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Comparison of the antioxidant activity of two Spanish onion varieties

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Abstract

Phenol content and antioxidant activity of two Spanish onion varieties, namely white onion and *Calçot de Valls,* have been studied. White onions contained higher phenol content than *Calçot* onions, with values which ranged from 2.57 ± 0.51 to 6.53 ± 0.16 mg gallic acid equivalents/g dry weight (GAE/g DW) and 0.51 ± 0.22 to 2.58 ± 0.16 mg GAE/g DW, respectively, depending on the solvent used. Higher phenol content was associated with higher antioxidant capacity. White onion extracts had the highest antioxidant activity at 86.6 ± 2.97 and 29.9 ± 2.49 μmol Trolox/g DW for TEAC and FRAP assays, respectively, while the values for the *Calçot* variety were 17.5 ± 0.46 and 16.1 ± 0.10 μmol Trolox/g DW.

The antioxidant capacity of freeze dried powder from both onion varieties was also tested in sunflower oil-in-water emulsions, and hydroperoxide formation was monitored during storage at 40 °C. In accordance with differences in phenol content, Spanish white onions had better antioxidant activity, while *Calçot* was only effective in the early stages of the oxidation process.

Keywords: White onion; *Calçot*; Extraction; Phenol content; Antioxidant capacity; Emulsion; Peroxide value

1. Introduction

Flavonoids, one group of polyphenolic compounds, are secondary metabolites widely distributed in the plant kingdom with a great variety of structures (Iwashina, 2000). They are important constituents of the human diet.

Plants of the *Allium* family are an important source of dietary flavonols (Tepe, Sokmen, Akpulat, & Sokmen, 2005; Yin & Cheng, 1998). Onion (*Allium cepa* L.), which is one of the most consumed vegetables, is known for its flavonoid content, contributing considerably to its dietary intake in many countries (Hertog, Hollman, & Katan, 1992; Sellappan & Akoh, 2002). Furthermore, onion is an important component of the Mediterranean diet and is commonly consumed uncooked, which minimises loss of flavonoids (Price, Bacon, & Rhodes, 1997). Flavonols, which are mainly present as glucosides of quercetin and kaempferol in onions, have been identified as contributing a major part of the antioxidant and health benefits of this food (Gennaro et al., 2002; Sellappan & Akoh, 2002).

The method used for the extraction of polyphenols from plant materials is important for the accurate quantification of antioxidant content and activity. This fact makes it difficult to compare data from the literature reports which vary in extraction solvent and conditions.

Several methods have been developed for determination of the polyphenol content and antioxidant activity of whole vegetable extracts, since the separation and analysis of pure antioxidants is difficult and less relevant commercially (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002; Stratil, Klejdus, & Kuban, 2006). The Trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) are commonly used to evaluate antioxidant activity *in vitro* (Benzie & Strain, 1996; Re et al., 1999). However, these chemical methods do not directly

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predict the activity of natural antioxidants in food, and other methods, which more directly evaluate the protection against oxidation in stored foods by measuring the inhibition of the formation of oxidation products, such as hydroperoxides, conjugated dienes or hexanal, are commonly used. The determination of antioxidant activity in model lipid emulsions has become one of the most relevant procedures to test the protective effect against lipid oxidation in food emulsions, such as mayonnaise and margarine (Almajano & Gordon, 2004).

The aim of this research is to quantify the total polyphenol content and antioxidant activity of two typical Spanish onion varieties: white onion, which is one of the most widely consumed varieties in Spain, and *Calçot de Valls*, which is typically found in the North-East part of the country. The effect of extraction solvent on yield of polyphenols has been studied. The antioxidant capacity of extracts has been analysed and compared by two spectrophotometric methods (TEAC and FRAP), and the antioxidant capacity of onion samples was determined by measurement of hydroperoxide formation in stored sunflower oil-in-water emulsions, in order to study effects in a model food system.

2. Materials and methods

2.1. Chemicals and reagents

The solvents methanol, ethanol, acetone, sodium carbonate and Folin–Ciocalteu reagent were analytical grade from Panreac (Barcelona, Spain).

Gallic acid (GA), rutin, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ), phosphate buffered saline, ferric chloride, potassium persulfate, Tween 20 and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma–Aldrich Company, Ltd. (Gillingham, UK).

