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Changes in the antioxidant activity and metabolite profile of three onion varieties during the elaboration of 'black onion'

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ABSTRACT

This study aims to investigate whether the heat treatment applied during the production of black onion, a novel derived product made from fresh onion, produces changes in the content of flavonoids, organosulfur compounds, organic acids, water soluble sugars and amino acids in three onion varieties ('Shallot', 'Chata' and 'Echalion'). The total flavonoid content decreased up to 12-fold in black onions compared with fresh onions while the quantities of isoalliin, the main organosulfur compound in black onions, drastically increased during the process. Moreover, the levels of fructose and glucose significantly increased during the elaboration process, contributing to the sweetness of black onions. The influence of heating on their antioxidant capacity showed a decreasing trend of the ORAC antioxidant activity of onion, while ABTS and DPPH did not show a clear tendency. These results present a comprehensive phytochemical characterization of black onions, highlighting the significant influence of the heating process on their phytochemical composition.

1. Introduction

The *Allium* genus belongs to the Liliaceae family, which includes garlic (*Allium sativum* L.), onion (*Allium cepa* L.), leeks (*Allium porrum* L.), chives (*Allium fistolosum* L.) and Shallots (*Allium ascalonicum* L.). All of these vegetables have been used historically for medicinal purposes for over 4000 years to treat a large number of conditions. Epidemiological evidence suggests that diets rich in *Allium* vegetables such as onion and garlic may decrease the risk of cancers (Nicastro, Ross, & Milner, 2015; Pourzand et al., 2016) and cardiovascular diseases (Bahadoran, Mirmiran, Momenan, & Azizi, 2017), in addition to protecting against the development of metabolic diseases such as diabetes (Akash, Rehman, & Chen, 2014). Moreover, there is substantial *in vitro* evidence showing that bioactive compounds present in onion or garlic have anti-inflammatory, anti-diabetic and anti-atherogenic properties (Santhosha, Jamuna, & Prabhavathi, 2013).

Onion in particular is a rich source of bioactive constituents, including flavonoids and organosulfur compounds, whose main components are thiosulfinates (aka alkane(ene) thial-S-oxide) and sulfur volatiles and which most of the biological health-promoting properties are attributed to. These compounds are formed upon damage or crushing of onions. After that, the enzyme alliinase is release from the vacuoles of cells and catalysed the cleavage of S-alk(en)yl-L-cysteine derivatives to sulfenic acid intermediates. These intermediates are highly reactive and rapidly produce thiosulfinate compounds via condensation reactions. The major onion thiosulfinate is isoalliin together with other precursor compounds such as S-methylcysteine sulfoxide (methiin) being precursors of a wide range of sensory-active and health-promoting compounds (Tapiero, Townsend, & Tew, 2004) including anti-inflammatory (Arreola et al., 2015), antimicrobial (Barba et al., 2014) and anti-obesity (Souza et al., 2011; Yoshinary, Shiojima, & Igarashi, 2012) effects. The subsequence condensation of sulfenic acid intermediates results into the formation of lachrymatory factor (thiopropanal S-oxide), thiosulfonates, bissulfines, sulphines including diallyl sulphide, diallyl disulphide and diallyl trisulfide, zwejebelanes, and cepaenes, all of which contribute to the flavour of onion (Nicastro et al., 2015) and have anti-cancer activity (Seki et al., 2008; Yang et al., 2006). Among flavonoids, high levels of quercetin and isorhamnetin glycosides have been found in onion (Lanzotti, 2006; Liguori et al., 2017).

Abbreviations: UHPLC-HRMS, ultra-high-performance liquid chromatography coupled to high resolution mass spectrometry; FA, formic acid; RH, Relative Humidity; DW, Dry Weight; GABA, Gamma-AminoButyric Acid.

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Onions are commonly consumed in prepared foods either fresh or after being subjected to a wide variety of cooking methods (Juániz et al., 2016). Among them, boiling, frying and griddling have been shown to induce significant changes in onion composition, bioactive compounds either being gained or lost, depending on the process (Kim, Lee, Shin, & Yoo, 2016). Moreover, one of the main advantages of cooking is to make onions more acceptable for consumers, eliminating their pungency and increasing their palatability and sweetness. In this sense, a novel commercially available product derived from onion, known as "black onion", has been developed by processing (aging) raw onion in a temperature- and humidity-controlled room without using any artificial additives. This aging process is a spontaneous fermentation for 30–60 days at 60–80 °C and high humidity (90% RH) (Chung, Kwon, Chung, & Chun, 2011). During the manufacturing process, a series of modifications to the compositional and sensory characteristics of the fresh product are produced. It has been shown that black onion differs significantly in terms of odour and aromas from the fresh onion, avoiding the pungency and burning characteristics of fresh onion mainly due to the decrease of different chemical families like sulfur and aromatic compounds, sulfur organic compounds and nitrogen oxides as stated by Wang et al. (Wang, Liu, Ma, Wang, & Zhao, 2015), with new aromas (like toffee and liquiorice) that are more palatable to many consumers. The final product has a more pleasing mouthfeel with fruit-like sweetness and improved organoleptic properties with less or non-stimulating smell compared with fresh onion (Ríos-Ríos, Montilla, Olano, & Villamiel, 2019). Black onion is considered a gourmet product that is consumed either directly as an aperitif or used in the haute cuisine for the preparation of sauces and seasoning, especially meats, salads and desserts. Moreover, due to the treatment, black onion increases its shelf-life avoiding spoilage during storage, a common issue in fresh vegetables. Likewise, previous studies have shown that black garlic, an analogous aged food product, presents higher nutritional characteristics than the fresh product (Jung et al., 2014). However, up to date there is no information about the nutritional and phytochemical composition of the aged black onion, available in the markets for the consumers. To the best of our knowledge there is only one study dealing with discrimination between onion samples at different processing times based on the content of volatile sulfur compounds (Wang, 2015).

The aim of this study was to determine the impact of the heat treatment on antioxidant activity and on flavonoids, organosulfur compounds, amino acids, organic acids and sugars profiles during the production of black onion from three different varieties of raw onion: 'Shallot', 'Chata' and 'Echalion'.

