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Antioxidant activity of Carob fruit extracts in cooked pork meat systems during chilled and frozen storage

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ABSTRACT

The purpose of this study was to evaluate the effect of adding condensed tannins in the form of non-purified (Liposterine®) or purified (Exxenterol®) extracts obtained from Carob fruit to prevent lipid cooked pork meat systems from oxidising during chilling and frozen storage. The antioxidant activity of these extracts was compared with that of α-tocopherol. Meat lipid alteration was evaluated as thiobarbituric acid reactive substances content (TBARS) and polar material-related triglyceride compounds followed by high-performance size-exclusion chromatography (HPSEC). TBARS levels were lower ($P < 0.05$) in samples containing Liposterine (LM), Exxenterol (EM), and α-tocopherol (TM) than in control sample (CM) under chilled storage. TBARS formation was similar ($P > 0.05$) for LM and EM but lower ($P < 0.05$) than for TM. Polar material increased several times in all samples, but significantly less in TM and EM than in LM. Thermal oxidation compounds determined by HPSEC were lower ($P < 0.05$) in EM than in LM or TM. The changes in polar material were proportionally smaller after six months frozen storage than after chilled storage, with Exxenterol displaying the highest antioxidant protection. Therefore Carob fruit extracts can be successfully used to reduce fat alteration in cooked pork meat at chilled and frozen temperatures.

1. Introduction

Modern trends towards convenience foods have resulted in an increase in production of precooked meat products that are easy to prepare and consume. Cooked meat is more susceptible than raw meat to lipid peroxidation during chilled and frozen storage. Lipid oxidation is the main cause of deterioration of cooked meat (Gray, Gomaa, & Buckley, 1996). The heating process leads to a dramatic increase of oxidative reactions in lipids in meat, which cause a warmed-over-flavour (WOF) in chilled cooked meat products. WOF is a fast-acting phenomenon due to heat-induced changes in muscle components, and its magnitude depends on a number of factors related to meat characteristics (e.g., lipid content, fatty acid composition, salt), the intensity of the heat treatment used, and the presence of antioxidants (Cross, Leu, & Miller, 1987). These oxidative reactions have adverse effects on quality, healthiness and acceptability and therefore hinder growth in sales of precooked muscle foods.

Synthetic and natural antioxidants have been successfully used to block or delay the oxidation process in meats (Cross et al., 1987). Due to the safety and toxicity problems of synthetic antioxidants, there is increasing interest in natural antioxidants (Ahn, Grün, & Fernando, 2002). Moreover, as well as increasing lipid stability, an antioxidant added to a food may act as an antioxidant in the body, thus reducing the risk of various diseases related to the production of free radicals (Boskou, 1999; Bravo, 1998). Thus, the addition of some bioactive components with antioxidant capacity to meat would make it a potential functional food (Arhiara, 2006; Fernández-Ginés, Fernández-López, Sayas-Barberá, & Pérez-Álvarez, 2005; Jiménez-Colmenero, Carballo, & Cofrades, 2001; Jiménez-Colmenero, Reig, & Toldrá, 2006). The role of these bioactive ingredients would presumably also be enhanced since the composition changes introduced to obtain a meat-based functional food (e.g., adding healthy unsaturated fatty acids) would alter the balance of oxidisable substrates, prooxidants and antioxidants, and this in turn would increase the rate and extent of oxidation (Jiménez-Colmenero, 2007).

