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Physicochemical characteristics of an α-linolenic acid and α-tocopherol-enriched cooked ham

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Abstract

Five batches of cooked hams were manufactured using pork legs enriched in polyunsaturated $n-3$ fatty acids and α-tocopherol from animals fed on diets with the same ingredients excepting the oil source [sunflower oil (batch control, C), linseed oil (L), 1/1 w/w linseed and olive oil (LO) and 20 (C, L and LO) or 200 (LOE and LE) mg/kg diet of α-tocopherol]. A final product was obtained enriched in $n-3$ fatty acids with a healthier polyunsaturated fatty acid $n6/n3$ ratio (<3) from all linseed oil-enriched batches than those of the Control (10.5). The only significant differences ($p < 0.05$) found in cooked hams were in the fatty acid composition and the α-tocopherol content.

Keywords: Cooked ham; Fatty acid; PUFAs n- 6/n- 3 ratio; α-tocopherol

1. Introduction

From the classical article of Ellis and Isbell (1926) until now, many investigations have been conducted to establish the effect of fat in the diet on the fat and meat characteristics of pork (Brooks, 1971; Morgan, Noble, Cocchi, & McCartney, 1992; Skelley et al., 1975). Many of this investigations have focused on the effects of pig diet polyunsaturated fatty acids on pork lipid and fatty acid composition. These efforts have mainly evaluated the possibilities of pork tissue enrichment in $n-3$ polyunsaturated fatty acids (Ahn, Lutz, & Sim, 1996; Cheriam & Sim, 1995; Fontanillas, Barroeta, Baucells, & Codony, 1997; Romans, Johnson, Wulf, Libal, & Costello, 1995; Van Oeckel, Casteels, Warnants, Van Damme, & Boucquè, 1996; Van Oeckel, Casteels, Warnants, & Boucquè, 1997) because of the beneficial effects, assigned to these fatty acids, on human health (Alexander, 1998; Rose & Connolly, 1999). Because of that, increase of the intake of $n-3$ PUFA foods has been recommended, establishing a $n-6/n-3$ PUFAs ratio less than 4 (British Nutrition Foundation, 1992) to improve the health status of the humans. One approach to reach this goal is the enrichment of pork and other meat from monogastric animals (i.e. poultry, rabbits), with $n-3$ PUFA. However, the modification of the pork fat fatty acid composition may lead to a higher susceptibility to lipid oxidation, which could negatively influence the meat quality. It has been shown that pork enrichment with vitamin E is an effective approach for controlling the lipid oxidation of liver, lard and meat (D'Arrigo, Hoz, Lopez-Bote, Cambero, Pin, & Ordoñez, 2002a, 2002b; Hoz et al., 2003) and meat products (Chizzolini, Novelli, & Zanardi, 1998).

In an attempt to improve the nutritive characteristics (enrichment of the $n-3$ fatty acids and α-tocopherol) of cooked ham with minor modifications to the chemical and sensory characteristics, the present research was conducted to evaluate the possibility of using of pork legs enriched in $n-3$ fatty acids and α-tocopherol in the manufacture of this meat product.

Abbreviations: PUFAs, polyunsaturated fatty acid; TBARs, 2-thiobarbituric acid-reactive substances; TPA, texture profile analysis.

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2. Materials and methods

2.1. Experimental design

Fifty Large White × Great York female pigs were fed with five different diets (D'Arrigo et al., 2002a, 2002b). All diets were formulated with the same ingredients except for the fat source (30 g/kg in all cases) and α-tocopherol. Dietary fat sources were sunflower oil (rich in C18:2$n-6$) for the control diet (C), linseed oil (L, rich in C18:3$n-3$) and a 1:1 (w/w) mixture of linseed oil and olive oil (LO, rich in C18:1$n-9$). Within each dietary fat treatment containing L, one group, as the control, was fed a basal level (20 mg α-tocopheryl acetate/kg diet) of vitamin E (Hoffman La Roche, Switzerland), and the others received a supplemented level (200 mg α-tocopheryl acetate/kg diet) of vitamin E (batches LOE and LE).

Animals were stunned, slaughtered and exsanguinated at a local slaughterhouse at 100.1 ± 7.09 kg live weight.

