Fat replacement and PUFA enrichment challenges in fermented sausage production

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Fat replacement and PUFA enrichment challenges in fermented sausage production

M Glisic[1], M Glisic[1], M Boskovic[1], M Z Baltic[1], D Trbovic[2], B Suvajdzic[1] and D Vasilev[1]

[1] Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Belgrade, Bulevar oslobodjenja 18, Belgrade, Serbia
[2] Institute of Meat Hygiene and Technology, Kacanskog 13, Belgrade, Serbia

E-mail: glisic.mica@gmail.com

Abstract. Pork backfat is traditionally used in the formulation of dry fermented sausages and contributes to the properties of the final product. In addition to its important technological function during ripening and drying processes, this fat significantly affects the appearance, texture, and formation of the characteristic flavour and aroma of dry fermented sausages, so its substitution in these products is a major challenge for the meat industry. In order to produce reduced-fat fermented sausages with improved fatty acid composition, 16% of pork backfat was replaced with inulin gelled emulsions of corn or rapeseed oil. The addition of emulsions led to a significant decrease in saturated fatty acids and increase in polyunsaturated fatty acids, n-6 and n-3 types ($P<0.05$). An improved n-6/n-3 ratio was observed only in inulin-rapeseed modified sausages (5.87). No signs of lipid oxidation measured by thiobarbituric acid reactive substance contents were detected in the modified sausages. However, the significantly higher total acid number and peroxide value in modified sausages ($P<0.05$) after ripening and 1-month storage indicate the greater susceptibility of these sausages to oxidation and lipolysis compared to control sausages.

1. Introduction

Europe is the major producer and consumer of dry fermented sausages which have a long tradition in Mediterranean countries, and a great variety of these products have been granted protected designation of origin (PDO) and/or protected geographical indication (PGI) labels [1]. Naturally fermented sausages are of great importance to the meat industry. These sausages are manufactured through traditional technologies from fresh pork meat and lard mixed with other ingredients (sugars, NaCl, black pepper, sweet and hot chilli pepper powder) that provide them with unique organoleptic and sensory profiles [2].

Even though fermented meat products have been consumed for centuries in different parts of the world and are considered to be one of the most valuable foods in human nutrition, the raw materials, meat and fat, used in the formulation of these products contain a higher proportion of saturated (SFA) than polyunsaturated fatty acids (PUFA) [3]. Numerous strategies have been developed in order to obtain new formulations of meat products with reduced SFA and cholesterol levels that provide different possibilities for the production of healthier fermented sausages [4]. When it comes to the enrichment of meat products with n-3 fatty acids, many studies were dedicated to a strategy that involved the increase in the proportion of these fatty acids (mostly α-linoleic acid) in animal feed and

the consequent production of meat and fats rich in PUFA [5,6]. Other studies were based on an increased PUFA fraction in meat products by replacing pork backfat with soybean, fish or linseed oil, in order to increase the PUFA/SFA and decrease n-6/n-3 ratios [3,7,8]. The first vegetable oil used for these purposes in Greek fermented sausages, Chorizo de Pamplona (traditional Spanish fermented sausages) and soujouk (popular Turkish dry fermented sausages), was olive oil rich in monounsaturated oleic acid (56-87% monounsaturated fatty acid (MUFA)) [9,10,11]. In addition to olive oil, many vegetable oils abundant in 16:0, 18:1n-9 and 18:2n-6 such as soybean, linseed, grapeseed and corn oils were used in fermented sausage reformulation [5,7,12,13,14]. However, there are no data on similar use of rapeseed oil in fermented meat products. Rapeseed oil has a good potential as a pork backfat substitute, since it has moderate levels of 18:2n-6 and 18:3n-3, is rich in 18:1n-9 and has a 18:2n-6/18:3n-3 ratio of 2:1, which is considered beneficial for human health [15].

In most of the previous studies, different water/emulsion systems were used for incorporation of oils into the meat matrix. However, in order to preserve the texture of the product, there was a constant need for stabilization and structural strengthening of these emulsions, so the use of gelling agents (konjac glucomannan, alginates, agar, j-carrageenan, inulin, gelatine) helped to overcome the problems of reducing the hardness and water holding capacity of various reduced and low-fat meat products [13,14,16,17,18]. In this sense, recently our group [19] developed a gelledemulsion containing 20% linseed oil, 20% inulin and 2% gelatine as a pork backfat replacer in dry fermented sausages.

The objective of this study was to investigate the effects of pork backfat substitution with inulin corn or rapeseed oil gelled emulsions on the fatty acid composition and lipid oxidation parameters of dry fermented sausages after the ripening process and 1-month storage period.

