LIPID OXIDATION IN DUTCH STYLE FERMENTED SAUSAGES WITH INCREASED LEVELS OF LINOLENIC ACID

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Introduction

In the 1970s researchers, struck by the low incidence of coronary artery disease among Greenland Inuit, associated this with the high content of *n*-3 polyunsaturated fatty acids (PUFAs) in their diet (Bjerregaard et al., 2000). *n*-3 PUFAs may also have a protective effect against breast and colon cancer (Rose and Connolly, 1999), rheumatoid arthritis and inflammatory bowel diseases (Alexander, 1998).

As meat (products), are some of the most important sources of dietary fat, modification of their lipid profiles by enhancing *n*-3 PUFAs, can help to improve the nutritional quality of the occidental diet (Ansorena and Astiasaran, 2004). Nutritional authorities recommend that a dietary *n*-6/*n*-3 PUFA ratio of less than 4 should be achieved and a polyunsaturated fatty acids/saturated fatty acids (P/S) ratio above 0.4 (Wood et al., 2004). Feeding animals PUFAs (basically *n*-3) had a positive effect on the fatty acid profile, but a negative effect on the sensory quality of the meat when added in high concentrations (D'Arrigo et al., 2002; Fontanillas et al., 1998; Hoffman et al., 2005; Hoz et al., 2004; Hoz et al., 2003; Matthews et al., 2000; Rey et al., 2001; Romans et al., 1995; SpechtOverholt et al., 1997).

The inclusion of vegetable oils in fermented sausages has been variously tested. Olive oil (Muguerza et al., 2003b; Muguerza et al., 2002; Muguerza et al., 2001; Severini et al., 2003), soybean oil (Muguerza et al., 2003a) and flaxseed oil (Ansorena and Astiasaran, 2004) were used as a source of monounsaturated fatty acids, to modify the P/S ratio, and to change both P/S and *n*-6/*n*-3 ratios, respectively. The main problem of increasing *n*-3 PUFAs in fermented sausages may arise from their susceptibility to oxidation.

Objective

The objective of this work was to evaluate the lipid modifications in dry-fermented sausages, when part of the animal fat was replaced by pre-emulsified flaxseed and canola

oil. Our work focuses on the changes in the P/S and *n*-6/*n*-3 ratios and on the development of lipid oxidation in the final product during storage.

Methodology

Sausage Preparation

Dutch style cervelat, a semi-dry fermented sausage, were manufactured at Wageningen University. Lean beef, pork backfat, flaxseed and canola oil were used as raw materials. The flaxseed and canola oil (both obtained from a local supermarket) were pre-emulsified with soy protein isolate (Hoogenkamp, 1989a,b). Seven formulations of sausages, about 1.5 kg each, were prepared. The control contained 70% beef and 30% pork backfat. The other formulations were produced with 27, 25.5 and 24% backfat and 3, 4.5 and 6% flaxseed or canola oil, respectively. This corresponds to a substitution of backfat with 10, 15 and 20% flaxseed or canola oil, respectively. The experimental design and the formulations are given in Table 1. The relative amounts of the other ingredients, added (g) per kg meat mixture, were: nitrite-curing salt (25); starter sausage (10); glucose (7); glutamate (2); white pepper (1.2); paprika (1); crushed pepper (1); ascorbic acid (0.5); mace (0.25); clove (0.16); and garlic powder (0.15).

Product doughs were manufactured according to a strictly standardized procedure and stuffed into 52 mm diameter cellulose-based casings. The sausages were fermented for three days at 25°C and dried at 15°C and 80–65% R.H. for 12 days at Meester-Stegeman C.V. (Deventer). To accelerate the rate of oxidation the sausages were next sliced (thickness 6 mm) and stored in a modified atmosphere containing 55% oxygen, 24% carbon dioxide and 21% nitrogen. The slices were packaged at Hanskamp Vers Vlees B.V. (Deventer). Samples from all formulations were taken for analysis at day 0 (= packaging day), 18, 30, 41, 55, 69 and 83 of storage in the dark at 5°C.

