"High in omega-3 fatty acids" Bologna type sausages stabilized with an aqueous-ethanol extract of *Melissa officinalis*.

Izaskun Berasategi[1], Sheila Legarra[1], Mikel García-Íñiguez de Ciriano[1], Sheyla Rehecho[2], Maria Isabel Calvo[2], Rita Yolanda Cavero[3], Íñigo Navarro-Blasco[4], Diana Ansorena[1]*, Iciar Astiasarán[1]

[1] Department of Nutrition, Food Science, Physiology and Toxicology, Faculty of Pharmacy, University of Navarra, Irunlarrea s/n, 31008-Pamplona, Spain.

[2] Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Navarra, Irunlarrea s/n, 31008-Pamplona, Spain.

[3] Department of Plant Biology (Botany), Faculty of Sciences, University of Navarra, Irunlarrea s/n, 31008-Pamplona, Spain.

[4] Department of Chemistry and Soil Science, Faculty of Sciences, University of Navarra. Irunlarrea s/n, 31008-Pamplona, Spain.

*Corresponding author: Tel.: +34 948 42 56 00 (ext. 6263); Fax: +34 948 42 56 49.

E-mail address: dansorena@unav.es

ABSTRACT

A new formulation of Bologna-type sausage enriched in ω-3 polyunsaturated fatty acids (PUFA) (8.75% linseed oil) was developed, using a lyophilized aqueous-ethanolic extract of *Melissa officinalis.* A comparison with the effectiveness of butylhydroxy anisole (BHA) synthetic antioxidant to decrease the oxidation of PUFAs was performed.

The formulation increased the ω-3 PUFAs content, especially α-linolenic acid, decreasing significantly the ω-6/ω-3 ratio from 17.3 to 1.9, and also the Atherogenic Index and Thrombogenic Index (0.38-0.31 and 1.03-0.54, respectively).

Modified sausages with BHA and *Melissa* extract showed significantly lower peroxides value (2.62 and 6.11meqO_2/kg) and thiobarbituric acid value (0.26 and 0.27mg malondialdehyde/kg) and higher antioxidant capacity (hydrophilic fraction ABTS: 0.45 and 0.74meq Trolox/g product; lipofilic fraction ABTS: 0.44 and 0.37meq Trolox/g product) than those without these ingredients (16.49meq O_2/kg, 2.08 mg malondialdehyde /kg, 0.26 and 0.27meq Trolox/g product, respectively). Sensorial tests showed that acceptability of the new formulations was similar to control products.

Keywords: meat-based functional foods; linseed oil; Melissa officinalis; natural antioxidant; antioxidant capacity; ω-3 PUFA.

1. INTRODUCTION

Melissa officinalis is one of the most used medicinal plants. A mixture of *Melissa officinalis* with other two herbs (*Morus alba* and *Artemisia capillaries*) has been found to regulate serum lipid profiles, adipose tissues mass and body weight in high –fat diet obese mice (Lee, *et al.,* 2008). Also, its beneficial effects related to neurological diseases have been widely studied (Brendler, *et al.,* 2005; Wheatley, 2005; Kennedy, Little & Scholey, 2004; Kennedy, Little, Haskell & Scholey, 2006). Moreover its neuroprotective properties have been demonstrated in *in vitro* cellular model with the PC12 (rat pheochromocytoma) cell line, as well as its neurological activities with methanolic extracts being more effective than aqueous extracts (López, Martín, Gómez-Serranillos, Carretero, Jager & Calvo, 2009). It seems clear that the effectiveness of *Melissa* in the prevention of neurological diseases, which are associated with oxidative stress, is related to its antioxidant capacity (Pereira *et al.,* 2009). Dastmalchi, Dorman, Oinonen, Darwis, Laakso & Hiltunen (2008) establishing the chemical composition and the *in vitro* antioxidative activity of a *Melissa officinalis* aqueous ethanolic extract pointed out that the extract may have the potential to prevent oxidative damage *in vivo* by preventing free-radical-mediated oxidative stress.

Bologna-type sausage, a cooked meat product, is very popular in Europe and it is one of the most consumed (Nowak, von Mueffling, Grotheer, Klein & Watkinson, 2008). Meat products are interesting protein and iron sources, however they usually also show relatively high amounts of fat, saturated fat and cholesterol, which have been related to some chronic diseases as cardiovascular diseases and cancer (Sieri et al., 2008; Siri-Tarino et al., 2010; Gonzalez and Riboli, 2010). In contrast, polyunsaturated fatty acids (PUFA), and especially ω-3 type PUFAs, have beneficial health effects (Simopoulos, 1997; Connor, 2000).

