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Impact of lipid content and composition on lipid oxidation and protein carbonylation in experimental fermented sausages

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ABSTRACT

This study aims to investigate the effect of lipid content (~4%, ~10% and ~15%) and composition (different lipid sources; animal fat and sunflower oil) on the oxidative stability of proteins and lipids in experimental fermented sausages. Increasing the lipid content of sausages enhanced the susceptibility of lipids to oxidation whereas the effect on the formation of specific carbonyls from protein oxidation was not so evident. Sausages manufactured with different lipid sources affected the susceptibility of lipids and proteins to oxidation as a likely result of the modifications in the fatty acid profile, as well as to the presence of antioxidant compounds. While the fatty acid profile had a major effect on the occurrence and extent of lipid oxidation, the presence of compounds with potential antioxidant activity may be more influential on the extent of protein carbonylation.

1. Introduction

Many sensory traits of fermented and dry-cured meat products depend on the content and composition of muscle lipids, including those affected by the onset of oxidative reactions (Gandemer, 2002). Lipid oxidation is responsible for rancidity which is considered unpleasant for consumers (Jeremiah, 2001). Several authors have found a connection between lipid oxidation and fat content in pork (Estévez, Morcuende, & Cava, 2003), liver pâtés (Estévez, Ventanas, & Cava, 2005) and dry-cured loins (Ventanas, Estévez, Andrés, & Ruiz, 2008). Moreover, lipid oxidation is primarily initiated in the highly unsaturated fatty acids components of membrane phospholipids (Mercier, Gatellier, Vincent, & Renerre, 2001) and thus increasing the degree of polyunsaturated fatty acids (PUFA) in meat products leads to a higher susceptibility to undergo lipid oxidation (Gandemer, 2002). Lipid oxidation is regarded as one of the main causes for functional, sensory and nutritional quality deterioration in meat and meat products (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998).

As a major component of muscle tissue, proteins play a decisive role in muscle foods regarding technological, nutritional and sensory aspects (Lawrie, 1998). Modification of the native structure and/or integrity of muscle proteins affect meat quality by modification of texture and colour (Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010; Utrera & Estévez, 2012). Protein oxidation, which causes multiple physico-chemical changes in proteins, could also affect meat quality (Lund, Heinonen, Baron, & Estévez, 2011).

The formation of carbonyl compounds is one of the most marked changes occurring during the oxidation of proteins (Estévez, 2011). Whereas the study of the interactions between oxidising lipids and proteins has been covered before, the application of the DNPH method (2,4-dinitrophenylhydrazine) provided limited insight on precise oxidation mechanisms (Estévez, 2011). On the contrary, the analysis of specific protein carbonyls has enabled the understanding of the mechanisms involved in the oxidative damage to proteins during meat processing (Ganhão, Morcuende, & Estévez, 2010; Utrera, Armenteros, Ventanas, Solano, & Estévez, 2012).

By using the DNPH method, several studies have reported the effect of different strategies against protein oxidation, such as modification of the composition of the muscle through dietary means or the addition of natural antioxidants in meat products (Estévez, Ventanas, & Cava, 2006; Ventanas, Estévez, Tejeda, & Ruiz, 2006; Ventanas, Ventanas, Tovar, García, & Estévez, 2007). However, to our knowledge, the influence of lipid content and composition in the formation of specific protein carbonyls in processed meat products has not been studied before.

The aim of this research was to study the impact of lipid content and lipid source on the oxidative stability of lipids and proteins in experimental fermented sausages. The effect of lipid content was

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studied by varying the level of fat (4%, 10% and 15%) while the effect of lipid composition was assessed by adding different lipid sources, namely back-fats from pigs with different feeding backgrounds, commercial lard and sunflower oil.

2. Materials and methods

2.1. Manufacture of fermented sausages

Formulation of experimental fermented sausages is reported in Table 1 (section a). Manufacture of sausages was run as follows: first, commercial lean pork was cleaned from external fat, chopped, minced and mixed in a vacuum cutter for 2 min. Subsequently the fat, sodium chloride and the additives were mixed for 2.5 min and finally glucono-delta-lactone (GDL) was added. Three types of sausages with increasing lipid content were manufactured using back fat from Iberian pigs fed extensively on acorns and pasture [~4%, ~10% and ~15%]. Moreover, four types of sausages varying in lipid composition were manufactured using animal fat [back-fat from Iberian pigs fed extensively on acorn and grass-MF; back fat from Iberian pigs fed on a concentrate–CF; and commercial Iberian lard–LF] and vegetable fat [sunflower oil–SOF]. These sausages were manufactured in order to obtain about 15% lipid content after the ripening process.

Finally, the batter was vacuum-packaged to remove air bubbles and stuffed into 6 cm diameter artificial collagen casings and the ripening of the experimental sausages was carried out in drying chambers for 15 days, the relative humidity ranging from 95% to 75% and the temperature from 22 °C to 12 °C. At the end of the ripening process the weight losses of the fermented sausages was 35% in average. The ripening process was fixed following the protocols developed for meat products containing GDL (Feiner, 2006).

2.2. Chemical composition of fermented sausages

Lipid content was determined by the Folch method (Folch, Lees, & Sloane Stanley, 1957), protein content by the Kjeldahl method (ISO 937, 1978), and moisture was determined by an AOAC method (AOAC, 2000). Twelve repetitions were performed per type of fermented sausage.