2. Material and methods

2.1. Chemicals

The reference flavonoid compounds rutin, luteolin, (–)-epicatechin, quercetin-3-*O*-glucoside, apigenin, quercetin, kaempferol-3-*O*-rutinoside, isorhamnetin, and the amino acids glycine, glutamic acid, leucine, isoleucine, proline, methionine, alanine, tyrosine, asparagine, lysine, histidine, ornithine, glutamine, serine, threonine, phenylalanine, tyrosine, aspartic acid, gamma-aminobutyric acid (GABA), arginine, valine and tryptophan were purchased from Sigma-Aldrich (Madrid, Spain). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), pyruvic, citric, quinic, succinic, fumaric, salicylic, oxalic, shikimic, malic, tartaric and ascorbic acids were obtained from Sigma-Aldrich (Madrid, Spain). Alliin, s-allyl-L-cysteine (SAC) together with sucrose, maltose, glucose and fructose, and formic acid were acquired from Sigma-Aldrich (Madrid, Spain). Ammonium formate, ammonium acetate and ethanol were obtained from Sigma-Aldrich. Acetronitrile and methanol were of LC-MS grade. Potassium phosphate monobasic was purchased from Panreac Química (Barcelona, Spain).

2.2. Materials and sample preparation

Ten kg of authenticated 'Shallot', 'Chata' and 'Echalion' onion varieties were obtained from a local supplier. The manufacturing process of black onion was carried out at IFAPA's facilities (Palma del Rio, Córdoba, Spain). These three onion varieties were selected based on the surface-volume ratio of the onion bulbs and their similar relative sizes, which makes the elaboration process of black onion more efficient. The heat equipment consisted of a food warming and heated holding cabinet (Edesa Industrial S.L., Mondragón, Spain) and the dryer equipment consisted of an ECO EVO drier (Tred Technology S.R.L., Ripalimosani, Italy). The manufacturing process involved the following stages: i) the onions were checked for defects such as injuries, scars or rots. Once the onions were deemed suitable, preparatory conditioning was carried out consisting of cleaning the outer layers and cutting the basal plate of the onion. ii) Heating process: in this phase, the bulbs were subjected to relative humidity conditions close to saturation (90% RH) and at a temperature between 65 and 70 °C in the presence of oxygen (20.9%) for 28 days. To study the evolution during the heating process, 0.5 kg of onion were taken at T0 (initial stage) and every 7 days until day 28: T1 (after 7 days), T2 (after 14 days), T3 (after 21 days) and T4 (after 28 days). A final phase involved applying a drying process to the T3 onion samples. The drying was performed with dehumidified hot air. To this end, the drier equipment first cooled the air to condense its humidity (final relative humidity of ~15%) and then the air was heated to 50 °C. This hot air was sent to the onion samples at high speed thought a turbine. The onion bulbs are dried after 24 h under these conditions. The samples from this stage are named T4S samples. Both the heating and the drying process of the three onion varieties were run in triplicate. All the onion samples were grinded to a final particle size of 10 μm using a cryogenic grinder with liquid nitrogen mill equipment (Freezer Mill model 6870, Fisher Scientific, Waltham, MA USA) and stored at −80 °C until analysis.

2.3. Analysis of flavonoids, amino acids and organosulfur compounds

Onion samples were extracted following the previously optimized procedure reported (Moreno-Rojas et al., 2018). Changes in (poly)phenols, amino acids and organosulfur compounds during the heating process were monitored using an UHPLC-PDA-MS system (Thermo Scientific, San José, CA, USA). The analysis of flavonoids, amino acids and organosulfur compound was carried out following a previously validated methodology (Moreno-Rojas et al., 2018). Figs. S1–S5 and S8, in Supported Information showed typical UHPLC-HRMS chromatograms of flavonoids, amino acids and organosulfur compounds in onion samples.

2.4. Extraction of organic acids

The extraction method followed the one previously described by Cuevas et al. (2015) with some modifications. 0.5 g of onion sample was mixed with 1 mL of deionized water with 3% phosphoric acid for 2 min at room temperature. The mixture was sonicated for 10 min and then centrifuged at 15,000 rpm for 15 min. The supernatant was collected and residues were re-extracted twice using 1 mL of the same solvent following the same protocol described previously. All the supernatants were pooled together and frozen at −80 °C until UHPLC-DAD analysis.

2.5. Analysis of organic acids

Organic acids were analysed by using an UHPLC-DAD (Thermo Scientific, San José, CA, USA) consisting of a HPLC pump, a DAD detector and an autosampler operating at 4 °C. The separation of organic acids

was performed on a 250 × 4.6 mm i.d. Synergi 4 μm Hydro-RP column (Phenomenex) and maintained at 22 °C. The mobile phase, 20 mM potassium phosphate, was pumped for 20 min at a flow rate of 0.7 mL/min using an isocratic method. The organic acids were identified by comparing the retention time and the maximum wavelength with pure organic acid standards at 254 nm for ascorbic acid and 220 nm for the rest of organic acids. Quantification was performed by reference to 0.1–100.0 mg/L calibration curves of their respective standards. The citric and fumaric acids co-eluted under this chromatographic condition and were quantified together. Fig. S6 in Supported Information showed a typical UHPLC-DAD chromatogram of organic acids in raw and black onion samples.

2.6. Extraction of simple sugars

Onion samples were extracted following the method previously described by Shanmugavelan et al. (2013) adapted to our samples: 0.5 g of sample was mixed with 1 mL of deionized water:ethanol (20:80, v/v) for 2 min at room temperature and the mixture was sonicated for 15 min and then centrifuged at 15,000 rpm for 15 min. The supernatant was collected and the residues were re-extracted twice using 1 mL of the same solvent following the same protocol previously described. All the supernatants were pooled together and frozen at −80 °C until HPLC-RID analysis.