Therefore, great efforts are being made to improve the lipid fraction of meat products, some of them attempting an increment in the PUFA content (Del Nobile, Conte, Incoronato, Panza, Sevi & Marino, 2009; Jiménez-Colmenero, 2007; Lee, Faustman, Djordjevic, Faraji & Decker, 2006; Martín, Ruiz, Kivikari & Puolanne, 2008; Pelser, Linssen, Legger & Houben, 2007; García-Íñiguez de Ciriano, Larequi, Rehecho, Calvo, Cavero & Navarro-Blasco, 2010a; García-Íñiguez de Ciriano et al., 2010b). One of the main problems dealing with these strategies is the higher susceptibility of PUFAs to oxidation processes (Gurr, Harwood & Frayn, 2002) what makes necessary the use of potent antioxidants in these products.

Synthetic antioxidants, butylhydroxy anisole (BHA), butylhydroxy toluene (BHT), sodium citrate, have been proved to be efficient and needed for stabilizing meat products rich in long chain ω-3 PUFAs (Lee, Faustman, Djordjevic, Faraji & Decker, 2006; Muguerza, Gimeno, Ansorena & Astiasaran, 2004; Valencia, Ansorena & Astiasaran, 2006, 2007). However, due to health risks associated with its use and the better perception of natural products by consumers, the use of plant extracts with significant antioxidant activity is being under research. Carob fruit extracts rich in condensed tannins and grape seed extracts were succesfully applied to reduce fat deterioration in cooked meat at chilled and frozen temperatures (Bastida, Sanchez-Muniz, Olivero, Perez-Olleros, Ruiz-Roso & Jimenez-Colmenero, 2009; Sasse, Colindres & Brewer, 2009). DeJong & Lanari (2009) reduced the formation of 2-thiobarbituric acid reactive substances in pre-cooked beef and pork by using waste waters of olive oil pomace, rich in hydroxy-tyrosol. Crude extract from *Eleutherine americana* was suggested as an efficient novel antioxidant to prevent lipid oxidation of meat products (Ifesan, Siripongvutikorn, Hutadilok-Towatana & Voravuthikunchai, 2009).

The antioxidant properties of methanolic and ethanolic extracts of *Melissa officinalis* have been already pointed out (Zandi & Arnadi, 2000; Ferreira, Proenca, Serralheiro & Araujo, 2006; López, Akerreta, Casanova, García-Mina, Cavero & Calvo, 2007). Aqueous ethanol extracts of these plants contain flavonoids and hydroxycinnamic acid derivates, known by their antioxidant capacity, being rosmarinic acid the major component (Dastmalchi, Dorman, Oinonen, Darwis, Laakso & Hiltunen, 2008).

García-Íñiguez de Ciriano et al. (2010a) proved that a lyophilized aqueous extract of *Melissa officinalis* was as efficient as BHA when controlling the thiobarbituric acid value (TBARs) formation in oil-in-water emulsions made with a mixture of algae and linseed oils. This aqueous extract efficiently controlled lipid oxidation in dry fermented sausages (García-Íñiguez de Ciriano et al., 2010b). Pereira *et al.* (2009) obtained lower amounts of phenolic compounds in ethanolic and methanolic extracts than in aqueous extracts from *Melissa officinalis*. However, it is well known that the different extraction conditions leads to different antioxidant capacity of the obtained extracts. Furthermore, there are anomalies in the correlation between antioxidant capacity and chemical composition of plant extracts (Ibarra, Cases, Bily, He, Bai & Roller, 2010).

Bologna-type sausages are subjected to a pasteurization process during processing, reaching Tª of 72-75ºC in the inner core of the products. This heat treatment, necessary from a microbiological and technological point of view, might have a negative influence on the lipid fraction and in the antioxidant capacity. Bastida, Sanchez-Muniz, Olivero, Perez-Olleros, Ruiz-Roso & Jimenez-Colmenero (2009) showed that heating process, 80ºC during 1 h, applied to cooked meat products (reaching 70ºC as internal temperature) leads to a high increase of oxidative reactions in products prepared without antioxidant, which cause a warmed-over-flavour (WOF) during chilling.

