Meat Science 90 (2012) 81–86

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Quality characteristics of Dutch-style fermented sausages manufactured with partial replacement of pork back-fat with pure, pre-emulsified or encapsulated fish oil

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ARTICLE INFO

Article history:
Received 18 August 2010
Received in revised form 31 May 2011
Accepted 1 June 2011

Keywords:
$n-3$ Fatty acids
Lipid oxidation
Fermented sausages
Fish oil
Encapsulation
$n-6/n-3$ Ratio

ABSTRACT

Dutch-style fermented sausages were manufactured with 15% and 30% pork back-fat substitution by pure or commercial encapsulated fish oil, either added as such or as pre-emulsified mixture with soy protein isolate. Adding commercial encapsulated fish oil was the most important factor influencing the chemical composition. The fat content was not significantly different between products ($p>0.05$). The $n-6/n-3$ ratio decreased from 8.49 in controls to 0.90–2.47 in modified products. Lipid oxidation parameters (propanal and hexanal) showed much higher values for sausages with pure fish oil than for products with encapsulated oil. For the latter, lipid oxidation was similar to controls. Products with encapsulated or pre-emulsified oil were significantly firmer than products from other treatments in physical and sensory analysis ($p<0.05$). Overall, it is technologically feasible to enrich dry fermented sausages with $n-3$ fatty acids from fish oil and the application of commercial encapsulated fish oil seems to be the best in retaining overall quality.
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1. Introduction

Although meat is an important source of protein, vitamins and minerals, meat products can also contain a high amount of fat, saturated fatty acids, salt and cholesterol (Jiménez-Colmenero, Carballo, & Cofrades, 2001). Consumers are becoming more aware of health impairing effects of these components, since fat and cholesterol are associated with obesity and an increased risk for cardiovascular diseases. Meat industries aim at producing meat products that have a healthier image. Meanwhile, the products should have acceptable technological and sensorial properties. One of the goals is to improve the quality by changing the quality of lipids. This can be done by replacing part of the saturated fatty acids with (poly)unsaturated fatty acids (Jiménez-Colmenero et al., 2001). Meat industries often aim at decreasing the ratio of $n-6/n-3$ fatty acids in order to create a healthier meat product. $n-3$ Fatty acids are known to have positive effects on human health, e.g., protecting against cancer and coronary heart disease. In contrast, $n-6$ fatty acids, which are present in sources such as seeds, vegetable oils, pork and poultry, tend to cause thrombosis and inflammation when consumed in excess (Covington, 2004; Simopoulos, 1991). A way to increase the amount of $n-3$ fatty acids is by adding oils with a high level of these fatty acids (e.g., linseed oil, rapeseed oil, fish oils) to meat products. Fatty fish and fish oil are examples of direct sources of two important $n-3$ fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Dry fermented sausages have been produced in various ways using oils high in $n-3$ fatty acids. It was found that the products became more susceptible to lipid oxidation due to a higher quantity of polyunsaturated fatty acids (Pelser, Linssen, Legger, & Houben, 2007; Valencia, Ansorena, & Astiasarán, 2006b). During the propagation stage of lipid oxidation free radicals are formed. Free radicals are transformed via primary oxidation products to secondary oxidation products. For $n-3$ and $n-6$ fatty acids propanal and hexanal are the major secondary products (Akoh & Min, 2008).

Besides lipid oxidation, the inclusion of oil instead of pork back-fat in the product may cause an unstable emulsion (Miklos, Xu, & Lametsch, 2011). Pre-treatment of oil can be a way to obtain a more homogenous and stable product of good quality (Zayas, 1997). Pre-emulsification and encapsulation of oil can be ways to achieve this. Earlier research has shown that addition of $n-3$ fatty acids from commercial encapsulated fish oil to fermented sausage is feasible (Pelser et al., 2007).

The objective of this study is to investigate the effect of the partial replacement of pork back-fat with pre-emulsified or commercial encapsulated fish oil on the extent of lipid oxidation in Dutch-style fermented sausages, mainly in terms of secondary lipid oxidation products (hexanal and propanal). In addition, this work focuses on the proximate composition of the final products and on several quality attributes analysed by instrumental and sensorial techniques.