2.7. Analysis of simple sugars

The identification and quantification of glucose, fructose and sucrose in onion samples were carried out using an HPLC-RID system (Perkin Elmer, Waltham, MA, USA) consisting of an HPLC pump, a RID detector, and an autosampler operating at 4 °C. The separation of the simple sugars was performed on a 250 × 4.6 mm i.d. Luna 5 μm NH_2 column (Phenomenex) and maintained at 40 °C. The mobile phases, A: deionized water and B: acetonitrile, (20% phase A–80% phase B) were pumped at a flow rate of 1.5 mL/min using an isocratic method for 15 min. The sugars were identified by comparing the retention times with pure reference standards. Quantification was performed by reference to the 0.3–50.0 mg/mL calibration curves of fructose and 0.3–10.0 mg/mL of glucose and sucrose. Fig. S6 in Supported Information showed a typical HPLC-IR chromatogram of simple sugars in onion samples.

2.8. Antioxidant activity by ABTS assay

The free radical scavenging activity was measured using the ABTS decolouration method (Re et al., 1999) with some modifications (Madrona, Pereira-Caro, Bravo, Mateos, & Espartero, 2011). The antioxidant activity was expressed as μM of Trolox equivalents per gram of fresh sample (μM TE/g FW). Each value is the average of three determinations.

2.9. Antioxidant activity by DPPH assay

Free radical DPPH (1,1-diphenyl-2-picryl-hydrazyl) scavenging capacity was determined by using the previously described methods Sánchez-Moreno, Larrauri, and Saura-Calixto (1998). The antioxidant activity was expressed as μM of Trolox equivalents per gram of fresh sample (μM TE/g FW). Each value is the average of three measurements.

2.10. Antioxidant activity by ORAC assay

The oxygen radical scavenging activity was measured by the ORAC assay according to the method previously published by Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002) and modified by Madrona et al. (2011). The final results were calculated according to Madrona et al. (2011). ORAC values are expressed as mM Trolox equivalents per gram of fresh sample (mM TE/g FW).

2.11. Statistical analysis

Statistical analyses were performed on the basis of three analytical replicates measured on each sample. A one-way ANOVA was carried out to assess for significant differences (significant model was accepted for a p-value <0.05) using the SPSS Statistic Program (v. 22). Next, Fisher's LSD pairwise comparison was performed on the data. A two-way ANOVA was used to compare the mean differences in the antioxidant activity during the heating process and to understand if there is an interaction between the heating process and the onion varieties using R software (v.3.5.0). Principal Component Analysis (PCA) was carried out as an unsupervised method using SIMCA software (v.15.0.2) to evaluate whether the changes on the profiles of flavonoids, organosulfur compounds, amino acids, organic acids and sugars were different enough to distinguish among process stages and onion varieties.

3. Results and discussion

3.1. Changes in flavonoids Profile.

A total of seven flavonoids were identified and quantified in the onion samples. Quercetin and quercetin derivatives including quercetin-3-*O*-glucuronide and two isomers of quercetin-diglucuronide were the main flavonoids in raw onion varieties (T0), accounting for 95.7, 97.2 and 97.6% of the total flavonoids in 'Shallot', 'Chata' and 'Echalion' varieties, respectively (Table 1). These results are in line with those reported by Juániz et al. (2016), who demonstrated that 90% of total flavonoids in raw onion were quercetin and quercetin derivatives. Other minor compounds such as isorhamnetin, luteolin and isorhamnetin glucoside were detected (Table 1). It is noteworthy that the 'Shallot', 'Chata' and 'Echalion' onion varieties showed similar concentrations of flavonoids. During the production of black onion (T4), the initial flavonoid content of the three onion varieties decreased up to 6-fold, 12-fold and 6-fold for 'Shallot', 'Chata' and 'Echalion' varieties, respectively. In general, the biggest differences were observed when comparing samples from T0 and T1, and further T1 and T2 intervals (Table 1). Of note is that isorhamnetin content increased in the T1 stage (7 days), arguably due to the hydrolysis of isorhamnetin glucosides during the heat treatment, and then decreased again in T2 (14 days) in all varieties. In stage T4S, quercetin was the major flavonoid present in the black onion samples, accounting for 93.7, 98.8 and 99.2% of the total flavonoid content in the 'Shallot', 'Chata' and 'Echalion' onion varieties, respectively. As Table 1 shows, 'Echalion' black onion showed a higher concentration of flavonoids with 41.10 mg/100 g DW, followed by the 'Shallot' variety with 36.80 mg/100 g DW. 'Chata' black onion had the lowest concentration (19.71 mg/100 g DW).

The significant decrease in flavonoid content during the production of black onion can be explained considering the heat conditions and incubation time. Cooking or heat treatments of onion samples has been shown to produce a significant increase in the concentrations of flavonoids in the final product since these treatments were performed for short periods of time. For instance, Sharma et al. (2015) evaluated the effect of different heating treatments on the flavonoid content of six onion varieties. They observed a positive effect of heat treatment on the total flavonoid content in five onion varieties when the maximum temperature was 120 °C. When heat treatment reached 150 °C, the total flavonoid content decreased drastically. Although the temperature used during black onion production was less aggressive than in other studies, the heat treatment was prolonged over time, thus affecting the final content of flavonoids of the black onion. Moreover, the losses in the phenolic content during the production of black onion are

Table 1
Concentration (mg/100 g DW) of flavonoids presented in onion samples at different stages during the production of black onion for three varieties (Shallot, Chata and Echalion). Data is expressed as mean values (n = 6).

