Reduced fat bologna sausages with improved lipid fraction

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ABSTRACT

BACKGROUND: This applied research was done in order to obtain cooked products (bologna sausages) with significantly lower amount of energy, total fat and saturated fat and higher amount of ω-3 fatty acids than conventional ones. Two subsequent experiments were performed.

RESULTS: Experiment 1 aimed a pork back-fat reduction and allowed to obtain sausages with 84 g kg^{-1} of fat and 1334 kcal kg^{-1}, without significant negative effects on sensory quality. Carrageenan was used as fat replacer. Experiment 2 aimed improving the lipid profile of the "energy-reduced" sausages previously developed, by a partial substitution of the pork back-fat with a linseed oil-in water emulsion (substitution levels: 25% to 100%). Using the 100% substitution level gave rise to products with 27 g kg^{-1} α-linolenic acid, and low saturated fat content (13.5 g kg^{-1}), showing good sensory results regarding taste, smell and texture. The use of antioxidant maintained low TBARs values in all formulations.

CONCLUSION**:** It is possible to obtain cooked meat products (bologna sausages) with low energy, low saturated fat and high amount of ω-3 fatty acids simultaneously, applying a combination of use of carrageenan, linseed oil emulsion and increment of water, without significant effects over the sensory quality. Functional products, interesting from a nutritional standpoint, were achieved.

Keywords: cooked sausages; meat-based functional foods; linseed oil; emulsion; reduced fat meat products; ω-3 PUFA.

Running title: “Functional” Bologna-type sausages with low fat

INTRODUCTION

Bologna sausage, a cooked meat product, is one of the most accepted processed meat products[1] and it is consumed and enjoyed worldwide.[2] Traditional formulations of this type of product include a rather high fat content and, in consequence, a high energy value (around 3000 kcal kg^{-1}).

The main strategies to decrease the energy value in this type of meat products consist on decreasing the amount of fat, increasing the amount of meat or water.[3] Fat is a critical part of the food matrix for achieving adequate flavour and texture properties.[2,4] Thus, any attempt leading to its reduction should take into account an evaluation of these properties. In general fat reduction has been joined with the addition of non-fat ingredients, like fiber[5-7] or vegetal proteins.[8-10] Also, carrageenan has been used for improving sensory properties of low fat meat products,[11] due to its water binding, thickening and gelling properties.[12] In cooked sliced meat products, carrageenan has been used to improve moisture retention, cooking yields, slicing properties, mouth-feel and juiciness.[13]

PUFAs and especially ω-3 PUFAs, have been demonstrated to have beneficial health effects.[14-16] ω-3 PUFA consumption improves vascular and cardiac hemodynamics, triglycerides, and possibly endothelial function, autonomic control, inflammation, thrombosis, and arrithmia.[17] More recently, nutritionists have focused on the type of PUFA and the dietary balance between ω-3 PUFA formed from α-linolenic acid (18:3) and ω-6 PUFA formed from linoleic acid (18:2).[18] A high ω-6/ ω-3 PUFA ratio has been considered a risk factor in cancer and coronary heart disease, as it has been associated to the formation of blood clots leading to a heart attack.[19] Consequently, different health authorities and scientific organizations now recommend an increase of the dietary intake of ω-3 PUFAs[20] and a growing trend to increase foods with these fatty

acids is noticed. WHO established ω-6/ ω-3 ratio between 1-4.[21] A viable technology to enrich meat products in PUFAs without radical changes of eating habits is to substitute, at least partially, the animal fat used in regular-fat formulations by emulsions elaborated with ω-3 PUFA oils.[22-26] Bologna sausages high in ω-3 PUFAs but with total fat amount similar to traditional formulations have been elaborated without technological problems in previous works.[27-29]

The objective of the present study was to obtain bologna sausages with low energy value and high in ω-3 PUFAs simultaneously. Technological and sensory aspects have been carefully analyzed in order to obtain commercially viable products.

MATERIALS AND METHODS

Materials

Pork shoulder-meat and back-fat were obtained from a local meat market. All the experiments were carried out with the same raw material. Linseed oil (Biolasi Productos Naturales, Guipúzcoa, Spain) was obtained in a local market. BDRom Carne (a mixture of typical aromatic compounds) and the red colorant Carmin de Cochenille 50% (E-120) were obtained from BDF Natural Ingredients S.L. (Girona, Spain). Satiagel (gelling refined carrageenan) was donated by Cargill S.L.U. (Martorell, Barcelona, Spain). Curavi (a mixture of curing agents: NaCl, E-250, E-252 and antioxidant E-331) and the rest of additives and spices used in the formulations were kindly donated by ANVISA (Arganda del Rey, Madrid, Spain). All the chemical reagents were obtained from Sigma-Aldrich Chemical Co. (MO, USA).

