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Characterisation of onion (*Allium cepa* L.) by-products as food ingredients with antioxidant and antibrowning properties

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Abstract

Processing and stabilising onion wastes (residues and surpluses of onion) could solve the environmental problem derived from a great onion wastes disposal. Moreover, obtaining stabilised onion by-products as natural antioxidant food ingredients could be advantageous to food industry, not only to improve the use of onion wastes but also to obtain new natural and functional ingredients. The aim of this study was to characterise onion by-products – juice, paste and bagasse – from two Spanish onion cultivars – 'Figueres' and 'Recas' – that have been stabilised by thermal treatments – freezing, pasteurisation and sterilisation – in order to evaluate the effect of the processing and stabilisation treatment on the bioactive composition, antioxidant activity and polyphenol oxidase (PPO) enzyme inhibition capacity. The results obtained triggered to choose one onion by-product offering better characteristics for its potential development as a food ingredient: source of antioxidant and antibrowning bioactive compounds. In this study it was shown that processing of 'Recas' onion wastes to obtain a paste (mixture content) and applying a mild pasteurisation were the best alternatives to obtain an interesting stabilised onion by-product with good antioxidant properties that made useful its use as functional food ingredient.
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Keywords: Onion by-products; Stabilisation treatments; Freezing; Pasteurisation; Sterilisation; Bioactive compounds; Antioxidant; Enzymatic browning; Functional food ingredient

1. Introduction

Onion (*Allium cepa* L.) is one of the major vegetable crops grown in Europe which production and cultivated area has increased constantly since 1998. More than 450,000 tonnes of onion wastes is produced annually in the European Union, mainly in UK, Holland and Spain. Nowadays, the food and agricultural products processing industries generate substantial quantities of phenolic-rich by-products, which could be valuable natural sources of antioxidants to be employed as ingredients. Some of these by-products have been the subject of investigations and have proven to be effective sources of phenolic antioxidants (Balasundram, Sundram, & Samman, 2006; Peschel et al, 2006).

There is a concern over the production of large quantities of industrial onion waste or by-products and its disposal. Onion wastes are not suitable for fodder, or landfill disposal due to the rapid growth of phytopathogens, e.g. *Sclerotium cepivorum* (white rot). Valorisation of by-products, particularly exploitation of them for profitable production of food-grade products will benefit the onion producers and processors (Lecain, Ng, Parker, Smith, & Waldron, 1999).

Processing and stabilising onion wastes (residues and surpluses of onion) could represent both advantages: a solution of the environmental problem derived from the great onion wastes disposal and the obtaining of stabilised onion by-products as natural antioxidant food ingredients. Spain is one of the major Mundial onion-producing coun-

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tries. It produced 936,827 tonnes of onion in a cultivated area of 21,324 hectares in 2003. Different varieties and cultivars of onion are spread out among all the regions of Spain, being Castilla-La Mancha, Levant and Andalusia the main producing areas. Catalonia produced 55,368 tonnes of onion in 2004. Onion industry produces wastes that yield an approximated 15% of the total production that is annually changeable. Therefore, this variability among harvests every year leads the industry to have an onion overproduction those years with a high volume of onion production. The 90% of onion produced in Catalonia is cultivated in Lleida. In Catalonia the production in 2004 was 18,250 tonnes of 'Recas' onion cultivar, 13,600 tonnes of 'Figueres' onion cultivar and 3000 tonnes of the rest of onion cultivars and varieties.

Onion nutritional composition is very complex. It has been shown that it is one of the major sources of dietary flavonoids in many countries. Specifically, onion has been characterised for its flavonol quercetin and quercetin derivates. Moreover, it is rich in other bioactive compounds such as frutooligosaccharides and sulfur compounds.

Epidemiological studies have indicated that the consumption of fruits and vegetables is associated with a reduced risk for the development of chronic diseases, such as cardiovascular disease and cancer. Phytochemicals, including phenolics and flavonoids, are suggested to be the major bioactive compounds contributing to the health benefits of fruits and vegetables (Yang, Meyers, Van der Heide, & Liu, 2004). Quercetin is one of the abundant flavonol-type flavonoids commonly found in vegetables and fruits (Moon, Nakata, Oshima, Inakuma, & Terao, 2000). Onion ranked highest in quercetin content in a survey of 28 vegetables and 9 fruits (Hertog, Hollman, & Venema, 1992). It shows a variety of pharmacological effects such as growth inhibition of tumour and microbial cells, reduction of cancer risk, scavenging of free radicals, and protection against cardiovascular disease, which are attributed to specific sulfur-containing compounds and flavonoids (Ly et al., 2005). In addition, onions have been found to have antioxidant properties in different *in vitro* models (Kim & Kim, 2006; Nuutila, Puupponen-Pimiä, Aarni, & Oksman-Caldentey, 2003).

A number of by-products have been previously studied as potential sources of antioxidants. In fact, an interesting approach to utilise by-products is their potential use as sources of natural compounds with high antioxidant activity (Larrosa, Llorach, Espín, & Tomás-Barberán, 2002). Onion wastes adequately processed and stabilised could be useful in the food industry as functional ingredients to be added to processed foods due to the increasing demand by consumers for substituting synthetic compounds by natural substances as food ingredients. Compounds of inherently natural origin would be widely accepted by consumers in the market (Jang, Sanada, Ushio, Tanaka, & Ohshima, 2002).