Many food sources can be used as natural antioxidants in meat products. Most of the antioxidant food materials that have been tested in meat products are of plant origin (fruit, vegetables, seeds, spices, etc.). The antioxidant activity of plant extracts containing phenolic compounds against lipid oxidation has been investigated in cooked meat products (Ahn et al., 2002; Murphy, Kerry, Buckley,

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& Gray, 1998; Rhee, 1987; Salminen, Estevez, Kivikari, & Heinonen, 2006; Vuorela et al., 2005). One of the dietary fibres with the highest polyphenol content is Carob bean fibre (Papagiannopoulos, Wollseifen, Mellenthin, Haber, & Galensa, 2004). The pulp of the Carob fruit contains sweet carbohydrate (40–50% dry matter) as well as dietary fibre, tannins, and polyphenols (Papagiannopoulos et al., 2004). Antioxidant activity of polyphenols has been reported in Carob pods (Kumazawa et al., 2002). Carob fruit extracts like Liposterine® and Exxenterol® contain condensed tannins in the form of oligomeric proanthocyanidin complexes (OPC) primarily known for their antioxidant activity (Bagchi, Krohn, Bagchi, Tran, & Stohs, 1997; Fine, 2000; Murray & Pizzorno, 1999; Ruiz-Roso, Pérez-Olleros, & Requejo, 2003). Liposterine has presented high antioxidant activity in various *in vitro* tests (Robert et al., 1990). Very recently, our group tested the effect of adding different amounts (from 50 to 1000 mg/kg oil) of Exxenterol to three different oils. Polar material-related triglyceride compounds (polar material), polymer and thermal oxidation formation and tocopherol degradation were significantly and dose-dependently inhibited by Exxenterol (Sánchez-Muniz et al., 2007a). Studies in laboratory animals and humans have shown that Carob fruit extracts like Liposterine present important hypocholesterolaemic properties (Pérez-Olleros, García-Cuevas, Ruiz-Roso, & Requejo, 1999; Ruiz-Roso, Requejo, Haya, & Pérez-Olleros, 2008a; Ruiz-Roso et al., 2003; Zunft et al., 2003).

With the ultimate aim of extending the shelf-life of cooked meat batters and achieving potential health benefits (functional meat), the present work examines the antioxidant activity of two different Carob fruit extracts (Exxenterol and Liposterine) added to meat batters during formulation, at functional doses (3 g/100 g) which have already been shown to exert hypocholesterolaemic effects (Ruiz-Roso et al., 2003, Ruiz-Roso, et al., 2008a). The antioxidant effects were evaluated in cooked meat systems (70 °C) and compared with the effects of α-tocopherol, during both chilling and frozen storage.

2. Material and methods

2.1. Meat raw materials and ingredients

Post-rigour pork meat (mixture of *Musculus biceps femoris*, *M. semimembranosus*, *M. semitendinosus*, *M. gracilis* and *M. aductor*) and fresh pork backfat were obtained from a local meat market. The meat was trimmed of visible fat and connective tissue. Meat and backfat were passed through a grinder with a 0.6 cm plate (Mainca, Granollers, Spain). Lots of approximately 500 g were vacuum packed, frozen and stored at −20 °C until used.

DL-α-Tocopherol 99.6% pure (DSM, Barcelona, Spain) was used. Two Carob fibre pulp extracts, "Liposterine®" and "Exxenterol®" (both from Exentia, Madrid, Spain) were also used. According to Ruiz-Roso, Requejo, Pérez-Olleros, Martin, and Haber (2008b), the major components of Liposterine are tannins 35%; cellulose 28%; hemicellulose 9%; lignine 7% (Fig. 1). The most noteworthy finding on the average composition of Exxenterol was the high percentage of non-extractable condensed tannins (85%) (Fig. 1).

2.2. Meat systems

Meat and backfat packages were thawed (approximately 18 h at 3 ± 2 °C). Three different products were prepared with the target final protein level of 17%. The procedure was as follows: 900 g raw meat was homogenised for 1 min in a chilled cutter (Stephan Universal Machine UM5, Stephan u. Söhne GmbH and Co., Hameln, Germany). Sodium chloride (2.0%) (Panreac Quimica, S.A. Barcelona, Spain), dissolved in 80 ml chilled water, was added to the meat and homogenised for 1 min under vacuum. Three meat samples containing 30 g/kg Exxenterol (EM), 30 g/kg Liposterine (LM) or 250 mg/kg α-tocopherol (TM) were prepared. Control meat samples (CM) with no added extracts or α-tocopherol were also prepared. In each case, the meat batter was homogenised again for 3 min. Mixing time was standardised to 5 min. The final batter temperature was below 14 °C in all cases. EM and TM samples were designed to contain 3 g/100 g of Carob fruit extracts, amounts that have been proven to exert hypocholesterolaemic effects in humans (Ruiz-Roso et al., 2003; Ruiz-Roso, et al., 2008a). Each product was prepared in duplicate.