The objectives of this work were to asses the nutritional benefits of a new formulation for a Bologna-type sausage enriched in ω-3 fatty acids from linseed oil and to analyze the efficacy of a lyophilized aqueous-ethanolic extract of *Melissa officinalis* as a natural antioxidant to prevent lipid deterioration. The influence of the heat treatment was also studied.

2. MATERIALS AND METHODS

2.1. Materials

Pork meat and back fat were obtained from a local meat market. Linseed oil (Biolasi Productos Naturales, Guipúzcoa, Spain) was obtained in a local market and *Melissa* dried leaves were purchased from Plantaron S.L. (Barcelona, Spain). 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). Curavi (a mixture of curing agents: NaCl, E-250, E-252 and antioxidant E-331) was kindly donated by ANVISA (Arganda del Rey, Madrid, Spain). All the chemical reagents were obtained from Sigma-Aldrich Chemical Co. (MO, USA).

Preparation of the lyophilized aqueous-ethanolic extract of Melissa officinalis

Aqueous-ethanolic extracts of *Melissa officinalis* were prepared as follows: 50 g of dried leaves were weighted and added to 500 ml of ethanol (50%). The mixture was subjected to boiling reflux during 30 minutes. Extraction process was repeated with another 500 ml of ethanol (50%), and both extracts were pooled together and completed with ethanol (50%) to a final volume of 1 L. Extracts were filtered using filter to remove

insoluble particles. Aqueous-ethanolic extraction was performed in triplicate. The extracts were lyophilized with a freeze-dryer-cryodo (Telstar, Barcelona, Spain), previous freezing at -80º C in a MDF-V5386S Ultra-Low-Temperature Freezer (Sanyo Electric Co., Ltd., Japan). 23 g of lyophilized material were obtained from 100 g of Melissa dried leaves. The lyophilized material was subsequently used as ingredient in the cooked product formulation.

2.2. Sausage formulation and processing

Four batches of Bologna-type sausages were manufactured in a pilot plant: *Control, Linseed, BHA* and *Melissa.* The total amount of each batch was 4 kg. Table 1 shows all ingredients of the control batch and of the 3 modified batches (Linseed, BHA and Melissa).

All ingredients were thoroughly minced in a chilled cutter for 1 minute at low speed and 2 minutes 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 minutes, the batters were stuffed in 6 cm diameter water impermeable plastic casings. A portion of the crude batter was separated from each batch in order to analyze each type of formulation before cooking. Samples were immediately frozen (-20ºC) and kept under vacuum until analysis. Sausages were cooked in a water bath at 80ºC for 1 h, until the core of the product reached 72ºC. Once heating was complete, the sausages were immediately cooled in a water bath for 2 h and stored frozen (-20ºC) under vacuum till analysis. The experiment was done in triplicate.

2.3. Chemical analysis

Characterization of antioxidant capacity of the lyophilized extract

Determination of Total phenolic content (TPC)

TPC of the lyophilized aqueous-ethanolic extract of *Melissa officinalis* was determined spectrophotometrically following the Folin-Ciocalteu colorimetric method as described in García-Herreros, García-Íñiguez de Ciriano, Astiasaran & Ansorena (2010). Dilutions of the lyophilized aqueous-ethanolic extract of *Melissa* ranging from 0.07 to 0.35 mg/ml were chosen in order to obtain absorbance readings within the standard calibration curve made from dilutions between 0.005 and 2 mg/ml of Gallic acid (GA). The reaction mixture was composed of 0.1 ml of suitable diluted sample, 7.9 ml of distilled water, 0.5 ml of Folin-Ciocalteu´s reagent, and 1.5 ml of 20 % sodium carbonate anhydrous solution (added 2 minutes after the Folin-Ciocalteu´s reagent). After the initial mixing, the tubes were allowed to stand at room temperature for 2 hours in the dark. The optical density of the blue-colored resulting solution was measured at 765 nm using a Lambda 5-UV-VIS spectrophotometer (Perkin Elmer, Paris, France). The total phenolic content was expressed as mg GA/g lyophilized extract, using the corresponding calibration curve and taking into account the concentration of the diluted extracts. Absorbance measurements were made in duplicate for each diluted solution.