Sausage formulation and processing

The experimental design consisted on two steps: 1st; a set of products with a progressive fat reduction (which was reached increasing the amount of water added and adding carrageenan) and 2nd; in the product that contained the lowest amount of fat from the previous experiment, a progressive substitution of the pork back fat by an emulsion rich in omega-3 fatty acids was attempted. Therefore, nine different formulations of bologna sausages were manufactured in a pilot plant in the two different experiments. Table 1 shows all ingredients of the nine types of products. In the first experiment, five formulations of bologna sausages were made (types 1, 2, 3, 4 and 5), with decreasing proportions of pork back-fat: from 350 g kg^{-1} (type 1), to 100 g kg^{-1} (type 5) while increasing proportions of ice. Type 1 was considered as control (traditional formulation), and no carrageenan was added. Types 2 to 5 incorporated 10 g kg^{-1} carrageenan. Once the formulation corresponding to type 5 was considered as viable, the second experiment was carried out. Four formulations of products were manufactured (5A-5D), with similar total fat content than type 5 (100 g kg^{-1}). Types 5A, 5B and 5C substituted a 25%, 50% and 75% of pork back fat by a linseed oil emulsion, respectively. Type 5D did not contain pork back fat and the fat was supplied by the emulsion. The emulsion was prepared with linseed oil according to a previously described procedure.[30] The fatty acid profile of the linseed oil used, expressed as g kg^{-1} of oil, was a follows: lauric (0.19), myristic (0.53), palmitic (56.8), palmitoleic (0.90), stearic (46.1) elaidic (1.10), oleic (191.2), vaccenic (7.49), t-linoleic (0.19), c-t linoleic (0.78), linoleic (136.1), araquidic (0.61), γ-linoleic (1.52), α-linolenic (527.5), behenic (1.38), eicosapentaenoic (1.18).[31] All these products also incorporated 10 g kg^{1} carrageenan.

All ingredients (including the emulsion, when used) were thoroughly minced in a chilled cutter for 1 min at low speed and for 2 min at high speed until a complete

emulsification of the mixture was obtained. After the application of a vacuum process to exclude oxygen from the mixture for 2 min, the batters were stuffed in 6 cm diameter water impermeable plastic casings. Sausages were cooked in a water bath at 80 ºC for 1 h, until the core of the product reached to 72 ºC. Once heating was completed, the sausages were immediately cooled in a water bath for 2 h and stored under refrigeration (4 ºC) until analyzed.

The two experiments were carried out in triplicates, and the analyses were carried out in three batches of each type of sausages.

Physicochemical analyses

The water activity (a_w) was determined using a Novasina aw-Center, (Novasina AG., Lanchen; Switzerland) and pH was determined with a pH-meter micropH 2000 with Needle electrode (Crison Instrument S.A., Barcelona). Total fat was determined by Soxhlet extraction with petroleum ether.[32] A mixture of chloroform:methanol was used for the extraction of lipids.[33] AOAC official method was used for protein determination.[34]

Thiobarbituric acid value (TBARs)

TBARs values were determined on fat basis according to the method described by Maqsood and Benjakul [35] with slight modifications. Briefly, the TBARs reagent was prepared by mixing 15% w/v trichloroacetic acid, 0.0375% w/v 2-thiobarbituric acid in 0.25N hydrochloric acid. The fat (0.5 g), 0.5mL of distillate water, 20 µL of BHT (1%) and the TBARs reagent (2 mL) were vortexed in a centrifuge tube immediately after combining, for 30 sec, placed in a boiling water bath for exactly 15 min and then cooled

in an ice bath to room temperature. Cyclohexanone (4 mL) and ammonium sulphate (1 mL, 4M) were added to the mixture and were vortexed for 30 sec. The mixture was centrifuged at room temperature at 4000 rpm for 10 min. The supernatant was collected and the absorbance was measured at 532 nm. A calibration curve TEP (tetraethoxypropane) was done for quantification purposes, using the same procedure as with the sample. Results were expressed in mg of malondialdehyde (MDA) equivalents kg^{-1} product.