2.2. Preparation of the cooked ham

Five different batches of cooked ham were manufactured, each comprising 10 different hams. All the cooked hams were manufactured on the same day using similar technology, ingredients and formulation. At 48 h post-mortem, the left legs were taken from the carcass and they were deboned and skin, tendons and fatty tissues removed. The boneless legs were pumped to 120% of their green weight with a brine solution, using a multi-needle brine injector. The brine solution was composed (g/l) of: sodium chloride 125, dextrose 48, phosphate 18, ascorbate 6, carrageenate 6, sodium nitrate 0.90 and sodium nitrite 0.30. The injected legs were massaged for 30 min in a meat tumbler (14 r.p.m.) and then stored at 2 °C for 24 h. Afterwards, they were massaged again for 30 min and stored at 2 °C for 24 h. After a final massage of 10 min, the legs were placed in pear-shaped ham moulds. Samples were cooked in a wet hot oven at 82 °C for 4.5 h until a core temperature of 70 °C was reached. After cooling, cooked hams were longitudinally cut. One portion (time 0) was immediately analysed for physicochemical, texture, colour and sensorial characteristics and the other one was vacuum-packed and stored at 2 °C for one month (time 1), and the same parameters were determined.

After the analyses for colour, texture and sensorial determinations, the cooked ham samples (times 0 and 1) were vacuum-packed, frozen at −18 °C and kept until chemical analysis.

2.3. Chemical analysis

Protein (Kjeldhal nitrogen), moisture (oven air-drying method) and ash (muffle furnace) were analysed following AOAC (1995) procedures. Water activity (a_w) was determined using a Decagon CX1 hygrometer (Decagon Devices Inc., Pullman, WA) at 25 °C.

Concentration of α-tocopherol was quantified as described by Rey, Lopez-Bote, Soares, and Isabel (1997). Analyses were carried out by reverse phase HPLC (HP 1050, Hewlett Packard, Waldbronn, Germany, equipped for separation with a C18 column RP-18, Hewlett Packard). The mobile phase was methanol:water (97:3 v/v) at a flow rate of 2 ml/min, and the detector was fixed at 292 nm.

Lipids of cooked ham were cold-extracted from the whole piece using the method of Hanson and Olley (1963) which involves the use of chloroform/methanol/water (1/1/1, v/v/v). Three hundred mg of lipids were methylated in the presence of 3 ml of sodium metal (0.1 N in methanol) and 3 ml of sulphuric acid (5% in anhydrous methanol) to obtain the fatty acid methyl esters (Sandler & Karo, 1992). The methyl esters were extracted with 3 ml of petroleum ether. Then, 1 μl was analysed using a Hewlett Packard HP-5890 (Avondale, PA, USA) gas chromatograph equipped with a flame ionization detector and a capillary column HP-Innowax (30 m × 0.32 mm id and 0.25 μm). Helium at 2.0 ml/min was used as the carrier gas and the split/splitless injector was used with a split/splitless ratio of 10/1. The temperature programme was as follows: injector and detector temperature 250 °C, the initial column temperature was 200 °C, which was kept for 2 min, 200–245 °C at 3.5 °C min^{-1}, held for 7 min. Fatty acid methyl esters were identified by comparison with standards run previously.

Lipid oxidation of the cooked ham was determined using the 2-thiobarbituric acid method (TBARs) described by Salih, Smith, and Dawson (1987). For that, 5 g of sample were homogenised in 15 ml of 0.38 M $HClO_4$ for 3 min in an ice bath. To avoid further oxidation, 0.5 ml of a 0.19 M BHT ethanolic solution were added. The homogenate was centrifuged (3000*g*, 5 min, 5 °C) and filtered through Whatman No. 54 paper. An aliquot (0.7 ml) was mixed with the same volume of a 0.02 M TBA solution and heated at 100 °C for 30 min. After cooling, the mixture was centrifuged at 3000*g* for 15 min at 5 °C. Finally, the absorbance was measured at 532 nm. Results were expressed as mg malonaldehyde/kg sample.