Portions of each meat batter (approximately 35 g) and replicate were placed in plastic containers (diam 3.4 cm, height 7.0 cm), hermetically sealed and heated to an internal temperature of 70 °C in a controlled water bath. The heating conditions were measured previously using thermocouples connected to a temperature recorder (Yokogawa Hokushin Electric YEW, Mod. 3087, Tokyo, Japan). After heating, containers were cooled immediately (3 °C). Half of the samples were stored at 3 °C, while the other half were immediately frozen and stored at −18 °C. Baselines for chilled and frozen samples were obtained after three days preparation.

2.3. Proximate composition

Moisture, protein, fat and ash contents of different meat samples were determined according to AOAC (2002) procedures. All determinations were done in triplicate.

2.4. Thiobarbituric acid reactive substances (TBARS) content

TBARS values were determined according to Serrano, Cofrades, and Jiménez-Colmenero (2006) in chilled samples (initial and after 10 and 20 days of storage) and in frozen samples (initial and after six months). The determinations were done in triplicate. TBARS values for each sample at a given time were used to calculate the inhibition of lipid oxidation as a percentage of control sample, which (%) = 100 × (control − treatment)/control (Tang, Kerry, Sheehan, Buckley, & Morrissey, 2001).

2.5. Polar material content

Meat product fat was extracted by the Bligh and Dyer (1959) method. Polar material contents in the extracted fat were determined in triplicate in chilled samples (initial and after 20 days of storage) and in frozen samples (initial and after six months) by silica column chromatography using a slight modification of the Dobarganes, Velasco, and Dieffenbacher (2000) method with a 90:10 (v/v) *n*-hexane/diethyl ether mixture to elute the polar fraction. About 0.5 g of the fat extracted from the different samples was analysed. Samples were passed through a 0.45 μm polypropylene filter (Econofilter, Agilent Technologies, Stuttgart, Germany).

2.6. High-performance size-exclusion chromatography (HPSEC)

To obtain further information about oxidative changes occurring during chilled and frozen storage, the polar fractions obtained as above were analysed by HPSEC (Dobarganes et al., 2000). Polar fractions (approximately 15 mg/ml in tethrahydrofuran) were applied to a 20 μl sample loop Water 501 chromatograph (Milford, Massachusetts). A Water 410 refractive index detector and two 300 mm × 7.5 mm id (5 μm particle size), 0.01 and 0.05 μm pL gel (polystyrene-divinylbenzene) columns (Hewlett–Packard, Palo Alto, CA, USA) connected in series were operated at 40 °C. Tetrahydrofuran HPLC-grade served as the mobile phase with a flow of 1 ml/min. All eluents and samples were precleaned by passing them through a 0.45 μm polypropylene filter (Econofilter, Agilent

