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Oxidation of lipids and proteins in frankfurters with different fatty acid compositions and tocopherol and phenolic contents

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Abstract

Lipid and protein oxidation and the stability of heme iron (HI) in refrigerated frankfurters (60 days/4 °C) were studied. Lipid oxidation was evaluated as TBA-RS and hexanal counts isolated with solid-phase microextraction (SPME), whereas protein carbonyls groups were quantified by following the DNPH coupling method. Frankfurters were produced using meat and fat from either extensively reared Iberian pigs (IF) or intensively reared white pigs (WF). Hybrid frankfurters (HF) manufactured with meat from white pigs and fat from Iberian pigs was also considered. Frankfurters had significantly different vitamin E and total phenolic contents with the IF having the highest antioxidant content, followed by HF and WF. Compared to WF, IF had significantly higher amounts of monounsaturated fatty acids (MUFA) and smaller of saturated (SFA) and polyunsaturated fatty acids (PUFA). HF presented an intermediate fatty acid composition between IF and WF. Results suggest an intense oxidative deterioration of frankfurters during refrigeration since PUFA were gradually degraded while oxidation products, such as TBA-RS and hexanal, were generated. Proteins were also damaged, since carbonyls derived from their oxidation and non-heme iron content increased during refrigeration, also suggesting oxidative instability of the heme molecule. IF showed a higher oxidative stability compared to WF, which could be explained by the different fatty acid composition and antioxidant status. Using fat from Iberian pigs for the production of HF improved the oxidative stability of frankfurters, with that being significantly higher than those from WF and IF. IF had a significantly higher amount of iron than had WF and HF which could have played a important role in the development of the oxidative reactions. Significant correlations were established between protein and lipid oxidation and protein oxidation and heme degradation, suggesting likely interactions between such processes.

Keywords: Lipid oxidation; Protein oxidation; Non-heme iron; Fatty acid composition; Tocopherols; Phenolics

1. Introduction

Lipid oxidation is one of the major factors reducing quality and acceptability of meat and fat products (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). The oxidation process involves the degradation of PUFA, vitamins and other tissue components and the generation of free radicals, which lead to the development of rancid odours and changes in colour and texture in foodstuffs (Kanner, 1994). Lipid oxidation is thought to promote the oxidative damage of proteins through the prooxidant activity of primary (hydroperoxides) and secondary (aldehydes, ketones) lipid oxidation products (Gardner, 1979; Kikugawa, Kato, & Hayasaka, 1999). Protein oxidation mainly occurs via free radical reactions in which peroxyl radicals generated in the first stages of PUFA oxidation can abstract hydrogen atoms from protein molecules, leading to the formation of protein radicals. The formation of non-covalent complexes between lipid oxidation products and reactive amino acids residues, as well as the presence of some particular metal, such as copper and iron, can also lead to protein radical generation (revised by Viljanen, Kivikari, & Heinonen, 2004a). Though relationships between lipid and protein oxidation have been established in microsomal,

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emulsion or liposome models (Batifoulier, Mercier, Gatellier, & Renerre, 2002; Howell, Herman, & Li-Chan, 2001; Viljanen et al., 2004a; Viljanen, Kylli, Kivikari, & Heinonen, 2004b), the development of protein oxidation reactions in food systems is still largely unknown. In addition, the effect of the oxidation of proteins on meat quality needs to be elucidated. Protein oxidation has been linked to a decrease of protein solubility and functionality and colour and texture changes in model systems (Howell et al., 2001; Karel, Schaich, & Roy, 1975). More recently, Mercier, Gatellier, and Renerre (2004) and ourselves (Estévez & Cava, 2004) have reported data on the oxidative stability of proteins in meat and liver products, establishing close relationships between lipid and protein oxidation phenomena. Nevertheless, the effect of antioxidant strategies (e.g. supplementation of antioxidants in animal diets, addition of antioxidants in muscle foods) on the quality and functionality of muscle proteins has been poorly studied.

As an oxidation promoter in animal foods, iron is thought to have a high catalytic activity (Kanner, 1994). However, knowledge of the approximate proportions between the chemical forms of iron is of great importance because of the strong differences displayed by heme iron (HI) and non-heme iron (NHI) in terms of promotion of lipid oxidation and bioavailability. NHI is considered one of the most important oxidation promoters in meat systems (Kanner, 1994) whereas HI is thought to be more bioavailable (Carpenter & Mahoney, 1992). An increase in the amount of NHI in meat systems has been considered to be a reflection of the decrease of heme iron (HI) as a consequence of the breakdown of the heme molecule during cooking or storage (Estévez & Cava, 2004; Lombardi-Boccia, Marfınez-Domínguez, & Aguzzi, 2002; Miller, Gómez-Basauri, Smith, Kanner, & Miller, 1994) and has been eventually linked to a disruption of the porphyrin ring of myoglobin (Estévez & Cava, 2004; Schricker, Miller, & Stouffer, 1982). However, the particular causes of the heme molecule disruption and the effects of the increase of non-heme iron in cooked products have not been considered.

