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Effect of two different red grape pomace extracts obtained under different extraction systems on meat quality of pork burgers

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ABSTRACT

The effect on meat quality (pH, microbial spoilage, lipid oxidation and colour coordinates) of two different types of red grape pomace extracts obtained by different extraction systems [(Grape pomace extract I, GPI (methanolic extraction + High-Low Instantaneous Pressure − HLIP—GPI) and Grape pomace extract II, GPII (methanolic extraction, GPII) at 0.06 g/100 g final product concentration] in pork burgers packed under aerobic conditions (4 °C) was assessed at 0, 3 and 6 days post-storage.

Based on the results the highest colour stability, lipid oxidation inhibition and the best global acceptability after 6 days of storage (data not shown) observed in burgers added with the GPI indicate that the new extraction system (HLIP) developed could be a valid alternative to optimize the purity of the grape pomace extracts in order to use them as preservative in meat foodstuffs.

1. Introduction

The prevention of oxidative deterioration in muscle foods is essential if their quality and shelf-life are to be guaranteed (Buckley, Morrissey, & Gray, 1995). Lipid oxidation generates a series of chemical reactions that can alter physico-chemical parameters, sensorial attributes (odour, colour, flavour) and shelf-life in meat and meat products (Liu, Lanari, & Schaefer, 1995). In pork, the lipid oxidation process can be especially intense due to its relatively high proportion of unsaturated fatty acids susceptible to oxidation (McCarthy, Kerry, Kerry, Lynch, & Buckley, 2001). Thus synthetic antioxidants (butylated hydroxyanisole, butylated hydroxytoluene or propyl gallate) have been successfully used in order to prevent such oxidation in fresh meats. Recently, it has been realized that these products may exhibit toxic properties in the human organism that counteract any beneficial effect for the consumer (Tamil Selvi, Joseph, & Jayaprakasha, 2003). This is one of the reasons for the increased demand for the healthy properties of the "*natural food ingredients*" of a plant origin (Rojas & Brewer, 2008). Among these, extracts rich in phenolic compounds, like those derived from the wine and juice industries, grape seeds and pomace, have been reported as good alternatives since they are available in plenty supply as industrial wastes and maintain a potential preservative effect (Baydar, Ozkan and Sagdiç, 2004). Many positive effects on human health have been described for polyphenols including anti-inflammatory, anti-carcinogenic, cardioprotective and vasodilatory properties (Bonilla, Mayen, Merida, & Medina, 1999).

The antioxidant capacity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms and chelate metal cations (Balasundram, Sundram, & Samman, 2006). Numerous authors mention the potent antioxidant effect of grape polyphenols (*Vitis vinifera*) in muscle food (pork: Carpenter, O'Grady, O'Callaghan, O'Brien, & Kerry, 2007; O'Grady, Carpenter, Lynch, O'Brien, & Kerry, 2008. beef: Rojas & Brewer, 2007, 2008; poultries: Brannan, 2009; Mielnik, Olsen, Vogt, Adeline, & Skrede, 2006; Sayago-Ayerdi, Brenes, & Goñi, 2009). Other studies (Castillo et al., 2000; Louli, Ragoussis, & Magoulas, 2004) have reported that the antioxidant components in grape residues can be changed or modified by pretreatment of the raw material and using different extraction procedures as well as purification and crystallization operations. The literature contains many mentions of different solvent-extraction processes for grape polyphenols: Baydar, Ozkan, and Sagdic (2004); Bonilla et al. (1999); Lapornik, Prosek, and Wondra (2005); Pekic, Kovac, Alonso, and Revilla (1998); Revilla, Ryan, and Martín-Ortega (1998). In addition Spigno and De Faveri (2007) remarked on the importance of systematic approaches to maximise the extract yield and purity of the antioxidant from grape pomace. Accordingly the present study focuses on a novel system for the mechanical

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destruction of the raw material, which involves sharp pressure changes to make the solvent-extraction method more efficient, thus increasing the purity of the extracts added to muscle foodstuffs. The technique is at present being perfected with different sub products on a semi-industrial scale in the Pilot Plant at the University (Saura, Martí, & Micol, 2008). The aims of this work were (1) to evaluate the effect of extracts from grape pomace extracted in different ways on the physico-chemical characteristics of pork burgers and (2) to evaluate the viability of using such extracts as natural preservatives in meat products.