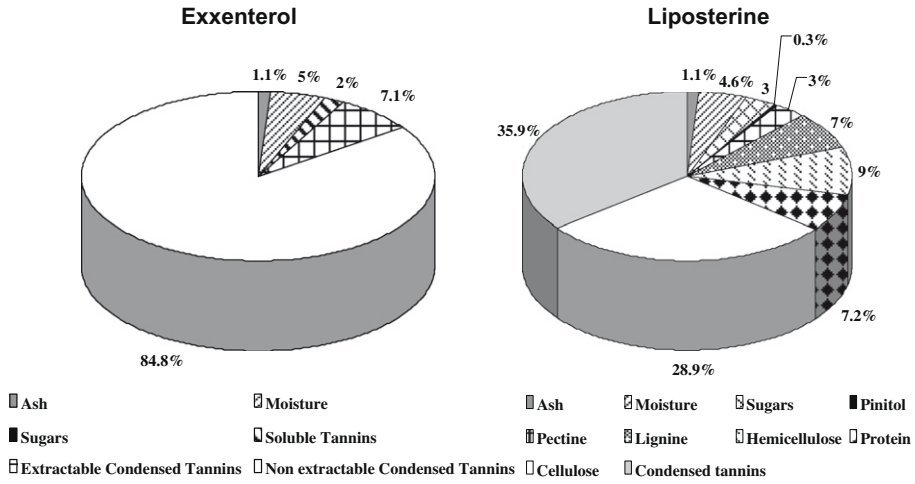

Fig. 1. Average composition of Liposterine and Exxenterol (Ruiz-Roso, et al., 2008b).

Technologies, Stuttgart, Germany). Thermal oxidation was quantified as the sum of oligomers, dimers and oxidised triglycerides, and hydrolytic alteration as the sum of diglycerides, monoglycerides and free fatty acids. A typical chromatogram obtained by HPSEC analysis is shown in Fig. 2.

2.7. Statistical analysis

Data were analysed using the SPSS 15.0 statistical package. The repeated measures test was used for statistical comparisons between samples. When significant interactions between samples and storage conditions (chilling or frozen) were found, the effect of each factor was tested by one-way ANOVA followed by the Bonferroni *post-hoc* test or paired Student-*t*-test. Differences were considered significant at $P < 0.05$.

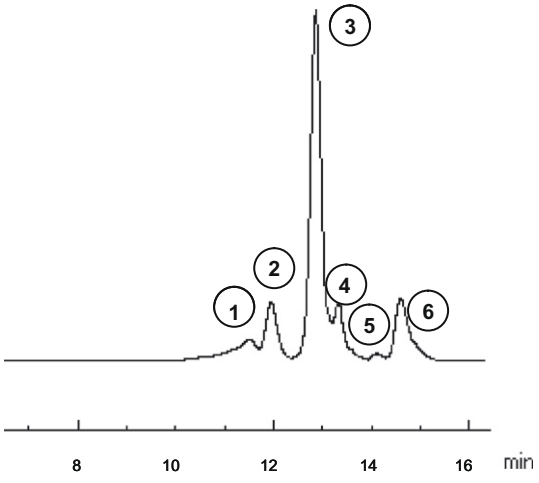

Fig. 2. Typical chromatograms of altered fat obtained by HPSEC. (1) Polymers of triglycerides; (2) dimers of triglycerides; (3) oxidised triglycerides; (4) diglycerides; (5) monoglycerides; and (6) free fatty acids.

3. Results

3.1. Proximate composition

Table 1 shows the proximate composition of the different meat samples. Protein, fat and ash contents were similar ($P > 0.05$) in all samples, while moisture was lower ($P < 0.05$) in LM and EM samples. Liposterine and Exxenterol were added in a proportion of 3%, which explains these results, at least in part. Carbohydrates, estimated by difference, were present at ~1% in LM and EM samples.

3.2. TBARS content

TBARS values were affected ($P < 0.001$) by a time * meat system interaction (Tables 2 and 3). In general, TBARS values increased during storage. Over the chilling storage period, TBARS values were higher ($P < 0.05$) in CM samples than in samples containing antioxidants (Table 2). TBARS contents of CM sample increased ($P < 0.05$) considerably during chilling storage but remained relatively low in TM, LM, and EM samples. In these meat systems, TBARS content had increased significantly by day 10 ($P < 0.05$) with no further increases between days 10 and 20 (Table 2). During frozen storage there was no change ($P > 0.05$) in the TBARS content of LM and EM samples (Table 3). On the other hand, TBARS significantly increased in CM and TM samples. CM samples presented the highest ($P < 0.05$) TBARS values after six months frozen storage.