ABTS method

For ABTS assay, the procedure described by Re, Pellegrini, Proteggente, Pannala, Yang & Rece-Evans (1999) was used, with some modifications detailed in García-

Herreros, García-Íñiguez de Ciriano, Astiasaran & Ansorena (2010). The concentrations of the dilutions of the lyophilized aqueous-ethanolic extract of Melissa were 0.147 mg/ml – 0.035 mg/ml. Results were finally expressed as mg Trolox/g lyophilized extract of *Melissa*. Absorbance measurements were made in duplicate for each diluted solution.

Determination of oxidation of Bologna-type sausages

Fat extraction

The method of Folch, Lees & Stanley (1957) was used for the extraction of fat.

TBARs (Thiobarbituric acid value)

TBARs values were determined on fat basis according to the method described by Masqsood & Benjakul (2010) 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, 20µL 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 minutes. 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 product.

POV Index (Peroxide index)

POV were determined according to the AOAC method (AOAC, 2002). Results were expressed in meq O_2/kg fat.

Determination of antioxidant capacity of Bologna-type sausages

The evaluation of the antioxidant capacity of the 4 types of Bologna-type sausages was done both in the hydrophilic and in the lipophilic fractions, using the ABTS method. Elimination of the water content of the meat batters by lyophilization was needed as a preparation step for obtaining the hydrophilic fraction.

The hydrophilic fraction was obtained as described by Wu, Duckett, Neel, Fontenot & Clapham (2008): lyophilized sample (1g) was extracted using 20% ethanol (40 mL) for 1 h at room temperature in a rotary shaker. The resulting homogenate was filtered through a paper filter and properly diluted for applying the ABTS method as previously described (note: adjustment of absorbance to 0.7 was done by dilution of ABTS in pure ethanol). The lipophilic fraction was obtained as described by Sacchetti, Di Mattia, Pittia & Martino (2008): samples (20 g) were added to 150mL of methanol:chloroform solution (2:1) kept in an ice bath and homogenized with an ultraturrax. The homogenate was put in an erlenmeyer, wrapped in an aluminum sheet and kept for 4h in a rotary shaker. The homogenate was filtered through filter paper, put in a funnel and added to 50 ml of physiologic solution (NaCl 0.9%) until the methanolic phase is transparent. The two separated phases (chloroform and methanol) were collected separately and ABTS was measured in each phase, being the sum of both results the value given as the antioxidant activity of the lipophilic fraction (Results were expressed in meq Trolox/g product).

Lipid fraction analysis

Fatty acids were determined in the lipid extracts by gas chromatography FID detection according to the procedure described by Valencia, O'Grady, Ansorena, Astiasaran & Kerry (2008).

Atherogenic Index (AI) and Thrombogenic Index (TI) were calculated according to Ulbricht & Southgate (1991):

AI = (C12:0 + C14:0 + C16:0)/ (ω-3PUFA + ω-6PUFA + MUFA)

TI = (C14:0 + C16:0 + C18:0)/ (0.5*MUFA + 0.5*ω-6PUFA + 3*3-nPUFA + (ω-3/ω-6))

2.4. Sensorial analysis

The sensorial acceptability of the Bologna type sausages was evaluated by an hedonic test. The test was carried out one day after the preparation of the products. Non-trained panellists were given 2 slices of 2mm thick products on a while plate and they were asked to score modified batches on a 1-9 continuous point scale in which the degree of acceptability for different attributes was evaluated: colour, taste, texture and aroma. Furthermore, overall acceptability was also assessed. A value of 1 corresponded to "extreme dislike" for each attribute and a value of 9 to "like extremely" Control samples were taken as the reference value, receiving a score of 5 points for every attribute.

2.5. Data analysis

Data were analyzed using *t* student test for the evaluation of the results obtained in each batch before and after the cooking process. A one way Anova test and the Tukey b posteriori test were used to determine significant differences among the different types of

Bologna-type sausages. SPSS version 15.0 was used (SPSS inc. Chicago, Illinois, USA). Significance level of $P \leq 0.05$ was used for all evaluations.