Determination of fatty acid composition

Fatty acids were determined in the lipid extract by gas chromatography. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters.[36] A Perkin-Elmer Autosystem XL gas chromatograph fitted with a capillary column SP™ - 2560 (100 m x 0.25 mm x 0.2 μm) and flame ionization detection was used. The temperature of the injection port was 250 ºC and of the detector was 260 ºC. The oven temperature was programmed at 175 ºC during 10 min and increased to 200 ºC at a rate of 10 ºC min^{-1}, then increased to 220 ºC at a rate of 4 ºC min^{-1}, which was kept for 15 min. The carrier gas was hydrogen, and the pressure was 20.5 psi. Split flow was 120 cms^{-1}. The identification of the fatty acid methyl esters was done by comparison of the retention times of the peaks in the sample with those of standard pure compounds (Sigma, St. Louis, MO, USA) and by spiking the sample with each standard individually. The quantification of individual fatty acids was based on the internal standard method, using heptadecanoic acid methyl ester (Sigma, St. Louis, MO, USA).

Texture

A Universal TA-XT2i texture analyzer was used to conduct the texture profile analysis.[37] Slices of sausage (6 cm diameter and 1 cm thickness) were compressed twice to 60% of their original height with a compression plate of 75 mm diameter. Force-time curves were recorded at a crosshead speed of 5 mms^{-1} and recording speed was also 5 mms^{-1}. Hardness (g), Springiness (mm), Cohesiveness, Gumminess (g) and Chewiness (gmm^{-1}) were evaluated. These parameters were obtained using the available computer software. Measurements were done in twelve different samples per type of product.

Colour

For colour measurement, samples were homogenized and introduced in a plate of 1 cm height. They were covered with a polyethylene film, with pressure to obtain a uniform, bubble-free surface. A digital colorimeter (Chromameter-2 CR-200, Minolta, Osaka, Japan) was used to obtain the reflectance spectra. Colour coordinates were obtained with the CIE L* a* b* system, using angle 10º and illuminant D65.[38] L*, a* and b* parameters indicate lightness, redness and yellowness, respectively. Chroma and Hue angles were calculated as follows:

$$Hue = arctg\left(\frac{b}{a}\right) \qquad Chroma = \sqrt{a^2 + b^2}$$

Measurements were done in four different samples per type of product.

Cooking yield (%)

The cooking yield was calculated as follows: (weight of sausage before cook – weight of sausage after cook) / weight of sausage before cook * 100. Mean values per type of product are discussed.

Sensory analysis

Two different types of sensory analysis were carried out to compare the sensory properties of the control and modified sausages. In both tests, samples were presented sliced (about 2 mm thick), on a white plate, at room temperature, in normalized cabinets, identified by a randomized three numbers code.

A triangular test was performed to determine the existence of perceptible sensory differences in colour, hardness, taste and appearance between the control (type 1) and the modified formulation (type 5). 10 trained panellists participated in the sessions. Each panellist was presented with three samples, of which two were identical, and asked to indicate which sample differed from the others. The number of correct answers was determined and data shown in the table corresponded to the mean value obtained for each type of product by the 10 panellists. According to the Spanish norm,[39] for a panel with 10 panellists the difference between samples was significant if the number of correct answers were 7 ($p<0.05$), 8 ($p<0.01$) and 9 ($p<0.001$).

Quantitative descriptive analysis (QDA) was used for describing the properties of products elaborated in the second experiment. 20 selected and trained panellists examined samples for colour intensity, smell, juiciness, hardness and taste. Type 5 product was considered as a control product in this experiment and a score of 5 points was arbitrary assigned for all parameters examined. The modified formulations (types 5A-5D) were scored by panellists from 1 (lowest intensity) to 10 (highest intensity) for every parameter. In this sense, modified formulations were compared to the control, noting in each case, higher or lower intensity for each parameter. Also, general acceptability was evaluated for each sample. This parameter was assessed scoring modified samples within a 0-10 scale.

Statistical analysis

Mean and standard deviation of data are shown in all tables. A one way Anova test and the Tukey *b* posteriori test were used to determine significant differences among the different types of bologna sausages. SPSS version 15.0 was used (SPSS inc. Chicago, Illinois, USA). Significance level of $p \leq 0.05$ was used for all evaluations.

RESULTS AND DISCUSSION

Experiment 1: Fat reduction

The first part of the work was designed to get a formulation with the minimum pork back fat content and maximum water content, maintaining adequate technological and sensory properties.