3.3. Polar material and alteration compounds

Polar material content was affected ($P < 0.001$) in chilled samples by a time * meat system interaction (Table 2). Initially polar material was significantly lower in samples containing antioxidants (TM, LM, and EM) than in the CM counterparts. No differences ($P > 0.05$) in polar material content were observed among TM, LM and EM samples. After 20 days refrigeration there was a sharp increase ($P < 0.05$) of polar material in all samples, but the increase in control sample (CM) was significantly higher ($P < 0.05$) than in samples containing α-tocopherol or Carob fruit extracts. EM and TM samples displayed similar values ($P > 0.05$), while LM was mid-way between those of CM and EM (Table 2). HPSEC data indicated that thermal oxidation in TM was similar ($P > 0.05$) to that in LM, but that hydrolysis was lower ($P < 0.05$) in TM than in LM or EM (Fig. 3).

Table 1
Proximate composition (g/100 g) of different cooked meat samples.[a]

	Moisture	Protein	Fat	Ash
Control meat sample (CM)	64.03 ± 1.51b	16.51 ± 1.02a	17.12 ± 1.35a	2.34 ± 0.25a
Tocopherol meat sample (TM)	64.00 ± 1.34b	16.37 ± 0.84a	17.38 ± 1.22a	2.35 ± 0.23a
Exxenterol meat sample (EM)	59.84 ± 0.99a	17.96 ± 0.78a	18.81 ± 1.35a	2.31 ± 0.17a
Liposterine meat sample (LM)	59.86 ± 1.12a	18.03 ± 0.88a	18.18 ± 1.35a	2.51 ± 0.21a

[a] Means ± standard deviation (SD). Different letters in the same column indicate significant differences ($P < 0.05$).

Table 2
Thiobarbituric acid reactive substances (TBARS, mg MDA/kg) and polar material (g/100 g fat) contents of different samples as affected by chilling storage (3 °C).[a]

Samples	Baseline	10 days	20 days	Time effect	Meat system effect	Time * meat system interaction
TBARS						
Control meat system	5.08 ± 0.16bA	14.58 ± 0.70cB	45.83 ± 2.00cC	<0.001	<0.001	<0.001
Tocopherol meat system	0.87 ± 0.2aA	2.64 ± 0.30bB	2.85 ± 0.20bB			
Exxenterol meat system	0.79 ± 0.15aA	1.31 ± 0.20aB	1.49 ± 0.15aB			
Liposterine meat system	0.88 ± 0.10aA	1.42 ± 0.20aB	1.47 ± 0.20aB			
Polar material						
Control meat system	8.3 ± 0.25bA		37.20 ± 1.29cB	<0.001	<0.001	<0.001
Tocopherol meat system	5.3 ± 0.25aA		22.86 ± 1.46aB			
Exxenterol meat system	4.8 ± 0.30aA		21.88 ± 1.55aB			
Liposterine meat system	5.2 ± 0.20aA		28.58 ± 0.75bB			

[a] Means ± standard deviation (SD). Different low case letters in the same column and different capital letters in the same row indicate significant differences ($P < 0.05$).

Table 3
Thiobarbituric acid reactive substances (TBARS, mg MDA/kg) and polar material (g/100 g fat) contents of different samples as affected by frozen storage (−18 °C).[a]

Samples	Baseline	Six months	Time effect	Meat system effect	Time * meat system interaction
TBARS					
Control meat system	3.08 ± 0.16bA	5.52 ± 0.03cB	<0.001	<0.001	<0.001
Tocopherol meat system	0.87 ± 0.2aA	2.44 ± 0.03bB			
Exxenterol meat system	0.79 ± 0.15aA	0.95 ± 0.01aA			
Liposterine meat system	0.88 ± 0.10aA	0.75 ± 0.01aA			
Polar material					
Control meat system	7.00 ± 0.30bA	19.50 ± 0.92cB	<0.001	<0.001	<0.001
Tocopherol meat system	4.47 ± 0.35aA	10.60 ± 0.46aB			
Exxenterol meat system	4.40 ± 0.26aA	11.25 ± 1.11aB			
Liposterine meat system	4.70 ± 0.20aA	16.45 ± 1.32bB			

[a] Means ± standard deviation (SD). Different low case letters in the same column and different capital letters in the same row indicate significant differences ($P < 0.05$).