3. RESULTS AND DISCUSSION

Oxidation

Measurements of Peroxide values (POV) and TBARs before and after the heat treatment were carried out in the four types of products made in this work (Fig. 1 and 2). Products enriched in PUFAs showed very different results for both parameters depending on the presence of extra-antioxidants in their composition. Before cooking, control and modified products without extra-antioxidants showed the highest amounts for POV (15 and 13 meq O_2/kg for control and modified products, respectively) and TBARs (0.61 and 1.36 mg MDA/kg respectively) pointing at a certain degree of oxidation already during chopping. Modified products with antioxidants showed much lower POV (<10) and TBARs (<1), without differences in TBARs between products with BHA and *Melissa* extracts. BHA batches showed the lowest statistically significant POV. BHA is a highly lipophilic radical quencher, which could explain its efficiency to stop lipid fraction oxidation, decreasing POV formation. Lee, Faustman, Djordjevic, Faraji & Decker (2006) observed higher amounts of lipid hydroperoxides in meat products (fresh patties) enriched in ω-3 PUFAs, compared to those with antioxidants, in agreement with the results shown in this paper.

After cooking, significant increases in TBARs were observed for every sample compared to non-cooked samples. Values found for batches with BHA (0.26 mg MDA/kg) and with *Melissa* extract (0.27 mg MDA/kg) were similar, and lower ($p<0.05$) than the

other batches (Fig. 2). These results suggest that both antioxidants are efficient and have similar behaviour during heat treatment. In the case of the Melissa extract, this antioxidant activity might be attributed to flavonoids and hydroxycinnamic acid derivates (Dastmalchi *et al.*, 2008).

With cooking, POV decreased in every product (Fig. 1), except in those PUFA enriched products that did not include BHA or *Melissa* extract, in which a slight increase was still observed. This decrease indicated that the peroxide compounds have partially reacted during the heat treatment giving rise to secondary oxidation products, which are responsible for the increases observed for TBARs. Also, the presence of antioxidants might have contributed to this reduction, neutralizing the peroxides formation that occurred during cooking in the batches enriched with PUFA and without extra-antioxidants. The highest amounts for both TBARs and POV after cooking corresponded to the enriched product without extra-antioxidant, pointing to a higher oxidation susceptibility for these products. Cáceres, García & Selgas (2008) found TBARs values of 0.37 mg MDA/kg in "mortadellas" prepared with fish oil, although in that work no differences were found in TBARs values of final products for control and fish oil added sausages.

Potential antioxidant activity

Besides the measure of the intensity of the oxidation process through the analysis of primary and secondary oxidation products, the analysis of the potential antioxidant activity was carried out. The results obtained before cooking showed that the radical scavenging activity (RSA) measured by the ABTS assay in the hydrophilic fraction extracted from the control products was much higher (4-fold) than that obtained for the lypophilic fraction (Fig. 3). Sacchetti, Di Mattia, Pittia & Martino (2008) measuring the total antioxidant

activity of poultry meat, observed, as well as in this paper, that the contribution of the hydrophilic fraction to the antioxidant capacity was much higher than that of the lipid soluble fraction. Meat is not considered as a source of dietetic antioxidants. However, some of its compounds such as the dipeptides, carnosine and anserine, or polyamines, and antioxidant enzymes are reported to be effective hydrophilic antioxidants whereas lypophilic antioxidants can also be present (α-tocopherol, carotenoids and ubiquinone) (Sachetti, Di Mattia, Pittia & Martino, 2008; Chan & Decker, 1994; Zhou & Deccker, 1999; Antonini, *et al.*, 2002). Among the three PUFA enriched batches, the one with *Melissa* extract showed the highest hydrophilic antioxidant activity (0.97 mEqTrolox/g product). Ibarra, Cases, Billy, He, Bai & Roller (2010) pointed out the interest of the extraction procedures applied to plants rich in antioxidants, on which depends the nature of the antioxidant composition and consequently, its efficacy. The *Melissa* lyophilized extract contained a polyphenol content of 385.4mg gallic acid equivalents/g dry extract, which might have contributed to the highest ABTS in the developed meat products. Every modified product (with and without extra antioxidants) showed higher amounts for ABTS in the lipophilic fraction than control ones, probably due to the supply of tocopherols and other lipidic compounds with antioxidant capacity from the linseed used in the emulsions. The use of antioxidants, both BHA and *Melissa*, significantly increased again the antioxidant activity.