Nowadays, one of the major concern for the food industry is to prevent the development of enzymatic browning prior to or during the processing of fruits and vegetables because of the alteration in the organoleptic and visual properties of the product. A quality loss is also a fact to take into account due to the phenolic compounds content decrease that occurs during the enzymatic browning (Tomás-Barberán & Espín, 2001). Recent studies have shown that sulfhydryl (SH or thiol) groups are good inhibitors of the enzyme PPO (Ding, Chachin, Ueda, & Wang, 2002), Therefore, it is assumed that the thiol compounds contained in onion might be the active components responsible for the PPO inhibitory effect of onion. Onion extracts could be used as natural food ingredients for the prevention of browning caused by PPO (Kim, Kim, & Park, 2005).

In this work, we attempt to evaluate onion by-products stabilised by different treatments in order to show their bioactive, antioxidant, and antibrowning properties. This would trigger to choose the onion by-product showing better characteristics for its potential use as antioxidant and antibrowning food ingredient.

2. Materials and methods

2.1. Chemicals

Acetonitrile and methanol were obtained from Labscan Ltd. (Dublin, Ireland). Di-sodium hydrogen phosphate anhydrous, sodium dihydrogen phosphate monohydrate, and sodium carbonate anhydrous were purchased from Merck KGaA (Darmstadt, Germany). Hydrochloric acid and *ortho*-phosphoric acid were purchased from Panreac Química, S. A. (Barcelona, Spain). Catechol, chlorogenic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), Folin–Ciocalteu's phenol reagent, polyvinylpolypyrrolidone, and quercetin were obtained from Sigma–Aldrich, Inc. (St. Louis, MO, USA).

2.2. Samples

2.2.1. Onion by-products. Processing and stabilisation treatments

'Figueres' and 'Recas' onion wastes from the harvesting period of 2005 (*Allium cepa* L. var. *cepa*) were supplied by a producing onion industry, CEBACAT (Asociación Catalana de Productores y Comercializadores de Cebolla) in Lleida (Catalonia, Spain). Their processing and stabilisation was held in The National Center for Food Technology and Safety (CNTA) in San Adrián (Navarra, Spain). Stabilised onion by-products analyses were performed in Instituto del Frío, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain.

Previously, onions wastes roots were removed and sheered with a 10×10 mm rack. Then, these onions were processed with a friction screw press to obtain the following three onion by-products: onion juice (the liquid fraction), onion paste (a mixture between the solid and the liquid fractions) and onion bagasse (the solid fraction).

Juice, paste, and bagasse by-products from 'Figueres' and 'Recas' onion cultivars wastes were packed into sterilisable bags (PET/ALU/OPA/PP, Amcor Flexibles Hispania S. A., Granollers, Barcelona, Spain) for the sterilisation and pasteurisation treatments; and into trays (PP/EVOH/PP, EDV, Llinars del Vallés, Barcelona, Spain) for the freezing treatment. Sterilisation (at 115 °C, 17–31 min) and pasteurisation (at 100 °C, 11–17 min) took place in a conventional autoclave. Sterilised and pasteurised onion by-products were stored at −4 °C until analysis. Freezing treatment (at −70 °C) was carried out in a liquid nitrogen cabinet (Frigothermic, model L.S.1, Martorell, Barcelona, Spain) until the product reached −18 °C. Frozen onion by-products were stored at −18 °C until analysis.

2.3. Analysis

Stabilised onion by-products were analysed for their bioactive composition, and their antioxidant and antibrowning properties.

2.3.1. Bioactive composition

2.3.1.1. Total phenols. Total Phenols were determined spectrophotometrically (Vinson, Hao, Su, & Zubik, 1998). Analyses were performed by visible spectrophotometry at 760 nm after reaction with Folin–Ciocalteu's reagent.

Juice (50 mL), paste or bagasse (10 g) plus 25 mL methanol/water (80:20, v/v) were homogenised in duplicate in an ultrahomogeniser (Omni mixer, model ES-270, Omni International Inc., Gainesville, VA, USA). Extracts were made up to 100 mL with methanol for juice and up to 50 mL for paste and bagasse. Next, they were introduced into test tubes and then 1.0 mL Folin–Ciocalteu's reagent and 0.8 mL sodium carbonate (7.5%) were added. The absorbance of all samples was measured at 760 nm after incubating at room temperature for 1 h. Results were calculated by a calibration curve obtained from chlorogenic acid and expressed as milligrams of chlorogenic acid equivalents (CAE) per 100 g of dry weight (dw).

2.3.1.2. Extraction, separation, identification and quantification of quercetin. Total quercetin was determined by high performance liquid chromatography (HPLC). The extraction was carried out according to the method by Hertog et al. (1992) with minor modifications.

2.3.1.2.1. Hydrolysis mixture. Juice (50 mL), paste or bagasse (10 g) plus 25 mL methanol/water (80:20, v/v) were mixed with 5 mL of a 6 M HCl solution. No antioxidants were added to the hydrolysis mixture. The hydrolysis was performed in duplicate. After refluxing at 90 °C for 4 h, the extract was allowed to cool, vacuum filtered, made up to 100 mL with methanol for juice and up to 50 mL for paste and bagasse, next sonicated. The extracts were filtered through a 0.45 μm membrane filter for organic solvents prior to injection. Duplicates of 20 μL for each extract were analysed by HPLC.