Compounds	T0	T1	T2	T3	T4	T4S
	Shallot' variety					
Quercetin	91.08 [a]	61.01 [b]	29.17 [d]	34.41 [d]	42.64 [c]	34.46 [d]
Isorhamnetin	3.31 [c]	9.51 [a]	4.17 [b]	2.53 [d]	1.61 [e]	1.81 [de]
Quercetin-3-O-glucoside	19.29 [a]	1.84 [b]	1.81 [b]	0.80 [c]	0.66 [c]	0.52 [c]
Luteolin	1.83 [a]	nq	nq	nq	nq	nq
Quercetin diglucoside	46.27 [a]	nq	nq	nq	nq	nq
Quercetin-4-*O*-glucoside	65.93 [a]	59.62 [b]	3.17 [c]	0.48 [c]	0.54 [c]	nq
Isorhamnetin-4́-*O*-glucoside	4.91 [a]	5.42 [a]	nq	nq	nq	nq
Total	**232.62 [a]**	**137.40 [b]**	**38.32 [c]**	**38.21 [c]**	**45.46 [c]**	**36.80 [c]**
	Chata' variety					
Quercetin	75.33 [a]	42.12 [b]	27.71 [c]	14.62 [d]	20.50 [cd]	19.47 [d]
Isorhamnetin	0.31 [d]	6.14 [b]	2.34 [c]	6.71 [a]	1.96 [c]	0.24 [d]
Quercetin-3-*O*-glucoside	8.56 [a]	0.72 [b]	0.44 [b]	0.20 [b]	nq	nq
Luteolin	1.96 [a]	0.26 [b]	nq	nq	nq	nq
Quercetin diglucoside	57.28 [a]	nq	nq	nq	nq	nq
Quercetin-4-*O*-glucoside	95.07 [a]	11.07 [b]	nq	nq	nq	nq
Isorhamnetin-4́-*O*-glucoside	4.64 [a]	nq	nq	nq	nq	nq
Total	**243.15 [a]**	**60.31 [b]**	**30.49 [c]**	**21.54 [c]**	**22.46 [c]**	**19.71 [c]**
	Echalion' variety					
Querctin	119.70 [a]	93.82 [b]	54.53 [c]	19.10 [e]	30.54 [de]	40.76 [cd]
Isorhamnetin	2.26 [c]	11.46 [a]	4.01 [b]	1.62 [c]	nq	nq
Quercetin-3-*O*-glucoside	13.19 [a]	0.85 [b]	1.51 [b]	0.34 [b]	0.28 [b]	0.34 [b]
Luteolin	0.88 [a]	nq	nq	nq	nq	nq
Quercetin diglucoside	37.48 [a]	nq	nq	nq	nq	nq
Quercetin-4-*O*-glucoside	74.64 [a]	19.32 [b]	3.40 [c]	nq	nq	nq
Isorhamnetin-4́-*O*-glucoside	3.00 [a]	nq	nq	nq	nq	nq
Total	**251.14 [a]**	**125.45 [b]**	**63.45 [c]**	**21.06 [d]**	**30.82 [d]**	**41.10 [cd]**

Different letters (one-way ANOVA) denote significant differences ($p < 0.05$) among the five stages for the same compound.

argue attributed to the oxidation of flavonoids to semiquinoid intermediates and the respective quinones, which normally react further with other quinones to produce dark melanin pigments (Friedman, 1996) or with proteins to produce dark polymers (Kroll, Rawel, & Rohn, 2003).

3.2. Changes in the amino acid profile

A total of 21 amino acids were identified and quantified in the onion samples. Arginine, glutamine, asparagine and glutamic acid were the main amino acids in the raw onion varieties (T0), accounting for the 80.5, 80.1 and 72.0% of the total amino acids in 'Shallot', 'Chata' and 'Echalion' varieties, respectively (Table S1, Supporting Information). These results are in line with those reported by Hansen (2001), who reported that glutamine, arginine, asparagine, glutamic acid and lysine are the main amino acids present in different parts of onions bulbs. The results from our study showed that arginine was the major amino acid in black onion for the three varieties, accounting for the 41.6, 40.1 and 31.1% of the total amino acid content, respectively. Black onions made with the 'Shallot' and 'Echalion' varieties showed lower concentrations of total amino acids compared with the 'Chata' variety (Table S1, Supporting Information).

Some specific compounds comprising phenylalanine, leucine, isoleucine, GABA, valine, proline, alanine, glycine, serine, aspartic acid and ornithine showed an increase during the production process of black onion (Table S1, Supporting Information). A similar behaviour was observed during the production of black garlic (Molina-Calle, de Medina, Priego-Capote, & de Castro, 2017). In agreement with those results, Kimura et al. (2017) showed that during the production of black garlic the amino acids leucine, isoleucine and phenylalanine increased 1.06-fold, 1.67-fold and 2.43-fold, respectively. On the contrary, there are some other amino acids, such as tryptophan, methionine, glutamic acid and glutamine, whose content decreased during the black onion production (Table S1, Supporting Information). Our results are in line with those found by Molina-Calle et al. (2017), who observed an important decrease in the levels of tryptophan during black garlic production. The three varieties showed a significant increase in GABA concentration during the heating process, 2-fold, 4-fold and 4-fold for the 'Shallot', 'Chata' and 'Echalion' varieties, respectively. GABA is a non-protein amino acid with health benefits that has a role in decreasing hypertension levels and cancer cell proliferation (Yoshimura et al., 2010; Oh & Oh, 2004). In addition, the important decrease in the glutamine content (more than 99% for the three varieties) observed in our study could be explained taking into account that free glutamine, together with asparagine and glutamic acid, are the major precursors of brown products in the Maillard reaction and are responsible for the dark appearance of black onion (Niquet & Tessier, 2007).

3.3. Changes in the organosulfur compound profile

A total of 27 organosulfur compounds were identified and quantified in onion samples. γ-Glutamyl-S-(1-propenyl) cysteine sulfoxide, γ-Glutamyl-S-(2-propenyl) cysteine sulfoxide, Isoalliin and γ–Glutamyl-S-(1-propenyl) cysteine are the main organosulfur compounds in raw onion varieties (T0), accounting for the 66.3, 53.8 and 53.7% of the total organosulfur compounds in 'Shallot', 'Chata' and 'Echalion' varieties, respectively (Table S2, in Supporting Information). These results are in line with those reported by Wiczkowski (2011), who reported that isoallin is the predominant flavour precursor in onion. Isoallin was also found to be the major organosulfur compound in black onion for the three varieties studied, accounting for 83.6, 82.4 and

83.8% of the total organosulfur compound content, respectively (Table S2, in Supporting Information).

