Scientia Agricola
http://dx.doi.org/10.1590/0103-9016-2014-0387

Influence of animal fat substitution by vegetal fat on *Mortadella*-type products formulated with different hydrocolloids

Erick Saldaña[1], Ana Lúcia da Silva Corrêa Lemos[2], Miriam Mabel Selani[1], Fernanda Papa Spada[1], Marcio Aurélio de Almeida[1], Carmen Josefina Contreras-Castillo[1]*

[1]University of São Paulo/ESALQ – Dept. of Agro-industry, Food and Nutrition, C.P. 09 13418-900 – Piracicaba, SP – Brazil.
[2]Institute of Food Technology, Av. Brasil, 2880 – 13070-178 – Campinas, SP – Brazil.
*Corresponding author <ccastill@usp.br>

Edited by: Luís Guilherme de Lima Ferreira Guido

Received November 21, 2014
Accepted May 19, 2015

ABSTRACT: Meat has played a crucial role in human evolution and is an important component of a healthy and well-balanced diet on account of its nutritional properties, its high biological value as a source of protein, and the vitamins and minerals it supplies. We studied the effects of animal fat reduction and substitution by hydrogenated vegetal fat, sodium alginate and guar gum. Fatty acid composition, lipid oxidation, color and instrumental texture as well as the sensorial difference between low, substituted-fat and the traditional formulations for mortadella-type products were analyzed. Both substitution and reduction of animal fat decreased the saturated fatty acids percentage from 40% down to 31%. A texture profile analysis showed differences between the formulations. Furthermore, lipid oxidation values were not significant for treatments as regards the type and quantity of fat used while the use of sodium alginate and guar gum reduced the amounts of liquid released after cooking. Animal fat substitution does cause, however, a difference in overall sensorial perception compared with non-substituted products. The results confirm the viability of substituting vegetal fat for animal fat.
Keywords: lipid profile, lipid oxidation, fat-replacers, fat vegetal, texture

Introduction

Meat plays a crucial role in human evolution and is an important component of a healthy and well-balanced diet on account of its nutritional properties, its high biological value as a source of protein, and the vitamins and minerals it supplies (Santos et al., 2013). However, recent studies have established a direct relationship between the consumption of meat products and the increase in risk of serious health disorders, such as colorectal cancer and coronary-heart diseases (Ferguson, 2010; Rodríguez-Carpena et al., 2012).

In Brazil, mortadella-type products number amongst the most widely produced meat products, and their consumption has become popular due mainly to their low cost, pleasant flavor, and the assortment of products available, which can be produced using a variety of fat types, since Brazilian legislation allows for broad classification (Guerra et al., 2011). Nowadays, consumers demand natural and healthy food products, including meat products, with better nutritional properties (Doménech-Asensi et al., 2013). Thus, the development of meat products with reduced fat levels, which are similar to traditional products with good consumer acceptability, is essential to the improvement of human health.

The use of animal fat is vital to the production of meat batters because it presents attractive sensory characteristics to the consumer. The substitution of animal fat by vegetal fat could be a good strategy for improving the nutritional quality of meat products, as it reduces the level of saturated fatty acids (SFA) and while increasing the level of polyunsaturated, both of which are essential to the prevention of heart diseases (Beiloune et al., 2014; Escrich et al., 2007). However, the incorporation of these vegetal fats may be associated with a reduction in quality due mainly to a significant oxidative instability. The oxidation of unsaturated lipid fractions along with oxygen presence during meat grinding and the adding salt in processing could have a negative impact on the quality of these products (Álvarez et al., 2011).

Hydrocolloids influence many of the functional properties of processed meat products (Chattong et al., 2007). They are commonly used in comminuted products as emulsifiers and "texture modifiers" (Fonkwe et al., 2003). The addition of hydrocolloid materials may structurally interfere with the cross-linking required for protein gel network formation (Pérez-Mateos et al., 2001).

In this context, this study aimed to study the influence of animal fat substitution by vegetal fat on mortadella-type products formulated with alginate, guar gum in terms of fatty acid composition, sensory characteristics and oxidative stability of lipids. The effectiveness of the quantification of thiobarbituric acid-reactive substances (TBA-RS) using conventional (spectrophotometric) and HPLC methods was also evaluated.

Materials and Methods

Materials

The commercial vegetal fat, alginate, guar gum, condiments and other additives were donated by local companies (São Paulo, Brazil). The beef meat was acquired from a local slaughterhouse (São Paulo, Brazil) and the animal fat (pork back fat, pork fat trim) and pork meat were donated by a local slaughterhouse

(Minas Gerais, Brazil). The expandable cellulose casings were donated by a local company (São Paulo, Brazil). For reasons of confidentiality, no further detail concerning brands of materials used can be provided.

Description and preparation of mortadella-type products

Six types of mortadella-type products were prepared with a reduction in and replacement of animal fat (50 % of pork back fat and 50 % of pork fat trim) by hydrogenated vegetal fat (sunflower, cotton and palm oils), with low levels of trans fatty acids (2g 100 g^{-1}). The fatty acid composition (g 100 g^{-1}) of the vegetal fat was as follows: 0.03 of C12:0, 0.3 of C14:0, 0.03 of C14:1, 0.03 of C15:0, 12.3 of C16:0, 0.12 of C16:1, 13.6 of C18:0, 60.8 of C18:1 (cis and trans), 11.4 of C18:2, 0.26 of C18:3, 0.6 of C22:0, 27 of SFA, 60 of monounsaturated fatty acid (MUFA) and 12 of polyunsaturated fatty acids (PUFA).

The formulations of mortadella-type products are reported in Table 1, and the manufacturing process of the product was carried out as follows: first, beef and pork meat and animal fat were stored at 4 °C and ground with a 20-mm disc. Subsequently, the meats were placed into the cutter. The following were added: salt, curing salt, phosphates, spices, garlic paste, maltodextrin and 50 % of the total ice required to reduce the mixture temperature to 0 °C and prevent protein denaturation. Afterwards, animal or vegetal fat, milk whey and hydrocolloids were added according to the different formulations. The remaining 50 % of the ice was then added and finally the antioxidants. The final temperature of the mixture obtained was 13 ± 0.8 °C.

Table 1 – Formulations (%) of Mortadella-type products with substitution of vegetal fat and reduction of animal fat.