The fat and energy content of the different types of products (1 to 5) is shown in Table 2. As there were no variations in the percentage of pork meat, only a decrease of fat content was observed (from 265.4 to 84.11 g kg^{-1}, in types 1 and 5, respectively) maintaining a mean protein content of 139 g kg^{-1}. Energy value consequently decreased as fat was reduced, from 2965 to 1339 kcal kg^{-1}. The current European regulation on nutrition claims[40] establish that, when the energy values are reduced by at least 30% compared with the traditional formulation (without the potential restriction of nutrient profiles), the products could be claimed as “light” or “energy-reduced”. That would be the case for the developed formulation types 3, 4 and 5, if no other nutrient profile restrictions are taken into account. In fact, the reductions achieved in these three formulations (3, 4 and 5) were of 31, 43 and 54% of energy, respectively, compared with the control product.

No differences were detected in the measures of pH and a_w in any of the fat-reduced products. a_w did not show significant differences among the different formulations with increasing water content as a consequence of the high water amounts of the formulations (588 g kg^{-1} type 1, 743 g kg^{-1} type 5), and pH ranged between 6.2-6.47. These were within a normal range for this type of products as reported by other researches[2,41,42] (Table 2). Also, minimal weight losses were observed after cooking (0.0 – 1.7%), confirming the water holding capacity of the new formulations and supporting their technological viability.

In order to perform a first approach to evaluate the consequences of the fat reduction over the sensory properties of the products, instrumental texture analysis and colour measurement were carried out (Table 2). Previous studies reported that, in meat products, textural properties are closely related to fat.[43-44] Hardness, cohesiveness, gumminess and chewiness values decreased significantly when the fat content decreased and water increased. However, springiness values were similar in all types. These results confirmed those found in fat-reduced bologna sausages[27] and in cooked meat batters[45], where a fat reduction was associated with a softer texture. In our case, the increasing amount of water substituting the fat was possible because of the addition of carrageenan.

The effect of fat reduction on instrumental colour parameters seems not to be consistent in the literature. The different processing conditions, type of products, use of fat replacers or additives might be behind the diversity of the effects found. The product with a major reduction of pork back-fat (Type 5) showed the highest value of Lightness (L*) and the lowest for Hue among all formulations in this experiment. That, as it will explain below, did not affect the sensory perception given by panellists. Instead, in

other studies performed with low-fat sausages, contrary results were found.[8,46] Other experiments in different fat content bologna sausages showed that increasing the fat content decreased the colour intensity and the shine of sausages, evaluated by a test panel.[28] These differences may be due to differences in product formulations and composition.

A triangular sensory analysis test was performed with the aim of analyzing whether the noticed differences in texture and colour using instrumental evaluation methods were significant from the sensory standpoint (Table 3). The comparison between the two extreme formulations revealed that panellists were not able to detect significant differences (p>0.05) between control batches (type 1) and batches with the greatest reduction of fat (type 5), for any of the descriptives (colour, hardness, taste and appearance). Hardness and taste allowed a correct identification of samples only in a 60% of cases, whereas for colour, this percentage was 20%. All these data suggested that the type 5 formulation was viable from the sensory quality point of view and formulation 5 was chosen for the second experiment, in which, besides the decrease in the fat content, an improvement of the lipid profile was attempted.

Experiment 2: Lipid profile improvement

On basis the fat reduced formulation (type 5) obtained in experiment 1 (550 g kg^{-1} pork meat, 100 g kg^{-1} pork back fat and 350 g kg^{-1} ice), different substitutions of pork back-fat by linseed oil-in-water emulsion were made, as it is shown in Table 1: from 25% substitution to 100% substitution. Again, no problems were found in the technological parameters with pH and a_w ranging among normal values for this type of products[2,41,42] (Table 4). Also, minimal weight losses were observed (0.5 – 1.0%). All

these formulations contained 10 g kg^{-1} of carrageenan which was obviously determinant for increasing not only the water holding capacity, but also for making possible to incorporate high amounts of emulsion without noticing texture defects.

As it was expected, very interesting nutritional advantages were achieved in the developed products. Values of 12.99, 22.44 and 28.80 g kg^{-1} of α-linolenic and 1.04, 1.68 and 2.30 g per 100 kcal, respectively, were detected in types 5B, 5C and 5D (Table 5). These values were all higher than the limit established by the Commission Regulation for the claim "high ω-3", which is 0.6 g α-linolenic per 100 g and 100 kcal. [40] Furthermore, EFSA proposed 2 g/day as the reference labelling intake value for α-linolenic acid.[47] Thus, one portion (50 g) of formulation 5D developed in this study contributed with a 72% of this recommendation.