A significant increase was observed in the concentration of organosulfur compounds between T0 and T1 for all varieties (24.4, 41.4 and 48.4% increase for 'Shallot', 'Chata' and 'Echalion' varieties respectively). Subsequently, a gradual decrease in the total concentration of these compounds was observed up to T4, this being more noticeable in the case of the 'Echalion' variety (Table S2, Supporting Information). During the heat treatment, the total content of organosulfur compounds showed a decrease of 31.2% for 'Shallot' variety, while the 'Chata' and 'Echalion' varieties showed an increase of 24.8 and 25.7% in the total content of organosulfur compounds, respectively. At T4S in particular, isoallin was the organosulfur compound with the highest concentration for all varieties, accounting for more than 80% of the total content of organosulfur compounds (703.55 mg/100 g DW, 1360.12 mg/100 g DW, and 1242.68 mg/100 g DW in 'Shallot', 'Chata' and 'Echalion' respectively). Furthermore, the process has an important impact on γ-glutamil-S-(1-propenyl)-L-cysteine sulfoxide (Fig. 1). This compound decreased 10-fold during the whole heating process for 'Shallot', 4-fold for 'Echalion' and 3-fold for the 'Chata' samples. Similar data were found for cycloalliin and γ-glutamil-S-(1-propenyl)-L-cysteine by Molina-Calle et al. (2017) during the production of black garlic. These authors suggested that the decrease in the organosulfur compounds during the heat treatment could be due to the formation of intermediate compounds such as thiosulfinates and the subsequent transformation to organosulfur volatiles (diallyl disulfides and diphenyl disulfides) (Molina-Calle, Priego-Capote, & Luque de Castro, 2017; Yu, Wu, Rosen, Hartman, & Ho, 1994).

3.4. Changes in the organic acid profile

The most abundant organic acids found in the raw onion samples were malic, tartaric and oxalic acids, which were present in the largest amounts in the T0 samples. The concentration of these organic acids increased significantly from T0 to T4S samples (Table 2), while the remaining organic acids did not present a clear trend during black onion production. In the last stage of the process (T4S), tartaric acid was the major organic acid quantified in the black onions, followed by malic acid (Table 2). The two organic acids accounted for 93.3% of the total content of organic acids in 'Shallot', 87.5% of the total content in

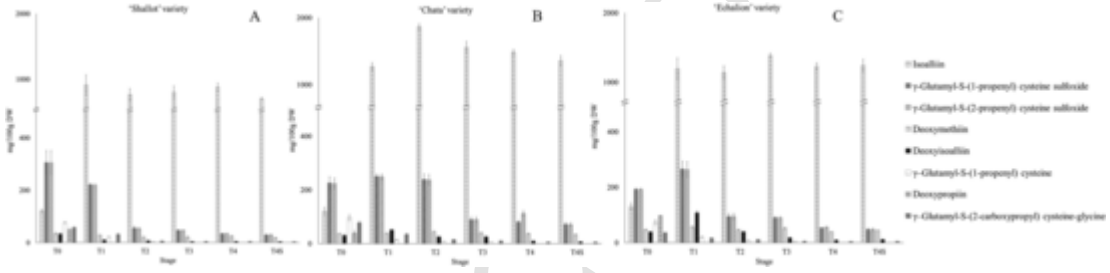

Fig. 1. Changes in organosulfur compound content during the production process of black onion for (A) raw 'Shallot' onion, (B) raw 'Chata' onion and (C) raw 'Echalion' onion. Data are expressed as mg/100 g DW as mean values (n = 3). Different letters (one-way ANOVA) denote statistically significant differences between the five stages.

Table 2
Concentration (mg/100 g DW) of organic acids presented in onion samples at different stages during the production of black onion for three varieties (Shallot, Chata and Echalion). Data is expressed as mean values (n = 6).

Compounds	T0	T1	T2	T3	T4	T4S
	Shallot' variety					
Oxalic Acid	131.00 ^{ab}	142.23 ^{a}	100.25 ^{c}	98.40 ^{c}	116.08 ^{b}	92.25 ^{c}
Tartaric Acid	236.21 ^{e}	2151.24 ^{a}	1645.54 ^{cd}	1549.31 ^{d}	1980.22 ^{b}	1717.82 ^{c}
Pyruvic Acid	19.94 ^{a}	15.54 ^{b}	12.28 ^{c}	9.97 ^{c}	19.83 ^{a}	16.32 ^{b}
Malic Acid	994.91 ^{c}	1685.51 ^{a}	1229.77 ^{b}	1213.97 ^{b}	1327.48 ^{b}	1012.86 ^{c}
Citric + Fumaric Acids	60.23 ^{a}	31.17 ^{b}	15.28 ^{e}	20.36 ^{d}	26.27 ^{c}	21.93 ^{d}
Succinic Acid	64.30 ^{a}	63.23 ^{a}	50.67 ^{b}	48.66 ^{b}	52.95 ^{b}	66.73 ^{a}
Total	**1506.59** ^{d}	**4088.91** ^{a}	**3053.79** ^{c}	**2940.67** ^{c}	**3522.83** ^{b}	**2927.92** ^{c}
	Chata' variety					
Oxalic Acid	364.46 ^{a}	219.58 ^{d}	282.25 ^{b}	240.92 ^{c}	238.43 ^{c}	240.04 ^{c}
Tartaric Acid	438.32 ^{c}	1933.63 ^{b}	2905.86 ^{a}	2936.68 ^{a}	2651.35 ^{a}	2869.62 ^{a}
Pyruvic Acid	52.39 ^{b}	53.31 ^{b}	62.90 ^{a}	31.21 ^{d}	43.35 ^{c}	27.60 ^{d}
Malic Acid	622.11 ^{a}	508.79 ^{a}	612.63 ^{a}	523.03 ^{a}	566.00 ^{a}	538.79 ^{a}
Citric + Fumaric Acids	52.05 ^{b}	42.01 ^{c}	32.69 ^{d}	34.75 ^{d}	72.41 ^{a}	28.91 ^{d}
Succinic Acid	124.78 ^{d}	117.97 ^{d}	225.08 ^{a}	227.97 ^{a}	167.19 ^{c}	189.57 ^{b}
Total	**1654.11** ^{c}	**2875.30** ^{b}	**4121.41** ^{a}	**3994.56** ^{a}	**3738.72** ^{a}	**3894.53** ^{a}
	Echalion' variety					
Oxalic Acid	101.44 ^{d}	171.66 ^{a}	118.40 ^{b}	112.91 ^{bc}	102.78 ^{cd}	115.47 ^{b}
Tartaric Acid	278.28 ^{c}	2708.32 ^{a}	2336.01 ^{b}	2826.94 ^{a}	2493.95 ^{b}	2827.04 ^{a}
Pyruvic Acid	1.33 ^{e}	4.74 ^{a}	4.42 ^{b}	3.61 ^{c}	3.22 ^{d}	4.77 ^{a}
Malic Acid	1781.34 ^{a}	1388.81 ^{c}	1042.31 ^{d}	1535.16 ^{bc}	1359.43 ^{c}	1574.68 ^{b}
Citric + Fumaric Acids	32.53 ^{b}	22.01 ^{d}	19.47 ^{e}	19.77 ^{e}	45.55 ^{a}	30.12 ^{c}
Succinic Acid	84.78 ^{a}	9.38 ^{e}	36.50 ^{d}	55.41 ^{c}	52.42 ^{c}	66.07 ^{b}
Total	**2279.70** ^{d}	**4304.91** ^{ab}	**3557.10** ^{c}	**4553.80** ^{a}	**4057.34** ^{b}	**4618.15** ^{a}