Component	AC	VC	RAF	RVF	RAFH	RVFH
Beef Forequarter	36	36	36	36	36	36
Pork Shoulder	30	30	30	30	30	30
Animal fat	16	0	8	0	8	0
Vegetable Fat	0	12	0	6	0	6
Water/Ice	13	15.2	19.5	20.5	19.5	20.5
Milk Whey	0	1.8	1.5	2.5	1.2	2.2
Sodium Alginate (Ibrac®)	0	0	0	0	0.25	0.25
Guar Gum (5000/Ibrac®)	0	0	0	0	0.05	0.05
Salt	0.8	0.8	0.8	0.8	0.8	0.8
Seasonings	1	1	1	1	1	1
Curing salt*	0.35	0.35	0.35	0.35	0.35	0.35
tripolyphosphate (Ibrac®)	0.35	0.35	0.35	0.35	0.35	0.35
paste garlic without salt	0.2	0.2	0.2	0.2	0.2	0.2
maltodextrin (Mor-rex 1940)	2	2	2	2	2	2
Antioxidant	0.35	0.35	0.35	0.35	0.35	0.35

AC= Animal Control: 100 % Animal Fat; VC= Vegetable Control: 100 % Vegetable fat; RAF= Reduced Animal Fat: 50 % Animal Fat; RVF= Reduced Vegetable Fat: 50 % Vegetable Fat; RAFH= Reduced Animal Fat with Hydrocolloids; RVFH= Reduced Vegetable Fat with Hydrocolloids. Hydrocolloids= alginate (0.25 %) and guar gum (0.05 %).*Mixture consisting of 90 % salt, 6 % sodium nitrite and 4 % sodium nitrate.

The dough was stuffed into expandable casings of cellulose with a 72-mm radius. In the formulations, fat was reduced by 62.5 % and up to 100 % of the animal fat was replaced.

The thermal treatment consisted of two steps. In the first step, the oven temperature was maintained at 50 °C for one hour with indirect steam and the chimney left open. In the second step, the temperature was kept at 60 °C for another hour using indirect steam and a closed chimney and was halted when the geometric center of the product reached 73 °C. After the cooking process, the products were cooled using water spray for 30 min before weighing and vacuum packing.

Analytical methods

Proximate composition – Moisture, fat content (Soxhlet) and total protein content (Kjeldahl) were determined following official methods (AOAC, 2005). All the analyses were carried out in triplicate.

Fatty acid profile – The fat was extracted following the method of Folch et al. (1957). Fatty acid methyl esters (FAMEs) were prepared from 50 mg of fat using the method described by Hartman and Lago (1973) with slight modifications. FAMEs were analyzed by gas chromatography equipped with a fused silica capillary column (30 m × 0.53 mm x 1.0 µm) and a flame ionization detector. A heating ramp was used in accordance with the following temperature program: 1) 180 °C for 3 min; 2) an increase to 220 °C at 5 °C min^{-1}; and 3) a final hold at 220 °C for 20 min. The injector and detector temperatures were 180 °C and 250 °C, respectively. Nitrogen was used as the carrier gas at a rate of 4 mL min^{-1} in a split injection mode in a ratio of 1:10. Samples (1 µL) were injected by an automatic injector. Individual FAMEs peaks were identified by comparing their retention times with those of the standards Mix C8-C22 (FAME Mix C8-C22, Supelco, USA). The results were expressed in grams per 100 g of FAMEs detected. All the analyses were carried out in triplicate.

Determination of TBA-RS numbers – TBA-RS were determined in triplicate using the method described by Vyncke (1970); Vyncke (1975) and Sørensen and Jørgensen (1996) with some modifications. For extraction, 5 g of the sample was homogenized in an Ultra Turrax at 10, 000 rpm for 30 s with 15 mL of a solution of 7.5 % of trichloroacetic acid (TCA), 0.1 % of propylgallate (PG) and 0.1 % of ethylene diamine tetraacetic acid (EDTA). After filtration with qualitative filter paper (12.5 mm), 5 mL of the filtrate was mixed with 5 mL of an aqueous solution (0.02 M of thiobarbituric acid (TBA)) in capped test tubes. The samples were incubated in a water bath at 100 °C for 40 min and then cooled in cold water. Absorbance was measured at 532 nm and 600 nm by a spectrophotometer against a blank containing 5 mL of the same TCA, PG and EDTA solution and 5 mL of

TBA solution. The difference (A 532 nm - A 600 nm) was used as absorbance values corrected for turbidity. The results were calculated from the standard curve of tetraethoxypropane (TEP) and expressed in terms of mg of malonaldehyde (MDA) per kg of sample. The R^2 of the calibration curve was 0.983.

Determination of malonaldehyde using HPLC – The method used for the extraction of malonaldehyde was the same as was used to determine TBA-RS. Once the malonaldehyde extracts were obtained, they were filtered through a 0.45 µm polytetrafluoroethylene (PTFE) membrane into autosampler vials and injected (20 µL) into an HPLC equipped with a fluorescence detector. The MDA-TBA complex was analyzed using a Zorbax plus column (C18, 4.6 mm × 250 mm, 5 µm). The elution process was isocratic using 85 % of sodium phosphate buffer (pH 7.0, 5 mM) and 15 % of acetonitrile at a rate of 1 mL min^{-1}. The detector wavelengths were set at 515 nm (excitation) and 543 nm (emission). The oven temperature was set to 40 C. A standard curve was made from TEP and the results expressed in terms of mg of malonaldehyde (MDA) per kg of sample. The R^2 of the calibration curve was 0.989.

Texture measurements – The texture profile analysis (TPA) was carried out at room temperature with a Texture Analyser as described by Bourne (2002). Six cylinders (2-cm diameter and 2-cm high) were taken from the samples and subjected to a two-cycle compression test. The samples were compressed to 30 % of their original height with a P-35 probe (long shaft, regular base) at a speed of 2 mm s^{-1}.

The following parameters were determined: hardness, maximum force during the first cycle of compression; springiness, the height at which the food recovers during the time that elapses between the end of the first cycle and the beginning of the second cycle; cohesiveness, extent to which the sample could be deformed prior to rupture (A2/A1, where A1 is the total energy required for the first compression, and A2 the total energy required for the second compression) and chewiness – i.e. the work required to masticate the sample for swallowing (Horita et al., 2011).

Color measurements – Instrumental color was determined by a Minolta colorimeter using the following parameters: L* (lightness), a* (redness) and b* (yellowness) (CIELAB). The parameters were calibrated in a standard white porcelain where Y = 93.7, x = 0.3160 and y = 0.3323 and with a measurement area of 8 mm in diameter, an observation angle of 10° and an illuminant D65. Three 2-cm thick slices from the middle region of each formulation were analyzed in triplicate.

pH – The pH was measured in triplicate using a pH-meter with a puncture electrode inserted into the final product at a temperature of 25 °C.

Cooking loss and emulsion stability – Product cooking loss was determined in triplicate and calculated as follows: Cooking loss = $[(W_b - W_a)/W_b] \cdot 100$, where W_b and W_a are the weights of the sample before and after cooking, respectively. The emulsion stability test was performed as described by Lin and Mei (2000) with some modifications. An aliquot of 50 g of emulsion mass was weighed in nylon/polyethylene bags resistant to high temperatures, vacuum sealed and placed in a water bath at 70 °C for one hour. Then the samples were cooled to 4 °C. The total amount of fluid released was expressed as a percentage of the sample weight. The test was performed five times for each formulation and was called "Total Fluid Release" in Table 3.