2.2. Preparation of onion samples

Two Spanish onion varieties (*Allium cepa L.*) were analysed. Spanish white onion (*var. Fuentes del Ebro*) and *Calot de Valls* were purchased in a local market. Samples were stored in the dark at −20 °C until their analysis.

Onions were skinned, chopped, blended and finally freeze-dried. The freeze-dried onions were ground in a mortar, in order to obtain a fine powder and were stored at 4 °C in the dark and in a dry atmosphere.

2.3. Extraction of polyphenols

Organic solvent (3 ml) was added to freeze-dried onion powder (0.3 g). After 30 min of extraction with magnetic stirring at 900 rpm, and 20 min in an ultrasonic bath, the extract was centrifuged at 3000 rpm. Extraction was performed twice more with magnetic stirring for 60 and 90 min, respectively. Finally, the three supernatants were combined, made up to 9 ml with solvent and stored at 4 °C in the dark. Light exposure was avoided during the extraction process. The extraction procedure was performed in triplicate.

Solvents used for the extraction were methanol, ethanol, acetone, these three solvents in water (50% and 75%), and water.

2.4. Determination of total phenol and flavonoid content

Total phenol content of each extract (TP) was determined in duplicate by the Folin–Ciocalteu method, as follows. An appropriate amount of the extract was diluted in distilled water to a final concentration of 2.5% (v/v). Folin–Ciocalteu reagent and 20% sodium carbonate solution were then added to obtain final concentrations of 4% and 12% (v/v), respectively. The mixture was allowed to stand in the dark for 1 h and finally diluted to an appropriate final volume with distilled water. Absorbance was measured at 765 nm using a Hewlett–Packard 8452A diode array spectrophotometer against a blank, containing distilled water instead of extract. Values were determined from a calibration curve prepared with gallic acid (ranging from 2 to 14 mg/l final concentration and $r^2 = 0.996$) and rutin (ranging from 1.25 to 15 mg/l final concentration and $r^2 = 0.995$). Results are expressed as mg of gallic acid equivalents/g dry weight (mg GAE/g DW) or mg of rutin equivalents/g of dry weight (mg rutin eq./g DW).

Flavonoid content was determined, as described by Bonvehi, Torrento, and Lorente (2001) with some modifications. An appropriate dilution of the extract was mixed with the same volume of 2% $AlCl_3$ in methanol solution (5% acetic acid in methanol). The mixture was allowed to react for 10 min and the absorbance was read at 430 nm against a sample blank without reactants. Values were determined from a calibration curve prepared with rutin (ranging from 10 to 50 mg/l final volume and $r^2 = 0.993$), and expressed as mg rutin eq./g DW.

2.5. Determination of antioxidant activity

2.5.1. Trolox equivalent antioxidant capacity method (TEAC)

The method used was based on that of Re et al. (1999). 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and potassium persulfate (7 mM ABTS and 2.45 mM potassium persulfate final concentration) were dissolved separately in water and then combined and made up to volume in a 10 ml volumetric flask. The mixed solution was transferred to an amber bottle, covered with aluminium foil and allowed to stand at room temperature for 12–16 h in the dark. The solution is stable for 3 days.

The $ABTS^{\bullet+}$ solution was diluted with PBS (pH 7.4, 1:100) and equilibrated at 30 °C, to an absorbance of 0.7 (±0.02) at 734 nm read using a Hewlett–Packard 8452A

diode array spectrophotometer. An appropriate dilution of the extract was added to $ABTS^{+}$ solution in the proportion of 1:100.

PBS (pH 7.4) was used as blank. After mixing, the absorbance at 734 nm was measured immediately and then, every minute for 5 min. Duplicate determinations were made for triplicate extractions. The percentage inhibition was calculated from the absorbance values at 5 min.

The relative change in sample absorbance, ΔA_{sample}, was calculated according to the following equation to correct for the solvent

$$\Delta A_{sample} = \frac{A_{t=0(sample)} - A_{t=5(sample)}}{A_{t=0(sample)}} - \frac{A_{t=0(solvent)} - A_{t=5(solvent)}}{A_{t=0(solvent)}}$$

Percent inhibition values were obtained by multiplying ΔA_{sample} values by 100. The TEAC value was determined from a Trolox calibration curve (ranging from 1 to 10 μM final concentration and $r^2 = 0.998$). Results are expressed as μmol of Trolox/g of DW.