Frankfurters are widely marketed and consumed meat products. The physicochemical characteristics of frankfurters are influenced by the quality of the ingredients, particularly, the meat and adipose tissue. Estévez, Morcuende, and Cava (in press) suggested using meat and adipose tissues from free-range-reared Iberian pigs for the production of high-quality frankfurters. According to the aforementioned paper, including 10% adipose tissue from Iberian pigs in commercial frankfurters enhanced their nutritional properties. Using tissues from Iberian pigs for the manufacture of cooked products would increase the benefits of the local industry, offering consumers a high quality product (Estévez, Morcuende, Ramirez, Ventanas, & Cava, 2004a). The large proportion of oleic acid (50–60%) in tissues from Iberian pigs is considered as one of the most peculiar quality traits and is caused by the intake of acorns by animals during traditional extensive rearing (Estévez et al., 2004a; Ruiz et al., 1998). The consumption of natural feeds by pigs is also associated with increasing levels in their tissues of minority substances with proven antioxidant activity, such as tocopherols (Cava, Ventanas, Tejeda, Ruiz, & Antequera, 2000; Daza, Rey, Ruiz, & López-Bote, 2005; Estévez et al., 2004a). In addition, the intake of grass and acorns by free-range reared pigs has recently been associated with the incorporation of plant phenolics in the animal tissues which could enhance their oxidative stability (González, Tejeda, Moltiva, & Romero, 2004). The differences between frankfurters from Iberian and white pigs in terms of their fatty acid composition and antioxidative status are expected to influence their oxidative deterioration during refrigerated storage (Estévez et al., in press). The amount of protein in some commercial frankfurters is even higher than the fat content (González-Viñas, Caballero, Gallego, & García-Ruiz, 2004), suggesting that the development of oxidative reactions will affect proteins as well as lipids, leading to a loss of quality. The occurrence of protein oxidation and the relationships between oxidative processes and the release of iron from myoglobin in frankfurters have not been previously described. The aim of the present work was to study the oxidative reactions in frankfurters elaborated with tissues from extensively reared Iberian and intensively reared white pigs with different fatty acid compositions and antioxidant status. Hybrid frankfurters elaborated with adipose tissue from Iberian pigs and meat from white pigs were also considered. Relationships between the different measured parameters were also established.

2. Material and methods

2.1. Animals and sampling

Seven Iberian pigs, commonly produced in the South-West of Spain and belonging to the Iberian pig pure breed, were free-range-reared and fed on natural resources (grass and acorns), following the traditional livestock farming for Iberian pigs. The animals were slaughtered at ~150 kg and an age of 12 months. Acorns (moisture: 46.10%, fat: 5.50%; protein: 4.31%) showed the following fatty acid profile (expressed as percentage of total fatty acids analyzed): palmitic acid (C16:0), 11.82%; stearic acid (C18:0), 0.56%; oleic acid (C18:1), 67.3%; linoleic acid (C18:2), 1 8.7%; linolenic acid (C18:3), 0.25%. The grass (moisture: 89.24%, fat: 6.26%; protein: 4.34%) fatty acid profile was as follows: C16:0, 14.0%; C18:0, 1.99%; C18:1, 5.24%; C18:2, 11.4%; C18:3, 57.8%.

Seven white pigs (Large-white × Landrace) were intensively reared under controlled conditions in a typical industrial livestock farm. The animals were fed ad-libitum on a mixed feed made with cereals and soya with no animal source of protein or fat and slaughtered at ~85 kg live weight and at the age of 7 months. The analysis of the mixed diet (moisture: 10.42%, fat: 2.94%; protein: 18.3%) revealed the following fatty acid profile: C16:0, 19.9%; C18:0, 8. 63%; C18:1, 32.8%; C18:2, 32.8%; C18:3, 2.45%.

Iberian pigs and white pigs were slaughtered at the same slaughter house one week apart. After slaughter and dress, a heterogeneous mixture of muscles derived from carcasses and adipose tissues (porcine back-fat) were vacuum-packaged and stored at −80 °C (less than 24 h from slaughter) until the manufacture of the frankfurters (less than 2 weeks).