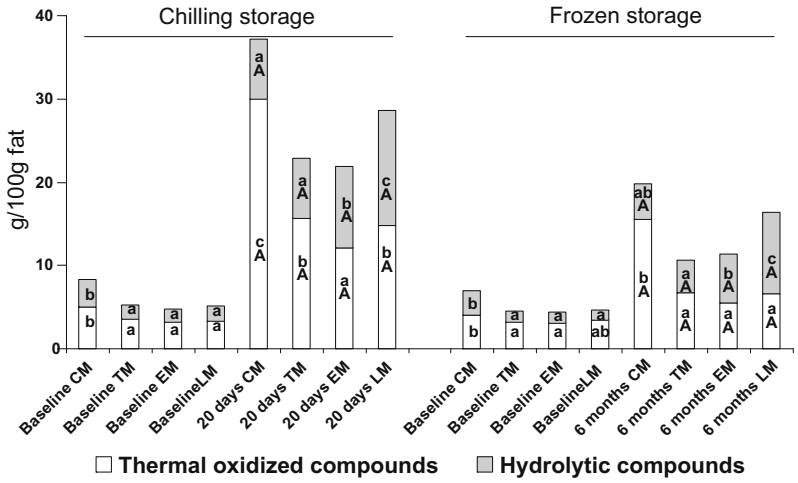

Fig. 3. Thermal oxidation and hydrolytic changes in different meat samples as affected by chilling (3 °C) and frozen (−18 °C) storage. CM, control meat system; TM, α-tocopherol meat system; EM, Exxenterol meat system; and LM, Liposterine meat system. Data bearing different lower-case letters for the same alteration compound and day of storage were significantly different (at least $P < 0.05$). Data bearing a capital letter for the same alteration compound and type of storage were significantly different (at least $P < 0.05$) from their respective baseline counterparts.

In frozen samples there was a significant interaction ($P < 0.001$) between storage time and meat system (Table 3). After six months frozen storage, TM, LM and EM samples displayed less ($P < 0.001$) polar material than CM sample. Polar material values were similar ($P > 0.05$) in EM and TM samples. After prolonged storage, Carob extracts significantly ($P < 0.05$) protected the meat system fat from lipid thermal oxidation (Fig. 2). The fat in EM sample presented less ($P < 0.05$) thermal oxidation than LM and TM samples at chilling temperatures but similar thermal oxidation ($P > 0.05$) at freezing temperatures (Fig. 3).

4. Discussion

The present data suggest that both Carob fruit extracts exerted antioxidant effects on cooked pork meat systems when added in amounts that have already demonstrated functional effects (Ruiz-Roso et al., 2003; Ruiz-Roso, et al., 2008a).

Meat systems presented a proximate composition consistent with meat product formulations. The high susceptibility of CM sample to lipid oxidation correlated closely with the meat system composition and processing. Like other processed meats, the meat systems formulated in this experiment contained relatively high fat percentages, which generally favour oxidation susceptibility. The meat samples were also prepared with added NaCl, and salting is known to increase the prooxidant activity of iron in myofibrillar foods (Decker & Xu, 1998; Murphy et al., 1998; Tang et al., 2001). Moreover, some additives (e.g., nitrites, ascorbic acids, etc.) with antioxidant activity were not employed in the present study. Sample preparation also included a major reduction in particle size (and hence loss of structural integrity) which increased the exposure of labile compounds to oxygen (Decker & Xu, 1998). Also, it is well known that heating accelerates oxidation in meats. Cooking disrupts cellular organisation and causes protein denaturation, resulting in loss of antioxidant enzyme activity and release of protein-bound iron (Decker & Xu, 1998). Heating during meat system preparation as in the present study (70 °C) could therefore also induce lipid oxidation. Lillard (1987) found that the oxidation rate was faster in meats cooked at 70 °C than in meats cooked at 80 °C or higher. Oxidation rates are lower in meat subjected to high temperatures and/or prolonged heating than in meat subjected to lower temperatures for shorter periods of time (Cross et al., 1987). This behaviour has been associated with the pro-oxidative activity of non-haeme iron, which catalyses lipid oxidation in this biological tissue, but also with protection against oxidation by the Maillard reaction products; these increase along with the heating temperature (Bailey, Shi-Lee, Dupuy, St. Angelo, & Vercellotti, 1987) while 70 °C is within the optimum temperature range for release of non-haeme iron (Cross et al., 1987). Heating of comminuted meat systems containing salt and relatively high concentrations of fat renders them prone to lipid oxidation, and so they are very suitable matrices in which to evaluate the antioxidant activity of tocopherol and Carob fruit extracts. It is known that proteins are also targets for oxygen reactive substances, and both lipid and protein oxidation can take place (Salminen et al., 2006). However, protein oxidation was not considered in the present study.