Sensory evaluation – For the sensory analysis, a Difference-from-Control test, was used as described by Meilgaard et al. (2006). The samples VC, RAF, RVF, RAFH and RVFH were evaluated in comparison with the AC sample, in duplicate, by a team of 20 panelists (55 % female and 45 % male). The panelists were recruited and selected from Campinas, SP, (Brazil), based on their consumption of the focal products and their sensory acuity. They assessed the six samples in a single session; 50 g of each sample were put in small plastic dishes identified with a random three-digit code and served following a balanced complete block experimental design (Meilgaard et al., 2006; Wakeling and MacFie, 1995). Panelists gave informed consent and were compensated for their participation.

A 7-point scale (1 = no difference to 7 = very large difference) was used to measure overall difference, seasoning difference, saltiness difference and firmness difference. The AC was the blind control (Lawless and Heymann, 2010). The evaluations were conducted in individual booths equipped with lighting supplied by fluorescent lamps and the Compusense® software, version 5.4 (Addinsoft, New York, USA).

Statistical analysis – The repeated measures test was used for statistical comparisons between samples. The data were evaluated through analyses of variance (ANOVA) and the averages were compared by Tukey's test using XLSTAT (Addinsoft, New York, EEUU). Differences were considered significant at a confidence level of 95 % ($p < 0.05$). For the sensory evaluation, differences were considered significant at a confidence level of 95 % ($p < 0.05$) using Dunnett's test. The relationships between sensory parameters were calculated using Pearson's correlation coefficients. Principal Component Analysis (PCA) was also carried out.

Results and Discussion

Proximate composition

Proximate analysis revealed a number of differences ($p < 0.05$) between the six formulations (Table 2). The differences in moisture content showed the ex-

istence of three groups. The first group consisted of AC and VC with 100 % of animal and vegetal fat in their formulations, respectively, containing around 61 % of moisture whereas the second group consisted of RAF and RAFH with 50 % of animal fat in their formulations, containing around 65 % of moisture. The third group consisted of RVF and RVFH with 37 % of vegetal fat in their formulations, which contained more than 67 % of moisture. Total lipid content varied between 10 % and 17 % showing differences ($p < 0.05$) between formulations. Although there was a difference ($p < 0.05$) in protein content of the formulations, though the variation between the values was only 1 % (ranging from 16 % to 17 %). These similar values occurred mainly because the six treatments were formulated with the same meat content. The ash content showed differences ($p < 0.05$) between formulations. The results of the present study are within the usual scope for this type of meat product (Horita et al., 2011).

pH, emulsion stability, instrumental color and texture

With the reduction and replacement of animal fat by vegetal fat in the formulations, the binding properties of water and fat were modified, changing the emulsion stability, color, pH and the textural properties of the product. According to Table 3, the fat reduction without the addition of hydrocolloids caused an increase in fluid release when the product was subjected to thermal treatment. This behavior usually occurs when fat is replaced by water. In such a case, since protein levels are constant, a decrease in protein concentration involved in the emulsion formation may have occurred, thereby reducing the binding properties of water and fat in products with a lower fat content. However, when sodium alginate and guar gum were added to the formulations, the fluid release was reduced, and this behavior is in agreement with other studies (Flores et al., 2007). The cooking loss showed differences ($p < 0.05$) between formulations. This result is certainly related to fat reduction and the presence of hydrocolloids in the product, once hydrocolloids became capable of holding water and fat, thereby reducing fluid release and consequently the cooking loss.

The TPA of the six formulations (Table 3) revealed differences ($p < 0.05$) for hardness, springiness, cohesiveness and chewiness. The RVFH formulation with 0.25 % of sodium alginate and 0.05 % of guar gum in its formulation had 16.45 N of hardness, a lower value compared with the VC and RVF formulations, but similar to the AC formulation. Similar behavior is reported by Marchetti et al., (2013) and is attributed to the increase in water holding capacity caused by the addition of sodium alginate and guar gum. Water provides less resistance to compression, leading to softer products (Youssef and Barbut, 2011). Thus, the presence of fiber could have caused a disruption in the protein-water and protein-protein network, leading to a decrease in the gel strength of the product (Lin et al., 1998). Different results were found by Mendoza et al. (2001), who reported that the addition of fiber (inulin) did not affect the hardness of sausages and by the study of Fernández-Ginés et al. (2003), who indicated that sausages with citrus fiber showed higher hardness values compared with the control. Both hardening and softening have been observed when fiber is added to various meat products, which can be due to the amount and type of fiber used (Fernández-Ginés et al., 2003).

Table 2 – Proximate composition of the formulations.

	Moisture	Lipid	Protein	Ash
	g 100 g^{-1}			
AC	61.03 ± 0.44^{c}	15.61 ± 0.15^{b}	17.22 ± 0.22^{ab}	3.24 ± 0.12^{b}
VC	61.12 ± 0.16^{c}	16.54 ± 0.14^{a}	16.55 ± 0.31^{bc}	3.29 ± 0.12^{ab}
RAF	65.20 ± 0.17^{b}	10.39 ± 0.22^{c}	17.47 ± 0.14^{a}	3.30 ± 0.14^{ab}
RVF	67.98 ± 0.11^{a}	9.90 ± 0.04^{c}	17.02 ± 0.16^{abc}	3.19 ± 0.08^{b}
RAFH	65.41 ± 0.09^{b}	9.97 ± 0.10^{c}	16.40 ± 0.19^{c}	3.59 ± 0.08^{a}
RVFH	67.37 ± 0.95^{a}	10.06 ± 0.33^{c}	16.86 ± 0.39^{abc}	3.34 ± 0.10^{ab}

Results are expressed as averages ± standard deviation. Averages followed by the same letter in the same column are not different ($p < 0.05$). AC= Animal Control: 100 % Animal Fat; VC= Vegetable Control: 100 % Vegetable fat; RAF= Reduced Animal Fat: 50 % Animal Fat; RVF= Reduced Vegetable Fat: 50 % Vegetable Fat; RAFH= Reduced Animal Fat with Hydrocolloids; RVFH= Reduced Vegetable Fat with Hydrocolloids. Hydrocolloids= alginate (0.25 %) and guar gum (0.05 %).

Table 3 – pH, emulsion stability, instrumental color and texture of products manufactured using vegetal fat as replacers of animal fat.