2.2. Frankfurter manufacture

The experimental frankfurters were manufactured in a pilot plant. The same formulation was used for all frankfurters. Three different types of frankfurters were considered, depending on the source of the raw material: frankfurters from Iberian pigs (IF), frankfurters from white pigs (WF) and hybrid frankfurters (HF) elaborated with meat from white pigs and adipose tissue from Iberian pigs. For the manufacture of the sausages, meat and adipose tissues from seven different animals were used for each of the groups (Iberian and white pigs). The ingredients were as follows per 100 g of elaborated product: 50 g meat, 10 g adipose tissue, 37 g distilled water, 2 g sodium caseinate, 1 g potato starch. Sodium chloride (2%), sodium di- and tri-phosphates (0.5%), sodium ascorbate (0.05%) and sodium nitrite (0.03%) (all from ANVISA, Madrid, Spain) were also added. Percentages of additives were calculated, based on the total amount of product. Following the aforementioned recipe, 1.3 kg of raw material was used for each group, to produce the experimental frankfurters. Firstly, the meat was chopped into small cubes (1 cm^3) and mixed with the sodium chloride, sodium nitrite and sodium ascorbate in order to allow the nitrification of the samples 2 h before the manufacture. Then, the meat was minced in a cutter (Foss Tecator Homogenizer, mod. 2094) for 2 min, together with the starch and 50% of the sodium caseinate, which was previously dissolved in water (75 °C). After that, the adipose tissue was added together with the remaining dissolved sodium caseinate and minced for 4 more minutes until a homogeneous raw batter was obtained. Finally, the mixture was stuffed into 18 mm diameter cellulose casings, handlinked at 10 cm intervals and given the thermal treatment in a hot water bath (80 °C/30′).

2.3. Refrigeration

In order to allow the development of oxidative reactions, the frankfurters were refrigerated stored (4 °C/60 days) in the darkness. Sampling was carried out at days 0, 20, 40 and 60 for analytical experiments, with day 0 being the day of the manufacture. After each refrigeration stage, frankfurters were frozen (−80 °C) until experiments were carried out.

2.4. Analytical methods

2.4.1. Compositional analysis of frankfurters

Moisture, total protein and ash were determined using AOAC methods (AOAC, 2000a, 2000b, 2000c). The method of Bligh and Dyer (1959) was used for isolating fat from frankfurters.

2.4.2. Iron analysis

Total iron was determined, following the procedure described by Miller et al. (1994). Non-heme iron (NHI) content was determined, following the method described by Rhee and Ziprin (1987). The amount of heme iron (HI) was calculated by difference between total and NHI. The amounts of iron were expressed as μg iron/g frankfurter.

2.4.3. Tocopherols content

α- and γ-Tocopherols were extracted from frankfurters according to the method described by Rey, López -Bote, Soares, and Isabel (1997). The analysis was carried by reverse phase HPLC (HP 1050, with a UV detector, HPIB 10) (Hewlett-Packard, Waldbronn, Germany).

2.4.4. Phenolic compounds content

The Folin Ciocalteau reagent was used for the quantification of total phenolics, as described by Turkmen, Sari, and Velioglu (2005) with minor modifications as follows: 0.5 g of frankfurter was homogenized with 10 mL of water, methanol or 80% methanolic water and centrifuged for 5 min at 3000 rpm and 4 °C. Phenolics were extracted from the pellets, following the same procedure. The supernatants were combined and 1 mL aliquot was mixed with 5 mL of Folin Ciocaltelu reagent (10% in distilled water) in test tubes. After 5 min, 4 mL of sodium carbonate (7.5% in distilled water) were added, the test tubes were cap-screwed and the samples allowed to stand for 2 h at room temperature in the darkness. A standard curve with ethanolic gallic acid (ranging from 0.625×10^{-3} mg/mL to 0.02 mg/mL) was used for quantification. Results were expressed as mg of gallic acid equivalents (GAE) per gramme of sample.

2.4.5. Fatty acid composition

Fatty acid methyl esters (FAMEs) were prepared by acidic esterification in the presence of sulfuric acid, following the method of López-Bote, Rey, Sainz, Gray, and Buckley (1997). FAMEs were analyzed using a Hewlett Packard, mod. HP-5890A, gas chromatograph, equipped with a flame ionization detector (FID). The derivatives were separated on a FFAP- TPA fused-silica column (Hewlett Packard 30 m long, 0.53 mm internal diameter and 1.0 μm film thickness). The injector and the detector temperatures were held at 230 °C. Oven temperature was maintained at 220 °C. The flow rate of the carrier gas (N_2) was set at 1.8 mL/min. Identification of FAMEs was based on retention times of reference compounds (Sigma). The quantification of fatty acids was carried out by using C13 as an internal standard. Results were expressed as g fatty acid 100 g^{-1} total fatty acid analyzed.

2.4.6. Protein oxidation measurement

Protein oxidation, as measured by the total carbonyl content, was assessed by the method of Oliver, Ahn, Moer-

man, Goldstein, and Stadtman (1987). Protein concentration was determined by spectrophotometry, using bovin serum albumin (BSA) as standard. The amount of carbonyls was expressed as moles-carbonyls/mg protein.

2.4.7. TBARS numbers

Thiobarbituric acid-reactive substances (TBARS) were determined using the method of Rosmini et al. (1996). Results were expressed as mg malondialdehyde (MDA)/kg frankfurter.