Chemical Analyses

Determinations were done: of moisture (AOCS., 1997a); of total fat (AOAC., 2002); of peroxide values (AOCS., 1997b); of thiobarbituric acid reactive substances (TBARS) (Juncher et al., 2000) and of protein (according to the Dumas method with a NA 2100 Protein analyser). The fatty acid compositions of the lipid fractions were determined as fatty acid methylesters (AOCS, 1997c). The hexanal content was determined by a GC static head space method (Shahidi and Pegg, 1994).

Statistical Analyses

Analysis of variance (ANOVA) and the Tukey test were used to determine significant differences ($p \leq 0.05$). Software used was SPSS version 10.0 (© 1999, SPPS inc., Chicago).

Results & Discussion

The formulations of the sausages, with a total added backfat plus oil of 30% in the meat mix, are presented in Table 1. The flaxseed or canola oil added in the modified products increased from 10 to 20% of the total added backfat.

Table 2 shows the percentages of moisture, fat and protein of the different products. There was no exudation of oil/fat in the treatments with substitution of pork backfat by pre-emulsified flaxseed and canola oil during the manufacturing process. Bloukas et al. (1997), Muguerza et al. (2001) and Vural (2003) found that the partial replacement of pork backfat with oil pre-emulsified with soy protein isolate resulted in significant differences in moisture content. In contrast, we did not find differences in the moisture content for the different treatments.

The fatty acid profiles of the various sausages are shown in Table 3. SFA content showed a progressive decrease from control by substitution of 0 to 20% of backfat by flaxseed or canola oil. The low amount of palmitic and stearic acid in flaxseed and canola oil are mainly responsible for these changes. The Σ MUFA showed a progressive decrease in the sausages with flaxseed oil (low content of oleic acid), whereas Σ MUFA in the sausages with canola oil (high oleic acid content) showed a progressive increase. All the modified products showed a progressive increase in the Σ PUFA, especially the sausages with flaxseed oil. The P/S ratio ratio increased from 0.32 in the control to 0.41–0.48 and to 0.49–0.70 in the sausages with canola and flaxseed oil, respectively. The increase in this ratio in relation to control is basically due to the increase in linolenic acid. Effectively, the *n*-6/*n*-3 ratio decreased from 11.29 in the control to 6.95–5.12 and to 1.93–1.05 in the sausages with canola and flaxseed oil, respectively.

Fig. 1 shows the peroxide values (P.V.) as a function of storage time of the different modified products. The P.V. of all the formulations were until day 55 quite similar, after that day the P.V. of the different products increased, especially for the sausages with flaxseed oil. After 83 days, the sausages with canola oil showed the same P.V. as the control. The sausages with flaxseed oil showed a higher increase of P.V. than the other formulations after that period of storage. Higher replacement of pork backfat by flaxseed oil, resulted in higher P.V. The high amount of linolenic acid in flaxseed oil is probably mainly responsible for these increments, because *n*-3 PUFAs are very susceptible to oxidation.

Secondary oxidation products (TBARS) were also determined (Fig. 2). After 45 days there was an increase in TBARS values of the sausages with flaxseed oil. All formulations with flaxseed oil had TBARS values around 7 μg malonaldehyde/g sausage after 83 days of storage. These values were higher than for the other formulations; the higher unsaturated character of flaxseed oil is probably mainly responsible for this effect. The TBARS values of the sausages with canola oil were comparable to the values of the control.

The measurable formation of hexanal, a specific secondary lipid oxidation product, in sausages with flaxseed oil starts around day 30 compared to day 60 for the sausages with canola oil (Fig. 3). The hexanal end-values of the flaxseed oil containing sausages were much higher than the values of the canola oil containing products. The formation of hexanal was in the control even higher than in the sausages with canola oil.