2.4. Texture analysis

Texture profile analysis (TPA) was used to evaluate the sausage texture (Bourne, 1978; Szczesniak, 1986), using the Stable Micro System Mod. TA-XTZi Texture Analyser (Stable Micro Systems, Godalming, England) equipped with a cylindrical probe P/25. This procedure involved cutting samples approximately 1.5 cm high and 2.5 cm wide after discarding the external layer (2 cm) of

the ham piece. Samples were tempered at room temperature and then compressed twice to 50% of their original height. The following parameters were determined: hardness (N), maximum force required to compress the sample (H); springiness (m), ability of sample to recover its original form after the deforming force was removed (S); adhesiveness ($N \times S$), area under the abscissa after the first compression; cohesiveness, extent to which the sample could be deformed prior to rupture (A2/A1, A1 was the total energy required for the first compression and A2 the total energy required for the second compression); gumminess (N), force to disintegrate a semisolid meat sample for swallowing ($H \times$ cohesiveness); chewiness (J), work to masticate the sample for swallowing ($S \times$ gumminess). To determine the maximum cutting force and the cutting work (Bourne, 1978) a reversible probe calibrated with 5 kg was used. Thirty slices of cooked ham were used per batch in the texture analysis (three slices per ham and 10 hams per batch).

2.5. Colour measurement

Colour measurements of the cooked ham surface were obtained using a tristimulus colorimeter (Minolta Chroma Meter CR300, Minolta Corporation, NJ). The L^* – (brightness), a^* – (redness) and b^* – (yellowness) values were measured four times on the surface of the sausages at three different analysis times (freshly cut sausage, 4 and 24 h after cutting). After the first colour measurement, the samples were kept at room temperature without protection.

2.6. Sensory analysis

To determine the possible sensory differences among the cooked hams, a triangle test (ISO, 1981a) was conducted. The cooked hams were evaluated by a panel of 15 tasters selected among the members of the Departamento de Nutrición, Bromatología y Tecnología de los Alimentos, who were previously trained in the sensory assessment of meat products. The evaluations were performed in individual booths built according to the criteria of the International Standards Organisation (ISO, 1981b). The tasters were given unsalted crackers and room temperature water to clean the palate between samples. To reduce fatigue, panel members conducted two sessions per day in which they were served four randomised samples per test session with a minimum of 1 h between sessions.

2.7. Statistical analysis

Data were analysed using the General Linear Model of SAS (2001). An individual ham was the experimental unit for analysis of all data. The comparative analyses between means were conducted using the Duncan multiple range test. Data were presented as the means of each group and the standard deviation (SD) of the mean.

3. Results and discussion

In a previous paper (Hoz et al., 2003), the effect of pig diet enrichment in $n-3$ fatty acids and α-tocopherol on pig muscle has been described. Results indicated that the PUFA $n-6/n-3$ ratio in pork tenderloin (*Psoas major*) was markedly modified by dietary linseed oil administration, which was due to the increase in the C18:3$n-3$ (and total $n-3$ fatty acids) content and the decrease in the C18:2$n-6$ (and total $n-6$ fatty acids) content ($p < 0.05$). The α-tocopherol level of the tenderloin was also dependent on diet concentration. In the present work, by using the raw pork legs from the same animals, we checked the use of this raw material in the production of cooked ham, taking into consideration the main physicochemical and sensory characteristics.

After the analysis of cooked ham from the five different experimental batches (C, L, LE, LO and LOE), vacuum stored for 0 and 1 month, no significant effect ($p > 0.05$) of vacuum-storage time was found in any parameter (chemical composition, fatty acid composition, physical or sensorial characteristics). All data were the averages of values obtained from samples vacuum-stored for 0 and 1 month. No differences were observed ($p > 0.05$) among the different cooked ham experimental batches in water, fat and ash contents, pH or water activity (a_w). The following average values were found: moisture 74.5 (% wet matter), fat 6.4 (% wet matter), ash 2.9 (% wet matter), pH 5.9 and a_w 0.980.

These figures were close to those described by Chan, Brown, Church, and Buss (1996) and Houben and Gerris (1998).

Several authors (Hoving-Bolink, Eikelenboom, van Diepen, Jongbloed, & Houben, 1998; Hoz et al., 2003) have previously observed that the addition of α-tocopherol to the animal feedstuff gives rise to a vitamin E accumulation in the pig muscle. Accordingly, the α-tocopherol contents (about 2.8 mg /kg wet matter) of cooked ham batches manufactured from legs of animals fed on diets supplemented with α-tocopherol (batches LE and LOE) were about 3-fold higher than those observed in batches C, L and LO (about 0.9 mg/kg wet matter). These results are in agreement with those of similar work carried out by Houben and Gerris (1998) although the absolute values reported by these authors for the α-tocopherol content of cooked hams from vitamin E-supplemented animals were slightly higher.