2.4.8. Hexanal analysis

The SPME fibre, coated with divinylbenzene-carboxen-poly(dimethylxilosane) (DVB/CAR/PDMS) 50/30 μm, was preconditioned prior to analysis at 220 °C during 45 min. Headspace (HS) sampling was performed, following a method previously described (Estévez, Ventanas, Ramirez, & Cava, 2004b). One gram of frankfurter was placed in a 2.5 mL vials and the SPME fibre exposed to the headspace of the pâté while the sample equilibrated during 30 min immersed in water at 50 °C. Analyses were performed on an HP5890GC series II gas chromatograph (Hewlett-Packard, USA) coupled to a mass-selective detector (Agilent model 5973). Volatiles were separated using a 5% phenyl–95% dimethyl polysiloxane column (Restek, USA) (30 m × 0.25 mm i.d., 1.0 mm film thickness). The carrier gas was helium at 18.5 psi, resulting in a flow of 1.6 mL min^{-1} at 40 °C. The SPME fibre was desorbed and maintained in the injection port at 220 °C during the entire chromatography run. The injector port was in the splitless mode. The temperature programme was isothermal for 10 min at 40 °C and then raised at the rate of 7 °C min^{-1} to 250 °C, and held for 5 min. The GC/MS transfer line temperature was 270 °C. The mass spectrometer operated in the electron impact mode with an electron energy of 70 eV, a multiplier voltage of 1650 V and collecting data at a rate of 1 scan s^{-1} over a range of m/z 40–300. Hexanal was identified by comparing its retention time with that from the standard compound. Results from the volatiles analysis were provided in area units (AU).

2.4.9. Data analysis

The results of the experiments were used as variables and analyzed by using the Analysis of Variance (ANOVA) (SPSS, 1997) in order to compare amongst types of frankfurters. The same statistical analysis was used to evaluate the effect of refrigerated storage on frankfurters. Statistical significance was set at 0.05.

3. Results and discussion

3.1. Proximate and fatty acid composition of frankfurters

The analysis of the proximate composition revealed no statistically significant differences among types of frankfurters since they had similar moisture, fat, protein and ash contents. IF however, had a significantly higher amount of iron than WF and HF. This result was expected since the meat from Iberian pigs contained a higher amount of iron than that from white pigs (Estévez et al., in press). Large differences among types of frankfurters were detected for most of the fatty acids analyzed (Table 1). IF had significantly smaller amounts of palmitic, stearic and total SFA than had WF. Compared to WF, IF contained significantly higher amounts of oleic and total MUFA. WF on the contrary, had larger amounts of PUFA, such as linoleic acid. In addition, WF contained significantly higher amounts of minority $n-3$ and $n-6$ long-chain PUFA than did IF. As expected, fatty acid composition of frankfurters reflected the fatty acid composition of the tissues used for their elaboration (Estévez et al., in press). As described in a previous paper (Estévez et al., 2004a), the differences are mainly explained by the different fatty acid compositions of the feeds given to the animals during the fattening period, and therefore, meat and adipose tissues from Iberian pigs reflected the fatty acid composition of the acorns which had high levels of oleic acid. The HF had an intermediate fatty acid composition, between IF and WF. Replacing 10% fat from white pigs with fat from Iberian pigs in the HF significantly influenced their fatty acid composition, significantly reducing the proportion of SFA and PUFA and increasing the percentages of oleic acid and MUFA compared to results from WF.

Table 1
Fatty acid composition of frankfurters[A]

	IF	WF	HF	SEM[B]	p[C]
C12	0.11b	0.13a	0.13a	0.00	0.004
C14	1.25b	1.36a	1.34a	0.01	0.000
C16	20.1c	23.5a	21.7b	0.38	0.000
C17	0.36c	0.63a	0.51b	0.03	0.000
C18	9.02c	13.9a	11.6b	0.53	0.000
C20	0.23	0.28	0.25	0.01	0.385
SFA	31.0c	39.8a	35.6b	0.96	0.000
C16:1 ($n-7$)	2.59a	2.62b	2.45c	0.02	0.000
C17:1 ($n-7$)	0.26c	0.42a	0.35b	0.02	0.000
C18:1 ($n-9$)	53.6a	42.9c	47.7b	1.16	0.000
C20:1 ($n-9$)	1.41a	1.06c	1.31b	0.04	0.000
C22:-1 ($n-9$)	0.04a	0.03.b	0.04a	0.00	0.000
MUFA	57.9a	47.1c	51.8b	1.18	0.000
C18:2 ($n-6$)	8.80c	10.8a	9.95b	0.21	0.000
C18:3 ($n-6$)	0.14c	0.20a	0.17b	0.01	0.000
C18:3 ($n-3$)	0.71	0.71	0.71	0.00	0.945
C20:2 ($n-6$)	0.51b	0.54a	0.55a	0.01	0.000
C20:3 ($n-3$)	0.06a	0.04b	0.05ab	0.00	0.025
C20:3 ($n-6$)	0.09b	0.11a	0.10ab	0.00	0.009
C20:4 ($n-6$)	0.41b	0.44a	0.38c	0.01	0.000
C20:5 ($n-3$)	0.15a	0.11c	0.14b	0.00	0.000
C22:2 ($n-6$)	0.03	0.05	0.05	0.01	0.176
C22:4 ($n-6$)	0.03	0.03	0.03	0.00	0.469
C22:5 ($n-3$)	0.09c	0.11a	0.10b	0.00	0.000
C22:6 ($n-3$)	0.09b	0.12a	0.13a	0.01	0.001
PUFA	11.1c	13.2a	12.4b	0.23	0.000

Different letters in the same row denote statistical differences among types of frankfurters. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

[A] g/100 g Fatty acids.