	AC	VC	RAF	RVF	RAFH	RVFH
Hardness^{A}	16.94 ± 0.34^{bc}	18.21 ± 0.21^{b}	18.57 ± 0.28^{ab}	18.23 ± 1.17^{b}	20.24 ± 0.8^{a}	16.45 ± 0.04^{c}
Springiness^{B}	0.89 ± 0.02^{b}	0.90 ± 0.02^{b}	0.96 ± 0.00^{a}	0.95 ± 0.02^{a}	0.93 ± 0.01^{ab}	0.94 ± 0.02^{a}
Cohesiveness^{B}	0.83 ± 0.02^{a}	0.79 ± 0.01^{b}	0.80 ± 0.01^{b}	0.78 ± 0.00^{b}	0.78 ± 0.01^{b}	0.77 ± 0.01^{b}
Chewiness^{B}	12.52 ± 0.37^{cd}	13.04 ± 0.41^{bcd}	14.26 ± 0.17^{ab}	13.58 ± 1.06^{abc}	14.65 ± 0.51^{a}	12.01 ± 0.26^{d}
L*	66.89 ± 0.39^{a}	67.34 ± 0.62^{a}	65.84 ± 0.32^{ab}	65.24 ± 0.97^{b}	65.92 ± 0.47^{ab}	65.25 ± 0.28^{b}
a*	12.63 ± 0.18^{bc}	12.07 ± 0.35^{c}	13.38 ± 0.09^{a}	12.84 ± 0.32^{ab}	13.03 ± 0.23^{ab}	12.92 ± 0.11^{ab}
b*	14.90 ± 0.22^{ab}	14.98 ± 0.06^{a}	14.69 ± 0.08^{ab}	14.48 ± 0.32^{b}	15.17 ± 0.14^{a}	15.1 ± 0.11^{a}
pH	6.76 ± 0.01^{a}	6.68 ± 0.02^{c}	6.72 ± 0.02^{ab}	6.70 ± 0.02^{bc}	6.66 ± 0.02^{bc}	6.70 ± 0.01^{c}
Total Fluid Released (%)	15.69 ± 0.43^{bc}	14.78±0.36^{bc}	16.15 ± 0.95^{b}	18.4±0.55^{a}	14.4 ± 0.38^{c}	14.97 ± 1.2^{bc}
Cooking loss (%)	0.74 ± 0.06^{a}	0.67 ± 0.05^{a}	0.49 ± 0.18^{b}	0.49±0.11^{b}	0.44 ± 0.15^{b}	0.46 ± 0.12^{ab}

Results are expressed as averages ± standard deviation. Values with a different letter (a–d) within a row are different ($p < 0.05$); ^{A}(N); ^{B}(Dimensionless). AC= Animal Control: 100 % Animal Fat; VC= Vegetable Control: 100 % Vegetal fat; RAF= Reduced Animal Fat: 50 % Animal Fat; RVF= Reduced Vegetable Fat: 50 % Vegetable Fat; RAFH= Reduced Animal Fat with Hydrocolloids; RVFH= Reduced Vegetable Fat with Hydrocolloids. Hydrocolloids= alginate (0.25 %) and guar gum (0.05 %).

Springiness varied from 0.89 to 0.96 and the results showed high values for the RAF, RVF, RAFH and RVFH formulations, while formulations with 100 % of fat (AC and VC) had low values, meaning that samples with high fat content have a low capacity for recovering their initial dimensions after the first deformation (Horita et al., 2011). The higher springiness values of treatments with hydrocolloids may be due to the fact that they may have altered the gel structure and increased binding within the product. Furthermore, according to Pietrasik and Duda (2000), fat reduction in meat products may result in products with higher springiness.

Cohesiveness indicates the degree of difficulty to break the internal structure of the product (Horita et al., 2011). In the experiment, cohesiveness varied from 0.77 to 0.83, and the AC formulation had the highest value among the treatments. Similar results were reported by Fernández-Ginéz et al. (2003), who verified lower values of cohesiveness in formulations with fiber addition and by Berasategi et al. (2014), who indicated that cohesiveness values decreased significantly when fat content decreased and water increased. Chewiness showed differences ($p < 0.05$) between treatments, ranging from 12.01 to 14.65. The RVFH formulation had the lowest value, which may be attributed to the addition of sodium alginate and guar gum that increased the water retention capacity of the treatments, decreased hardness and, consequently, led to a lower value for chewiness.

The preparation of mortadella-type products with the reduction and substitution of vegetal fat for animal fat changed ($p < 0.05$) the objective color measurements (Table 3). In reduced fat treatments, with or without hydrocolloids, a slight decrease in luminosity (L*) and a slight increase in redness (a*) compared to the controls (AC and VC) were observed. The yellowness (b*) increased in products with guar gum and sodium alginate. Color variation between formulations may be the result of the amount of animal fat (light color) and oil distribution in the actomyosin matrix during processing (cuttering and mixing) which increased the surface of the fat particles and altered the color after cooking (Álvarez et al., 2011). For the pH, formulations with vegetal fat addition had values close to 6.70 while the treatments with animal fat had values from 6.66 and 6.76. Although pH values presented significant effects of the treatments, variations of 0.1 can be considered marginal and not significant on a practical level.

Fatty acids profile

Ninety percent of all fatty acids are represented by palmitic acid, stearic acid, oleic acid and linoleic acid (Table 4). As expected, the reformulation caused important changes in the fatty acids profile. The incorporation of vegetal fat significantly decreased total SFA content (Table 4). In the treatments with vegetal fat (VC, RVF and RVF), palmitic acid (C16:0) decreased 9 perceptual points (PP) and stearic acid (C18:0) increased 1 PP compared with animal fat formulations. Clearly, the addition of vegetal fat affected SFA ($p < 0.05$), reflecting its particular composition. Consequently, the reduction in SFA

Table 4 – Fatty acid profile (g 100 g^{-1}) of products manufactured using vegetal fat as replacers of animal fat.