2.3.1.2.2. HPLC procedure. The analytical HPLC system employed consisted of a Hewlett-Packard (Palo Alto, CA, USA) Model 1050 coupled with a quaternary solvent delivery pump and equipped with an autosampler (G1329A ALS) with a 20 μL sample loop and a Hewlett-Packard 1040A rapid scanning UV–vis photodiode array detector. Separation of flavonoids was performed on a reverse-phase Zorbax Eclipse XDB C_{18} Hypersil ODS (5 μm) stainless steel column (250x4.6 mm i.d., 5 μm particle size) (Agilent, Spain). The mobile phase was deionised Milli-Q water adjusted to a pH 2.5 with *ortho*-phosphoric acid (solution A) and acetonitrile (solution B). The program began with a gradient elution from 90% to 65% A, and from 10% to 35% B for 20 min, followed by a gradient from 65% to 90% A, and from 35% to 10% B for the next 5 min. The flow rate was fixed at 1 mL/min and runs were monitored with the UV–vis photodiode array detector which was set at 370 nm. The data were stored and processed using a Hewlett-Packard (Palo Alto, CA, USA) ChemStation and related software. Identification of the quercetin was carried out by HPLC by comparing the retention time and UV–vis absorption spectrum with those of the quercetin standards. The quantification was achieved by the absorbance recorded in the chromatograms relative to the external standards of flavonoids previously referred to. Total quercetin content was expressed as milligrams of total quercetin per 100 g of dry weight (dw).

2.3.2. Scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical

Antioxidant activity was determined by the measurement of the DPPH· radical scavenging (Sánchez-Moreno, Larrauri, & Saura-Calixto, 1998).

2.3.2.1. Extraction. Juice (50 mL), paste or bagasse (10 g) plus 25 mL methanol/water (80:20, v/v) were mixed with 5 mL of a 6 M HCl solution. No antioxidants were added to the hydrolysis mixture. The hydrolysis was performed in duplicate. The refluxing period in this case was 2 h.

2.3.2.2. DPPH· radical scavenging capacity. The determination of the radical scavenging capacity was evaluated with the stable radical DPPH·. The method is described extensively elsewhere (Sánchez-Moreno, Plaza, de Ancos, & Cano, 2003). The parameters EC_{50}, which reflects 50% depletion of initial DPPH· radical and the time needed to reach the steady state at EC_{50} concentration (T_{EC50}) were calculated. The antiradical efficiency ($AE = 1/EC_{50} \times T_{EC_{50}}$), a parameter that combines both factors, was also calculated.

2.3.3. Polyphenol oxidase (PPO) inhibition assay

Minor modifications of the Kim et al. (2005) research were carried out in order to evaluate the inhibitory effect of onion stabilised by-products extracts on avocado polyphenol oxidase.

2.3.3.1. Avocado PPO extraction. Avocados (*Persea americana* Miller var. *americana* 'Fuerte') were purchased from a Spanish local supermarket. They were peeled, cut into small pieces and frozen with liquid nitrogen. Afterwards, the frozen pieces were grinded and homogenised into a blender (Osterizer, NC, USA) and stored at −20 °C until their analysis.

Avocado frozen powder (2 g) was mixed with polyvinylpolypyrrolidone (PVPP) (0.8 g) and homogenised in an ultrahomogeniser with 20 mL of a sodium phosphate buffer solution (0.1 M, pH 6.5) for 3 min. The homogenate was centrifuged at 9500*g* for 15 min at 4 °C. The supernatant was collected and filtered through a six coat cheese cloth. The filtered was used as avocado PPO enzyme extract throughout this experiment. All steps were carried out at 4 °C.

2.3.3.2. Onion extracts preparation. The previous step was to freeze-dry the onion by-products in a lyophilizer (model Lyoalfa, Telstar, S. A., Barcelona, Spain). Freeze dried onion by-products (1.2 g) were homogenised with distilled water (20 mL) for 3 min in the ultrahomogeniser. The homogenate was centrifuged at 17,500*g* for 20 min at 4 °C. The supernatants were vacuum filtered through a 0.45 μm membrane filter. Each extraction was prepared in duplicate.

2.3.3.3. PPO inhibition assay. The PPO activity was assayed with 0.07 M catechol as a substrate by a spectophotometric procedure (Kim et al., 2005).

Polyphenol oxidase activity was assayed using the stabilised onion by-products extracts (1 mL), the PPO avocado extract (0.1 mL) and a solution of 0.07 M catechol (1 mL) in a sodium phosphate buffer (0.05 M, pH 6.5) (0.9 mL). The total volume of the PPO inhibition assay was 3 mL. Firstly, the inhibition reaction mixture (stabilised onion by-product extracts and PPO extract) was incubated for 5 min at 25 °C. Immediately after, the rest of the reactants were added. Absorbance at 420 nm was monitored at 25 °C for 30 s.

The results were expressed as relative enzymatic activity (REA): the percentage of PPO activity were measured and extrapolated to 100% REA (in percentage, %). Thus, REA represents the residual PPO activity reached after adding different onion by-products as natural inhibitors to the model solution.

2.3.4. Statistical analysis

Results were given as mean ± standard deviation of six independent determinations. One-way analysis of variance (ANOVA) was used to compare the means. Differences were considered significant at $P < 0.05$. All statistical analyses were performed with Statgraphics Plus 5.1 (Statistical Graphics Corporation, Inc., Rockville, MD, USA).

3. Results and discussion

The following results are exposed regarding different onion by-products within the same stabilisation treatment. Thus, discussion will compare the bioactive composition (total phenols and total quercetin), the antioxidant activity, and the inhibition PPO capacity parameters in 'Recas' and 'Figueres' frozen, pasteurised and sterilised onion by-products (juice, paste and bagasse).

3.1. Bioactive composition (total phenol and total quercetin content)

From a nutritional point of view, it is desirable to minimise the loss of the biological activity of onion by-products throughout processing by controlling all the technological and stabilisation parameters involved in all operations of the process. Therefore, to obtain a representative onion by-product offering better characteristics as a food ingredient it is crucial to focus on the type of onion by-product and on the stabilisation treatment applied.