[B] Standard error of the means.

[C] Statistical significance.

3.2. Antioxidant content of frankfurters

Results from the quantification of tocopherols and total phenolics in frankfurters are shown in Table 2. IF presented higher levels of α- and γ-tocopherol than did WF, which is consistent with previous reports of tocopherol contents in the tissues (meat and adipose tissue) from outdoor-reared Iberian pigs and white pigs reared indoors (Cava et al., 2000; Daza et al., 2005; Estévez et al., 2004a). The HF had an intermediate amount of tocopherols, suggesting that the addition of 10% fat from Iberian pigs to these frankfurters increased α- and γ-tocopherol levels compared to those in WF. The high content of tocopherols in tissues and meat products from outdoor-reared Iberian pigs has been profusely described in previous works, and considered as one of the most appreciated quality traits (Cava et al., 2000; Daza et al., 2005; Estévez et al., 2004a) as long as tocopherols enhance the oxidation stability of the meats and meat products, improving their nutritional and technological properties (Morrissey et al., 1998; Rey, Isabel, Cava, & López -Bote, 1998). The α- and γ-tocopherol contents in animal tissues reflect the tocopherol concentration of the diets (Daza et al., 2005), and therefore, the high levels of tocopherols in the grass and acorns with which Iberian pigs were fed explain the high levels of such substances in their tissues. According to Cava et al. (2000) and Daza et al. (2005) meat from Iberian pigs fed on natural resources (grass and acorns) had similar or even higher tocopherol levels than those fed with diets supplemented with α-tocopherol up to 200 mg/kg. Furthermore, acorns have been shown to be important sources of γ-tocopherol for extensively reared pigs and the presence of such a tocopherol isomer in pig muscles is almost restricted to tissues from pigs fed with acorns (Daza et al., 2005; Rey et al., 1998), which is in agreement with results obtained in the present work. The relatively small amounts of tocopherols in WF were expected since the white pigs were fed with a mixed diet without access to fresh materials.

The amount of total phenolics varied considerably, depending on the solvents used for their extraction, ranging from 0.52 to 1.09 g/100 g in IF, from 0.46 to 0.85 g/100 g in WF and from 0.46 to 0.89 g/100 g in HF. Regardless of the type of extraction, the amount of total phenolics in IF was significantly higher than in either WF or HF. The information concerning the occurrence of phenolic compounds in animal tissues is extremely scarce since such compounds are widespread in the plant kingdom and, therefore, their presence in animal tissues is principally relegated to the intake of fresh natural resources and the subsequent accumulation in animal tissues. The intake of grass and acorns by Iberian pigs could explain the higher amount of phenolic compounds in their tissues and elaborated frankfurters than in those from white pigs. In fact, Cantos et al. (2003) have recently reported elevated polyphenol levels in acorns. In agreement with the present results, González et al. (2004) have recently reported significantly higher amounts of phenolic compounds in adipose tissue from Iberian pigs fed exclusively on natural resources (grass and acorns) than those fed with mixed diets. Amongst phenolic compounds, some particular polyphenols derived from plants, are substances with proven antioxidant activity and the presence of such compounds in the animal tissues could protect them and their transformed products from oxidative deterioration.

Table 2
Tocopherol and total phenolic contents of frankfurters

	IF	WF	HF	SEM[D]	p[E]
Tocopherol[A]					
α-Tocopherol	3.72a	1.31c	2.74b	0.28	<0.001
γ-Tocopherol	0.23a	0.05b	0.15a	0.02	0.001
Total phenolics[B]					
Water[C]	1.04a	0.66b	0.79b	0.05	<0.001
Methanol	1.09a	0.85b	0.89b	0.04	0.012
80% Methanol water	0.52a	0.46b	0.46b	0.01	0.045

Different letters in the same row denote statistical differences among types of frankfurters.
[A] mg/kg Frankfurter.
[B] g/100 g Frankfurter.
[C] Solvents used for phenolic compounds extraction.
[D] Standard error of the means.
[E] Statistical significance.