Part of an explanation for the reduced lipid oxidation in the canola oil containing products may be an antioxidant effect of soy protein isolate (Bloukas et al., 1997) in combination with the relatively high content of vitamin E in this oil.

Conclusions

In the manufacture of Dutch style fermented sausages, up to 20% of pork backfat can be substituted with flaxseed or canola oil, pre-emulsified with soy protein isolate. The addition of flaxseed and canola oil progressively increased the PUFA/SFA ratio and decreased the *n*-6/*n*-3 ratio leading to values closer to those considered optimal. The addition of canola oil, did not reduce the shelf life in terms of lipid oxidation, however, the addition of flaxseed oil showed an increased lipid oxidation during storage. Further research is needed to get more insight in the sensory and physical characteristics of the products.

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Tables and Figures

Table 1. Experimental design and raw materials used

Treatments[a]	Backfat level[b] (%)	Oil replacing level (%)	Materials in g/kg of meat mixture					
			Beef	Pork backfat	Flaxseed oil	Canola oil	ISP[c]	Water[c]
Control	30	0	700	300	–	–	–	–
F10	30	10	700	270	30	–	3.0	24.0
F15	30	20	700	255	45	–	4.5	36.0
F20	30	30	700	240	60	–	6.0	48.0
C10	30	10	700	270	–	30	3.0	24.0
C15	30	20	700	255	–	45	4.5	36.0
C20	30	30	700	240	–	60	6.0	48.0

[a]F10, F15 and F20, substitution of pork backfat with 10, 15 and 20% flaxseed oil, respectively; C10, C15 and C20, substitution of pork backfat with 10, 15 and 20% canola oil, respectively.

[b]On the day of preparation.
[c]ISP, isolated soy protein. ISP and Water added on top.

Table 2. Mean percentages of moisture, fat and protein of the different final products (*N*=2)

	Control	F10[a]	F15	F20	C10	C15	C20
Moisture	33.3	32.3	33.3	30.8	33.5	32.4	32.8
Fat	40.4	38.6	40.5	40.8	40.4	38.4	39.3
Protein	23.0	24.0	24.1	25.5	24.3	25.6	26.1

[a]See corresponding subscript Table 1.

Table 3. Fatty acid content at the time of packaging for seven types of sausages (g/100 g fat)

Fatty acid		Control	F10[a]	F15	F20	C10	C15	C20
Caproic	10:0	0.08[b]a	0.04b	0b	0b	0b	0b	0b
Lauric	12:0	0.40a	0.37a	0.37a	0.32a	0.28a	0.37a	0.32a
Myristic	14:0	2.91a	2.63ab	2.49bd	2.37cd	2.60bc	2.41bd	2.26d
Palmitic	16:0	24.46a	22.92b	22.08bc	20.96de	22.92b	21.90cd	20.79e
Palmitoleic	16:1	3.05a	2.69bc	2.53cd	2.49de	2.73b	2.52ce	2.39de
Margaric	17:0	0.59a	0.55ab	0.51bc	0.50c	0.53ac	0.51bc	0.49c
Stearic	18:0	12.85a	12.06ab	11.86ab	11.21bc	11.76ab	11.35b	10.94c
Oleic	18:1	42.04c	39.65d	38.48de	37.31e	43.76b	44.63ab	45.97a
Linoleic	18:2	12.37e	12.53de	13.27c	12.72d	13.49bc	13.94ab	13.99a
Linolenic	18:3	1.10d	6.49c	8.42b	12.12a	1.94d	2.32d	2.73d
Arachidic	20:0	0.16a	0.08a	0a	0a	0a	0.05a	0.11a
Behenic	22:0	0a	0.01a	0a	0a	0a	0a	0a
Σ SFA[c]		41.45	38.64	37.30	35.36	38.08	36.59	34.92
Σ MUFA		45.09	42.34	41.00	39.80	46.48	47.14	48.36
Σ PUFA		13.46	19.02	21.69	24.84	15.43	16.26	16.73
P/S		0.32	0.49	0.58	0.70	0.41	0.44	0.48
n-6/*n*-3		11.29	1.93	1.58	1.05	6.95	6.01	5.12

[a]See corresponding subscript Table 1.
[b]Values with different letters within a row are significantly different ($P<0.05$; $N=4$).
[c]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; P/S, polyunsaturated fatty acids/saturated fatty acids.