2. Material and methods

2.1. Grape samples and extraction procedures

To carry out the present analysis methanol solvent (HPLC-gradient grade PAI-ACS, Panreac, Barcelona, Spain) was used. Two different types of natural extracts from industrial red grape pomace (*V. vinifera* var. Monastrell, Murcia, Spain) were considered according to the extraction method used: Grape pomace extract Type I (High-Low Instantaneous Pressure − HLIP + methanolic extraction − GPI) and Grape pomace extract Type II (methanolic extraction, GPII). Prior to the alcoholic extraction procedure the extract GPI was processed, following an innovative method that consists of modifying the pomace tissues to increase their permeability to the extraction solvents by sudden pressures changes. The technique is based on the rapid expansion under controlled conditions that occur in the treatment chamber, which produces a dramatic pressure change from 1×10^5–4×10^5 Pa (N/m^2) (pressure generated by water vapour) to 5×10^3 Pa (N/m^2). This fast change in pressure in less than 1 s breaks tissues and creates microchannels through which the extraction solvent penetrates. In order to obtain the grape skin extract the following procedure was carried out: A l0 g sample of dry skin was obtained by grinding dry skin with and without HLIP treatment and then mixing the sample in a 500 ml flask with 100 ml of methanol and stirring at room temperature for 10 min. The sample was then filtered through paper filter by gravity (once). The retentate cake was washed with 100 ml of methanol at room temperature for 10 min, and was again extracted as in the first extraction procedure. In a third extraction step, was added again 100 ml of methanol and shaken at room temperature for 10 min. It was then filtered as in the previous steps. Finally, the entire volume of the three extractions was mixed together in a flask adapted to a rotary evaporator (IKA, Königswinter, Germany) at 50 °C and 200 rpm in order to evaporate the methanol and obtain a dry sample. All the solids in the flask were removed with a known quantity of distilled water.

2.1.1. Measurement of the Trolox equivalent antioxidant capacity (TEAC)

The antioxidant capacity of the extracts was determined as radical scavenging activity. The Trolox equivalent antioxidant capacity (TEAC) assay, which measures the reduction of the radical cation of ABTS by antioxidants following the procedure described by Re et al. (1999). (ABTS+) assay [2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid)] was produced by reacting ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–24 h before use. The ABTS + solution was diluted with ethanol or water (lipophilic and hydrophilic assay, respectively) to an absorbance of 0.70 (±0.02) at 734 nm. For the photometric assay 1 ml of the ABTS + solution and 100 μL antioxidant solution were mixed and measured immediately after 3 min at 734 nm. Determinations were carried out by triplicate and the results were expressed as mmol/L of Trolox.

2.1.2. Total polyphenols index

The total polyphenols index T.P.I. was ascertained by measuring the optical density (OD 280) against the distilled water of the sample diluted 101 times, at 280 nm, under a 10 mm quartz cuvette in according to Ribéreau-Gayon, Glories, Maujean, and Dubourdieu (1998a, 1998b).

2.1.3. Total anthocyanins

A sample of 0.5 ml was taken from each extract and diluted to a volume of 5 ml with 10 ml/100 ml ethanol. A 0.25 ml aliquot of each diluted sample was subsequently added to 0.25 ml of 0.1 ml/100 ml HCl in 95 ml/100 ml ethanol, and 4.55 ml of 2 ml/100 ml HCl. Each sample was vortexed and allowed to stand for 15 min. The absorbance was measured in a 1 cm quartz cuvette at 520 nm using a Beckman DU 640 spectrophotometer (Beckman Coulter, California, USA). Total anthocyanins (mg/L) were quantified from a standard curve with malvidin-3-glucoside (in 10 ml/100 ml ethanol).

2.2. Sample preparation

A total of 120 burgers were distributed into three different batches established [control group (no extracts), GPI and GPII (with 0.06 g/100 g final product of the extract Type I and II, respectively)] and each one was carried out by replicated (20 burgers per batch × 3 different batches × 2 replicates each). Burgers were prepared according to a homemade formulation with the following ingredients: 70 g/100 g lean pork, 30 g/100 g back fat and 2 g/100 g salt. In a refrigerated room at 12 °C, the meat was minced (5 mm) using a P3298 cutter (Braher International, San Sebastian, Spain) and then mixed for 10 min using an RM-60 Mixer (Mainca Granollers, Spain). Burgers (100 g each) were formed with a conventional burger-maker (Juan Martínez Pérez Ltd., Murcia, Spain) before being packaged in clear polystyrene trays BA-85 (Sena, Sociedad de Envases Alimentarios, Aduna, Spain) and covered with a polypropylene film with an oxygen permeability of 6000–8000 cm^3/m^{-2} at standard temperature and pressure (Raelma Industries, Madrid, Spain). Samples were stored at 4 °C for 0, 3 and 6 days in a display cabinet illuminated with white fluorescent light (620 lux) simulating retail display conditions. Measurements.