2.5.2. Ferric reducing antioxidant power method (FRAP)

The FRAP method was performed as described by Benzie and Strain (1996) with some modifications. The FRAP reagent was prepared with acetate buffer (300 mM, pH 3.6), 2,4,6-tripyridyl-s-triazine (TPTZ) (10 mM in HCl, 40 mM) and $FeCl_3$ (20 mM). The proportions were 10:1:1 (v:v:v), respectively.

A suitable dilution of the extract was added to the FRAP reagent (1:30, v:v) and incubated at 37 °C for 5 min. Then, the absorbance at 593 nm was determined with a Hewlett–Packard 8452A diode array spectrophotometer. The analysis was performed in duplicate for each triplicate extract and values were determined from a calibration curve of Trolox (ranging from 3 to 20 μM final concentration; $r^2 = 0.989$). The results are expressed as μmol of Trolox/g DW.

2.5.3. Antioxidant evaluation in oil-in-water emulsion

Oil-in-water emulsions were prepared with 1% of Tween 20 as emulsifier and 10% of sunflower oil, previously filtered through alumina (Panreac), as described by Yoshida (1993), in order to remove the tocopherols. The oil was added dropwise to the aqueous sample containing emulsifier cooled in an ice-bath, while sonicating for 5 min in total.

Freeze-dried powder of both kinds of samples were added directly to the emulsion and homogenised, obtaining final concentrations of 10, 20 and 30 mg/ml. For control, no sample was added.

All emulsions were stored in triplicate in 25 ml amber bottles in the dark and allowed to oxidise at 40 °C.

Peroxide value (PV) was measured periodically using aliquots of 0.005–0.1 g of each sample and determined by the ferric thiocyanate method (Frankel, 1998), after calibrating the procedure with a series of oxidised oil samples analysed by the AOCS Official Method Cd 8-53.

2.6. Statistical analysis

Data were analysed by STATGRAPHICS Plus 5.1 for Windows (Stat Point Inc., Herndon, VA) and expressed as means ± standard deviations. Any significant difference between solvents and samples were determined by one-way analysis of variance and Tukey HSD multiple range test, considering differences significant at $p < 0.05$.

3. Results and discussion

3.1. Polyphenol content

The total phenol content (TP) of the onion extracts determined by the Folin–Ciocalteu method was in the range 2.57 ± 0.51 mg GAE/g DW to 6.53 ± 0.16 mg GAE/g DW for the Spanish white onions and 0.51 ± 0.22 to 2.58 ± 0.16 mg GAE/g DW for the *Calçot de Valls* onions. Therefore, the TP of the white onions was significantly higher than that of the *Calçot de Valls* onions (Table 1). Results obtained for these varieties are consistent with those reported for other onion varieties from other countries. The TP of the *Calçot de Valls* onions was similar to the range reported by Stratil et al. (2006) in unhydrolysed extracts. Several onion varieties contained TP in a similar range to that of the Spanish onions (Sellappan & Akoh, 2002).

For both varieties the phenol content of the extracts was dependent on the solvent used and its polarity. For both onion varieties, ethanol (75%), methanol (100%) and methanol (75%) gave the highest yield of TP, without significant differences between them. Extraction with acetone (100%) and ethanol (100%) gave the lowest yield of phenols ($p < 0.05$). Furthermore, pure solvent systems gave lower phenol yields than aqueous-organic solvents, except for methanol (100 %). In the case of the *Calçot de Valls* onions, TP of the extracts varied less with solvent than for the Spanish white onions.

Ethanol (70% or 75%) is commonly used to extract plant materials for phenol analysis (Bahorun, Luximon-Ramma, Crozier, & Aruoma, 2004; Djeridane et al., 2006). Yu, Ahmedna, and Goktepe (2005) found that methanol (80%) and ethanol (70%) were better solvents than H_2O for the extraction of polyphenols from the *Moringo oleifera* tree and from peanut skins. Aqueous methanol is one of the most commonly used solvents for the extraction of flavonoids present in onions and other vegetables (Hertog et al., 1992; Nuutila, Puupponen-Pimia, Aarni, & Oksman-Caldentey, 2003) while absolute methanol is also frequently selected for extraction of *Allium* species (Tepe et al., 2005).