	AC	VC	RAF	RVF	RAFH	RVFH
C10:0	0.06 ± 0.001^{b}	0.02 ± 0.001^{e}	0.06 ± 0.001^{b}	0.04 ± 0.010^{c}	0.06 ± 0.002^{a}	0.03 ± 0.001^{d}
C12:0	0.06 ± 001^{bc}	0.04 ± 0.001^{d}	0.06 ± 001^{b}	0.06 ± 0.001b^{c}	0.07 ± 0.001^{a}	0.06 ± 0.001^{c}
C14:0	1.25 ± 0.003^{b}	0.59 ± 0.007^{e}	1.25 ± 0.001^{b}	0.82 ± 0.010^{c}	1.41 ± 0.021^{a}	0.78 ± 0.015^{d}
C14:1	0.04 ± 0.001^{bc}	0.03 ± 0.001^{c}	0.04 ± 0.001^{bc}	0.07 ± 0.003^{a}	0.08 ± 0.013^{a}	0.06 ± 0.011^{b}
C15:0	0.10 ± 0.001^{ab}	0.08 ± 0.002^{b}	0.10 ± 0.001^{ab}	0.12 ± 0.002^{a}	0.14 ± 0.002^{a}	0.10 ± 0.000^{a}
C16:0	24.71 ± 0.013^{a}	15.56 ± 0.017^{d}	24.71 ± 0.005^{a}	17.10 ± 0.074^{b}	24.60 ± 0.046^{a}	16.70 ± 0.042^{c}
C16:1 (n-7)	2.30 ± 0.004^{b}	0.75 ± 0.010^{e}	2.30 ± 0.002^{b}	1.12 ± 0.013^{c}	2.46 ± 0.007^{a}	1.06 ± 0.018^{d}
C17:0	0.46 ± 0.005^{b}	0.21 ± 0.002^{e}	0.46 ± 0.001^{b}	0.30 ± 0.004^{c}	0.50 ± 0.001^{a}	0.27 ± 0.001^{d}
C18:0	12.58 ± 0.001^{b}	13.81 ± 0.007^{a}	12.59 ± 0.013^{b}	13.8 2± 0.013^{a}	12.52 ± 0.029^{b}	13.66 ± 0.062^{a}
C18:1 (n-9)	47.49 ± 0.011^{d}	57.85 ± 0.179^{a}	47.49 ± 0.013^{d}	55.92 ± 0.068^{c}	47.48 ± 0.005^{d}	56.68 ± 0.051^{b}
C18:2 (n-6)	9.18 ± 0.005^{c}	10.37 ± 0.056^{a}	9.18 ± 0.005^{c}	9.65 ± 0.032^{b}	8.67 ± 0.008^{d}	9.78 ± 0.017^{b}
C18:3 (n-3)	0.32 ± 0.003^{a}	0.19 ± 0.012^{c}	0.31 ± 0.004^{a}	0.22 ± 0.004^{b}	0.30 ± 0.008^{a}	0.22 ± 0.003^{b}
C22:0	0.67 ± 0.005^{a}	0.24 ± 0.015^{e}	0.66 ± 0.008^{ab}	0.29 ± 0.006^{c}	0.64 ± 0.001^{b}	0.27 ± 0.019^{d}
SFA	39.89 ± 0.028^{a}	30.56 ± 0.051^{d}	39.89 ± 0.024^{a}	32.55 ± 0.110^{b}	39.94 ± 0.102^{a}	31.87 ± 0.141^{c}
MUFA	49.84 ± 0.016^{d}	58.64 ± 0.190^{a}	49.83 ± 0.015^{d}	57.12 ± 0.084^{c}	50.03 ± 0.025^{d}	57.79 ± 0.081^{b}
PUFA	9.50 ± 0.009^{c}	10.56 ± 0.068^{a}	9.49 ± 0.010^{c}	9.88 ± 0.036^{b}	8.98 ± 0.015^{d}	10.00 ± 0.021^{b}
n-3	0.32 ± 0.003^{a}	0.19 ±0.012^{c}	0.31 ± 0.004^{a}	0.22 ± 0.004^{b}	0.30 ± 0.008^{a}	0.22 ± 0.003^{d}
n-6	9.18 ± 0.005^{c}	10.37 ± 0.056^{a}	9.18 ± 0.005^{c}	9.65 ± 0.032^{b}	8.67 ± 0.008^{d}	9.78 ± 0.017^{b}
n6/n3	29.02 ± 0.296^{c}	53.53 ±3 .075^{a}	29.33 ± 0.425^{c}	43.45 ± 0.729^{b}	28.42 ± 0.748^{c}	43.59 ± 0.588^{b}
PUFA/SFA	0.238 ± 0.000^{d}	0.346 ± 0.002^{a}	0.238 ± 0.000^{d}	0.303 ± 0.002^{c}	0.225 ± 0.000^{e}	0.311 ± 0.004^{b}

Results are expressed as averages ± standard deviation. Values with a different letter (a–e) within a row are different ($p < 0.05$). AC= Animal Control: 100 % Animal Fat; VC= Vegetable Control: 100 % Vegetable fat; RAF= Reduced Animal Fat: 50 % Animal Fat; RVF= Reduced Vegetable Fat: 50 % Vegetable Fat; RAFH= Reduced Animal Fat with Hydrocolloids; RVFH= Reduced Vegetal Fat with Hydrocolloids. Hydrocolloids= alginate (0.25 %) and guar gum (0.05 %). SFA= saturated fatty acids, MUFA= monounsaturated fatty acid, PUFA= polyunsaturated fatty acids.

in products with vegetal fat increased the percentage of MUFA and PUFA in 10 and 1 PP, respectively. For MUFAs, oleic acid increased 10 PP while for PUFAs, linoleic acid increased 1 PP compared to the treatments with animal fat. The fatty acid profile is very important from the nutritional viewpoint, since it allows for quantifying the SFA, MUFA and PUFA. The SFA increases the low density lipoproteins (LDL), and according to Rodríguez-Carpena et al. (2012), the intake of SFA could lead to an increase in cholesterol levels in the blood. The unsaturated fatty acids are composed of MUFA and PUFA, and according to Mattson and Grundy (1985), MUFAs show better effects than PUFAs in terms of LDL reduction.

The nutritional quality of the lipid fraction of food can be evaluated through the PUFA/SFA ratio. Increases in this ratio could lead to a reduction in total cholesterol in the blood plasma (McAfee et al., 2010). The products formulated with vegetal fat had increases ($p < 0.05$) in the PUFA/SFA ratio compared to products prepared with animal fat; none of the formulations reached the level of 0.4 (minimum recommended value) (Wood et al., 2004). This behavior may be attributed to the characteristically low PUFA/SFA ratio in meat (0.1) (Webb and O'Neill, 2008), which implied an imbalance. Furthermore, hydrogenated vegetal fat does not have a high content of PUFAs. Thus, if the objective is to reduce and replace the animal fat with vegetal fat, it is necessary to not only reduce the SFA, but also to increase the PUFA, especially the n-3 type (Ospina-E. et al., 2011).

PUFAs are very important in meat products because n-6 and n-3 fatty acids cannot be synthesized by humans; n-6 fatty acid can be elongated to araquidonic acid (C20:4, n-6) which acts as a precursor of eicosanoids (Webb and O'Neill, 2008). N-3 fatty acids play a key role in diet in terms of inflammatory reduction, HDL increase and LDL reduction (Johnston, 2009). Another index based on the PUFA amount which evaluates the quality of the lipid fraction in food is the n-6/n-3 ratio (Rodríguez-Carpena et al., 2012) which may cause cardiovascular diseases at high levels (Simopoulos, 2002). In this research, the relationship n-6/n-3 significantly increased with the addition of vegetal fat to the formulation. Therefore, it is not an adequate replacement of animal fat not only because of the PUFA/SFA ratio, but also because of the n-6/n-3 ratio.