The following measurements were carried out in raw samples burgers:

- pH using a penetrating electrode adapted to a portable Crison 507 pH-meter (08328, Alella, Barcelona, Spain).
- Colour values, lightness (L^*) redness (a^*) and yellowness (b^*) using a colorimeter CR 400 (Minolta Ltd., Milton Keynes, United Kingdom) calibrated against a standard white tile.
- Microbiological analysis: Samples were blended (Stomacher IUL Instruments, GMBH, Königswinter, Germany) and diluted in peptone water (Oxoid Ltd. CM0087, Basingstoke, Hampshire, United Kingdom). Total Viable Count (TVC) and Psychrophile (PHY) spoilage were evaluated using a plate count agar (Oxoid Ltd. CM0325, Basingstoke, Hampshire, United Kingdom), while for the Total Coliform Count (TCC) a chromogenic *Escherichia coli*/coliform medium (Oxoid Ltd. CM956, Basingstoke, Hampshire, United Kingdom) was used. For TVC and TCC, incubation was at 37 °C for 48 h (Incubator Heraeus S.A. Boadilla, Madrid, Spain) and for PHY, at 4 °C for 10 days.
- Thiobarbituric acid reactive substances (TBARS) level (mg malondialdehyde/kg sample) was analysed by duplicate as described by Botsoglou et al. (1994). Two grams of sample were homogenized with an acid solution [8 ml of aqueous dissolution of acid TCA (5 ml/100 ml) (trichloroacetic acid) (Scharlau

Chemie S.A. Barcelona, Spain) and 5 ml of BHT (0.8 ml/100 ml) (butylhydroxytoluene) (Scharlau Chemie S.A. Barcelona, Spain) in hexane (Mallinckrodt Baker B.V. Deventer, Holland)] by using an Ultra-Turrax T25 (IKA – Labortechnik, Staufen, Germany) during 1 min at 9.500 rpm. After that the samples were centrifuged during 10 min at 1.028 g (Centrifugal Kubota 2010; Kubota Corporation, Tokyo, Japan). Later, the supernatant was removed and the volume completed until 10 ml with the TCA solution. After centrifugation (1.028 g during 10 min), aliquots of 2.5 ml were taken from each sample by triplicate and adding 1.5 ml of thiobarbituric solution (Acros Organics Geel, Belgium). All samples were put in a water bath at 70 °C during 30 min. After incubation, the samples were fastly cooled in a water bath until room temperature (22 ± 2 °C). Absorbancies were measured using a spectrophotometer (Pye Unicam Ltd., Cambridge, United Kingdom) at 532 nm. TBARS were calculated using a 1, 1, 3, 3-tetraethoxypropane standard curve.

2.3. Data analysis

The data were analysed using an analysis of variance to determine the effect of the type of burger (control, GPI and GPII) and the storage time (from 0 to 6 days) on meat quality. The total number of samples was 120 (20 burgers per batch × 3 different batches × 2 replicates each). When differences among elaboration groups were significant ($P < 0.05$) a Tukeys' test at a significance level of $P < 0.05$ was carried out to check the differences between pairs of groups. Data were analysed using the SPSS 15.0 version statistical package.

3. Results and discussion

The antioxidant activity (mmol/L Trolox), Total polyphenols index and Total Anthocyanins (mg/L) for both extracts were showed in Table 1.

The total polyphenols content was higher in GPI than in GPII. The previous process carried out to methanolic extraction had an evident effect on the grape extract composition. It seems that the rapid expansion under controlled conditions that occur in the treatment chamber with the novel technology plays an important role on the final concentration of the phenolic profile. This is also evident on the anthocyanins content which was higher in the extract GPI than in the GPII.

The GPII extract showed a total polyphenols content similar to those found by others authors in red grape extracts (Revilla et al., 1998), while the anthocyanins content of both extracts was much higher than found by same authors.

Lastly, the antioxidant activity determined of 141.81 vs 63.36 mmol/L Trolox for GPI and GPII respectively, clearly remarks important differences between extracts. pH and microbial growth of raw pork burgers.