In our work, total phenols and total quercetin content were measured in onion by-products in order to evaluate their bioactive composition.

Regarding different onion by-products within the same stabilisation treatment our results were the following:

Frozen 'Recas' paste showed the highest total phenol content among all the frozen 'Recas' by-products analysed. Frozen 'Figueres' onion by-products showed significantly different ($P < 0.05$) total phenol content among them (Table 1). Frozen 'Recas' paste was also the onion by-product which reached the highest total quercetin content (4431.21 ± 415.23 mg/100 g dw) among all the stabilised

Table 1
Bioactive compounds and antioxidant activity of frozen onion by-products[a]

By-product	Cultivar	Total phenols (mg CAE/100 g dw)	Total quercetin (mg/100 g dw)	EC_{50} (g dw/g DPPH•)	$T_{EC_{50}}$ (min)
Juice	'Figueres'	118.56 ± 4.01Aa	57.90 ± 13.13Aa	10.75 ± 0.17Ca	31.12 ± 3.55Aa
	'Recas'	183.96 ± 23.74Ab	214.64 ± 18.22Ab	12.35 ± 0.38Bb	45.33 ± 1.34Cb
Paste	'Figueres'	238.95 ± 43.62Ba	671.48 ± 51.54Ca	1.76 ± 0.007Ba	22.23 ± 4.07Aa
	'Recas'	441.31 ± 50.93Cb	4431.21 ± 415.23Cb	4.05 ± 0.04Ab	28.15 ± 0.16Aa
Bagasse	'Figueres'	407.64 ± 32.02Cb	600.72 ± 7.24Ba	1.47 ± 0.39Aa	63.35 ± 7.98Bb
	'Recas'	330.40 ± 10.81Ba	2230.89 ± 277.25Bb	4.12 ± 0.34Ab	36.27 ± 4.64Ba

[a] Values are means ± SD, $n = 6$. Means within a column with different capital letters in different by-products for the same cultivar are significantly different at $P < 0.05$. Means within a column with different small letters in the same by-product for different cultivars are significantly different at $P < 0.05$.

Table 2
Bioactive compounds and antioxidant activity of pasteurised onion by-products[a]

By-product	Cultivar	Total phenols (mg CAE/100 g dw)	Total quercetin (mg/100 g dw)	EC_{50} (g dw/g DPPH·)	$T_{EC_{50}}$ (min)
Juice	'Figueres'	128.23 ± 33.7Aa	23.13 ± 4.10Aa	3.37 ± 0.03Ba	58.75 ± 3.29Bb
	'Recas'	151.03 ± 10.71Aa	31.44 ± 2.02Ab	3.96 ± 0.04Cb	52.47 ± 0.37Aa
Paste	'Figueres'	143.01 ± 7.55Aa	131.98 ± 13.68Ba	1.33 ± 0.32Aa	52.86 ± 2.17Aa
	'Recas'	329.77 ± 83.49Bb	195.17 ± 7.27Bb	2.30 ± 0.04Ab	52.98 ± 1.04Aa
Bagasse	'Figueres'	143.55 ± 11.13Aa	212.19 ± 29.19Ca	3.21 ± 0.011Bb	54.99 ± 1.94ABa
	'Recas'	453.29 ± 29.36Cb	721.37 ± 4.94Cb	2.65 ± 0.020Ba	61.18 ± 0.17Bb

[a] Values are means ± SD, $n = 6$. Means within a column with different capital letters in different by-products for the same cultivar are significantly different at $P < 0.05$. Means within a column with different small letters in the same by-product for different cultivars are significantly different at $P < 0.05$.

Table 3
Bioactive compounds and antioxidant activity of sterilised onion by-products[a]

By-product	Cultivar	Total phenols (mg CAE/100 g dw)	Total quercetin (mg/100 g dw)	EC_{50} (g dw/g DPPH·)	$T_{EC_{50}}$ (min)
Juice	'Figueres'	153.15 ± 39.28Aa	11.83 ± 0.11Aa	32.48 ± 2.83Bb	43.89 ± 3.96Ba
	'Recas'	213.79 ± 31.08Aa	79.08 ± 7.81Ab	14.86 ± 0.41Ca	57.25 ± 3.23Cb
Paste	'Figueres'	416.21 ± 38.53Ba	260.17 ± 3.30Ba	4.07 ± 0.09Ab	27.05 ± 2.58Aa
	'Recas'	591.25 ± 21.01Cb	489.78 ± 9.48Bb	3.34 ± 0.02Ba	43.01 ± 0.18Ab
Bagasse	'Figueres'	220.51 ± 37.30Aa	310.92 ± 36.38Ca	6.13 ± 0.40Ab	29.78 ± 0.53Aa
	'Recas'	398.79 ± 26.61Bb	724.72 ± 5.78Cb	2.61 ± 0.05Aa	51.23 ± 0.54Bb

[a] Values are means ± SD, $n = 6$. Means within a column with different capital letters in different by-products for the same cultivar are significantly different at $P < 0.05$. Means within a column with different small letters in the same by-product for different cultivars are significantly different at $P < 0.05$.

by-products analysed. Frozen 'Figueres' onion by-products did not reached such accused total quercetin content (Table 1).

Pasteurised 'Recas' bagasse showed higher total phenol content than those shown by pasteurised 'Recas' paste or juice. Pasteurised 'Figueres' by-products did not show significantly difference ($P > 0.05$) in their total phenol content among them (Table 2). Likewise, pasteurised 'Recas' bagasse was the onion pasteurised by-product which showed the highest total quercetin content (721.37 ± 4.94 mg/100 g dw) followed by 'Recas' paste or juice, significantly different ($P < 0.05$) among them. Pasteurised 'Figueres' by-products were also significantly different ($P < 0.05$) among them regarding total quercetin content. Pasteurised 'Figueres' bagasse showed the highest total quercetin content (212.19 ± 29.19 mg/100 g dw) followed by pasteurised 'Figueres' paste and juice (Table 2).