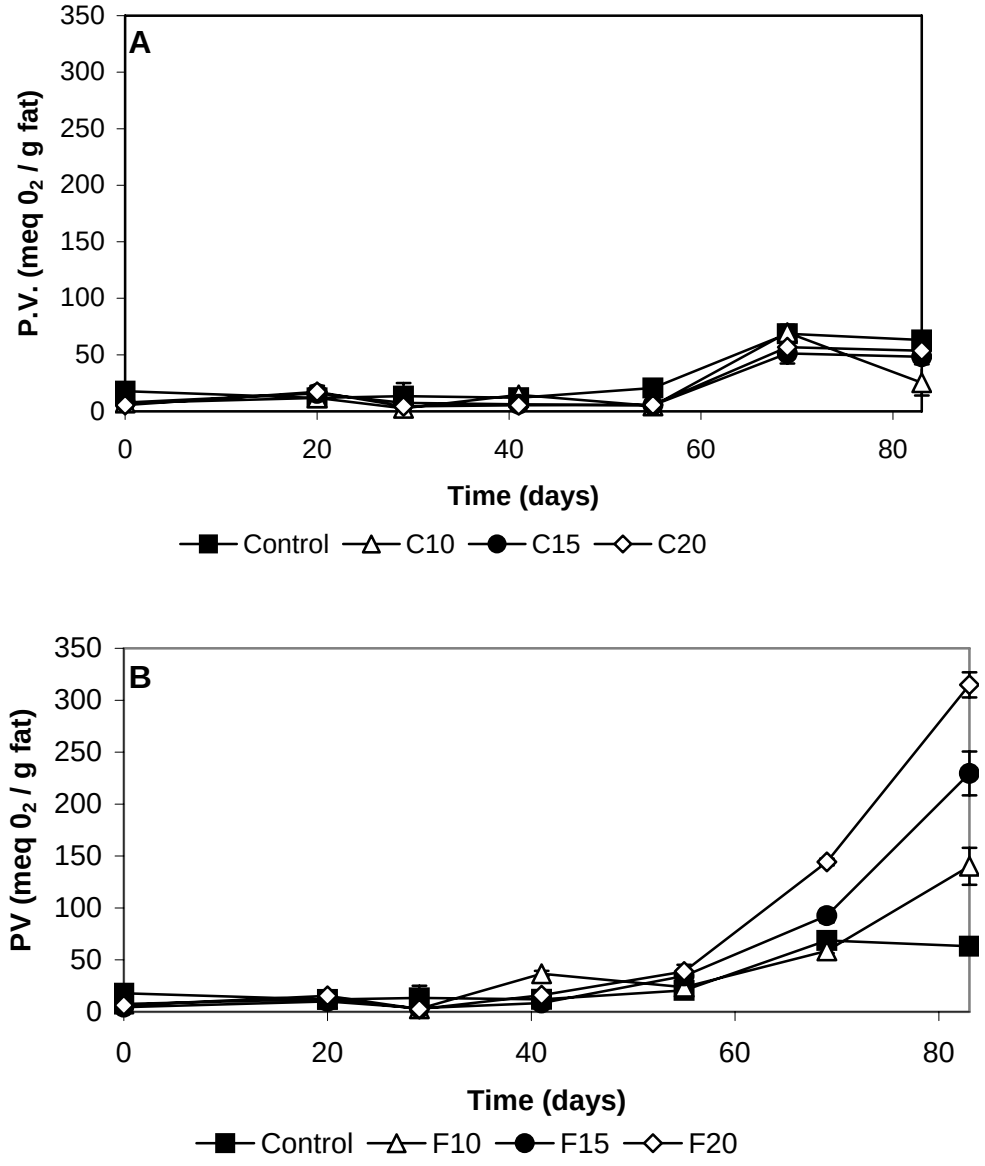

Fig. 1. Peroxide values of the different formulations during storage (*N*=4). A: Products with canola oil. B: Products with flaxseed oil. See further first subscript Table 1.