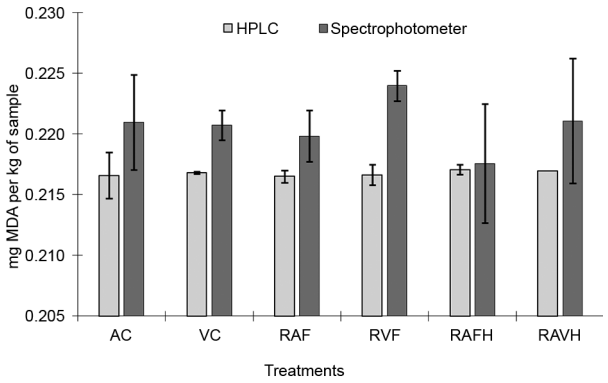

Figure 1 – TBA-RS values of the six types of products (mg malonaldehyde per kg of sample). AC= Animal Control: 100 % Animal Fat; VC= Vegetable Control: 100 % Vegetable fat; RAF= Reduced Animal Fat: 50 % Animal Fat; RVF= Reduced Vegetable Fat: 50 % Vegetal Fat; RAFH= Reduced Animal Fat with Hydrocolloids; RVFH= Reduced Vegetal Fat with Hydrocolloids. Hydrocolloids= alginate (0.25 %) and guar gum (0.05 %).

Oxidative stabilization and malonaldehyde measurement

The measurement of TBA-RS was taken and all formulations showed oxidation values below 0.5 mg malonaldehyde per kg of sample (Figure 1), which is the maximum level allowed for processed meat products because it produces a rancid odor and taste that can be detected by the consumer (Rodríguez-Carpena et al., 2012).

Both the reduction and replacement of animal fat by hydrogenated vegetal fat had no effect on TBA-RS ($p > 0.05$), thus lipid oxidation was not influenced. This behavior may be explained by the composition of vegetal fat which has a predominance of MUFAs, and low quantities of PUFAs, that are more sensitive to lipid oxidation. However, the AC formulation presenting more fat than RAF, RVF, RAFH and RVFH, did not show higher TBA-RS values ($p > 0.05$), since animal fat presents SFA as major fatty acids.

In this study, the quantification of MDA was determined in two ways: the spectrophotometric and HPLC methods. The spectrophotometric method is the more commonly used in quantifying MDA, but it is not specific for this compound. Thus, to obtain a specific quantification of MDA, this investigation evaluated the content of malonaldehyde using HPLC.

Although no difference ($p > 0.05$) between methods was observed (Figure 1), the content of malonaldehyde spectrophotometrically determined was slightly higher and presented a higher standard deviation than the results obtained by HPLC. These results are in agreement with those reported by Papastergiadis et al. (2012).

The overlapping of the chromatograms from the MDA-TBA products of the six formulations is presented in the Figure 2, where the retention time for the formation of the maximum peak was about 6.5 min at a flow rate of 1 mL min^{-1}.

Sensory evaluation

A difference ($p < 0.05$) between the AC and VC was found for the overall difference attribute, while for the other attributes there was no difference ($p > 0.05$) between formulations (Table 5). This result shows that the six formulations evaluated were very similar and that, in general, the reduction of fat and addition of hydrocolloids did not affect the sensory characteristics studied here. The PCA was carried out using a correlation matrix on sensory attribute data to explain which attribute within the four evaluated had a closer relationship with the over-

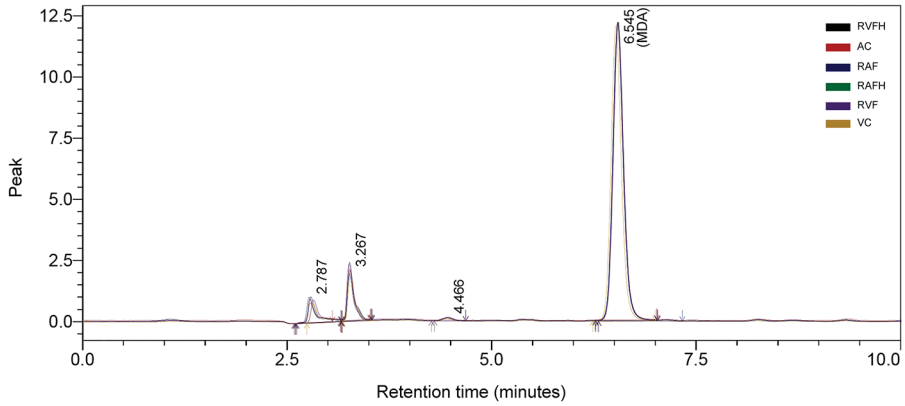

Figure 2 – HPLC chromatograms of MDA-TBA-RS complex (retention time 6.545 min) of the six products. AC= Animal Control: 100 % Animal Fat; VC= Vegetable Control: 100 % Vegetable fat; RAF= Reduced Animal Fat: 50 % Animal Fat; RVF= Reduced Vegetable Fat: 50 % Vegetable Fat; RAFH= Reduced Animal Fat with Hydrocolloids; RVFH= Reduced Vegetal Fat with Hydrocolloids. Hydrocolloids= alginate (0.25 %) and guar gum (0.05 %).

Table 5 – Mean values of the sensory evaluation of Mortadella-type products.

	Overall difference	Seasoning flavor	Saltiness	Firmness
VC	3.0 ± 1.2[b]	3.4 ± 1.0[a]	3.5 ± 1.1[a]	3.9 ± 0.8[a]
RAF	2.1 ± 0.9[a]	4.1 ± 1.0[a]	4.0 ± 0.8[a]	4.1 ± 0.8[a]
RVF	2.2 ± 1.1[a]	3.8 ± 0.8[a]	3.9 ± 0.6[a]	3.9 ± 0.8[a]
RAFH	2.2 ± 1.0[a]	4.0 ± 1.0[a]	3.9 ± 0.8[a]	4.0 ± 0.7[a]
RVFH	2.3 ± 1.1[a]	4.1 ± 1.0[a]	3.7 ± 0.7[a]	3.7 ± 0.7[a]
LSD (5 %)	0.71	0.64	0.54	0.5

Results are expressed as averages ± standard deviation. Values with a different letter (a–b) were different ($p < 0.05$) by Dunnett's test. LSD = Least Significant Difference. AC= Animal Control: 100 % Animal Fat; VC= Vegetable Control: 100 % Vegetable fat; RAF= Reduced Animal Fat: 50 % Animal Fat; RVF= Reduced Vegetable Fat: 50 % Vegetable Fat; RAFH= Reduced Animal Fat with Hydrocolloids; RVFH= Reduced Vegetable Fat with Hydrocolloids. Hydrocolloids= alginate (0.25 %) and guar gum (0.05 %).

all difference, and to explain why AC and VC differ ($p < 0.05$). The two first principal components explain 72 % and 22 % of the variation of the experimental data.