Different letters (one-way ANOVA) denote significant differences ($p < 0.05$) among the five stages for the same compound.

'Chata' and 95.3% of the total content in 'Echalion'. These results are in line with those obtained by Colina-Coca, de Ancos, and Sánchez-Moreno (2014) in the first stage of the heat process. In addition, regarding the pyruvic acid content, our three varieties of black onion samples are classified as low pungency, according to Dhumal, Datir, and Pandey (2007).

3.5. Changes in the sugar profile

The total sugars content in the black onions of the three varieties studied increased significantly with regard to the fresh onions. More specifically, a significantly increase in fructose levels was found in the black onions compared with raw onions in all the varieties, representing up to 10-fold, 2-fold and 5-fold increases for the 'Shallot', 'Chata' and 'Echalion' varieties, respectively (Fig. 2). In general, the fructose content increased greatly from T0 to T2, with a concomitant decrease in sucrose levels, and from then on the amount of fructose was constant until the end of the production process. Similar behaviour was observed for the glucose content in all the onion varieties (Fig. 2). These results are in line with those obtained by Martínez-Casas et al. (2016), who showed a 10-fold increase in fructose content during black garlic production. The substantially increased levels of fructose and glucose in black onions are arguably due to the potential hydrolysis of fructans during de thermal process of black onion production (Yuan, Sun, Chen, & Wang, 2016). In addition, the sweetness of fructose is more than twice that of glucose and 1.7 times that of sucrose. These results are further evidence that the sweetness of black onion is mainly due to the increase in the level of fructose during the heat treatment (Yuan, Sun, Chen, & Wang, 2018; Zhang et al., 2015). The increase in glucose content during the process may also contribute to a greater or lesser extent to the characteristic sweetness of black onion (Yuan et al., 2018; Zhang et al., 2015; Edwards, Rossi, Corpe, Butterworth, & Ellis, 2016).

It is worth noting that at the beginning of the process, the 'Chata' variety presented much higher glucose and fructose contents than the 'Shallot' and 'Echalion' varieties. During thermal processing, the increase in these simple sugars was more marked in the 'Shallot' and 'Echalion' varieties, which could lead to an effect on their final taste that conditions the final sensorial acceptance of the product.

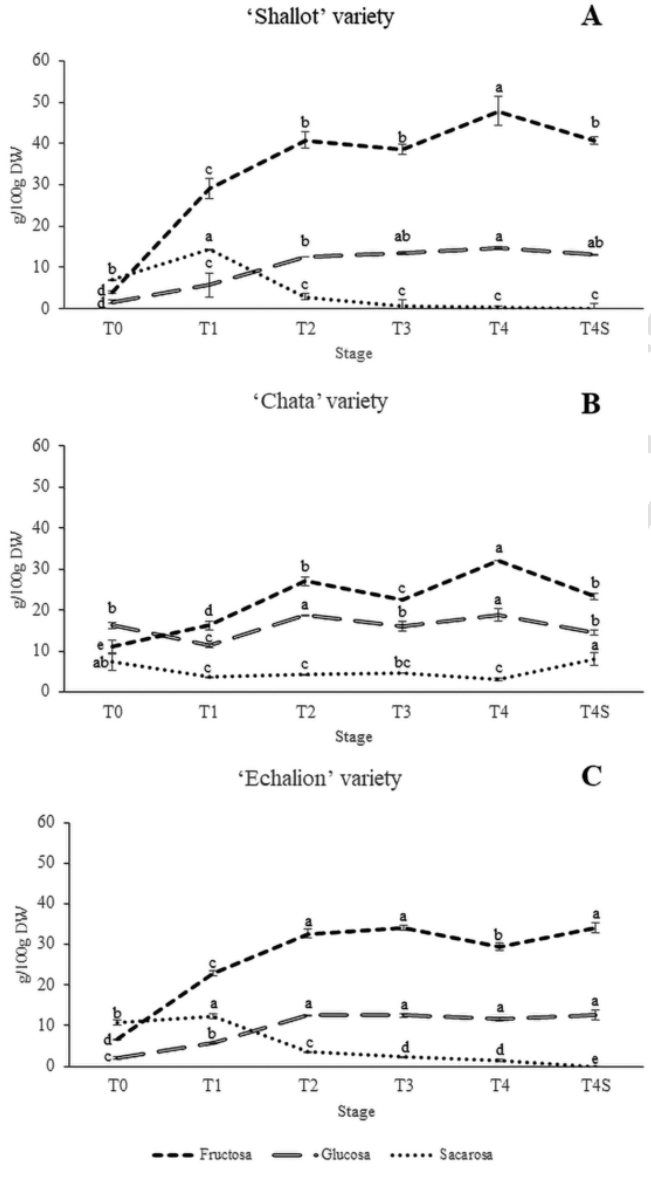

Fig. 2. Evolution of simple sugars during production process of black onion for (A) raw 'Shallot' onion, (B) raw 'Chata' onion and (C) raw 'Echalion' onion. Data are expressed as g/100 g DW as mean values (n = 3). Different letters (one-way ANOVA) denote statistically significant differences between the five stages.