Vitamin E has been used as an antioxidant in meat systems, and nutritional and technological strategies have been used to increase the concentration of vitamin E in meat products. The effect of dietary vitamin E supplementation on lipid oxidation in meat products has been widely reported (Kerry, Buckley, & Morrissey, 2000; Morrissey, Buckley, & Galvin, 2000). Lipid stability in meat products can also be improved by adding vitamin E during the manufacturing process. Whang, Aberle, Judge, and Peng (1986) showed that α-tocopherol (100 and 200 mg/kg) slowed down oxidation in cooked pork stored at either 4 or −20 °C. Miles, McKeith, Betchel, and Novakofski (1986) reported that α-tocopherol was effective in controlling lipid oxidation of restructured pork during chilling storage at 4 °C for 16 days. However, Channon and Trout (2002) reported that tocopherols (ranging 0–1000 mg/kg) had an inconsistent effect on rancidity development in pork products (cured and cooked sausages and uncured and cooked pork roast) during frozen storage, a finding that was related to the very low lipid oxidation in products without the antioxidant. Ahn et al. (2002) reported that vitamin E (200 mg/kg) improved the oxidative stability of cooked ground beef. Georgantelis, Ambrosiadis, Katikou, Blekas, and Georgakis (2007) observed that α-tocopherol (115 mg/kg) produced an antioxidant effect in fresh pork sausage stored for 20 days at 4 °C. The present results agree with those reported by several authors describing α-tocopherol as an effective antioxidant during chilling and frozen storage of cooked pork meat products. TM sample were not as susceptible to lipid oxidation as the control sample (CM) because the rate of lipid oxidation inhibition (based on TBARS results) was 90%. Unpublished data from our group suggest that the tocopherol concentrations in the cooked meat systems were very stable during the first few days but decreased by about 50% during the third week in chilling storage. Moreover, tocopherol protects meat fat from oxidation and hydrolytic alterations under frozen storage.

Polar material determination has proven to be one of the most specific methods to analyse fat and oil alterations (Dobarganes et al., 2000; Sánchez-Muniz, 2006; Sánchez-Muniz & Bastida, 2003). The polar material content in the fat of all meat samples increased significantly during storage. The absence of added antioxidant would explain why TBARS and polar material contents were higher in CM than in the other meat systems. The antioxidant properties of natural plant extracts have been examined in several meat products such as precooked roast beef slices (Murphy et al., 1998), cooked ground beef (Ahn et al., 2002), cooked turkey meat (Nan, Kim, Ahn, & Lee, 2004) and cooked pork meat patties (Salminen et al., 2006). The present results suggest that the tested Carob fruit extracts protected the cooked pork meat batters from oxidative deterioration. These effects may be associated with the presence of some compounds with antioxidant properties in the Carob fruit extracts assayed. Meat samples with added Liposterine or Exxenterol exhibited inhibition of oxidation based on TBARS results higher than 94%. Liposterine and Exxenterol contain very high concentrations of condensed tannins in the form of OPCs, which are primarily known for their antioxidant activity (Fine, 2000). Robert et al. (1990) have shown in various *in vitro* tests that Liposterine has high antioxidant activity. Moreover, our research group (Sánchez-Muniz et al., 2007a) has found that when added to olive oil, sunflower oil or a homogeneous blend of these oils, Exxenterol exerts a powerful antioxidant effect at frying temperatures. The polar material and polymers in those oils were significantly and dose-dependently inhibited by Exxenterol. In fact, polar material and polymer formation at 180 °C were inhibited by at least 44% in olive oil or sunflower oil when 1 g/kg oil of this Carob fruit extract was added. Moreover, the tocopherol degradation was also dose-dependently inhibited (Sánchez-Muniz et al., 2007a). Those results clearly suggest that this non-extractable tannin-rich fibre can be successfully employed as an additive to prolong oil shelf-life at frying temperatures.