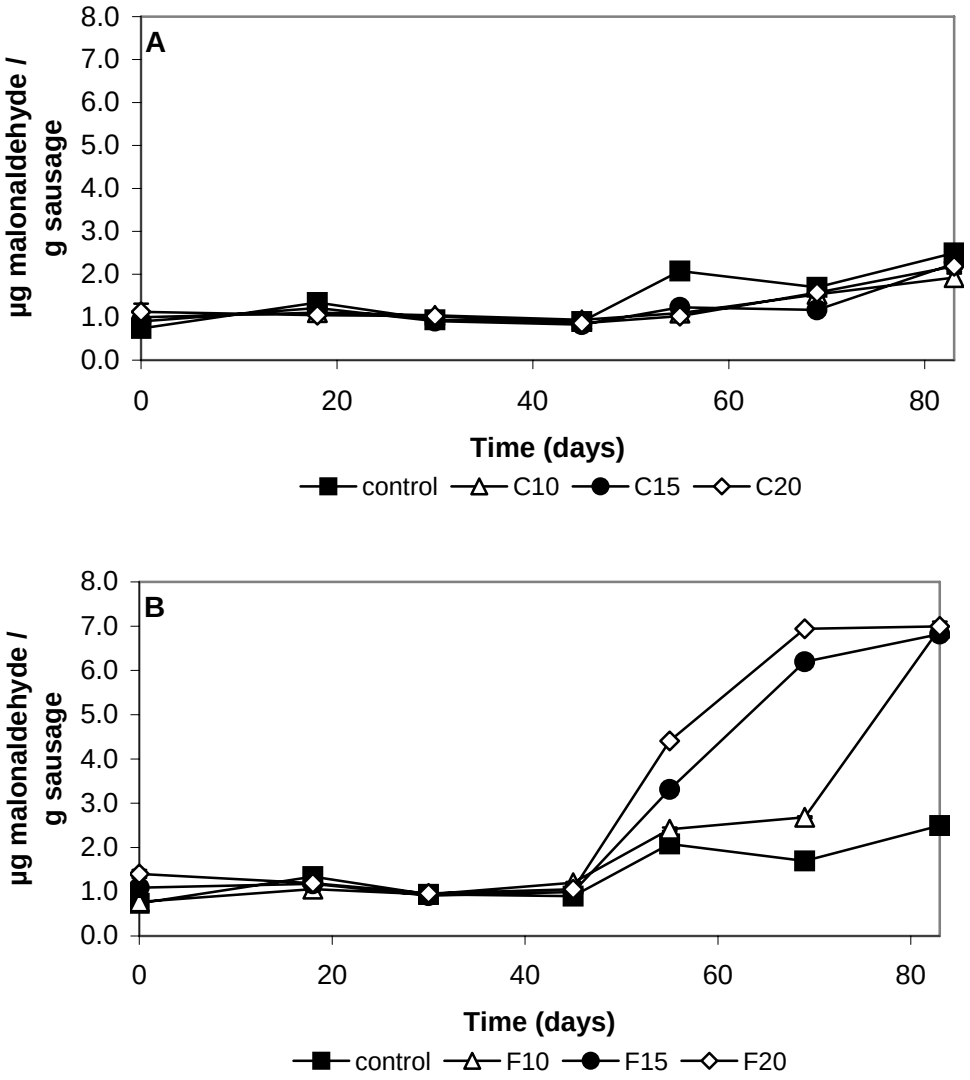

Fig. 2. TBARS values of the different formulations during storage (*N*=2). See further subscript Fig. 1.