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doi:10.1016/j.meatsci.2011.06.001

2. Materials and methods

2.1. Sausage preparation

Dutch-style, semi-dry fermented sausages were prepared at Wageningen University (The Netherlands). Lean beef, pork back-fat, pure fish oil and a commercial encapsulated fish oil were used as raw materials. The lean beef and pork back-fat were provided by Kaldenberg Slagerijen (Herwijnen, The Netherlands). Commercial encapsulated fish oil (31.9% fat; 11.9% protein; 2.8% moisture; 2.6% ascorbic acid; 1.5% ash) (Vana-Sana NG Codliver supplied by FrieslandCampina Kievit, Meppel, The Netherlands) was used as well as the pure oil (Omega-360 Pure 22 supplied by Denomega Nutritional Oils, Gamle Fredrikstad, Norway). The pure and commercial encapsulated oil were either pre-emulsified or not with soy protein isolate (SPI) (Dutch Protein & Services, Tiel, The Netherlands). Water (approximately 60 °C) and SPI were mixed with an Ultraturrax T25 basic (IKA-Werke, Staufen, Germany) for 2 min at a ratio of 8:1. Next, ten parts of oil were added and emulsified for 3 min. After emulsifying, the mixture was cooled to room temperature (approximately 25 °C).

Ten formulations of fermented sausages were prepared, including two control products. The partial replacement of pork back-fat corresponded to weight substitutions of pork back-fat by 15% and 30% with fish oil. The experimental design and raw materials of the sausages are shown in Table 1. Meat–oil mixtures of approximately 1.2 kg were prepared, in such a way that the absolute amounts of beef, pork back-fat and pure oil were equal for each batch. The additional water added together with the SPI is assumed to evaporate during the drying/ripening process. The control was produced using 70% beef and 30% pork back-fat. Four products were produced with 15% back-fat substitution and four products with 30% back-fat substitution by fish oil. Four products were produced with a commercial encapsulated oil containing about 32% fish oil and four products with pure fish oil. The amounts of other ingredients in all formulations, expressed per kg of meat mixture, were: nitrite-curing salt (0.6% nitrite), 25 g; glucose, 7 g; glutamate, 2 g; white pepper, 1.2 g; paprika powder, 1 g; crushed black pepper, 1 g; ascorbic acid, 0.5 g; starter culture LS 25 (Gewürzmüller, Korntal-Münchingen, Germany), 0.25 g; mace powder, 0.25 g; clove powder, 0.16 g; and garlic powder, 0.15 g.

Frozen beef and pork back-fat were cut and the beef was chopped in a FA-20 cutter (Stephan Nederland, Almelo, The Netherlands) at low speed for 1–2 min and 30 s at high speed and mixed with other ingredients, except for nitrite-curing salt. The pork back-fat was added and the meat mixture was chopped for 1 min at high speed. The meat mixture, pure oil, pre-emulsified and/or encapsulated oil and nitrite-curing salt were added in an N-506 Hobart mixer (Hobart MFG. Co., Troy, USA). Mixing was done for 3 min and stopped 3 times in between to help mixing manually. The final dough was transferred to a plastic vacuum bag and air was removed to total vacuum with an Alvac-1-90 vacuum apparatus (Stephan Nederland). Next, the meat was stuffed into collagen-cellulose casings with a diameter of 85 mm, using a Dick TWF-12 stuffer (Friedr. Dick GmbH & Co. KG, Deizisau, Germany).