3.6. Changes in the total antioxidant capacity

In order to evaluate the changes in the antioxidant capacity of black onion during different stages of the production process, three different antioxidant activity measurements were performed: ABTS, DPPH and ORAC assays. At time T0, the 'Shallot' and 'Echalion' onions showed higher antioxidant activity measured by ABTS and ORAC assays than the 'Chata' variety. Meanwhile, the DPPH assay showed that the three varieties presented similar antioxidant activity (Fig. 3A and B). With regard to the heating process, the three varieties showed different trends with regard to antioxidant activity measured by ABTS. The 'shallot' variety showed a decrease in the antioxidant activity from T0 to T1, reaching the initial value at T2 and then remaining stable to T4S. Meanwhile, the 'Chata' variety showed a significant increase in the ABTS values from T0 to T2, and then these values decreased up to T4S. In general, the antioxidant activity measured by the ABTS assay did not show significant differences between the T0 and T4S stages for the 'Shallot' and 'Chata' varieties. In the case of the 'Echalion' variety, the antioxidant activity gradually increased from T0 to T4S, showing a significant difference if the T0 stage is compared with T4S.

DPPH assays showed a marked decrease in the antioxidant activity values from the initial stage (T0) up to T1 for the 'Shallot' and 'Chata' varieties. From T1 to T2, the DPPH values showed an increase and remained stable until T4 and T3 for the 'Shallot' and 'Chata' varieties, respectively. From these stages, the DPPH values significantly decreased up to the final stage. In general, the antioxidant activity measured by DPPH assay decreased from T0 to T4S for the 'Shallot' and 'Chata' varieties. It is noteworthy that the 'Echalion' variety did not show important variations in the DPPH values during the heating treatment.

The ORAC assays showed an appreciably decrease in the antioxidant activity from the initial stage (T0) up to T1 for the three varieties. From T1 to T4, although there were slight oscillations in the antioxidant activity values, they all remained under the values of the initial stage (T0). In general, the antioxidant activity by ORAC assay greatly decreased from T0 to T4S in all the varieties.

It is worth mentioning that the differences in the results from the three antioxidant activity assays (ORAC, ABTS and DPPH) could be explained taking into account the chemistry principles upon which the antioxidant assays are based (Ou et al., 2002). For example, the ORAC assay typically measures peroxyl-radical scavenging with a more relevant significance, while ABTS and DPPH involve organic radicals who presented large compound with steric issues (Craft, Kerrihard, Amarowicz, & Pegg, 2012). Our findings are in agreement with those of

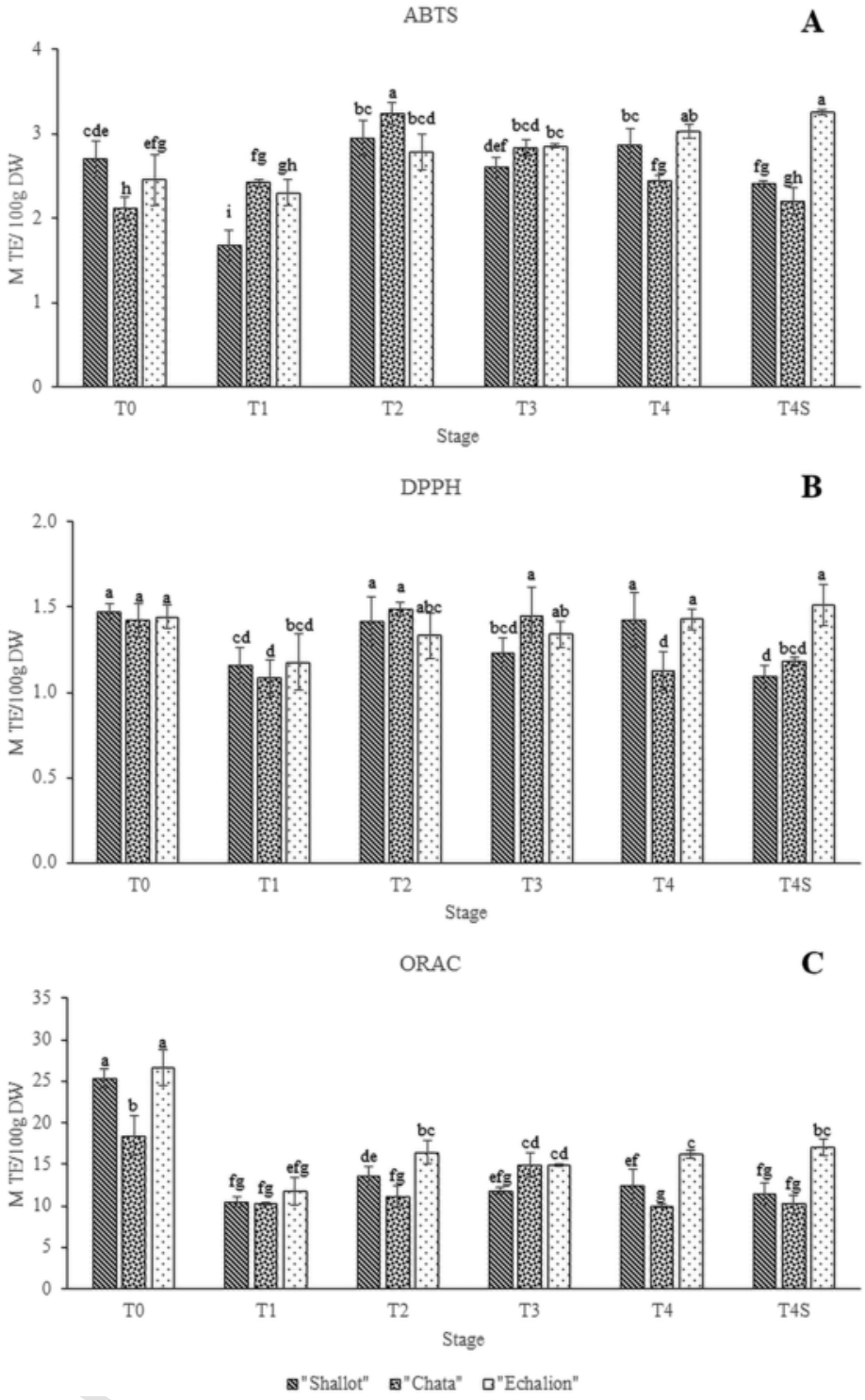

Fig. 3. Antioxidant activity for 'Shallot', 'Chata' and 'Echalion' varieties during production process of black onion measured by (A) ABTS, (B) DPPH and (C) ORAC assays. Data are expressed M TE/100 g DW as mean values (n = 9). Different letters (Two-Ways ANOVA) denote statistically significant differences between the five stages.