Table 2 shows the pH values (mean ± s.d.) of the three types of burgers packed under aerobic conditions for 6 days of storage. The values ranged between 5.4 and 5.5 and no significant differences were found due to the effect of the type of burger (control, GPI, GPII) or the storage time (0, 3, 6 days) in agreement with other studies on raw pork patties (Carpenter et al., 2007; O'Grady et al., 2008). Similarly, others like Han and Rhee (2005) found no significant differences in raw ground beef with added herbal extracts. However, in the present study slight differences were observed on day 3 since the burgers containing the grape pomace extract Type I (GPI) reached lower pH values ($P < 0.05$) than the control samples (5.44 vs 5.52, for GPI and C, respectively). Ahn, Grun, and Mustapha (2004) obtained similar data for beef patties treated with grape seed extract, an effect they attributed to the high phenolic acid level of the extracts which may also explain our results.

Microbial growth (log c.f.u./g) of Total Viable Count (TVC), Psychrophile (PHY) and Total Coliform Count (TCC) in the three types of burger packed under aerobic conditions for 6 days are shown in Table 3. Generally, the addition of red grape pomace extracts did not affect the spoilage of burgers in any of the three groups. Furthermore the growth values from 0 to 3 days of storage were below the 10^6–10^7 c.f.u./g considered the limit of microbial meat spoilage (ICMSF, 1986). Other authors (Díaz, 2005) found similar results (4–5 log c.f.u./g) for Total Viable Counts in beef burgers treated with grape seed extracts during 6 days of storage. However, in the present study an unacceptable level of spoilage (10^6–10^7 c.f.u./g) was observed in all the analysed samples at 6 days

Table 1
Antioxidant capacity, total polyphenols index and total anthocyanins of the two red grape pomace extracts (*Vitis vinifera* var. Monastrell).

Extracts	Total polyphenols index	Total anthocyanins (mg/L)	Antioxidant capacity (mmol/L Trolox)
GPI ($n = 3$)	546.00 ± 16.16	1783.50 ± 45.48	141.81 ± 8.07
GPII ($n = 3$)	256.50 ± 10.17	816.06 ± 17.76	63.36 ± 4.55

Table 2
pH values (mean ± s.d.) of raw pork burgers stored in aerobic packaging for 0, 3 and 6 days under retail display conditions.

Type of burger	Analysis time (in days)			*p*-value
	0	3	6	
C ($n = 40$)	5.47 ± 0.02	5.52 ± 0.16[a]	5.46 ± 0.06	0.487
GPI ($n = 40$)	5.47 ± 0.02	5.44 ± 0.01[b]	5.41 ± 0.01	0.172
GPII ($n = 40$)	5.56 ± 0.00	5.52 ± 0.02	5.56 ± 0.01	0.152
p-value	0.079	0.053	0.381	

C: control sample (no extracts); GPI: extract Type 1; GPII: extract Type 2 (0.06 g/100 g of final product of GPI and GPII, respectively).
NS: not significant.
[a, b]: Different superscripts in the same column indicate significant differences between samples from C vs GPI groups.

Table 3
Total Viable Count (TVC), Psychrophile (PSY) and Total Coliform Count (TCC) (mean ± s.d.) in raw pork burgers stored in aerobic packaging for 0, 3 and 6 days under retail display conditions.

Growth (u/g)	Type of burger	Analysis time (in days)			*p*-value
		0	3	6	
TVC	C ($n = 40$)	4.3 ± 0.1^{X}	5.7 ± 0.0^{Y}	6.5 ± 0.1^{Z}	0.0001
	GPI ($n = 40$)	4.5 ± 0.0^{X}	5.7 ± 0.1^{Y}	6.5 ± 0.1^{Z}	0.004
	GPII ($n = 40$)	4.2 ± 0.0^{X}	5.2 ± 0.0^{Y}	6.7 ± 0.0^{Z}	0.0001
	p-value	0.304	0.053	0.529	
PSY	C ($n = 40$)	4.2 ± 0.1^{X}	5.7 ± 0.1^{Y}	6.3 ± 0.1^{xYA}	0.0001
	GPI ($n = 40$)	4.5 ± 0.0^{X}	5.6 ± 0.0^{Yu}	6.6 ± 0.0^{xZu}	0.001
	GPII ($n = 40$)	4.2 ± 0.0^{X}	5.2 ± 0.0^{Yw}	6.3 ± 0.0^{yYBw}	0.001
	p-value	0.231	0.117	0.016	
TCC	C ($n = 40$)	4.2 ± 0.0^{yXa}	5.5 ± 0.1^{Y}	6.3 ± 0.1^{Z}	0.0001
	GPI ($n = 40$)	4.6 ± 0.0^{xXbu}	5.4 ± 0.1^{Y}	6.0 ± 0.0^{Zu}	0.003
	GPII ($n = 40$)	4.2 ± 0.0^{yXw}	4.9 ± 0.0^{Y}	6.4 ± 0.0^{Zw}	0.0001
	p-value	0.001	0.130	0.259	