Sterilised 'Recas' paste showed higher total phenol content than sterilised 'Recas' bagasse and juice. In the same way, sterilised 'Figueres' paste showed higher total phenol content than sterilised 'Figueres' bagasse or juice (Table 3). Sterilised 'Recas' bagasse showed significantly higher ($P < 0.05$) total quercetin content (724.72 ± 5.78 mg/100 g dw) than sterilised 'Recas' paste or juice. Sterilised 'Figueres' by-products had the same behaviour than pasteurised 'Recas' by-products, being sterilised 'Figueres' bagasse the by-product which reached the highest total quercetin content (310.92 ± 36.38 mg/100 g dw) followed by paste or juice (Table 3). Sterilised onion by-products did not show significant differences ($P < 0.05$) compared to pasteurised ones, being 'Recas' and 'Figueres' bagasses the by-products showing the highest total quercetin content followed by 'Recas' and 'Figueres' pastes or juices.

Generally, stabilised by-products from 'Recas' onion cultivar had a significantly higher ($P < 0.05$) bioactive composition (total phenols and quercetin) than those by-products from 'Figueres' cultivar (Tables 1–3).

Concerning total phenol content among 'Recas' onion by-products analysed, sterilised and frozen pastes showed the highest values followed by pasteurised 'Recas' bagasse. In addition, freezing and pasteurisation stabilisation treatments did not rend significantly differences ($P > 0.05$) in total phenol content when 'Recas' paste was analysed.

Referring to total quercetin content in 'Recas' onion by-products, it was shown that frozen 'Recas' paste had the highest content among all the stabilised pastes analysed, followed by pasteurised and sterilised 'Recas' bagasses which did not show significant differences ($P > 0.05$). Pasteurised and sterilised 'Recas' paste were significantly different ($P < 0.05$) to frozen 'Recas' paste.

Our results showed that 'Recas' onion cultivar and paste by-product were one of the best choices due to their higher bioactive content. In this work, it has been shown that bioactive compounds are highly concentrated in those onion by-products containing more wall cells like paste or bagasse than in juice which loss a great proportion of wall cells during its process. Moreover, the by-products from the colourful cultivar 'Recas' showed moderately high bioactive compounds values. Previous works stated that onion unutilised outer layers of a red variety had the higher contents of total phenols followed by a continuous decrease towards the inner part of the bulb. They were a rich source of quercetin with high antioxidant activity and showed significant protection of DNA damage caused by free radicals (Prakash, Singh, & Upadhyay, 2007).

Stabilisation treatments applied would have to be carefully chosen. These treatments not only would have to maintain as higher bioactive composition as possible but also ensure the safety and stability of these onion by-products during its whole self-life. In our work, sterilisation was a thermal treatment (115 °C) which provoked a higher phenol release compared with the pasteurisation (100 °C), and freezing (−18 °C). This fact is in agreement with previous works that attribute to the thermal treatment an increase in the release of bioactive compounds from the cell walls of the onion skin or the onion outer tissues (Lombard, Peffley, Geoffriau, Thompson, & Herring, 2005). In addition, Kim et al (2006) showed that the total phenol content of grape seed extracts was significantly increased by heat treatments, indicating that phenolic compounds in these extracts were liberated by heat treatments. Onions contain large amounts of quercetin glycosides and they are often subject to thermal processes in food production. The thermal treatment led to a degradation of the quercetin glycosides. The main product is the aglycone quercetin, which remained stable during further roasting (180 °C) (Rohn, Buchner, Driemel, Rauser, & Kroh, 2007). Thus, this flavonol may be stable at the 115 °C temperature applied in the sterilisation and it would be stable at lower temperatures applied in pasteurisation or freezing treatments.

Analysing the applied stabilisation treatments is crucial to choose one treatment that does not involve microbiological risk in order to develop a safe food ingredient. In our study, freezing was a treatment which may not be chosen as a stabilisation treatment due to the microbiological risk it could involve (data not shown). In addition, sterilisation may produce caramelised compounds in the onion by-products stabilised by this treatment. This fact could influence on their nutritional composition by causing a great loss in the bioactive composition measured, total quercetin content indeed. By contrast, pasteurisation as a mild thermal treatment would represent the best choice to stabilise onion by-products maintaining mainly intact their bioactive composition. Our results showed that this stabilisation treatment caused a low decrease in the total phenols and quercetin content measured in the onion by-products analysed (compared to freezing or sterilisation).

3.2. Antioxidant activity (DPPH· stable radical scavenging)

Several radical scavenging parameters were measured: EC_{50}, $T_{EC_{50}}$, and antiradical efficiency (AE). The AE was calculate in order to evaluate the total antioxidant activity, this parameter combines both factors (EC_{50} and $T_{EC_{50}}$) (Sánchez-Moreno et al., 1998).

Regarding AE as antioxidant parameter and comparing the effect of the onion processing within the same stabilisation treatment the results were the following:

Frozen 'Recas' paste ($8.7 \pm 0.003 \times 10^{-3}$) showed a significantly higher ($P < 0.05$) AE value than frozen 'Recas' bagasse ($3.4 \pm 0.58 \times 10^{-3}$) or juice ($1.7 \pm 0.12 \times 10^{-3}$). When analysing 'Figueres' onion cultivar, frozen paste ($25.8 \pm 4.85 \times 10^{-3}$) showed significantly higher ($P < 0.05$) AE value than frozen 'Figueres' bagasse ($9.5 \pm 6.36 \times 10^{-3}$) or juice ($3.0 \pm 0.14 \times 10^{-3}$) (Fig. 1a).