The first principal component (Factor 1) (Figure 3A) was positively correlated with the attributes' differences in seasoning flavor, saltiness and firmness, while it was negatively correlated with the attributes' overall difference. Furthermore, the principal component 2 (Factor 2) was positively correlated with the attributes' overall difference, difference in saltiness and firmness. The correlation matrix of all the attributes evaluated had a module close to the unit, suggesting an acceptable explanation of the experimental variation of all the attributes in the two principal components considered.

The AC, RAF and RAFH samples were located on the right side of principal component 1 (Figure 3B), indicating a higher intensity in the attributes positively correlated with this principal component (differences in seasoning flavor, saltiness and firmness) and negatively correlated with the overall difference. As regards principal component 2, the RVF, RAF, AC and VC samples, located in the upper side of principal component 2, had a

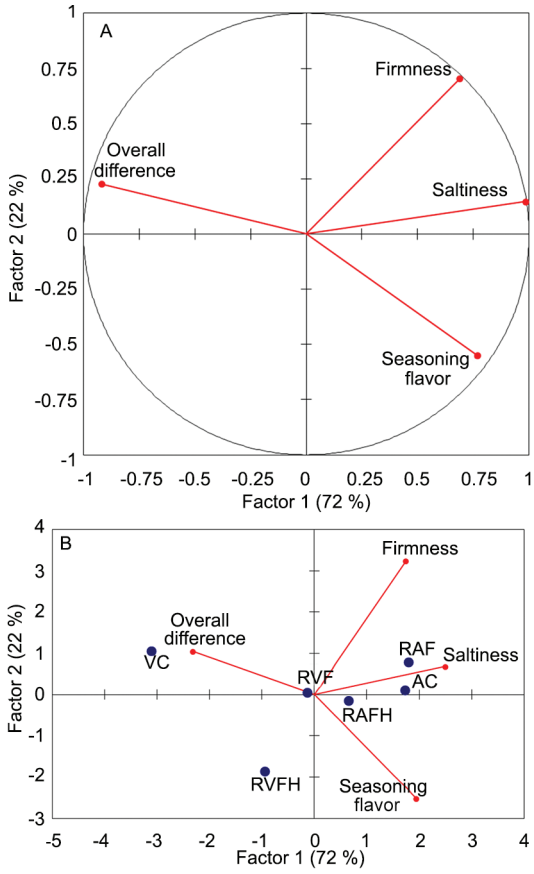

Figure 3 – Principal Component Analysis in a correlation matrix of the attributes evaluated. A: representation of the attributes. B Biplot with representation of attributes and samples. AC= Animal Control: 100 % Animal Fat; VC= Vegetal Control: 100 % Vegetable fat; RAF=educed Animal Fat: 50 % Animal Fat; RVF= Reduced Vegetable Fat: 50 % Vegetab Fat; RAFH= Reduced Animal Fat with Hydrocolloids; RVFH= Reduced Vegetable Fat with Hydrocolloids. Hydrocolloids= alginate (0.25 %) and guar gum (0.05 %).

high intensity with the attributes' overall difference, and differences in saltiness and firmness while RVFH and RAFH samples were negatively correlated with principal component 2, showing low intensity for these attributes. The difference ($p < 0.05$) between VC compared to the control formulation (AC) is due to the fact that these treatments are at the opposite ends of principal component 1 (Figure 3).

The results of this research highlight the importance of the reformulation of meat products using unsaturated vegetal fats and hydrocolloids. The evaluation of the functional effect of the reformulated products and the development of further studies on the sensory characteristics and consumer studies to verify the scope of the reformulation is recommended.

Conclusions

The development of mortadella-type products with reduction and replacement of animal fat by vegetal fat, sodium alginate and guar gum resulted in meat products with improved nutritional properties as a consequence of an increase in monounsaturated fatty acids. Changes in the composition had no effect on lipid oxidation, which is extremely interesting. The physicochemical properties were altered, thereby affecting the instrumental texture, but the use of hydrocolloids helped to reduce this effect. Animal fat substitution causes difference in overall sensorial perception compared with non-substituted products.

Acknowledgements

Erick Saldaña thanks the "Ministerio de Educación del Perú" for the scholarship granted by the program "Programa Nacional de Becas y Crédito Educativo" (PRONABEC). The authors are grateful to the São Paulo Research Foundation (FAPESP) for funding project n° 2013/05359-7. Helpful comments from two anonymous reviewers of the first version of the manuscript are also gratefully acknowledged.

References

Álvarez, D.; Delles, R M.; Xiong, Y.L.; Castillo, M., Payne, F.A.; Laencina, J. 2011. Influence of canola-olive oils, rice bran and walnut on functionality and emulsion stability of frankfurters. LWT - Food Science and Technology 44: 1435-1442.

Association of Official Analytical Chemists - International [AOAC]. 2005. Official Methods of Analysis. 18ed. AOAC, Gaithersburg, MD, USA.

Beiloune, F.; Bolumar, T.; Toepfl, S.; Heinz, V. 2014. Fat reduction and replacement by olive oil in bologna type cooked sausage: quality and nutritional aspects. Food and Nutrition Sciences 5: 645-657.

Berasategi, I.; García-Íñiguez de Ciriano, M.; Navarro-Blasco, I.; Calvo, M.I.; Cavero, R.Y.; Astiasarán, I.; Ansorena, D. 2014. Reduced-fat bologna sausages with improved lipid fraction. Journal of the Science of Food and Agriculture 94: 744-751.

Bourne, M. 2002. Food texture and Viscosity: Concept and Measurement: Academic Press, San Diego, CA, USA.

Chattong, U.; Apichartsrangkoon, A.; Bell, A.E. 2007. Effects of hydrocolloid addition and high pressure processing on the rheological properties and microstructure of a commercial ostrich meat product "Yor" (Thai sausage). Meat Science 76: 548-554.

Doménech-Asensi, G.; García-Alonso, F.J.; Martínez, E.; Santaella, M.; Martín-Pozuelo, G.; Bravo, S.; Periago, M.J. 2013. Effect of the addition of tomato paste on the nutritional and sensory properties of mortadella. Meat Science 93: 213-219.

Escrich, E.; Moral, R.; Grau, L.; Costa, I.; Solanas, M. 2007. Molecular mechanisms of the effects of olive oil and other dietary lipids on cancer. Molecular Nutrition & Food Research 51: 1279-1292.

Ferguson, L.R. 2010. Meat and cancer. Meat Science 84: 308-313.