The flavonoid content of the white onion extracts was also determined, to compare with TP. The results confirmed a literature report (Yang, Meyers, Van der Heide, & Liu, 2004) that flavonoids represent the main group of phenolic compounds in white onion. This can be seen in Fig. 1, with values ranging from 0.56 ± 0.06 to

Table 1
Polyphenol content and antioxidant activity of onion extracts

Solvent	White onion			Calçot		
	Polyphenol content (mg GAE/g DW)	Antioxidant activity (μmol Trolox/g DW)		Polyphenol content (mg GAE/g DW)	Antioxidant activity (μmol Trolox/g DW)	
		TEAC	FRAP		TEAC	FRAP
Water	4.76 ± 0.27^{c}	63.1 ± 3.52^{b}	17.8 ± 0.40^{de}	2.11 ± 0.24^{c}	17.5 ± 0.46^{a}	6.66 ± 1.19^{d}
Methanol (%)						
100	6.33 ± 0.36^{a}	81.1 ± 3.35^{a}	24.9 ± 2.49^{a}	2.58 ± 0.16^{d}	12.4 ± 2.00^{ab}	12.7 ± 0.29^{b}
75	6.31 ± 0.21^{a}	77.6 ± 2.17^{a}	23.1 ± 1.35^{a}	2.51 ± 0.03^{d}	15.1 ± 5.09^{ab}	16.1 ± 0.10^{a}
50	$5.15 \pm 0.06^{b,c}$	62.7 ± 1.82^{b}	19.3 ± 0.51^{cd}	2.48 ± 0.07^{cd}	14.6 ± 2.90^{ab}	12.8 ± 0.28^{b}
Ethanol (%)						
100	4.30 ± 0.13^{d}	49.2 ± 2.31^{c}	16.0 ± 0.60^{e}	1.20 ± 0.32^{b}	3.86 ± 0.89^{cd}	2.73 ± 0.55^{c}
75	6.53 ± 0.16^{a}	86.6 ± 2.97^{a}	23.1 ± 0.59^{ab}	2.41 ± 0.08^{cd}	9.10 ± 0.58^{bc}	8.19 ± 1.41^{c}
50	4.97 ± 0.61^{bc}	63.2 ± 1.54^{b}	18.6 ± 2.85^{cd}	2.48 ± 0.29^{cd}	9.59 ± 0.59^{bc}	6.53 ± 0.33^{c}
Acetone (%)						
100	2.57 ± 0.51^{e}	6.13 ± 0.68^{d}	2.95 ± 0.58^{f}	0.51 ± 0.22^{a}	2.32 ± 0.96^{d}	1.31 ± 0.56^{d}
75	5.70 ± 0.28^{b}	66.1 ± 4.03^{b}	20.9 ± 0.85^{bc}	2.14 ± 0.01^{c}	9.85 ± 0.40^{bc}	6.71 ± 0.28^{c}
50	5.27 ± 0.19^{bc}	63.0 ± 5.77^{b}	19.7 ± 1.79^{cd}	2.31 ± 0.11^{cd}	10.1 ± 0.38^{bc}	6.30 ± 0.20^{c}

Data expressed as means ± SE of duplicate analyses of each triplicate extraction. Values in the same column with different letters present significant differences $p < 0.05$.

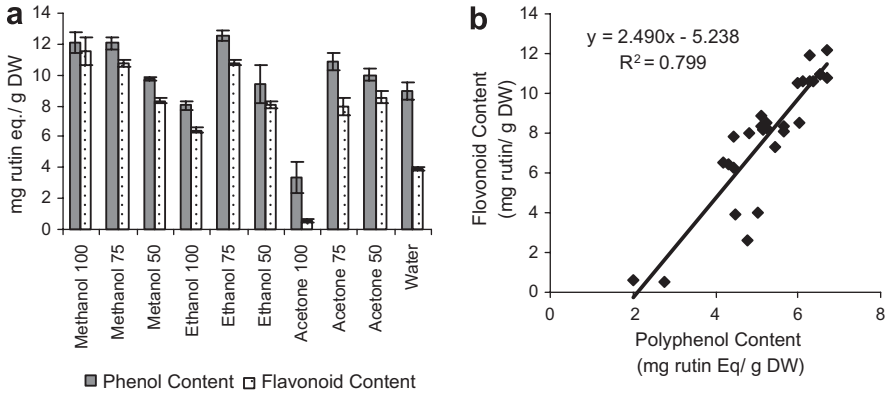


Fig. 1. (a) Polyphenol content and flavonoid content of white onion extracts. (b) Correlation between polyphenol content and flavonoid content.