2. Materials and Methods

2.1. Preparation of dry fermented sausages

Conventional dry fermented sausages (C) were prepared with 35% lean beef, 40% pork meat and 25% pork backfat, while in reduced-fat fermented sausages, 16% of pork backfat was replaced with inulin corn oil gelled emulsion (IC) or inulin rapeseed oil gelled emulsion (IR). To all three formulations, 23 g salt, 0.32 g curing salt, 4 g spice mixture (Cajna nova, Raps GmbH, Austria) and 0.25 g preparation of the ripening culture (FLORA ITALIA LC SafePro®, Chr. Hansen, Denmark) were added.

Inulin corn and rapeseed oil gelled emulsions were prepared in two phases according to Glisic *et al*. [19] with 150 g/kg water, 200 g/kg commercial corn (Uvita d.o.o., Serbia) or rapeseed oil (Suncokret d.o.o., Serbia) and 30 g/kg soybean lecithin for oil pre-emulsion, and 350 g/kg water, 20 g/kg pork gelatine and 250 g/kg inulin powder for inulin gelled suspension.

Meat, pork backfat and frozen inulin gelled emulsions were comminuted and mixed with all other ingredients in the cutter, after which the mixtures were stuffed into 50 mm diameter collagen casings and sausages were submitted to fermentation, cold smoking and ripening as previously described by Glisic *et al*. [19]. After ripening, sausages were aerobically packaged and stored at 4°C.

2.2. Chemical analysis

The fatty acid composition was determined in the lipid extracts of the sausages by gas chromatography. Hexane/isopropanol mixture (ASE 200; Dionex, Dreieich, Germany) was used for the accelerated solvent extraction of total lipids [20]. After evaporation of solvent until dryness under a stream of nitrogen, trimethylsulfonium hydroxide was used for fatty acid methyl ester (FAMEs) preparation [21]. FAMEs were separated and quantified by a gas chromatograph Shimadzu 2010 (Kyoto, Japan) with a cyanopropyl HP-88 capillary column (100 m × 0.25 mm × 0.20 μm) and flame ionisation detector. The identification of FAMEs was done by comparison of the relative retention times of the peaks in the samples with those of standard pure compounds (Supelco 37 Component FAME Mix, Supelco, Bellefonte, PA, USA).

Acid values and peroxide values were respectively determined using standard methods ISO 660 [22] and ISO 3960 [23]. Thiobarbituric acid reactive substances (TBARs) were determined according to the combined method of Tarladgis *et al*. [24] and Holland [25], and are expressed as mg malonaldehyde/kg sample.

2.3. Statistical analysis

The entire trial was performed in triplicate. Six randomised sausages from each batch were analysed after the ripening period (day 28) and 1-month storage (day 58). Statistical analyses of the results were conducted using the software GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). The results were expressed as meanstandard deviation. The differences between sausage were appraised by one-factor analysis of variance (ANOVA) with Tukey's multiple comparison test, while Student's *t*-test was performed to compare the content of docosanoic (C22:0), docosapentaenoic (C22:5n-3) and tetracosanoic fatty acid (C24:0) in two formulations after ripening. Differences considered significant if $P<0.05$.

3. Results and Discussion

3.1. Fatty acid composition

Substitution of pork backfat with 16% inulin gelled emulsion of corn or rapeseed oil affected the fatty acid profile of modified sausages (Table 1). The content of myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), arachidic (C20:0), eicosenoic (C20:1) and eicosadienoic (C20:2) fatty acids was higher in IC and IR sausages ($P<0.05$). The most abundant fatty acid in IR sausages was oleic acid (C18:1cis-9). IR sausages also differed from C and IC sausages in the amount of α-linoleic acid (C18:3n-3) ($P<0.05$). Similar changes in the fatty acid profile, especially in regard to the α-linoleic acid content, were observed in sausage with inulin gelled linseed emulsion [19]. In IC sausages, the addition of emulsion significantly increased the content of linoleic acid (C18:2n-6) (20.44 g/100 g) as a consequence of its high proportion in corn oil [26]. These sausages also had the highest content of n-6 dihomo-γ-linoleic acid (C20:3n-6). Docosanoic (C22:0) and tetracosanoic acids (C24:0) were detected only in reformulated products with emulsions. The contents of SFA were lower, while the PUFA and n-6 were higher in IC and IR sausages, and all parameters differed significantly in relation to C. The n-3 content significantly increased only in IR sausages (2.56), with the lowest n-6/n-3 ratio in these sausages (5.87) and the highest in IC sausages (19.57). The results from the present study are supported by other authors who also successfully improved the fatty acid profile and the nutritive value of modified fermented sausages by adding different vegetable oils in liquid, encapsulated, emulsified or gelled forms [5,7,8,12,13,14,16,18]. There are only a few studies concerning rapeseed oil emulsions as fat substitutes in meat batter for cooked sausages [27,28,29], while there are no data on the use of such emulsions in dry fermented sausage production. These results show the improvement in fatty acid composition of our fermented sausages with rapeseed oil addition, while IC sausages, apart from the observed differences in PUFA, as a result of an increase in n-6 content did not have a favourable n-6/n-3 ratio, with an increase of about 1.72-fold compared to control sausages.