To the best of our knowledge the evidence as to the effect of Carob fruit extract on triglyceride hydrolysis is very weak. However, given that LM and EM samples at chilling storage and LM sample at frozen storage displayed greater hydrolysis than CM (Fig. 3), it seems possible that the Carob fruit extracts, and particularly Liposterine, enhanced enzymatic hydrolysis, which in turn would increase the amount of hydrolytic compounds of triglycerides. Future studies to prove this hypothesis would be useful.

Moreover, the observed polar material increases were due more to increases in hydrolytic compounds than in thermally oxidised compounds. Librelotto, Bastida, Zulim-Botega, Jiménez-Colmenero, and Sánchez-Muniz (2009) suggested that some residual hydrolytic activity might still remain in cooked walnut enriched-meats, thus triggering the release of free fatty acids from oxidised and non-oxidised triglycerides.

Fats containing >25% polar material are nutritionally unacceptable (Sánchez-Muniz, 2006; Sánchez-Muniz & Bastida, 2003, 2006). According to our data, after 20 days at chilling temperatures the TM and EM samples would still have been acceptable, while the control sample was unacceptable and LM even less so. However, after six months, all the frozen meats would have been acceptable, with TM and EM presenting ~50% less polar material than CM.

The decrease in thermal oxidation observed in the present study with the addition of Carob fruit extract is also of nutritional interest because thermal oxidation products are potentially toxic (Dobarganes, Márquez-Ruiz, Berdeaux, & Velasco, 1999; Sánchez-Muniz, 2006; Sánchez-Muniz & Bastida, 2006). These are compounds which are actively digested and their products absorbed (González-Muñoz, Bastida, & Sánchez-Muniz, 1998; Sánchez-Muniz, Bastida, & González-Muñoz, 1999). Thus, the addition of Exxenterol and Liposterine to such meat systems would presumably reduce their potential toxicity by limiting the possibility of their forming during preparation and increasing during chilling or frozen storage. Fig. 3 shows that with the exception of chilled CM sample at day 20, all the tested meat systems presented a fraction with thermal oxidation compounds <18 g/100 g fat. Moreover, in frozen samples with the different test products added, the oxidation fraction ranged 5–7 g/100 g fat. Oligomer plus dimer contents were very low in all the assayed meat samples (data not shown). A 10–16% polymer cut-off point has been accepted for discarding of fats and oils (Sánchez-Muniz & Bastida, 2003; Sánchez-Muniz, Bastida, Márquez-Ruiz, & Dobarganes, 2007b). These findings clearly suggest that the addition of Carob extract would help to improve the fat stability and toxicological safety of these meat systems.

In conclusion, the addition of Carob extracts as functional ingredients during meat system preparation exerts antioxidant effects on such foods when stored at chilling and freezing temperatures. Moreover, meat could be a useful vector through which to assure consumption of OPC-rich condensed tannins. Future studies could usefully test the effect of such extracts on more complex meat formulations (e.g., with healthy unsaturated fatty acids and/or nitrites) to determine any antirancidity and functional effects of meat with these condensed tannin-rich Carob extracts added.

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