C: control sample (no extracts); GPI: extract Type 1; GPII: extract Type 2 (0.06 g/100 g of final product of GPI and GPII, respectively).
[x, y]: Different superscripts in the same column indicate significant differences by effect of the antioxidant.
[X, Y, Z]: Different superscripts in the same row indicate significant differences by effect of the storage time for the same type of burger.
[a, b]: Different superscripts in the same column indicate significant differences between samples from C vs GPI groups.
[A, B]: Different superscripts in the same column indicate significant differences between samples from C vs GPII groups.
[u, w]: Different superscripts in the same column indicate significant differences between samples from GPI vs GPII groups.

possibly related with the low dose of extract added since the antimicrobial effect of these natural extracts decreases when they are applied to food systems (Ahn, Grun and Mustapha, 2007). On the other hand microbial growth in the three batches increased with storage time in agreement with the observations of Rodríguez (2006) in pork burgers with grape extracts at 300 ppm. Antioxidants did not prevent spoilage during storage completely, indicating that the packaging systems as well may been important in patties preservation as has been discussed by Mitsumoto, O'Grady, Kerry, and Buckley (2005).

3.1. Lipid oxidation and colour stability of raw pork burgers

Table 4 illustrates the effect of the type of burger and storage on the lipid oxidation levels (thiobarbituric acid reactive substances TBARS, measured as mg malondialdehyde [(MDA)/kg^{-1})] in raw pork burgers stored under aerobic conditions for 6 days.

The initial lipid oxidation level (at day 0) ranged from 0.5 to 1 mg MDA/kg^{-1} ($P < 0.05$) in the three groups (C, GPI, GPII). In control burgers the TBARS value was double that of the burgers with the GPI extract (1.07 vs 0.54 for C and GPI, respectively, $P < 0.05$) due to the positive effect of grape polyphenols compounds in meat preservation. Similar results were found by Sayago-Ayerdi et al. (2009) in raw chicken meat with added grape antioxidant dietary fibre (0.8–1 mg MDA/kg^{-1}). In pork patties Mitsumoto et al. (2005) found that lipid oxidation was retarded by using natural antioxidants, since TBARS initial values in meat with tea catechins were of 0.2–0.5 mg MDA/kg^{-1} which is comparable to that reported in the present research (0.5–0.8 mg MDA/kg^{-1}, Table 4). A stronger antioxidative effect was also observed with the GPI than with the GPII since a lower malondialdehyde concentration was reached in the GPI burgers group (0.54 vs 0.78, for GPI and GPII, respectively, $P < 0.05$). The same trend was observed at day 3 and 6 of storage with significant differences between groups ($P < 0.05$). Therefore, at day 3 the control and GPII burgers showed markedly higher MDA concentrations while lipid oxidation was minimal in the GPI meat. Grape polyphenols, more specifically, the anthocyanins act as antioxidants by donating hydrogen to highly reactive radicals preventing free radical formation (Lapornik et al., 2005). The GPI extract contained the highest polyphenol (546) and total anthocyanins (1783.5 mg/L) levels, compared with 256.5 and 816.06 mg/L in GPII, which is directly related with the observed higher antioxidant capacity, double in the case of the extract GPI compared with GPII (141.81 vs 63.36 mmol/L Trolox for GPI and GPII, respectively) (Table 1) and, consequently, with the intense antioxidative effect of the first extract (Caillet, Salmieri, & Lacroix, 2006). On day 6 the TBARS level for the burgers containing extract GPI was between 2 and 7 times lower than in the GPII and control burgers, respectively which demonstrates the intense antioxidant effect of the GPI extract in pork burgers. The new extraction procedure proposed in this study seems to increase the effectiveness of the grape pomace antioxidant compounds extraction method.