Pasteurised and frozen 'Recas' paste had similar AE values ($P > 0.05$). In addition, pasteurised 'Recas' paste was more efficient scavenging radicals than pasteurised 'Recas' bagasse or juice ($8.0 \pm 0.3 \times 10^{-3}$ *vs.* $6.1 \pm 0.06 \times 10^{-3}$ and $4.8 \pm 0.1 \times 10^{-3}$, respectively). Pasteurised 'Figueres' paste ($15.0 \pm 4.2 \times 10^{-3}$) showed significantly higher AE value than pasteurised 'Figueres' bagasse ($5.6 \pm 0.2 \times 10^{-3}$) or juice ($5.0 \pm 0.3 \times 10^{-3}$). Thus, pasteurised 'Figueres' paste ($15.0 \pm 4.2 \times 10^{-3}$) showed the highest value

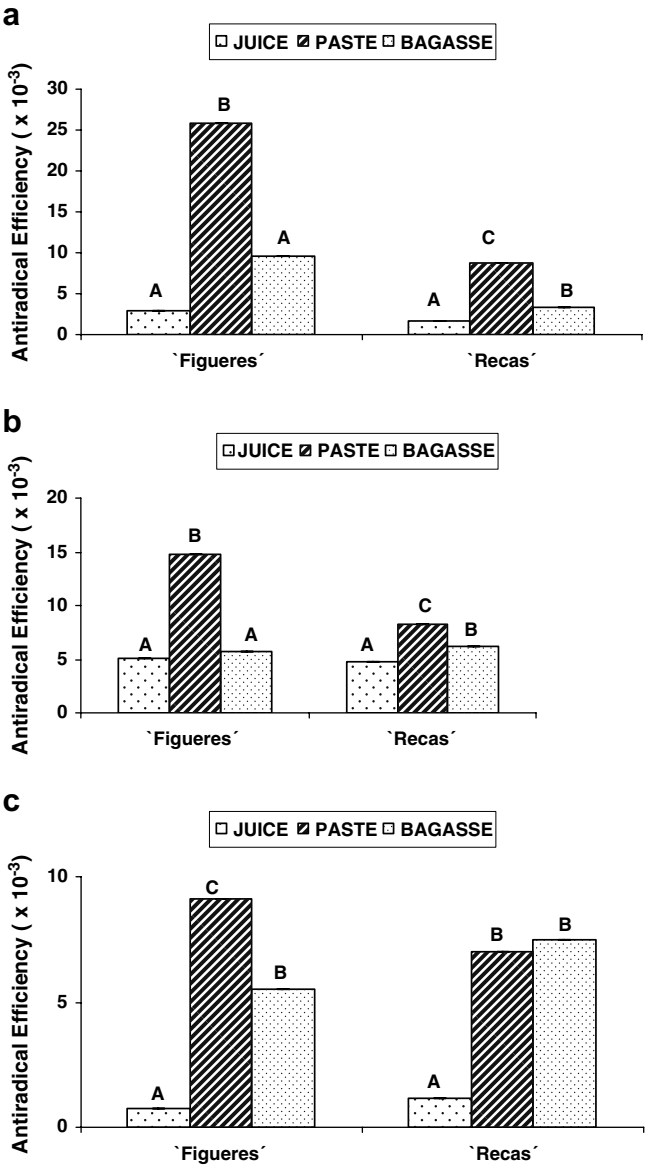

Fig. 1. Antiradical efficiency (AE) of frozen onion by-products (a), pasteurised onion by-products (b), and sterilised onion by-products (c). Bars with different capital letters in different by-products for the same cultivar are significantly different at $P < 0.05$. AE expressed as $1/[EC_{50}$ (g dw/g DPPH·) $\times T_{EC_{50}}$ (min)].

among all the pasteurised onion by-products analysed ('Recas' and 'Figueres') (Fig. 1b).

There were not significantly differences ($P > 0.05$) between sterilised 'Recas' paste ($7.0 \pm 0.6 \times 10^{-3}$) and bagasse ($7.5 \pm 0.007 \times 10^{-3}$) AE values. The AE value shown by sterilised 'Recas' juice ($1.17 \pm 0.03 \times 10^{-3}$) was significantly lower ($P < 0.05$) than sterilised 'Recas' paste ($7.0 \pm 0.6 \times 10^{-3}$) and bagasse ($7.5 \pm 0.007 \times 10^{-3}$). Sterilised 'Figueres' paste ($9.1 \pm 0.6 \times 10^{-3}$) AE value was significantly higher than sterilised 'Figueres' bagasse and juice ($5.5 \pm 0.5 \times 10^{-3}$, and $0.72 \pm 0.04 \times 10^{-3}$, respectively) (Fig. 1c).

In general, pastes from the two onion cultivars assayed showed higher antiradical efficiency values. Therefore, they showed better characteristics as potential antioxidant food ingredients. Pasteurised and frozen 'Recas' pastes reached the following AE values: $8.0 \pm 0.3 \times 10^{-3}$ and $8.7 \pm 0.003 \times 10^{-3}$, respectively. These values were significantly higher than that found in sterilised 'Recas' paste ($7.0 \pm 0.6 \times 10^{-3}$).