Fatty acid methyl esters (FAMEs) were prepared by acidic transesterification in the presence of sulphuric acid (5% sulphuric acid in methanol) (Sandler & Karo, 1992). FAMEs were analysed by gas chromatography using a Hewlett–Packard HP-5890A gas chromatograph, equipped with an on-column injector and a flame ionisation detector, using a polyethyleneglycol capillary column (Supelcowax-10, Supelco, Bellefonte, PA) (60 m × 0.32 mm i.d. × 0.25 μm film thickness). Gas chromatograph oven programme temperatures were as follows: initial temperature of 190 °C, 2 °C/min to 235 °C; 15 min at this temperature and thereafter 6 °C/min to 250 °C, and then kept for an additional 20 min. Injector and detector temperatures were 250 °C. Carrier gas was helium at a flow rate of 0.8 ml/min. Individual FAME peaks were identified by comparison of their retention times with those of standards (Sigma, St. Louis, MO). Tridecanoic acid was used as internal standard. Eight repetitions were performed per type of fermented sausage and results are expressed as percentage of the total fatty acids analysed.

2.3. Lipid oxidation measurements

Lipid oxidation was evaluated by assessing the formation of hexanal by static headspace analysis (Ventanas et al., 2006) and also by monitoring malondialdehyde (MDA) formation by means of TBARS (Salih, Smith, Price, & Dawson, 1987). Hexanal concentration was quantified by headspace-SPME and GC/MS. A gas chromatograph Hewlett–Packard 5890 serie II was used, coupled to a mass selective detector (Hewlett–Packard HP-5791 A). One gramme of minced sample was weighed into a 4 ml vial. All vials were closed with a Teflon/silicone septum. An SPME fibre (50/30 μm divinylbenzene-carboxen-polydimethylsiloxane coating) was inserted through the septum and exposed to the headspace of the vial. Vials were pre-conditioned for 15 min at 37 °C. Extraction was carried out at 37 °C for 5 min in a water bath. After extraction, the SPME fibre was immediately transferred to the injector of the chromatograph, which was in splitless mode at 270 °C. The separation of the volatile compounds was performed on a 5% phenyl–methyl silicone (HP-5) bonded phase fused silica capillary column (Hewlett– Packard, 50 m × 0.32 mm i.d, film thickness 1.05 μm), operating at 6 psi of column head pressure. The oven programme was: 45 °C for 10 min, 10 °C min^{-1} to 200 °C, 15 °C min^{-1} to 250 °C, and held 250 °C for 10 min. The transfer line to the mass spectrometer was maintained at 270 °C. The mass spectra were obtained using a mass selective detector by electronic impact at 70 eV, a multiplier voltage of 1756 V and collecting data at a rate of 1 scan s^{-1} over the *m/z* range 30–500. Hexanal was identified by comparing their mass spectrum and linear retention index with that of the standard. Results are given in area units (AU). Five repetitions per type of sausage were performed.

TBARS were assessed following the method developed by Salih et al. (1987). The product (2.5 g) was dispensed in cone plastic tubes and homogenised with 7.5 ml of perchloric acid (3.86%)

Table 1
(a) Formulation (%) and (b) fatty acid profile (means ± standard deviation) of fermented sausages with different lipid content and various lipid sources MF (back-fat from pigs fed on acorns and grass), CF (back-fat from pigs fed on concentrate), LF (commercial lard) and SOF (sunflower oil). Results are expressed in %.

(a) Formulation	Lipid content				Lipid source				
	~4%	~10%	~15%		MF	CF	LF	SOF	
Lean pork	96.84	92.93	88.11		88.11	88.11	89.00	89.00	
Animal fat/vegetable fat	0.00	3.90	8.73		8.73	8.73	7.83	7.83	
Sodium chloride	2.10	2.10	2.10		2.10	2.10	2.10	2.10	
Additives									
Glucono-delta-lactone	1.00	1.00	1.00		1.00	1.00	1.00	1.00	
Sodium nitrite	0.015	0.015	0.015		0.015	0.015	0.015	0.015	
Ascorbic acid	0.05	0.05	0.05		0.05	0.05	0.05	0.05	
(b) Fatty acid profile	~4%	~10%	~15%	*P*	MF	CF	LF	SOF	*P*
ΣSFA	43.04 ± 1.06^{a}	38.23 ± 0.77^{c}	41.45 ± 0.57^{b}	***	41.45 ± 0.57^{b}	47.74 ± 1.91^{a}	47.34 ± 1.17^{a}	22.01 ± 2.82^{c}	***
ΣMUFA	43.07 ± 0.73^{b}	48.98 ± 0.57^{a}	48.97 ± 0.58^{a}	***	48.97 ± 0.58^{a}	44.74 ± 1.49^{b}	41.95 ± 1.04^{c}	30.23 ± 3.37^{d}	***
ΣPUFA	13.79 ± 0.49^{a}	12.71 ± 0.37^{b}	9.49 ± 0.49^{c}	***	9.49 ± 0.49^{b,c}	7.48 ± 2.71^{c}	10.66 ± 0.53^{b}	47.39 ± 1.40^{a}	***

SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids. $^{a-c}$Means with different superscript differ significantly. Statistical significance $^{***}P < 0.001$.