3.3. Lipid oxidation during refrigerated storage of frankfurters

TBARS numbers gradually increased in experimental frankfurters during 60 days of refrigerated storage at 4 °C (Fig. 1). Significant changes ($p < 0.05$) were detected for MDA content between day 0 and day 60 for IF (from 0.37 to 0.94 mg MDA/kg frankfurter), WF (from 0.49 to 1.12 mg MDA/kg frankfurter) and HF (from 0.25 to 0.78 mg MDA/kg frankfurter). The hexanal counts also increased after 60 days of refrigerated storage (Fig. 2). As expected, a statistically significant ($p < 0.01$) correlation ($R^2 = 0.55$) was detected between TBARS and hexanal contents. These data revealed that, regardless of the addition of substances with proven antioxidant activity, such as nitrite and phosphates, considerably intense lipid

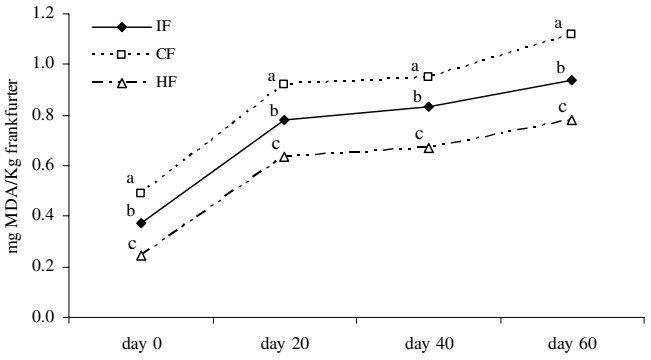

Fig. 1. Evolution of TBARS numbers during refrigerated storage of frankfurters (significant differences, $p < 0.05$, among types of frankfurters within a day of storage are denoted by different letters).

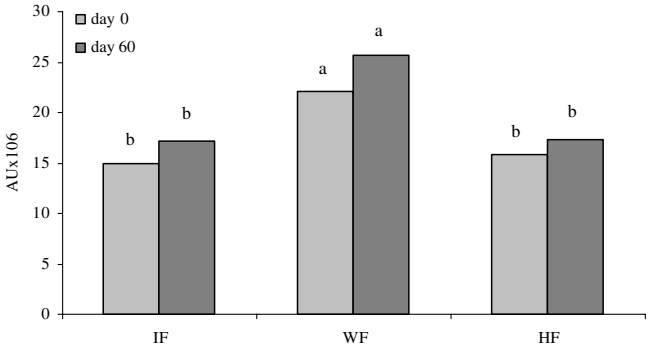

Fig. 2. Increase of hexanal counts during refrigerated storage of frankfurters (significant differences, $p < 0.05$, among types of frankfurters within a day of storage are denoted by different letters).

oxidation processes affected frankfurters during refrigeration. Based on findings by Gray and Pearson (1987), rancid flavour is initially detected in meat products with TBARS values between 0.5 and 2.0. Furthermore, Boles and Parrish (1990) reported that a warmed-over flavour (WOF) could be perceived in meat products at TBARS values above 1.0. On the other hand, the hexanal is mainly generated as a consequence of the oxidative decomposition of PUFA and has been related to rancid odours and used as an indicator of lipid oxidation (Shahidi & Pegg, 1993). Throughout the entire storage period, TBARS numbers in WF were significantly higher than in the IF. The chromatographic areas for hexanal were also significantly higher in WF than in the IF at days 0 and 90 of storage. These results agree with those obtained in previous works devoted to the comparison of the lipid oxidative stability between cooked meats and liver products from Iberian and white pigs (Estévez & Cava, 2004; Estévez et al., 2004b). The different fatty acid composition, with higher amount of PUFA in WF, could explain these findings. High levels of PUFA in frankfurters have previously been associated with high oxidative instability during storage (Jeun-Horng, Yuan-Hui, & Chun-Chin, 2002). On the other hand, Bloukas and Paneras (1993) improved oxidative stability of frankfurters by increasing the amount of MUFA (by replacing pork back fat with olive oil), which is in agreement with results from the present work. In addition, the higher amounts of antioxidants, such as tocopherols and phenolic compounds, in IF might also have an influence. The relationship between the nutritional background (pasture- and mixed diet finishing) and the fatty acid profile and oxidation stability of liver, pork and their based products is profusely documented (Cava et al., 2000; Daza et al., 2005; Rey et al., 1998; Ruiz et al., 1998). Including 10% adipose tissue from Iberian pigs in HF led to a remarkable improvement of their oxidative stability, since HF had significantly smaller TBARS values and hexanal counts than WF. This improvement was achieved by the modification of the fatty acid composition of the frankfurters, significantly increasing MUFA contents and reducing PUFA content. In addition, the adipose tissue from Iberian pigs was a source of tocopherols for HF, which likely enhanced their oxidative stability. Surprisingly, HF had even smaller TBARS values than had IF. This could be due to the different iron contents between types of frankfurters. The meat and meat products from Iberian pigs contain higher amounts of iron than those from white pigs (Estévez et al., 2004a) which imply enhanced nutritional properties. On the other hand, iron is considered a potent oxidation promoter and, from that point of view, high levels of that metal in meat from Iberian pigs increase its oxidative instability. The manufacture of frankfurters with meat from white pigs and adipose tissue from Iberian pigs lead to a product with improved fatty acid composition and high levels of antioxidants without increasing the iron levels which explains its high lipid oxidative stability.