11.5 ± 0.93 mg rutin eq/g DW and a good correlation between TP and flavonoid content was found $r^2 = 0.799$.

3.2. Antioxidant activity

The TEAC method is frequently used for determination of antioxidant activity. It is based on the radical-scavenging capacity of antioxidants. The $ABTS^{\cdot+}$ radical, which has a maximum absorbance at 764 nm, can be quenched by antioxidants to its colourless form. However, the $ABTS^{\cdot+}$ radical can also degrade under experimental conditions and the antioxidant activity of samples can be overestimated. A correction for the spontaneous degradation of $ABTS^{\cdot+}$ should be included, in order to obtain accurate data.

White onion extracts had a significantly higher antioxidant activity than those of *Calçot* ranging from 6.13 ± 0.68 to 86.6 ± 2.97 μmols Trolox/g DW and 2.32 ± 0.96 to 17.5 ± 0.46 μmol Trolox/g DW, respectively, which is consistent with the phenol content of the two varieties. Extracts prepared with ethanol (75%), methanol (100%) and methanol (75%) had the maximum antioxidant activity while acetone (100%) and ethanol (100%) gave extracts with the lowest antioxidant activity. Water extracts of *Calçot* also showed good antioxidant activity. The antioxidant capacity of the extracts was only weakly dependent on the solvent used, if at least 50% of water was present. FRAP analysis confirmed the effects of solvent on antioxidant activity of extracts.

The difference between the TP of vegetable varieties is reflected in the antioxidant activity of extracts, as reported previously (Sellappan & Akoh, 2002). The antioxidant capacity of *Calçot* extracts determined by this method is in good agreement with recent reports for onions from other countries (Bahorun et al., 2004; Nuutila et al.,

2003). However, the TEAC values of Spanish white onion extracts are slightly higher than those reported in another study (Stratil et al., 2006). Differences in cultivation conditions, or different extraction procedures or experimental conditions can contribute to differences in the literature data.

The FRAP assay is commonly used for assessing antioxidant activity, since it has high sensitivity, and is rapid and inexpensive. At low pH (optimum pH 3.6) Fe^{3+}–TPTZ complex is reduced by antioxidants to its intense blue coloured form Fe^{2+}–TPTZ which has maximum absorbance at 593 nm.

In agreement with findings from the TEAC assay, the FRAP values were higher for white onion extracts, ranging from 2.95 ± 0.58 to 24.9 ± 2.49 μmol Trolox/g DW, than for *Calçot* extracts which ranged from 1.31 ± 0.56 to 16.1 ± 0.10 μmol Trolox/g DW. The FRAP values were in good agreement with literature reports for onion samples (Bahorun et al., 2004; Ou et al., 2002).

The TEAC and FRAP values of the onion extracts correlated well, with $r^2 = 0.862$ (Fig. 2). However, the TEAC values were much higher than the FRAP values, which is in agreement with other reports, where various antioxidant methods were compared (Stratil et al., 2006). Several studies have compared different methods, to evaluate the antioxidant activity of samples, although a general consensus has not yet been established. Some authors find similar values between the methods while others report noticeable differences between them or a dependency on the type of food sample. Bahorun et al. (2004) found higher values for Mauritanian vegetables by the FRAP method than by the TEAC method, which is in disagreement with these results. Quercetin glycosides present in onions appear to have high radical-scavenging activity but relatively low reducing activity, compared with other phenolics, as can also be seen in results obtained by Stratil et al. (2006) where radical-scavenging activity of onion extracts was four times higher than reducing capacity. Interference by other substances (e.g., carotenoids, ascorbic acid or saccharides) may also contribute to differences from studies with other plant materials.

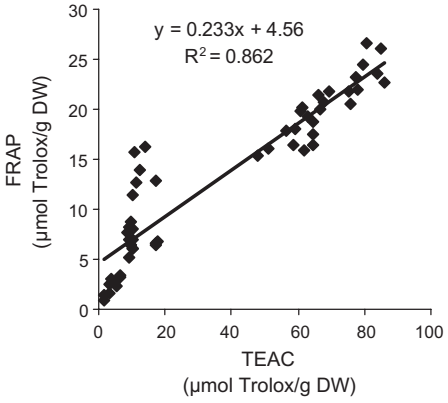


Fig. 2. Correlation between antioxidant capacity evaluated by TEAC and FRAP.