Although it has been described that the heat treatment reduces the vitamin E content in foods (Gregory,

1996) no losses were found by Liu, Scheller, Schaefer, Arp, and Williams (1994) in cooked ham manufacture. The same fate of the α-tocopherol was observed in the present work since the contents of this compound in the different experimental cooked hams were similar to those of tenderloin (*Psoas major*) muscle from the same pigs (Hoz et al., 2003).

The TBARs values of cooked ham of all batches (C, L, LE, LO and LOE) were not affected ($p > 0.05$) by either vacuum-storage or dietary treatment (data not shown). The TBARs mean values were about 0.2.

The fatty acid composition of experimental cooked hams is shown in Table 1. No effect of vacuum-storage of cooked ham was observed. In all batches, the major fatty acid was C18:1$n-9$, accounting for more than 40%. Significant differences ($p < 0.05$) among batches were found for four fatty acids (C18:2$n-6$, C18:3$n-6$, C18:3$n-3$ and C20:4$n-3$). The enrichment of the animal diet with linseed or linseed and olive oil significantly increased ($p < 0.05$) the percentage of C18:3$n-3$ (5–7-fold) and C20:4$n-3$ (4–5-fold). The total content of the $n-3$ family fatty acids may be arranged as follows: L and LE > LO and LOE > C, which corresponded with the pig diet C18:3$n-3$ content (Hoz et al., 2003). The most abundant fatty acid of the $n-3$ family was the C18:3$n-3$, with values close to 5.1% in batches L and LE, about 3.3% in LO and LOE and 0.7% in C. Also, an important modification of the PUFAs $n-6/n-3$ ratio was observed with values decreasing from 10.5 (batch C) to about 2.6 (LO and LOE) and 2.0 (L and LE). The enrichment in $n-3$ fatty acids was concomitant with the decrease of the 18:2 $n-6$ level.

No effect of the diet ($p > 0.05$) on L^*-, a^*- and b^*-values of the cooked ham was observed just after cutting the samples for analysis. However, a clear influence of the air exposure after cutting (0, 4 and 24 h) was observed in all batches, which is usual in meat products (Perlo et al., 1995) although the colour changes after air exposure were similar in all the batches. In a similar study, dealing with the effect of dietary supplementation with vitamin E on colour stability of packed, sliced pasteurized ham, Houben and Gerris (1998) found, in

Table 1
Fat (% wet matter) and fatty acid composition (g/100 g total fatty acids) of the whole piece of experimental cooked hams[a]