The correlation between antioxidant capacity and bioactive composition (total phenols and flavonoids) has been widely studied (Aviram & Aviram, 2002; Pyo, Lee, Logendra, & Rosen, 2004; Sánchez-Moreno et al., 2003; Sellappan & Akoh, 2002). Nuutila et al. (2003) found an observable correlation between high radical scavenging/antioxidant activity and high amounts of total phenolics and flavonoids of the onion extracts, resulting the phenolic compounds of *Allium* plants contribute to their antioxidative properties. Moreover, these authors showed that the skin extracts of onion possessed the highest activities (Nuutila et al., 2003). Thus, in our work the correlation between antioxidant capacity and bioactive composition was also studied in the two onion cultivars by-products assayed.

Concerning 'Recas' paste, our results showed that the EC_{50} parameter and total phenols were inversely correlated in the frozen and sterilised 'Recas' paste ($r = -0.9552$; $P = 0.0001$ *vs.* $r = -0.8196$; $P = 0.0068$, respectively). EC_{50} parameter and total quercetin showed a significant inverse correlation in frozen and sterilised 'Recas' paste ($r = -0.9832$; $P = 0.0001$ *vs.* $r = -0.9495$; $P = 0.0001$, respectively). A significant inverse correlation between the EC_{50} parameter and total phenols ($r = -0.7566$; $P = 0.0183$) was shown in pasteurised 'Recas' paste. Probably other bioactive compounds that have not been analysed in this research would be responsible of the antioxidant capacity found in pasteurised paste.

Antioxidant capacity of onion has been widely studied. There have been shown different antioxidant capacities among different cultivars or varieties (Aoyama & Yamamoto, 2007; Benkeblia, 2005; Nuutila et al., 2003; Yang et al., 2004). Moreover, it has been elucidated an increasing antioxidant activity from the inner to the outer part of the onion (Kim et al., 2006; Ly et al., 2005; Suh, Lee, Cho, & Chung, 1999). In concordance, our results showed that there was a difference between the two cultivars analysed 'Figueres' and 'Recas'. Generally, 'Recas' onion by-products assayed offered better radical scavenger properties than 'Figueres' onion by-products. In addition, by-products with a higher content of outer parts of onion (paste and bagasse) showed higher antioxidant activity than juices.

Processing and stabilising onion wastes may have an impact on the antioxidant activity measured. As different thermal treatments applied to onion caused a lost in the free radical scavenging properties found in this fresh vegetable, the temperature used to stabilise onion by-products must be carefully controlled in order not to lose the potential antioxidant properties of these by-products (Agostini, Jimenez, Ramón, & Gómez, 2004; Fu, 2004; Kawamoto, Sakai, Okamura, & Yamamoto, 2004; Yin & Cheng, 1998).

In our study, pasteurised 'Recas' paste offered better characteristics than pasteurised 'Recas' bagasse or juice as antioxidant food ingredient due to the lower concentration (EC_{50}) needed to scavenge the stable radical DPPH$^{\cdot}$ (Table 2). In this context, it is important to take into account that onion by-products have been used to increase antioxidant characteristics in tomato juice (Larrosa et al., 2002).

Pasteurisation was a mild treatment that did not reach the high temperatures found when sterilisation was applied, maintaining better the antioxidant properties of the by-products analysed.

3.3. Antibrowning activity (polyphenol oxidase inhibition assay)

The use of natural inhibitors of PPO is still stimulated by the need to replace sulfating agents in order to prevent or minimize the loss of fresh or processed foodstuffs (Billaud, Brun-Mérimée, Louarme, & Nicolas, 2004). From a technological point of view, it would be conceivable to use natural antibrowning agents in processed fruits provided that their safety is assessed and their commercial feasibility is demonstrated. Among the numerous compounds capable of reducing enzymatic browning and/or oxidoreductase activity, onion has been found to have bioactive compounds with such properties (Eissa, Fadel, Ibrahim, Hassan, & Abd Elrashid, 2006).

In this work, PPO activities of avocado fruit were significantly reduced by the different onion by-products analysed. In order to measure their antibrowning capacity, we compared the onion by-products within the same stabilisation treatment and the results showed the following behaviour:

Frozen 'Recas' paste reduced significantly ($P < 0.05$) the avocado PPO activity (57.08%) followed by frozen 'Recas' juice and bagasse, averaging 39.69%. By contrast, when 'Figueres' by-products were analysed, frozen bagasse was the by-product with a significantly higher ($P < 0.05$) inhibitory enzymatic effect (55.82%), followed by 'Figueres' frozen paste (34.51%) and juice (29.25%) (Table 4).

Pasteurised 'Recas' paste and juice reduced PPO activity 53.49% and 65.52%, respectively meanwhile pasteurised

Table 4
Inhibitory enzymatic effect of onion by-products[a]

Antibrowning agent		Relative enzymatic activity (REA, %)	
Onion by-product	Stabilisation treatments	Cultivar	
		'Figueres'	'Recas'
Juice	Freezing	70.75 ± 3.25Bb	60.38 ± 3.00Ca
	Pasteurisation	51.81 ± 4.91Ab	34.48 ± 1.81Aa
	Sterilisation	63.51 ± 4.84Bb	40.36 ± 2.50Ba
Paste	Freezing	65.49 ± 3.27Bb	42.92 ± 3.40Ba
	Pasteurisation	67.18 ± 4.57Bb	46.50 ± 2.78Ba
	Sterilisation	31.86 ± 1.84Ab	10.29 ± 0.81Aa
Bagasse	Freezing	44.18 ± 9.70Aa	60.23 ± 1.96Bb
	Pasteurisation	72.53 ± 7.04Ba	86.08 ± 9.24Ca
	Sterilisation	61.22 ± 2.68Bb	27.32 ± 3.41Aa

[a] Values are means ± SD, $n = 6$. Means within a column with different capital letters in different by-products for the same cultivar and stabilisation treatment are significantly different at $P < 0.05$. Means within a column with different small letters in the same by-product and stabilisation treatment for different cultivars are significantly different at $P < 0.05$.