Dudonne, Vitrac, Couriere, Woillez, and Mérillon (2009), who reported that 30 plant extracts showed different antioxidant activities with various reaction mechanisms. Moreover, the different trends regarding antioxidant activity could also be attributed to the increase in or the formation of specific compounds with a different number of hydrogen atoms. In this regard, the thermal treatment to produce black onion broke the glucosides of flavonoids to form aglycones, which possess different antioxidant properties. Additionally, the Maillard reaction products formed by the non-enzymatic browning, including a wide variety of intermediates products formed during the Maillard reaction and, ultimately, melanoidins, could be other factors in the different antioxidant activity trends. Thus, different classes of compounds during the stages of the heating process may have different contribution to ORAC, DPPH and ABTS values (Bernaert, De Loose, Van Bockstaele, & Van Droogenbroeck, 2013). Therefore, antioxidant profiles must be based on a variety of types of antioxidant activities because reactive

oxygen species are formed by a number of different mechanisms and can be determined by a variety of techniques.

3.7. Overall changes and remarks

With the aim of determining at a glance the influence of the heating process during the production of different varieties of black onion on their levels of flavonoids, organosulfur compounds, amino acids, organic acids and sugar, a multivariate treatment of the data was used applying PCA (principal component analysis) (Fig. 4). Fig. 4A shows the score plot according to the two principal components selected (PC). PC1, accounting for 46% of the total variance, shows a clear discrimination among samples based on the different stages of the heating process, separating between T0 samples (initial stage) and the rest (T1 to T4S). Fewer differences were found between T1 to T4S. Thus, these results highlight that the major changes in the phytochemical composition took place during the first stage of the black onion process. In addition, the most significant variables that contribute to sample discrimination were glucose, fructose, isoallin and tartaric acid (Fig. 4B), linked to the dramatic increase in their concentration from the T0 to T1 stages. Some amino acids, including asparagine, glycine, GABA, proline, alanine and valine, also contributed to the differentiation between onion samples during the black onion production. PC2, which explained 29% of the total variance, showed a clear discrimination of black onion samples at the last stage of the heating process based on onion varieties. For instance, black onion from the 'Shallot' variety is different to that from the 'Chata' and 'Echalion' varieties. The loading plot reveals that fructose, glucose and malic acid contributed largely to this separation.

4. Conclusions

The heating process applied to black onion production, consisting of controlling the humidity and temperature conditions (90–95% RH and 65–70 °C) for 28 days, induces several phytochemical modifications in three onion varieties, which results in a loss of total flavonoids and increases in organosulfur compounds, predominantly isoalliin, but also fructose, glucose and tartaric acid. These changes are influenced by the sensitivity of the individual compound to modification or degradation under the heating process, although others parameters such as pH, the presence of oxygen during the process along with the presence of other phytochemicals in the matrix also impact on their alterations.

Black onion from the 'Shallot' variety presented a different phytochemical profile compared with those obtained from the 'Chata' and 'Echalion' varieties. Indeed, during thermal processing, the increase in simple sugars is more marked in the 'Shallot' variety, which could affect the final taste that conditions the final sensorial acceptance of the product. It is important to highlight that black onion from all the varieties presented isoalliin as the main bioactive compound. Future research should focus on understanding the bioavailability and biological effects of the isoalliin present in black onion as there is no data on the bioavailability and bioactivity of this organosulfur compound in the literature.

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Uncited reference

Martínez-Casas et al. (2017).

CRediT authorship contribution statement

Alicia Moreno-Ortega: Investigation, Writing - original draft. **Gema Pereira-Caro:** Investigation, Writing - review & editing. **José Luis Ordóñez:** Investigation. **José Manuel Muñoz-Redondo:** Formal analysis. **Rafael Moreno-Rojas:** Writing - review & editing. **Jesús Pérez-Aparicio:** Resources. **José Manuel Moreno-Rojas:** Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

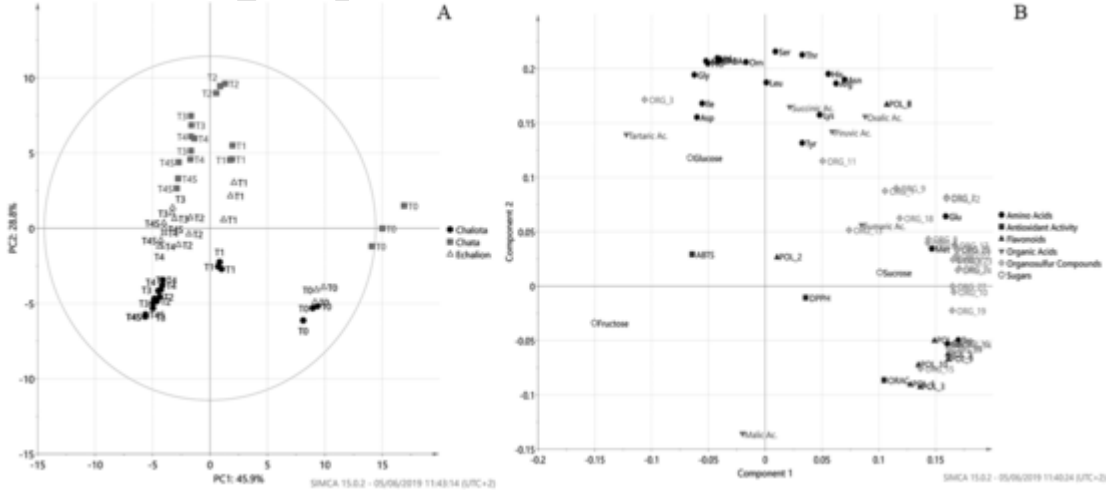

Fig. 4. Scores (A) and loadings (B) of PCA comparing data from samples of three different onion varieties during black onion production.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125958.

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