A progressive and significant decrease in SFA and trans fatty acids was detected with the increasing percentage of the added emulsion. In the last formulation (type 5D) the SFA content was a 45% of the initial formulation (type 5), containing only 13.21 g kg^{-1} SFA. The total amount of SFA and trans was 13.52 g kg^{-1}, that it is below the upper limit established by UE Regulation for the claim "low saturated fat", which is established in 15g kg^{-1}. [40]

Other parameters related to the fatty acid profile also improved with the proposed modifications. PUFA/SFA ratio increased almost 6 fold from type 5 to type 5D (0.51 to 2.88) and PUFA+MUFA/SFA increased from 1.82 to 4.43. Also, the ω-6/ω-3 ratio decreased significantly from types 5 to 5D (13.47 to 0.39), being much lower than the recommended values (4-5),[48] and contributing to decrease this ratio in the diet. These results can be considered as successful from a nutritional point of view.

In this experiment TBARs measurement were carried out in order to control the potential higher susceptibility of the new formulated products to lipid oxidation as a

result of the increment in the PUFAs. The obtained results (below 0.25 mg kg-1) allowed us to conclude that no oxidation problems were noticed (Figure 1). The total substitution of pork back-fat with linseed oil-in-water emulsion showed statistically higher TBARs values compared to the lower substitution levels. However, despite this slight increase, these values can still be considered very low, taking into account that even levels of 4-6 mg MDA kg^{-1} in sliced bologna sausages vacuum-packed did not reveal rancid taste appreciation.[49] In consequence, despite the high unsaturation degree of this formulation, the oxidative stability was maintained by the use of antioxidant.

When colour instrumental data were analyzed, results showed for L* a similar evolution to that observed in experiment 1. Effectively, lightness increased significantly with the percentage of emulsion in the product. In the case of redness (a*) and yellowness (b*) a dose dependant trend was also observed, decreasing and increasing, respectively, with the incorporation of the emulsion. All these changes were, in this case, reflected on the colour intensity appreciated by the sensory panel (Table 6). Thus, scores given by panellists for colour intensity in products 5A and 5D were 3.6 and 2.7, respectively, which clearly indicated a colour defect that should be corrected with the use of an adequate mixture of colorants. As colorants are used in traditional formulation of this type of product this fact does not seem to be a significant problem. Other works found similar effect over colour with substitutions of animal fat with vegetable oils in different meat products.[22,45,50] This fact can be probably related to the much smaller oil globules diameter, which reflect more light than the larger animal fat globules.[48] Other authors did not detect changes in colour parameters in formulations with vegetable oils.[24,51,52]

In relation to textural properties, the effects of the new formulations were lower than in experiment 1. An increase of instrumental hardness was detected with the emulsion percentages increasing (Table 4) however, scores 5.56-4.75 in the sensory analysis (Table 6), without noticing significant differences with the control product (type 5). Effectively, the increases in emulsion percentages corresponded with increases in hardness, reaching in type 5D products values for hardness similar to those obtained for type 2 (products with 25% of pork back-fat). Thus, the incorporation of the emulsion counteracted the hardness decrease observed when the fat decreased in the first experiment. Replacing pork back fat with linseed oil-in-water emulsion also modified other textural properties of products in a dose-dependent trend (Table 4). This might occur, as in the analysis of colour, as a consequence of creating much smaller fat globules in the oil containing products, contributing to increase the consistency.[45] In other studies made in different cooked meat products, similar results had been found.[27,48,50] Generally, frankfurters made with any of the different oil-in-water emulsions presented higher hardness, cohesiveness and chewiness than control products.

The proportion between fat and emulsion seems to be a critical factor for hardness. The higher surface area when using emulsion, covered by proteins, allowed more bonding to the matrix and resulted in firmer products (higher resistance to compression).[45]

Other sensory descriptives dealing with taste, smell or flavour did not show significant differences among the five types of products (Table 6). Moreover, general acceptability of the emulsion containing products was always higher than 7, pointing to the sensory viability of the new formulations. Furthermore, panellists were asked to describe any non-characteristic smell or taste in the products, and no special notes were

reported. In this sense, different studies where linseed has been introduced to swine diets at different levels reported controversial results. Concentrations of α-linolenic acid up to 39 g kg^{-1} fat, did not lead to adverse effects from the sensory stand point,[53-55] whereas others showed some flavour modifications.[56]

In conclusion, bologna sausages with low energy, low saturated fat and high amount of ω-3 fatty acids, can be developed using linseed oil instead of animal fat, adding high amount of water and using carrageenan as stabilizer. The obtained products comply more accurately to the current dietary guidelines, without significant effects over the sensory quality.

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