Cooking did not affect the antioxidant activity of the lipophilic fraction except in products with *Melissa* extract where a slight decrease was detected. In the case of the hydrophilic fraction, a significant decrease in the ABTS values was found in every product. Wu, Duckett, Neel, Fontenot & Clapham (2008) observed that cooking beef decreased the hydrophilic ORAC, probably because of protein denaturation and the degradation of

antioxidant compounds. However, those authors found an increase in the lipophilic ORAC of cooked beef samples compared to raw samples, being related by the authors to an increase in the bioavailability of lipophilic antioxidants such as β-carotenoids. When total antioxidant activity is taken into account (Fig. 3) it is clear that modified products needs the presence of antioxidants to maintain and even increase the endogenous antioxidant activity. Moreover, the efficacy of *Melissa* extract over the BHA can be observed both, before and after cooking.

Beneficial lipid profile

Regarding the fatty acid profile, modified products could be considered as healthier products than the traditional ones (Table 2). From the 25 different fatty acids analyzed, only 3 did not shown significant differences among products.

The total amount of trans fatty acids was about 1% in all products. The greatest change was observed for α-linolenic acid, as a consequence of the addition of linseed oil. A 10-fold increment in this fatty acid was observed in modified cooked products in relation to the control ones, without significant differences between Linseed batch and Melissa batch. No effect of the use of antioxidants was noticed over the linolenic acid content, despite the higher oxidation degree in non-antioxidant containing products. Small and not quantitatively relevant differences were noticed for oleic and linoleic acids among the 3 modified products, which did not significantly affect their nutritional value.

According to the Commission Regulation EU nº116/2010, all types of modified products could claim that they are "high in omega-3 fatty acids", as they contain more than 0.6 g α-linolenic acid per 100 g. Effectively, the amount of α-linolenic C18:3 (ω-3) in control products was 0.27 g/100g, whereas the amounts in modified products were 2.31-

2.57 g/100g. This fatty acid has been associated with a reduced risk of cardiovascular disease and it is a nutritionally essential one required for synthesis of important fatty acids and eicosanoids. Current recommendations for total omega-3 fatty acids suggest daily intakes for adults of 2g/day without making a distinction between α-linolenic and long chain PUFA (EFSA, 2005). In consequence, a portion of 50g of modified products would supply approximately 1.2g, which means a 60% of these daily recommendations. Furthermore, SEN (Sociedad Española de Nutrición- Spanish Nutrition Society) (Roset, 2010) suggests that α-linolenic acid should cover a 0.5% of the total daily energy value, which would be 1.1g/day, in a 2000 kcal-diet. So, the mentioned portion of modified products would cover 100% of this recommendation.

As a consequence of the α-linolenic increment, the two major fatty acids present in a traditional formulation, oleic acid and palmitic acid, significantly decreased in modified products. Palmitic acid decreased a 12%, on average, and oleic acid decreased between 7 and 10%. These modifications were obviously reflected in changes of some interesting ratios. Wood *et al.* (2004) reported a recommended PUFA/SFA ratio above 0.4, whereas other studies pointed out that this ratio should reach the range 1-1.5 to be considered favourable to reduce the risk of cardiovascular disease (Kang *et al.,* 2005). Polyunsaturated/saturated fatty acids ratio (PUFA/SFA) increased from 0.49 in control products to 0.77-0.84 in modified ones, which were closer to recommended values. More relevant changes were observed for ω-6/ω-3 ratio between control (17.27) and the rest of the products (1.84-1.96). The current recommendation for this ratio according to nutritional guidelines is around 4 (Simopoulos, 2002). These results can be considered as successful from a nutritional point of view. López-López, Cófrades, Ruíz-Capillas & Jimenez-Colmenero (2009) developing functional frankfurters with different edible seaweed and

algae oil obtained PUFA/SFA ratios around 0.39-0.28, and ω-6/ω-3 ratios around 1.79-4.65, the lattets being quite similar to these obtained in this work. Delgado-Pando, Cófrades, Ruíz-Capillas & Jimenez-Colmenero (2010) developed low-fat frankfurters with healthier lipid combination. In this work they substituted all of pork fat of control frankfurter with an oil-in water emulsion, which contained olive oil, linseed oil and fish oil. They obtained frankfurters with 17.70g α-linolenic per 100 g of fat. The decrease of SFA and the increase of PUFAs, specially the increase of ω-3 PUFAs, were very significant.