Table 1. The fatty acid composition of control and fermented sausages with two different inulin gelled emulsions ripened for 28 days expressed in grams of fatty acid per 100 g of product

Fatty acid	Control sausages	Sausages with corn oil emulsion	Sausages with rapeseed oil emulsion
C14:0	1.43±0.03 [a]	0.91±0.04 [b]	0.80±0.07 [c]
C15:0	0.07±0.005 [a]	0.09±0.009 [b]	0.08±0.005 [b]
C16:0	24.69±0.42 [a]	19.12±0.48 [b]	18.58±0.37 [b]

C16:1	2.39±0.07 [a]	2.14±0.08 [b]	2.00±0.06 [c]
C17:0	0.36±0.005 [a]	0.38±0.03 [a]	0.38±0.016 [a]
C18:0	10.01±0.83 [a]	7.60±0.16 [b]	7.68±0.24 [b]
C18:1cis-9	44.37±0.81 [a]	44.65±0.50 [a]	50.25±0.20 [b]
C18:1cis-11	2.88±0.06	nd	nd
C18:2n-6	11.03±0.25 [a]	20.44±0.81 [b]	14.87±0.32 [c]
C20:0	0.22±0.01 [a]	0.29±0.027 [b]	0.32±0.009 [c]
C18:3n-3	0.46±0.04 [a]	0.57±0.05 [a]	1.75±0.09 [b]
C18:3n-6	0.03±0.005 [a]	0.03±0.005 [a]	0.03±0.005 [a]
C20:1	0.84±0.04 [a]	1.30±0.10 [b]	1.33±0.02 [b]
C20:2	0.36±0.07 [a]	0.57±0.01 [b]	0.55±0.02 [b]
C22:0	nd	0.25±0.02 [a]	0.21±0.03 [b]
C22:1+C22:4	0.27±0.05 [a]	0.23±0.02 [a]	0.26±0.05 [a]
C20:3n-6	0.08±0.01 [a]	0.88±0.09 [b]	0.09±0.01 [a]
C20:3n-3	0.10±0.03 [a]	0.09±0.03 [a]	0.13±0.02 [b]
C20:5n-3	0.38±0.018 [a]	0.06±0.005 [b]	0.16±0.031 [c]
C22:5n-3	nd	0.10±0.03 [a]	0.20±0.40 [b]
C22:6n-3	0.04±0.01 [a]	0.29±0.03 [b]	0.31±0.06 [b]
C24:0	nd	0.06±0.005 [a]	0.04±0.009 [b]
SFA	36.78±1.23 [a]	28.68±0.45 [b]	28.09±0.38 [b]
MUFA	50.48±0.96 [a]	48.09±0.58 [b]	53.58±0.14 [c]
PUFA	12.74±0.26 [a]	23.25±0.99 [b]	18.35±0.43 [c]
n-6	11.13±0.26 [a]	21.35±0.85 [b]	14.98±0.32 [c]
n-3	0.98±0.06 [a]	1.10±0.13 [a]	2.56±0.13 [b]
n-6/n-3	11.36±0.48 [a]	19.57±1.70 [b]	5.87±0.26 [c]

Means±SD with different lower-case superscript letters in the same row indicates differences (P<0.05); nd - not detected

3.2. Lipolysis and lipid oxidation

The lipid oxidation parameters of control sausages and sausages with corn and rapeseed oil inulin gelled emulsions are shown in Table 2. It is noticeable that reformulation led to differences in the degree of lipolysis after ripening. Specifically, the total acid number was significantly higher in reformulated sausages compared to C sausages (P<0.05), where IC sausages had the highest value (1.28 mg KOH/g) that differed from that of IR sausages (P<0.05). During storage, this value increased in all sausages, and the differences between modified sausages and control sausages remained (P<0.05), while the modified sausages did not differ among themselves for this parameter. The results obtained show the formulations with inulin gelled emulsions of oils rich in PUFA were more susceptible to lipid oxidation due to the generation of larger amounts of free fatty acids [30]. In our previous study, we also found a higher total acid number in sausages with inulin gelled emulsion of linseed oil [19]. Unlike our current results, Muguerza *et al*. [7] did not find any difference in the acid number of fermented sausages with increasing amounts of soybean oil added. Apart from higher PUFA content in modified sausages, these variations could be explained by different factors that affect the process of fatty acid formation by tissue and microbial lipases in dry fermented sausages, such as salt content, temperature and decrease in the number of microorganisms, or microbial lipolytic enzyme activity due to the decrease of a_w values [31].