	Experimental cooked ham[a]				
	C	L	LE	LO	LOE
Fat (% wet matter)	6.22 ± 1.12	5.96 ± 1.81	6.29 ± 1.36	6.74 ± 1.50	6.89 ± 1.97
Fatty acid (g/100 g total fatty acids)					
C12:0	0.10 ± 0.01	0.08 ± 0.02	0.09 ± 0.01	0.08 ± 0.04	0.08 ± 0.04
C14:0	1.00 ± 0.15	0.93 ± 0.16	1.05 ± 0.05	0.97 ± 0.15	0.85 ± 0.20
C16:0	21.5 ± 1.03	21.3 ± 2.29	20.9 ± 1.46	20.9 ± 1.67	20.2 ± 1.24
C16:1*n*9	0.39 ± 0.04	0.34 ± 0.05	0.34 ± 0.03	0.34 ± 0.03	0.34 ± 0.02
C16:1	1.96 ± 0.30	1.80 ± 0.32	2.26 ± 0.12	1.84 ± 0.43	1.64 ± 0.19
C18:0	11.2 ± 1.00	11.9 ± 2.22	10.9 ± 0.47	11.4 ± 1.13	13.5 ± 2.13
C18:1*n*9/1*n*7	42.5 ± 1.52	41.3 ± 3.83	42.7 ± 1.96	45.7 ± 2.37	44.2 ± 1.78
C18:2*n*6	16.7 ± 2.68a	13.0 ± 0.76b	12.4 ± 0.95b	11.7 ± 1.31b	11.8 ± 0.85b
C18:2*n*3	0.09 ± 0.06	0.08 ± 0.02	0.08 ± 0.02	0.07 ± 0.01	0.05 ± 0.02
C18:3*n*6	0.09 ± 0.02a	0.06 ± 0.01b	0.04 ± 0.03b	0.04 ± 0.01b	0.07 ± 0.02b
C18:3*n*3	0.70 ± 0.12c	5.08 ± 0.81a	5.26 ± 0.31a	3.35 ± 0.54b	3.21 ± 0.22b
C18:4*n*3	0.1 ± 0.02	0.08 ± 0.04	0.09 ± 0.03	0.09 ± 0.02	0.09 ± 0.02
C20:0	0.18 ± 0.04	0.21 ± 0.04	0.16 ± 0.02	0.20 ± 0.04	0.22 ± 0.05
C20:1*n*9	0.82 ± 0.08	0.81 ± 0.09	0.83 ± 0.12	0.73 ± 0.22	0.91 ± 0.09
C20:3*n*9	0.78 ± 0.15	0.59 ± 0.05	0.64 ± 0.29	0.56 ± 0.09	0.60 ± 0.10
C20:4*n*6	0.89 ± 0.25	0.64 ± 0.19	0.54 ± 0.20	0.72 ± 0.37	0.66 ± 0.25
C20:4*n*3	0.12 ± 0.02c	0.63 ± 0.20b	0.70 ± 0.05a	0.54 ± 0.07b	0.54 ± 0.07b
C20:5*n*3	0.08 ± 0.02	0.06 ± 0.01	0.08 ± 001	0.07 ± 0.02	0.07 ± 0.02
C22:1*n*9	0.07 ± 0.01	0.07 ± 0.03	0.05 ± 0.01	0.05 ± 0.02	0.06 ± 0.02
C22:4*n*6	0.11 ± 0.05	0.12 ± 0.03	0.13 ± 0.05	0.11 ± 0.04	0.16 ± 0.10
C22:5*n*3	0.38 ± 0.11	0.57 ± 0.23	0.49 ± 0.10	0.43 ± 0.15	0.62 ± 0.16
C22:6*n*3	0.24 ± 0.08	0.25 ± 0.09	0.21 ± 0.05	0.20 ± 0.09	0.21 ± 0.11
Total SAFA	34.1 ± 1.85	34.5 ± 4.15	33.1 ± 1.59	33.5 ± 1.81	34.8 ± 2.95
Total MUFA	45.7 ± 1.56	44.4 ± 4.08	46.2 ± 2.25	48.7 ± 2.28	47.2 ± 1.86
Total PUFA	20.2 ± 2.98a,b	21.2 ± 1.03a	20.8 ± 0.69a	17.9 ± 1.99c	18.1 ± 1.33b,c
*n*6	17.8 ± 2.85a	13.8 ± 0.91b	13.1 ± 0.22b	12.6 ± 1.43b	12.7 ± 0.91b
*n*3	1.69 ± 0.42c	6.75 ± 0.96a	6.93 ± 0.24a	4.74 ± 0.61b	4.78 ± 0.39b
*n*6/*n*3	10.5 ± 2.60a	2.10 ± 0.42b	1.91 ± 0.05b	2.67 ± 0.16b	2.66 ± 0.08b

a–d Means of experimental cooked ham bearing different letter within the same row are significantly different ($p < 0.05$).

[a] Mean values of vacuum-packed cooked ham stored during 0 and 1 month at 2 °C manufactured with legs from pigs fed on diets: C = control (30 g/kg sunflower oil), L = 30 g/kg linseed oil, LE = 30 g/kg linseed oil + 200 mg/kg α-tocopheryl acetate, LO = 15 g/kg linseed oil + 15 g/kg olive oil, LOE = 15 g/kg linseed oil + 15 g/kg olive oil + 200 mg/kg α-tocopheryl acetate.

general, similar results to our own, concluding that dietary enrichment with vitamin E of pigs destined for the manufacture of pasteurized ham products does not appear to offer significant advantage over currently used feeding regimens.

Textural features were not affected by the cooked ham enrichment in $n-3$ PUFAs since no significant differences in the results of the TPA, cutting force and cutting work among the different experimental cooked hams were found (data not shown).

Similarly, triangular test of the sensory analysis (data not shown), did not show any significant differences ($p > 0.05$) among cooked ham batches.

It can be concluded that it is possible to manufacture cooked ham from pig legs enriched in $n-3$ PUFA without adverse effects on its composition, lipid stability, textural or sensory properties. Since the cooked ham is a heat-treated meat product, usually vacuum-packed and stored under refrigeration, it might be unnecessary to add vitamin E to the pig diet because it is not probably the cause of development of lipid oxidation.

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