Table 4
Lipid oxidation values (mean ± s.d) (mg malondialdehyde/kg^{-1}) of raw pork burgers stored in aerobic packaging for 0, 3 and 6 days under retail display conditions.

Type of burger	Analysis time (in days)			*p*-value
	0	3	6	
C ($n = 40$)	1.07 ± 0.08^{ayZ}	5.41 ± 0.45^{xYaA}	7.18 ± 0.46^{xXaA}	0.0001
GPI ($n = 40$)	0.54 ± 0.05^{bxYu}	0.43 ± 0.08^{zYbu}	1.22 ± 0.16^{yXbu}	0.029
GPII ($n = 40$)	0.78 ± 0.01^{xyYw}	2.76 ± 0.02^{yXBw}	2.92 ± 0.10^{yXBw}	0.0001
p-value	0.039	0.001	0.0001	

C: control sample (no extracts); GPI: extract Type 1; GPII: extract Type 2 (0.06 g/100 g of final product of GPI and GPII, respectively); NS: not significant.
[x, y]: Different superscripts in the same column indicate significant differences by effect of the antioxidant.
[X, Y, Z]: Different superscripts in the same row indicate significant differences by effect of the storage time for the same type of burger.
[a, b]: Different superscripts in the same column indicate significant differences between samples from *C* vs *GPI* groups, respectively ($P < 0.05$).
[A, B] : Different superscripts in the same column indicate significant differences between samples from *C* vs *GPII* groups, respectively ($P < 0.05$).
[u, w] : Different superscripts in the same column indicate significant differences between samples from *GPI* vs *GPII* groups, respectively ($P < 0.05$).

On the other hand, the storage also affected the MDA concentration and values increased with storage time in all the burgers groups ($P < 0.001$, 0.05 and 0.001, respectively, for C, GPI, GPII) in agreement with other researches (beef: Brannan & Mah, 2007; mutton: Jayathilakan, Sharma, Radhakrishna, & Bawa, 2007 or goat: Han & Rhee, 2005).

The ratio of TBARS for control burgers ranged from 5 to 7 mg MDA/kg^{-1} of meat at 3 and 6 days of storage which is much higher than the values reported by Sayago-Ayerdi et al. (2009) after almost two weeks of storage in raw chicken burgers (around 1–2 mg MDA/kg^{-1}). This fact may be related with the higher fat content of pork in comparison with poultry meat (Yen, Biing-Hwan, & Davis, 2008). Others like Bastida et al. (2009) found similar TBARS values in pork patties (control) that also included 2% salt in its formulation. Others like Houben, Eikelenboom, and Hoving-Bolink (1998) reported lower TBARS values in minced pork (0.05–0.18 at 0 and 3 days, respectively) but in not salted samples (Rojas & Brewer, 2007).

The lowest MDA concentration was reached in GPI burgers at day 3 (0.4 mg MDA/kg meat) which confirms the potent effect of the grape seed extracts in the inhibition of lipid oxidation in pork patties like previously were described in cooked pork samples by others (Brannan & Mah, 2007; Rojas & Brewer, 2007).

Colorimetric parameters (L^*, a^*, b^* coordinates, mean ± s.d.) of the three types of burger packed under aerobic conditions for 6 days of storage are shown in Table 5. Lightness (L^* coordinate) was lower (darker meat) on day 6 in burgers with added extract Type I (41.93) than in the control samples (46.14) ($P < 0.05$). Both of the red grape pomace extracts obtained in the present research (GPI and GPII) are highly rich in total anthocyanins but the highest content was described for GPI. This fact could be responsible for the darker colour of the raw GPI patties since these molecules act as potent natural colourants with a positive nutritional and therapeutic effect on human organism derived from its antiradical and antioxidant effect (Balasundram et al., 2006; Lapornik et al., 2005; Revilla et al., 1998). In contrast, others researches (Carpenter et al., 2007; O'Grady et al., 2008) did not find any significant differences in L^* values following the addition of grape seed extracts although they point out that such extracts are colour enhancers of red meat and its derivates. A similar L^* value evolution was also described by Ahn et al. (2007) in beef burgers treated with grape seed extract, which was attributed to the antioxidative effect of the extract, rich in a variety of polyphenolics compounds with free radical scavenging action (Caillet et al., 2006). Also L^* increased with time in control samples ($P < 0.05$), possibly due to the absence of any meat preservative in the burgers and faster meat pigment oxidation (Rojas & Brewer, 2007).