Other fatty acids ratios have also been proposed to evaluate and characterize the health related aspects of dietary fat (Ulbricht & Southgate, 1991). The index of atherogenicity (AI) and the index of thrombogenicity (TI) take into account the different effects of the different fatty acids on cardiovascular risk. The lipid profile achieved in new formulations contributed to decrease both indexes, from 0.38 to around 0.31 the AI, and from 1.03 to 0.54 the TI (Table 2), which was considered positive from the health standpoint.

Sensorial analysis

In order to assess the acceptability of the linseed containing products, an hedonic sensorial test was carried out. Sensorial analysis results of the new formulations revealed very promising data (Figure 4). In fact, no significant differences were observed for texture and aroma for all linseed containing products compared to control ones. A slightly lower score was noticed for the batch with Melissa extract, for colour and taste acceptability, that however did not affect general acceptability scores, which gave statistically similar results to control products.

In summary, Bologna-type cooked products enriched in linseed oil were successfully stabilized using a lyophilized aqueous-ethanolic extract of *Melissa officinalis*. Stability of the new formulation was equivalent as that used with the artificial antioxidant BHA.

ACKNOWLEDGEMENTS

We thank the “Programa Consolider-Ingenio 2010 CARNISENUSA CSD2007-00016”, the “Proyecto AGL2008-01099/ALI” (Ministerio de Ciencia e Innovación), and “Plan Investigador de la Universidad de Navarra” (PIUNA) for their contribution to the financial support of this work. I. Berasategi is grateful to Gobierno de Navarra (Departamento de Innovación, Empresa y Empleo) and to “Asociación de Amigos de la Universidad de Navarra” for the grants received. We are also grateful to Dr. Mohino (ANVISA).

4. BIBLIOGRAPHY

Antonini, F., Petruzzi, E., Pinzani, P., Orlando, C., Poggesi, M., Serio, M., Pazzagli, M., & Masotti, G. (2002). The meat in the diet of aged subjects and the antioxidant effects of carnosine. *Archives of Gerontology and Geriatrics,(8)* , 7-14.

AOAC. (2002). Determination of peroxide content. 965.33.12.

Bastida, S., Sanchez-Muniz, F., Olivero, R., Perez-Olleros, L., Ruiz-Roso, B., & Jimenez-Colmenero, F. (2009). Antioxidant activity of Carob fruit extracts in cooked pork meat systems during chilled and frozen storage. *Food Chemistry, 116*(3), 748-754.

Brendler, T., Gruenwald, J., Kligler, B., Keifer, D., Abrams, T. R., Woods, J., Boon, H., DeFraneo Kirkwood, C., Basch, E., Ulbricht, C., & Hackman, DA. (2005). Lemon balm (*Melissa officinalis L.*): An evidence-based systematic review by the natural standard research collaboration. *Journal of Herbal Pharmacotherapy, 5*(4), 71-114.

Cáceres, E., Garcia, M., & Selgas, M. (2008). Effect of pre-emulsified fish oil - as source of PUFA n-3 - on microstructure and sensory properties of mortadella, a Spanish bologna-type sausage. *Meat Science, 80*(2), 183-193.

Chan, K., & Decker, E. (1994). Endogenous skeletal-muscle antioxidants. *Critical reviews in food science and nutrition, 34*(4), 403-426.

Connor, W. (2000). Importance of n-3 fatty acids in health and disease. *Proceedings of the 58th flax institute of the united states,* 225-225.

Dastmalchi, K., Dorman, H., Oinonen, P., Darwis, Y., Laakso, I., & Hiltunen, R. (2008). Chemical composition and in vitro antioxidative activity of a lemon balm (*Melissa officinalis L.*) extract. *Food science technology, 41*(3), 391-400.

DeJong, S., & Lanari, M. (2009). Extracts of olive polyphenols improve lipid stability in cooked beef and pork: Contribution of individual phenolics to the antioxidant activity of the extract. *Food Chemistry, 116*(4), 892-897.

Del Nobile, M., Conte, A., Incoronato, A., Panza, O., Sevi, A., & Marino, R. (2009). New strategies for reducing the pork back-fat content in typical Italian salami. *Meat Science, 81*(1), 263-269.

Delgado-Pando, G., Cofrades, S., Ruiz-Capillas, C., & Jiménez-Colmenero, F. (2010). Healthier lipid combination as functional ingredient influencing sensory and technological properties of low-fat frankfurters. *European journal of lipid science and technology, 112*(8), 859-870.