The good correlations between phenol content and TEAC, and FRAP values ($r^2 = 0.936$ and $r^2 = 0.881$, respectively) confirm that phenols are mainly responsible for the antioxidant activity of extracts (Fig. 3). Several studies have reported a good correlation between the phenol content of plant extracts and antioxidant activity (Bahorun et al., 2004; Djeridane et al., 2006), but other studies report a poor correlation (Sellappan & Akoh, 2002). Flavonols, which are known for their antioxidant activity, are the phenolics mainly present in *Allium* samples. Quercetin or its glycosylated forms are found in high amounts in onions and contribute antioxidant and antibacterial properties (Shon, Choi, Kahng, Nam, & Sung, 2004; Yin & Cheng, 1998).

Differences in antioxidant activity determined by different methods emphasise the importance of using several methods to assess this parameter, in order to obtain accurate data and to improve comparison with other literature, as previously reported by Stratil et al. (2006).

3.3. Antioxidant evaluation in oil–water emulsions

Lipid oxidation is mainly responsible for off-flavour development in fatty foods, so phenolic antioxidants should be studied in suitable food model systems.

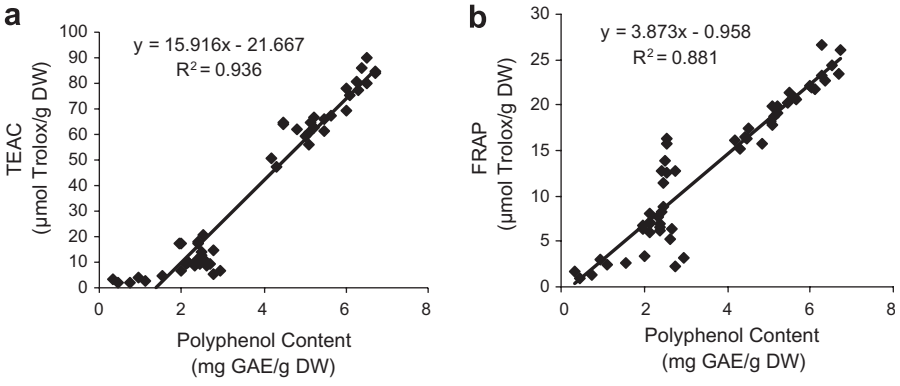


Fig. 3. Correlation between polyphenol content and antioxidant capacity of both onion varieties: (a) phenol content vs. TEAC and (b) phenol content vs. FRAP values.

In the present study peroxide formation has been determined in sunflower oil-in-water emulsions incubated at 40 °C. Different amounts of dry onion powder of both Spanish varieties (10, 20, 30 mg/ml emulsion) were added to test their antioxidant activity.

Fig. 4 shows hydroperoxide formation during emulsion storage. Samples from *Calçot* onions showed little antioxidant activity but white onion extracts were more effective.

In order to allow statistical analysis of the data, the time to reach PV = 10, 20, 30 and 40 meq/kg has been calculated (Fig. 5). Both onion varieties were effective in retarding the formation of hydroperoxides, until the PV reached 10 and 20 meq/kg. However, no significant differences were found between the control and *Calçot* samples at higher PVs which show that these extracts only inhibited oxidation in the early stages of emulsion deterioration. White onion samples were shown to be more effective than *Calçot* ones in retarding peroxide formation, even at higher PV values.

The antioxidant effect of *Calçot* in emulsions at 40 °C did not increase with concentration ($p < 0.05$). White onion strongly retarded hydroperoxide formation, and the effect increased with concentration up to 30 mg/ml.

The higher antioxidant activity of white onion powder in emulsions was in good agreement with the higher TP and antioxidant capacity values of these extracts.