The redness value (a^* coordinate) did not vary due to the type of burger (control, GPI, GPII) but decreased with storage time from 0 to up 6 days in control and GPII groups ($P < 0.001$ and 0.05, for C and GPII, respectively). The same results were reported by Cheng, Wang, and Ockerman (2007) in pork patties with different types of antioxidant, suggesting that pigment oxidation can still occur even if antioxidants are added. However no significant variation in the a^* coordinate value was observed in the burgers containing extract I. The colour stability of the a^* coordinate during storage in

Table 5
Colorimetric coordinates values (mean ± s.d.) in raw pork burgers stored in aerobic packaging for 0, 3 and 6 days under retail display conditions.

Coordinates	Type of burger	Analysis time (in days)			*p*-value
		0	3	6	
L^*	C ($n = 40$)	43.78 ± 0.70^{XY}	43.03 ± 0.88^{Y}	46.14 ± 0.17^{xXa}	0.022
	GPI ($n = 40$)	40.53 ± 1.11	43.56 ± 3.01	41.93 ± 0.03^{yb}	0.578
	GPII ($n = 40$)	43.50 ± 0.31	45.07 ± 0.58	43.47 ± 1.79^{xy}	0.569
	p-value	0.081	0.637	0.020	
a^*	C ($n = 40$)	17.03 ± 1.06^{X}	9.84 ± 0.52^{Y}	8.65 ± 0.42^{Y}	0.0001
	GPI ($n = 40$)	13.22 ± 0.33^{w}	9.53 ± 1.72	9.58 ± 0.10	0.131
	GPII ($n = 40$)	17.29 ± 0.87^{Xu}	11.13 ± 1.18^{Y}	9.66 ± 0.91^{Y}	0.024
	p-value	0.103	0.571	NS	
b^*	C ($n = 40$)	7.81 ± 0.33^{xa}	6.02 ± 0.71	7.20 ± 0.41^{xa}	0.092
	GPI ($n = 40$)	5.20 ± 0.71^{yb}	5.62 ± 0.29	2.91 ± 0.56^{yb}	0.075
	GPII ($n = 40$)	7.27 ± 0.74^{xy}	6.20 ± 0.84	5.66 ± 0.32^{x}	0.362
	p-value	0.040	0.894	0.004	

C: control sample (no extracts); GPI: extract Type 1; GPII: extract Type 2 (0.06 g/100 g of final product of GPI and GPII, respectively).
[x, y]: Different superscripts in the same column indicate significant differences by effect of the antioxidant.
[X, Y]: Different superscripts in the same row indicate significant differences by effect of the storage time for the same type of burger.
[a, b]: Different superscripts in the same column indicate significant differences between samples from C vs GPI groups.
[u, w]: Different superscripts in the same column indicate significant differences between samples from GPI vs GPII groups.

GPI burgers could be related with the intense antioxidant effect of this compound, since lipid oxidation and myoglobin deterioration are highly linked and the secondary products generated in lipid oxidation processes (e.g. aldehydes) strongly promote colour deterioration (Luciano et al., 2009). This fact shows the important pigment colour protective effect of the Type I extract compared with the GPII extract (Table 1).

In addition lower b^* values (yellowness) were observed in GPI burgers (5.20 and 2.91 at 0 and 6 days of storage, $P < 0.05$, 0.01, respectively). Phenolic compounds in this extract act as potent free radical scavengers of the iron from the myoglobin, delaying the lipid oxidation process but promoting a decrease in b^* value, as previously described by Mitsumoto et al. (2005) in raw chicken patties with added tea catechin. No significant differences in b^* coordinate were found by effect of the storage time.

4. Conclusions

Briefly, the addition of extracts (GPI, GPII) did not affect the pH value or the microbial spoilage in raw pork burgers at the 0.06 g/100 g concentration tested in the current research. However, the highest colour stability and the lowest lipid oxidation values were described in burgers containing extract Type I which showed a potent antioxidant effect. It seems that the new extraction procedure [(methanolic extraction + High-Low Instantaneous Pressure (HLIP))] permits recovery of a high purity extract which does not adversely affect the sensorial quality of the meat system (data not shown). Grape pomace extracts could be a low-cost antioxidant source, but further researches must be lead in this sense to evaluate the optimal concentration to obtain a potent antimicrobial activity without changing the quality attributes of the product.

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