Chemistry, 54*, 3462–3471.

Kamat, J., Boloor, K., Devasagayam, P. A., & Venkatachalam, S. R. (2000). Antioxidant properties of *Asparagus racemosus* against damage induced by irradiation in rat liver mitochondria. *Journal of Ethnopharmacology, 71*, 425–435.

Larrauri, J. A., Rupérez, P., & Saura-Calixto, F. (1997). Mango peel fibres with antioxidant activity. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung, 205*, 39–42.

Lecumberri, E., Mateos, R., Izquierdo-Pulido, M., Rupérez, P., Goya, L., & Bravo, L. (2007). Dietary fibre composition, antioxidant capacity and physico-chemical properties of a fibre-rich product from cocoa (*Theobroma cacao* L.). *Food Chemistry, 104*, 948–954.

Llobera, A., & Cañellas, J. (2007). Dietary fibre content and antioxidant activity of Manto Negro red grape (*Vitis vinifera*): Pomace and stem. *Food Chemistry, 101*, 659–666.

Makris, D. P., & Rossiter, J. T. (2001). Domestic processing of onion bulbs (Allium cepa) and asparagus spears (*Asparagus officinalis*): Effect on flavonol content and antioxidant status. *Journal of Agricultural and Food Chemistry, 49*, 3216–3222.

Marín, F. R., Soler-Rivas, C., Benavente-García, O., Castillo, J., & Pérez-Álvarez, J. A. (2007). By-products from different citrus processes as a source of customized functional fibres. *Food Chemistry, 100*, 736–741.

Nijveldt, R. J., VanNood, E., VanHoorn, D. E., Boelens, P., VanNorren, K., & VanLeeuwen, P. (2001). Flavonoids: A review of probable mechanisms of action and potential applications. *American Journal of Clinical Nutrition, 74*, 418–425.

Plate, A. Y. A., & Gallaher, D. D. (2005). The potential health benefits of corn components and products. *Cereal Foods World, 50*, 305–314.

Rodríguez, R., Guillén, R., Heredia, A., Fernandez-Bolaños, J., & Jiménez, A. (1999). Postharvest changes on cell wall and peroxidases of white asparagus (*Asparagus officinalis* L.). *Acta Horticulturae, 479*, 477–482.

Rodríguez, R., Jaramillo, S., Guillén, R., Jiménez, A., Fernandez-Bolaños, J., & Heredia, A. (2005a). Cell wall phenolics of white and green asparagus. *Journal of the Science of Food and Agriculture, 85*, 971–978.

Rodríguez, R., Jaramillo, S., Rodríguez, G., Espejo, J. A., Guillén, R., Fernandez-Bolaños, J., et al. (2005b). Antioxidant activity of ethanolic extracts from several asparagus cultivars. *Journal of Agricultural and Food Chemistry, 53*, 5212–5217.

Rodríguez-Arcos, R., Smith, A. C., & Waldron, K. W. (2004). Ferulic acid cross-links in asparagus cell wall in relation to texture. *Journal of Agricultural and Food Chemistry, 52*, 4740–4750.

Sanz, T., Salvador, A., Jiménez, A., & Fiszman, S. M. (2008). Yogurt enrichment with functional asparagus fibre. Effect of extraction method on rheological properties, colour, and sensory acceptance. *European Food Research and Technology, 227*, 1515–1521.

Serpen, A., Capuano, E., Fogliano, V., & Gokmen, V. (2007). A new procedure to measure the antioxidant activity of insoluble food components. *Journal of Agricultural and Food Chemistry, 55*, 7676–7681.

Shiomi, N., Benkeblia, N., Onodera, S., Omori, T., Takahashi, N., Fukushima, M., et al. (2007). Saccharide and fructooligosaccharide contents, and invertase, 1-KHE, 1-SST, 1-FFT and 6G-FFT activities in green asparagus spears during storage: Effects of temperature and spear portion. *Journal of Applied Glycoscience, 54*, 187–194.

Smoczkiewicz, M. A., Nitschke, D., & Wieladek, H. (1982). Microdetermination of steroid and triterpene saponin glycosides in various plant materials. I. Allium species. *Mikrochimica Acta, II*, 43–53.

van den Heuvel, E. G. H. M., Muys, T., Dokkum, W. v., & Schaasfma, G. (1999). Oligofructose stimulates calcium absorption in adolescents. *American Journal of Clinical Nutrition, 69*, 544–548.

Wang, T.-S., Lii, C.-K., Chang, J.-Y., Kuo, C.-I., & Chen, H.-P. (2007). Anti-Fenton reaction activity of three taxa of water yam (*Dioscorea alata* L.). *International Journal of Food Science and Technology, 42*, 1107–1113.

Wang, M., Tadmor, Y., Wu, Q.-L., Chin, C.-K., Garrison, S. A., & Simon, J. E. (2003). Quantification of protodioscin and rutin in asparagus shoots by LC/MS and HPLC methods. *Journal of Agricultural and Food Chemistry, 51*, 6132–6136.

Wiboonpun, N., Phuwapraisirisan, P., & Tip-pyang, S. (2004). Identification of antioxidant compounds from *Asparagus racemosus*. *Phytotherapy Research, 18*, 771–773.

Yu, S., Chee-Kok, C., Chi-Tang, H., Wei, M., Garrison, S. A., Ho, C., et al. (1996). Antitumor activity of the crude saponins obtained from asparagus. *Cancer Letters, 104*, 31–36.

Yuan, X., Wang, J., & Yao, H. (2005). Feruloyl oligosaccharides stimulate the growth of *Bifidobacterium bifidum*. *Anaerobe, 11*, 225–229.

Zafar, T. A., Weaver, C. M., Zhao, Y., Martin, B. R., & Wasttney, M. E. (2004). Nondigestible oligosaccharides increase calcium absorption and supress bone resorption in ovariectomized rats. *Journal of Nutrition, 134*, 399–402.

Zhao, Z., & Moghadasian, M. H. (2008). Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review. *Food Chemistry, 109*, 691–702.