Table 2. Lipid oxidation parameters of three different fermented sausage formulations after ripening (d 28) and after 1-month storage (d 58)

Days	Sausages	The total acid number (mg KOH/g)	Peroxide value (mmol/kg)	TBARs (mg malonaldehyde/kg)

28	C	0.74±0.06 ^{a}	0.06±0.02 ^{a}	0.10±0.01 ^{a}
	IC	1.28±0.09 ^{b}	0.21±0.01 ^{b}	0.03±0.01 ^{b}
	IR	1.15±0.05 ^{c}	0.31±0.08 ^{c}	0.04±0.01 ^{b}
58	C	0.91±0.01 ^{A}	0.32±0.07 ^{A}	0.87±0.14 ^{A}
	IC	1.48±0.07 ^{B}	0.95±0.05 ^{B}	0.05±0.01 ^{B}
	IR	1.44±0.07 ^{B}	0.87±0.22 ^{B}	0.07±0.02 ^{B}

Means±SD with different lower-case superscript letters in the same column indicates differences (P<0.05) after ripening (d 28); Means±SD with different upper-case superscript letters in the same column indicates differences (P<0.05) after storage (d 58); C; control sausages; IC; sausages with inulin corn oil gelled emulsion; IR; sausages with inulin rapeseed oil gelled emulsion; TBARs; thiobarbituric acid reactive substances

Since lipolysis induces the oxidation and production of peroxides, similar changes were observed in the peroxide values of modified sausages. The peroxide number was significantly higher in IC and IR sausage formulations then control for both periods, after ripening (day 28) and storage (day 58) (P<0.05). Previously published data showed also a higher peroxide number in fermented sausages with linseed oil gelled emulsion (0.29 mmol/kg) [19]. In contrast, in the study Muguerza *et al*. [7], the peroxide number was below the level of detection in sausages with added soybean oil and vitamin E. However, Alejandre *et al*. [14], in fermented sausages with konjac gel linseed oil emulsion as a fat substitute, recorded a peroxide number in the range of 0.33 to 0.38 mmol/kg, which is higher than in control and modified sausages from the present study after the ripening. These lower values of the peroxide number as well as the additional lipid stability of vegetable fats in our sausages with emulsions could be explained by the presence of soy lecithin for which it has been shown to exhibit good antioxidant properties [32].

It is interesting that during ripening and storage, reformulation did not lead to an increase in TBARs. IC and IR formulations had even significantly lower TBARs values than control (P<0.05). These results are in accordance with other authors who determined TBARs below 1 mg malonaldehyde/kg in oil-modified fermented sausages after ripening and storage [5,8,14]. It can be considered that the stability of lipid oxidation and TBARs levels, especially during storage, in these fermented sausages is ensured by the addition of nitrite (nitrite content was significantly higher in IC and IR sausages compared to control – data not shown), using soy lecithin to prepare oil pre-emulsion and temperature of the refrigerator. Refrigeration temperatures reduce the rate of chemical reactions [33], nitrites react with C=C bonds of unsaturated fatty acids [34], and soy lecithin has an antioxidant effect in dry fermented sausages [32]. Lipid oxidation of unsaturated fatty acids is one of the main factors affecting the quality of meat and meat products, since it can lead to the development of off-odour and rancid taste due to the formation of compounds such as n-alkenals and dienals, drip losses, discolouration, nutrient loss, reduction of shelf-life and accumulation of mutagens and carcinogenic compounds [35]. It is confirmed that lipid oxidation products are associated with the aetiology of various neurodegenerative and cardiovascular diseases, as well as some types of cancers [13]. Considering this, it is important not only to improve the nutritional value of meat products but also to minimize the degree of lipid oxidation.

4. Conclusion

Taking into account established eating habits and consumers' preferences for recognizable meat products, the results of this study show it is possible to formulate such product with reduced SFA, increased PUFA content and a low n-6/n-3 ratio. Reformulated sausages could be a good alternative to classic foods that are sources of dietary long-chain PUFAs. Furthermore, the inulin gelatine network and soy lecithin have the potential to be a good oil-stabilizing matrix that could reduce lipid oxidation in PUFA-enriched dry fermented sausages. However, since this oil carrier was not that effective in sausages with linseed oil gelled emulsion, further research is needed to confirm these findings.

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