The sausages were fermented for 6 days at a temperature decreasing from 25 °C to 18 °C at approximately 1.2 °C/day. The relative humidity during fermentation decreased from 98% to 80% at approximately 3%/day. During 15 days of drying, temperature and relative air humidity were kept constant at 16 °C and 76–78% respectively, as used by Stegeman meat producer in Deventer, The Netherlands. Possible drip of fat from the sausage was collected in a bucket hanging under each sample. To accelerate the rate of lipid oxidation the sausages were cut in slices of 6 mm thick. Packaging was carried out at ProMessa meat producer in Deventer, The Netherlands, who also supplied the packaging material. Two slices were placed in a transparent plastic box made of amorphous polyethylene terephthalate and polyethylene (A-PET-PE) with a thickness of 0.8 mm and a volume of approximately 1.8 l. The lidding film used was LINtop LBA (LINPAC Plastics Pontivy, Noyal-Pontivy, France) with a thickness of 40.0 μm and oxygen transmission rate of less than 8 cm^3/m^2/day (at 50% RH and 23 °C). The boxes were stored in the dark at 7 °C with a modified atmosphere containing 65% oxygen (to stimulate lipid oxidation), 26% carbon dioxide and 9% nitrogen. Directly after fermentation and drying samples were tested for lipid oxidation as well as quality properties. Lipid oxidation parameters were determined every two weeks during storage.

Table 1
Experimental design and raw materials used.

Treatments[a]	Materials in g/batch						
	Beef	Pork back-fat	Fish oil	Encapsulated fish oil[b]	SPI[c]	Water	Total
C1	840	360	–	–	–	–	1200
C2	840	360	–	–	–	–	1200
F15	840	306	54	–	–	–	1200
F15P	840	306	54	–	5.4	43.2	1248.6
F30	840	252	108	–	–	–	1200
F30P	840	252	108	–	10.8	86.4	1297.2
E15	840	306	–	168	–	–	1314
E15P	840	306	–	168	5.4	43.2	1362.6
E30	840	252	–	336	–	–	1428
E30P	840	252	–	336	10.8	86.4	1525.2

[a] C1, control first charge; F15 and F30 series, prepared with 15% and 30% fish oil respectively; C2, control second charge; E15 and E30 series, prepared with 15% and 30% encapsulated fish oil respectively; P, indicating pre-emulsification.
[b] Commercial encapsulated fish oil consists of 31.9% oil.
[c] SPI, soy protein isolate.

2.2. Chemical analyses

After ripening, pH measurements were carried out with a digital pH meter Inolab pH Level1 (Wissenschaftlich Technische Werkstätten, Weilheim, Germany). 7.0 g of sample was homogenised with 7.0 ml of distilled water with help of an Ultraturrax T25 basic (IKA-Werke) for 1 min.

Moisture was determined in triplicate according to the American Oil Chemistry Society (AOCS, 1997). The protein content was determined in triplicate by the DUMAS method (FlashEA 1112 series Nitrogen Analyzer, Thermo Fisher Scientific, Interscience, Breda, The Netherlands) (Yeh, 1966). The total fat was quantified in triplicate as described by Folch, Lees, and Stanley (1957). The fatty acid composition of the lipid fractions as well as the fatty acid composition of the fish oil as such was determined in triplicate by preparing fatty acid methyl esters, as described by the International Organization for Standardization International Standards, 1978. Standards used for identification were FAME MIX RM6 (Supelco O7631-1AMP, Sigma-Aldrich, St. Louis, USA) and PUFA No. 3 from Menhaden Oil (Supelco 47085-U, Sigma-Aldrich). The gas chromatograph (Focus GC Interscience, Thermo Fisher Scientific, with Triplus Autosampler) was equipped with a flame ionisation detector (FID) and for analysis a capillary column of 30 m × 0.25 mm ZB-Wax 0.25 μm film thickness was used (Phenomenex, Torrence, PA, USA). The oven temperature was programmed to increase from 160 °C (hold for 1 min) to 250 °C (hold for 1 min) at 4 °C/min. The temperatures of the injector and detector were 250 °C and 270 °C respectively. Furthermore, samples were analysed with split injection (helium carrier gas flow 1 ml/min; split ratio 1:10). The injection volume was 1.0 μl.

The oxygen content in the headspace of the package was measured in triplicate with a PBI Dansensor (Checkmate II, PDI Dansensor A/S, Ringsted, Denmark) every two weeks at time of sampling. Hexanal and propanal contents were determined in triplicate by a GC static headspace procedure. The gas chromatograph (Focus GC Interscience, Thermo Fisher Scientific, with Triplus Autosampler) was equipped

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