The antioxidant activity of onion is consistent with literature reports. Nuutila et al. (2003) observed that onion

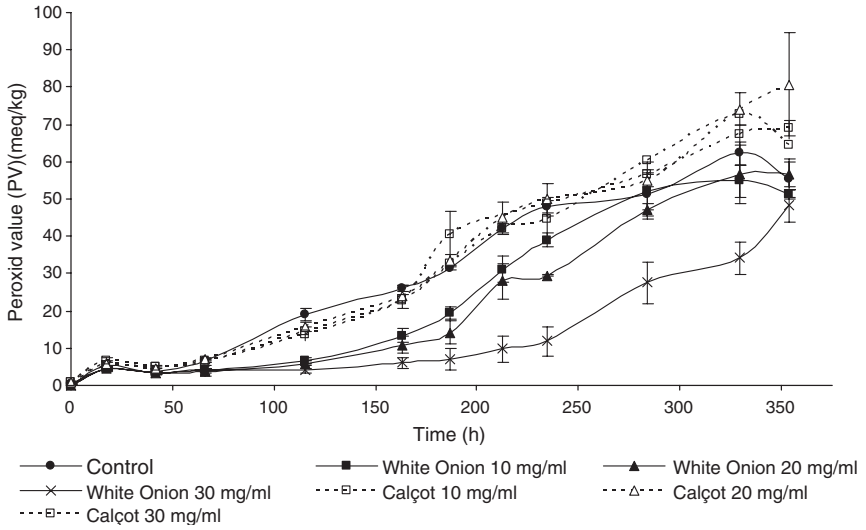
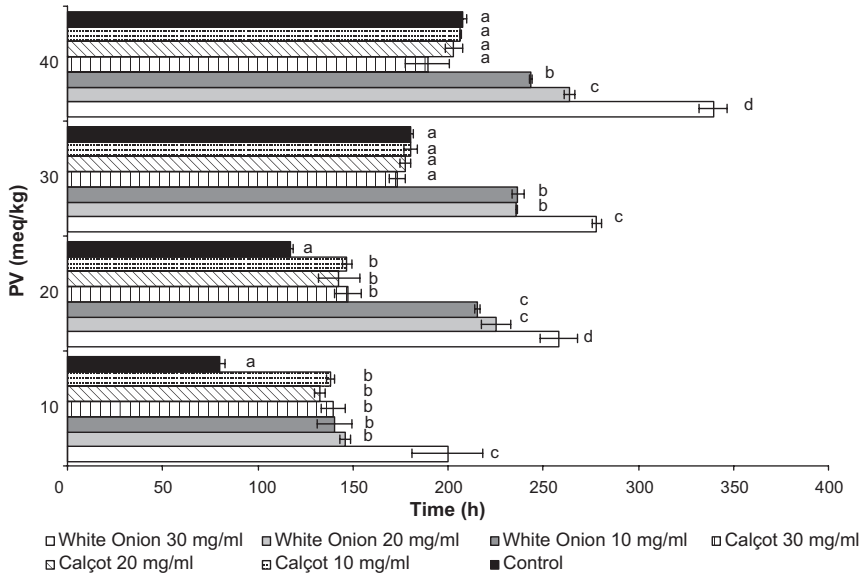


Fig. 4. Effects of onion powder on peroxide value (PV) formed in stored emulsions at 40 °C.



Samples with different letters denote significant differences ($p < 0.05$)

Fig. 5. Time for samples to reach different peroxide values.

extracts and some flavonols were effective in inhibiting peroxidation of rat hepatocytes. They also reported a good correlation between radical-scavenging activity and protection against oxidation. Quercetin and kaempferol, two of the most abundant flavonols in onion (Lanzotti, 2006), are known as good inhibitors of lipid oxidation. Aglycones of both flavonols are more active than their glycosylated forms, due to the presence of free hydroxyl groups. The antioxidant activity may be due to both free radical scavenging and metal chelation. Onion antioxidant activity has also been reported by Yin and Cheng (1998) in liposome preparations, although onion showed a weaker effect than other *Allium* members such as garlic. Recently, Navas, Carrasquero-Duran, and Flores (2006) found no effect of onion extracts on oxidation of corn oil in induced oxidations and they confirmed that garlic extracts were more effective than onion extracts.

4. Conclusion

Ethanol (75%), methanol (100%) and methanol (75%) were the most efficient solvents for phenol extraction from Spanish onion varieties. Regardless of the solvent used, the *Calçot de Valls* variety contained lower concentrations of phenolic compounds than Spanish white onions. There was a good correlation between phenol content and antioxidant capacity. In general extracts with higher amounts of phenolic compounds showed higher TEAC and FRAP values.

Dry powder of both onion varieties retarded oxidation in an oil-in-water emulsion system. In accordance with the higher phenol content, white onions were more effective than *Calçot* samples in retarding oxidation. The effect of white onion increased with concentration. In conclusion, onion can be a useful natural antioxidant in fatty foods.

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