'Recas' bagasse reduced it 13.92%. Pasteurised 'Figueres' juice reduced PPO activity 48.19%, and pasteurised 'Figueres' bagasse and paste did not show significantly difference ($P > 0.05$) among them. Pasteurised 'Recas' paste inhibitory capacity towards avocado PPO was higher (53.49%) than the capacity shown by pasteurised 'Figueres' paste (32.82%) (Table 4).

Sterilised 'Recas' paste reduced PPO activity 89.71% meanwhile sterilised 'Recas' bagasse and juice did it 72.68% and 59.64%, respectively. Sterilised 'Figueres' paste reduced PPO activity 68.14%. Sterilised 'Figueres'bagasse and juice reduced it 38.78% and 36.49%, respectively.

Interestingly, in our work it was shown the same behaviour by paste onion by-products when pasteurisation or sterilisation were applied. However, sterilised by-products showed more accused inhibitory effect than pasteurised ones. Thus, sterilised 'Recas' paste inhibitory capacity towards avocado PPO was higher (89.71%) than the capacity shown by pasteurised 'Recas' paste (53.49%) (Table 4).

The percentage of relative enzymatic activity found when pasteurised (110 °C, 11–17 min) 'Recas' paste (46.50%) was used as an antibrowning agent was similar to that found (45.9%) by Kim et al. (2005) when using heated onion extracts (100 °C, 10 min).

Technological and stabilisation processes applied to onion may influence significantly on their PPO inhibition capacity. Higher antibrowning activity was found in sterilised by-products followed by pasteurised and frozen ones. Sterilised 'Recas' and 'Figueres' pastes showed a high antibrowning effect reducing the PPO activity in 89.71% and 68.14%, respectively.

Recent studies have shown that sulfhydryl (SH or thiol) compounds are good inhibitors of the enzyme PPO (Ding et al., 2002; Jang et al., 2002; Martínez & Whitaker, 1995; Negishi & Ozawa, 2000). Onions are rich in two chemical compounds flavonoids and alk(en)yl cysteine sulfoxides (ACSO) (Griffiths, Trueman, Crowther, Thomas, & Smith, 2002). Therefore, it is generally assumed that sulfur compounds of low molecular weight contained in onions are responsible of the PPO inhibition.

It has been shown that heated onion extracts were more effective in prevention of pear and banana browning than fresh onion extracts (Kim et al., 2005; Lee, 2007). In our work, we have studied the effect caused by the temperature used to stabilise onion by-products on avocado PPO inhibition. The onion processing used to obtain the different onion by-products was also studied.

The positive effect of a temperature rise in onion extracts towards different fruits or vegetables PPO inhibition has been widely studied (Ding et al., 2002; Hosoda & Iwahashi, 2002; Kim et al., 2005; Lee et al., 2002). Moreover, a synergic effect among sulfur compounds (contained in onions), Maillard compounds and caramelisation products formed at high temperatures had also been postulated and studied in several researches (Billaud et al., 2004; Cheriot, Billaud, Maillard, & Nicolas, 2007; Gruber, Vieths, Wangorsch, Nerkamp, & Hofmann, 2004; Kim et al., 2005; Wagner, Reichhold, Koschutnig, Chériot, & Billaud, 2007).

Results of our research were in concordance with the researches previously cited. Generally, 'Recas' cultivar displayed PPO inhibiting properties more potent than that found in 'Figueres' cultivar in all the stabilisation treatments and onion by-products assayed. A temperature rise offered better antibrowning properties in all onion by-products assayed standing out paste. Data suggested that thermal treatments (pasteurisation and sterilisation) were mainly responsible of the avocado polyphenol oxidase inhibition, whereas non-thermal treatments (freezing) did not show such accused effect. Interestingly, it was shown that sterilised 'Recas' paste was the by-product with the strongest PPO inhibitory effect among all the onion by-products analysed.

Therefore, stabilising onion by-products by sterilisation would offer better antibrowning properties to these potential food ingredients than pasteurisation or freezing. By contrast, applying sterilisation as a stabilisation treatment would have the added problem of caramelisation and it might show the disadvantages exposed above. Thus, pasteurisation could represent a better choice in order to develop a food ingredient with an interesting added antibrowning property. Moreover this thermal treatment would maintain the safety of the food ingredient.

4. Conclusions

After analysing bioactive composition, antioxidant activity, and polyphenol oxidase inhibition capacity in the stabilised onion by-products from both cultivars ('Figueres' and 'Recas'), it was concluded that those by-products obtained from the 'Recas' onion cultivar showed better characteristics. Pasteurisation (100 °C, 11–17 min) applied as stabilisation treatment kept bioactive and technological characteristics of onion by-products. This treatment did not trigger the adverse effects caused by thermal

sterilisation such as caramelisation. Thus, pasteurised 'Recas' paste was chosen to be the most appropriate onion by-product for developing an antioxidant food ingredient among all the onion by-products analysed. It showed several advantages: a remarkable antioxidant activity (AE), a moderate high bioactive composition (total phenols and quercetin), and an excellent antibrowning effect from a technological point of view.

By-products derived from the manipulation and preparation of onion for its marketing involves a great economic loss for that sector food industry. From this study, it could be concluded that there is a real possibility of using those onion by-products for developing natural food ingredients with functional properties.

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