3.4. Protein oxidation during refrigerated storage of frankfurters

The accumulation of protein oxidation products was noted in refrigerated stored frankfurters (Fig. 3). The amount of carbonyls significantly increased ($p < 0.05$) from 3.7 to 5.4 nmoles carbonyls/mg protein, from 5.5 to 6.5 nmoles carbonyls/mg protein and from 3.2 to 4.8 nmoles carbonyls/mg protein in IF, WF and HF, respectively. Compared to WF, IF and HF had smaller amounts of carbonyls on all days of analysis. These results agree with those obtained, for lipid oxidation, suggesting a possible relationship between lipid and protein oxidation. In fact, statistically significant correlations were found between protein oxidation and TBARS ($R^2 = 0.75$; $p < 0.01$) and between protein oxidation and hexanal ($R^2 = 0.68$; $p < 0.01$) with those correlation coefficients being higher that that between TBARS and hexanal contents (Table 3). Mercier, Gatellier, and Renerre (1995) originally reported a relationship between lipid and protein oxidation in beef muscles. Accordingly, Viljanen et al. (2004a, 2004b) and discussed, in detail, the likely relationships between the oxidation of some particular proteins (BSA, lactalbumin and casein) and the development of lipid oxidation in liposomes. We have recently reported statistically

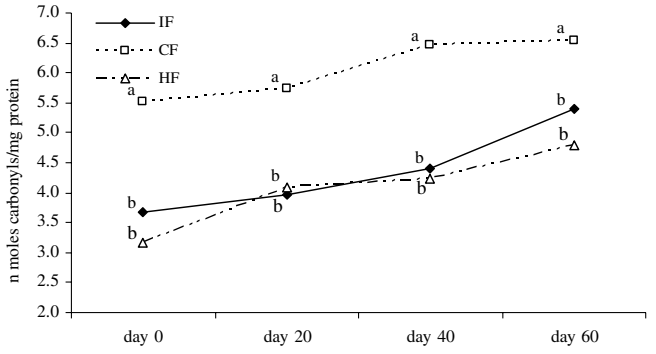

Fig. 3. Evolution of carbonyl content during refrigerated storage of frankfurters (significant differences, $p < 0.05$, among types of frankfurters within a day of storage are denoted by different letters).

Table 3
Pearson's correlation coefficients (R^2)[a]

	R^2
TBARS vs. hexanal	0.55*
TBARS vs. Pox[b]	0.75*
TBARS vs. NHI	0.67*
Pox vs. NHI	0.51*
Pox vs. hexanal	0.68*
NHI vs. hexanal	0.51*

[a] $n = 10$ Frankfurters for correlation coefficients taken from measurements at days 0 and 90 of storage.
[b] Protein oxidation as assessed by total carbonyl content.
* $p < 0.01$.

significant correlations between TBARS values and carbonyls contents in refrigerated-stored liver pâtés (Estévez & Cava, 2004). A causal relationship between lipid and protein oxidation is probable since primary and secondary oxidation products can interact with proteins leading to protein radicals (Gardner, 1979). The differences between frankfurters could also be explained by the likely protective effect of tocopherols and phenolics from IF and HF and the large differences in fatty acid composition between types of frankfurters. In agreement with the present results, Mercier et al. (2004) reported smaller amounts of carbonyls in beef from cows furnished with pasture than in those furnished with mixed diets, suggesting the protective role of vitamin E from the grass against the oxidation of proteins. The antioxidant activity of plant phenolics against protein oxidation has also been described in model systems. Viljanen et al. (2004b) described the antioxidant activity of berry phenolics in proteins from liposomes whereas we have (Estévez, Morcuende, Ventanas, & Cava, 2004c) described the protective effect of added sage and rosemary essential oils against protein oxidation in liver pâtés. Phenolic compounds can inhibit the oxidation of proteins by retarding the lipid oxidative reactions and by binding to the proteins and by forming complexes with them (Siebert, Troukhanova, & Lynn, 1996). Though a loss of protein functionality, associated with protein oxidation, has been described (Howell et al., 2001; Karel et al., 1975), scarce information is available on the impact of protein oxidation on meat product quality.