Fernandez-Gines, J.M.; Fernandez-Lopez, J.; Sayas-Barbera, E.; Sendra, E.; Perez-Alvarez, J.A. 2003. Effect of storage conditions on quality characteristics of bologna sausages made with citrus fiber. Journal of Food Science 68: 710–715.

Flores, M.; Giner, E.; Fiszman, S.M.; Salvador, A.; Flores, J. 2007. Effect of a new emulsifier containing sodium stearoyl-2-lactylate and carrageenan on the functionality of meat emulsion systems. Meat Science 76: 9-18.

Folch, J.; Lees, M.; Sloane-Stanley, G. 1957. A simple method for the isolation and purification of total lipids from animal tissues. The Journal of Biological Chemistry 226: 497-509.

Fonkwe, L.G.; Narsimhan, G.; Cha, A.S. 2003. Characterization of gelation time and texture of gelatin and gelatin–polysaccharide mixed gels. Food Hydrocolloids 17: 871-883.

Guerra, I.C.D.; Félex, S.S.S.; Meireles, B.R.L.M.; Dalmás, P.S.; Moreira, R.T.; Honório, V.G.; Milani, R.F.; Benevides, S.D.; Queiroga, R.C.R.E.; Madruga, M.S. 2011. Evaluation of goat mortadella prepared with different levels of fat and goat meat from discarded animals. Small Ruminant Research 98: 59-63.

Hartman, L.; Lago, R.C. 1973. Rapid preparation of fatty acid methyl esters from lipids. Laboratory Practice 22: 475-476.

Horita, C.N.; Morgano, M.A.; Celeghini, R.M.S.; Pollonio, M.A.R. 2011. Physico-chemical and sensory properties of reduced-fat mortadella prepared with blends of calcium, magnesium and potassium chloride as partial substitutes for sodium chloride. Meat Science 89: 426-433.

Johnston, C. 2009. Functional foods as modifiers of cardiovascular disease. American Journal of Lifestyle Medicine 3: 39-43.

Lawless, H.T.; Heymann, H. 2010. Sensory Evaluation of Food: Principles and Practices. Springer, New York, NY, USA.

Lin, K.C.; Keeton, J.T.; Gilchrist, C.L.; Cross, H.R. 1988. Comparisons of carboxymethyl cellulose with differing molecular features in low fat frankfurters. Journal of Food Science 53: 1592-1595.

Lin, K.W.; Mei, M.Y. 2000. Influences of gums, soy protein isolate, and heating temperatures on reduced-fat meat batters in a model system. Journal of Food Science 65: 48-52.

Marchetti, L.; Andrés, S.C.; Califano, A.N. 2013. Textural and thermal properties of low-lipid meat emulsions formulated with fish oil and different binders. LWT - Food Science and Technology 51: 514-523.

Mattson, F.H.; Grundy, S.M. 1985. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. Journal of Lipid Research 26: 194-202.

McAfee, A.J.; McSorley, E.M.; Cuskelly, G.J.; Moss, B.W.; Wallace, J.M.W.; Bonham, M.P.; Fearon, A.M. 2010. Red meat consumption: an overview of the risks and benefits. Meat Science 84: 1-13.

Meilgaard, M.C.; Carr, B.T.; Civille, G.V. 2006. Sensory Evaluation Techniques. CRC Press, Boca Raton, FL, USA.

Mendoza, E.; García, M.L.; Casas, C.; Selgas, M.D. 2001. Inulin as fat substitute in low fat, dry fermented sausages. Meat Science 57: 387-393.

Ospina-E., J.C.; Sierra-C, A.; Ochoa, O.; Pérez-Álvarez, J.A.; Fernández-López, J. 2011. Substitution of saturated fat in processed meat products: a review. Critical Reviews in Food Science and Nutrition 52: 113-122.

Papastergiadis, A.; Mubiru, E.; Langenhove, H. van; De Meulenaer, B. 2012. malondialdehyde measurement in oxidized foods: evaluation of the spectrophotometric Thiobarbituric Acid Reactive Substances (TBARS) test in various foods. Journal of Agricultural and Food Chemistry 60: 9589-9594.

Pérez-Mateos, M.; Hurtado, J.L.; Montero, P.; Fernández-Martín, F. 2001. Interactions of κ-carrageenan plus other hydrocolloids in fish myosystem gels. Journal of Food Science 66: 838-843.

Pietrasik, Z.; Duda, Z. 2000. Effect of fat content and soy protein/carrageenan mix on the quality characteristics of comminuted, scalded sausages. Meat Science 56: 161-188.

Rodríguez-Carpena, J.G.; Morcuende, D.; Estévez, M. 2012. Avocado, sunflower and olive oils as replacers of pork back-fat in burger patties: effect on lipid composition, oxidative stability and quality traits. Meat Science 90: 106-115.

Santos, B.A.; Pollonio, M.A.R.; Cruz, A.G.; Messias, V.C.; Monteiro, R.A.; Oliveira, T. L.C.; Faria, J.A.F.; Freitas, M.Q.; Bolini, H.M.A. 2013. Ultra-flash profile and projective mapping for describing sensory attributes of prebiotic mortadellas. Food Research International 54: 1705-1711.

Simopoulos, A.P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine & Pharmacotherapy 56: 365-379.

Sørensen, G.; Jørgensen, S. 1996. A critical examination of some experimental variables in the 2-thiobarbituric acid (TBA) test for lipid oxidation in meat products. Zeitschrift für Lebensmittel-Untersuchung und Forschung 202: 205-210.

Vyncke, W. 1970. Direct determination of the thiobarbituric acid value in trichloracetic acid extracts of fish as a measure of oxidative rancidity. Fette, Seifen, Anstrichmittel 72: 1084-1087.

Vyncke, W. 1975. Evaluation of the direct thiobarbituric acid extraction method for determining oxidative rancidity in mackerel (Scomber scombrus L.). Fette, Seifen, Anstrichmittel 77: 239-240.

Wakeling, I.N.; MacFie, H.J.H. 1995. Designing consumer trials balanced for first and higher orders of carry-over effect when only a subset of k samples from t may be tested. Food Quality and Preference 6: 299-308.

Webb, E.C.; O'Neill, H.A. 2008. The animal fat paradox and meat quality. Meat Science 80: 28-36.

Wood, J. D.; Richardson, R.I.; Nute, G.R.; Fisher, A.V.; Campo, M.M.; Kasapidou, E.; Sheard, P.R.; Enser, M. 2004. Effects of fatty acids on meat quality: a review. Meat Science 66: 21-32.

Youssef, M.K.; Barbut, S. 2011. Fat reduction in comminuted meat products-effects of beef fat, regular and pre-emulsified canola oil. Meat Science 87: 356-360.