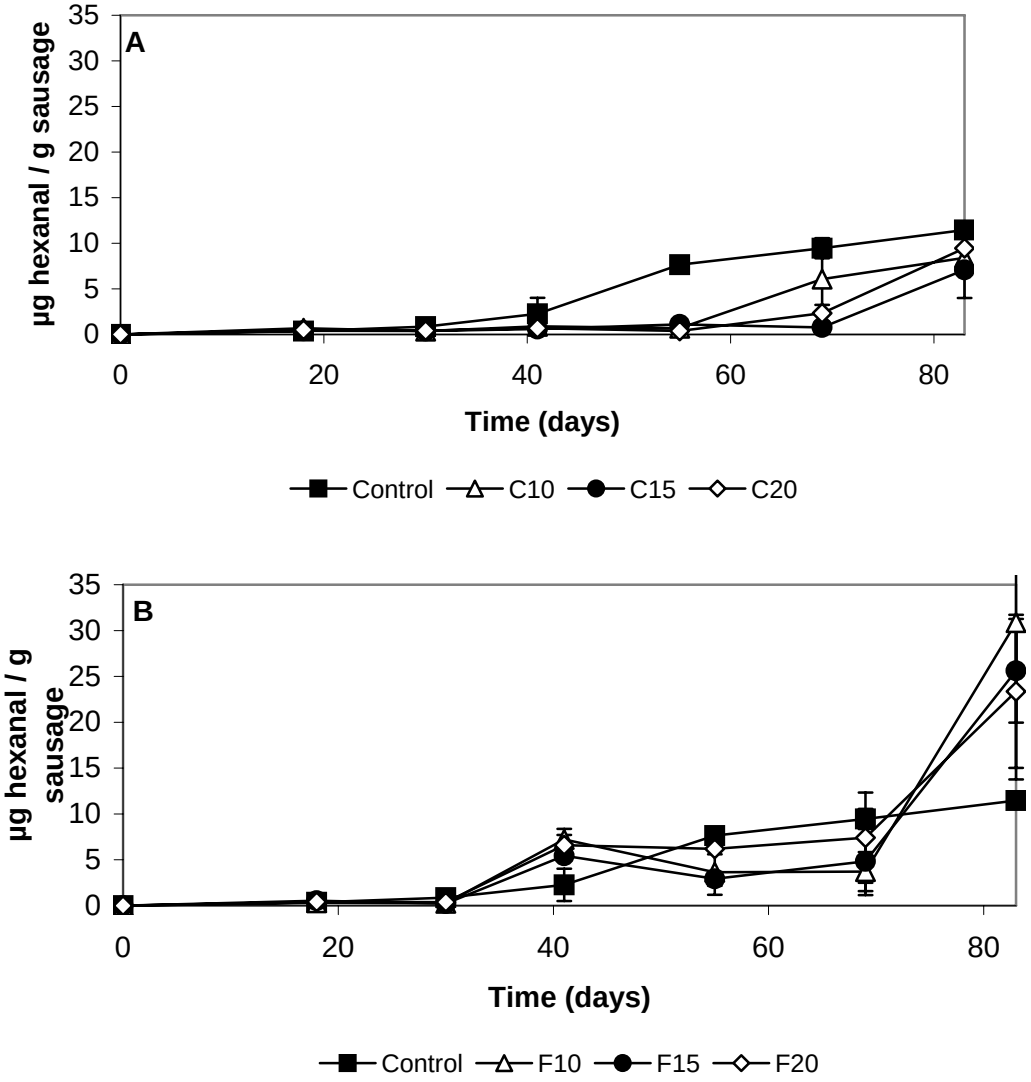

Fig. 3. Hexanal values of the different formulations during storage (*N*=2). See further subscript Fig. 1.