3.5. Release of iron from heme during refrigerated storage of frankfurters

In the present work, the amount of HI gradually decreased in frankfurters during storage (Fig. 4(a)) and, as a likely reflection of this fact, the amount of NHI steadily increased (Fig. 4(b)). The breakdown of the heme molecule and the release of iron from the porphyrin ring have been reported to occur as a consequence of the high temperatures during cooking (Lombardi-Boccia et al., 2002; Miller et al., 1994). The evolution of non-heme iron content suggests that the heme degradation occured throughout the refrigerated storage. In accordance with our results,

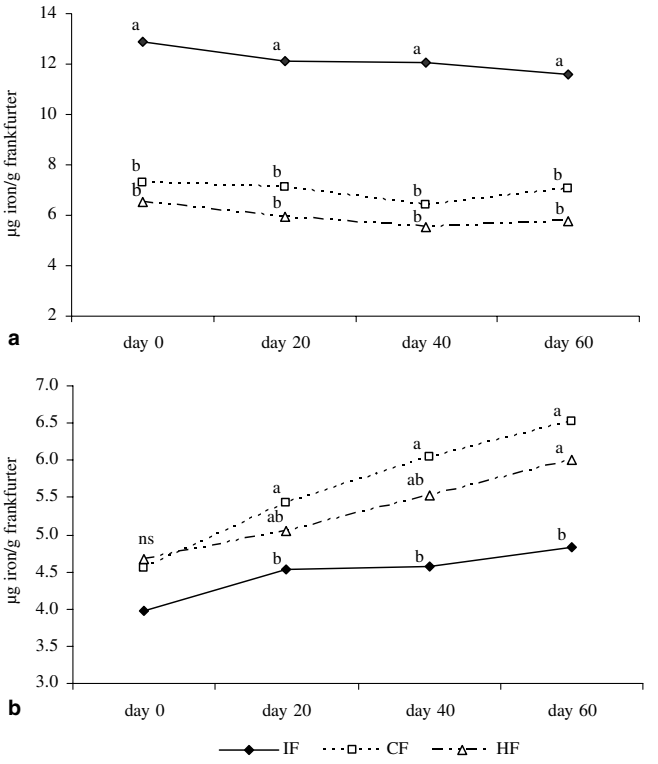

Fig. 4. Evolution of heme (a) and non-heme iron (b) contents during refrigerated storage of frankfurters (significant differences, $p < 0.05$, among types of frankfurters within a day of storage are denoted by different letters).

Gomez-Basauri and Regenstein (1992) reported the rapid breakdown of the heme molecule during refrigerated storage of cod and mackerel flesh. Studying the heme stability in ground cooked pork, Miller et al. (1994) suggested a relationship between the release of iron from the heme molecule during refrigerated storage and the disruption of the porphyrin ring. Purchas, Rutherfurd, Pearce, Vather, and Wilkinson (2004) reported similar conclusions, studying variations in the form of iron during refrigerated storage of beef and lamb meat. Though the precise causes of the heme degradation were not elucidated, release of iron from the heme molecule has been related to oxidative reactions. In fact, Miller et al. (1994) described the use of the NHI quantification during refrigerated storage of meats as a sensitive, reliable and consistent analysis for the evaluation of lipid oxidative changes. Accordingly, the aforementioned authors and ourselves (Estévez & Cava, 2004) reported significant correlations between NHI content and TBARS which is in agreement with the correlations found in the present study between TBARS and NHI ($R^2 = 0.67$; $p < 0.01$) and between hexanal counts and NHI ($R^2 = 0.51$; $p < 0.01$). In addition, a significant correlation was found between the carbonyl content derived from protein oxidation and the NHI ($R^2 = 0.51$; $p < 0.01$). Though relatively small, this correlation reasonably suggests that the oxidative deterioration of some particular proteins, such as myoglobin, could promote the degradation of the

heme group and the subsequent release of iron. In fact, results from the release of iron are consistent with those previously reported on the oxidative stability of frankfurters since WF had (from day 20 to day 60) significantly higher amounts of NHI than had IF. The accurate knowledge of the levels of the chemical forms of iron in meat products is of a great important from nutritional and technological points of view. From a nutritional poinl of view, HI has a higher bioavailability than NHI and represents the primary source of iron in the human diet (Carpenter & Mahoney, 1992). Consequently, the degradation of heme iron would reduce the nutritional value of the frankfurters in terms of iron bioavailability. IF contained, during the entire storage period, a significantly higher amount of HI than did WF and HF, which represents an important nutritional benefit. This difference is mainly explained by the higher amount of total iron in IF since the amount of NHI was similar among types of frankfurters. On the other hand, iron is considered as one of the most important oxidation promoters in meat systems (Kanner, 1994). The forms of NHI, including ferritin, lactoferrin, cytosolic iron-dependent enzymes and low molecular weight (LMW) chelatable iron ions, enhance lipid peroxidation in meat to a higher extent than HI (Kanner, 1994). Consequently, the increase of NHI content, as a result of the release of the iron from the heme group, would increase the oxidative instability of the frankfurters, promoting the formation of further TBARS, hexanal and carbonyls from proteins.

4. Conclusions

Frankfurters made with tissues from free-range-reared Iberian pigs, exhibited a higher oxidative stability than those made with tissues from intensively reared white pigs. The higher levels of MUFA, lower levels of PUFA and the presence of antioxidants probably protected lipids and proteins from 'Iberian' frankfurters against oxidative reactions. Using 10% adipose tissue from Iberian pigs improves the quality of frankfurters from white pigs, modifying their fatty acid composition and enhancing their oxidative stability.

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