EFSA. (2005). Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to nutrition claims concerning omega-3 fatty acids, monounsaturated fat, polyunsaturated fat and unsaturated fat.

Ferreira, A., Proenca, C., Serralheiro, M., & Araujo, M. (2006). The in vitro screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. *Journal of ethnopharmacology, 108*(1), 31-37.

Folch, J., Lees, M., & Stanley, G. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry, 226*(1), 497-509.

Garcia-Hereros, C., Garcia-Iniguez, M., Astiasarán, I., & Ansorena, D. (2010). Antioxidant activity and phenolic content of water extracts of borago officinalis l.: influence of plant part and cooking procedure. *Italian journal of food science, 22*(2), 156-164.

García-Íñiguez de Ciriano, M., Larequi, E., Rehecho, S., Calvo, M., Cavero, R., Navarro-Blasco, I., Astiasarán, I., & Ansorena, D. (2010a). Selenium, iodine, omega-3 PUFA and natural antioxidant from *Melissa officinalis L.*: A combination of components from healthier dry fermented sausages formulation. *Meat Science, 85*(2), 274-279.

García-Íñiguez de Ciriano, M., Rehecho, S., Calvo, M., Cavero, R., Navarro, I., Astiasarán, I., & Ansorena, D. (2010b). Effect of lyophilized water extracts of *Melissa officinalis* on the stability of algae and linseed oil-in-water emulsion to be used as a functional ingredient in meat products. *Meat Science, 85*(2), 373-377.

Gonzalez, C. A., & Riboli, E. (2010). Diet and cancer prevention: Contributions from the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *European journal of cancer, 46*(14), 2555-2562.

Gurr, M., Harwood, J., & Frayn, K. (2002). Lipid biochemistry. *Lipid biochemistry,* Ed. 5, xvi + 320pp.

Ibarra, A., Cases, J., Bily, A., He, K., Bai, N., Roller, M., Coussaert, A., & Ripoll, C. (2010). Importance of Extract Standardization and In Vitro/Ex Vivo Assay Selection for the Evaluation of Antioxidant Activity of Botanicals: A Case Study on Three *Rosmarinus officinalis L.* Extracts. *Journal of medicinal food, 13*(5), 1167-1175.

Ifesan, B., Siripongvutikorn, S., Hutadilok-Towatana, N., & Voravuthikunchai, S. (2009). Evaluation of the Ability of Eleutherine americana Crude Extract as Natural Food Additive in Cooked Pork. *Journal of Food Science, 74*(7), M352-M357.

Jiménez-Colmenero, F. (2007). Healthier lipid formulation approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meat fats. *Trends in food science technology, 18*(11), 567-578.

Kang, D.H., Kang, S.J., Song, J.M., Choi, K.J., Hong, M.K., Song, J.K., Park, S.W., & Park, S.J. (2005). Efficacy of myocardial contrast echocardiography in the diagnosis and risk stratification of acute coronary syndrome. *The American Journal of Cardiology, 96*(11), 1498-1502.

Kennedy, D., Little, W., Haskell, C., & Scholey, A. (2006). Anxiolytic effects of a combination of *Melissa officinalis* and *Valeriana officinalis* during laboratory induced stress. *Phytotherapy research, 20*(2), 96-102.

Kennedy, D., Little, W., & Scholey, A. (2004). Attenuation of laboratory-induced stress in humans after acute administration of *Melissa officinalis* (lemon balm). *Psychosomatic medicine, 66*(4), 607-613.

Lee, J., Chae, K., Ha, J., Park, B., Lee, H., Jeong, S., Kim, M.Y., & Yoon, M. (2008). Regulation of obesity and lipid disorders by herbal extracts from *Morus alba, Melissa officinalis*, and *Artemisia capillaris* in high-fat diet-induced obese mice. *Journal of ethnopharmacology, 115*(2), 263-270.

Lee, S., Faustman, C., Djordjevic, D., Faraji, H., & Decker, E. (2006). Effect of antioxidants on stabilization of meat products fortified with n-3 fatty acids. *Meat Science, 72*(1), 18-24.

López, V., Akerreta, S., Casanova, E., Garcia-Mina, J., Cavero, R., & Calvo, M. (2007). In vitro antioxidant and anti-rhizopus activities of lamiaceae herbal extracts. *Plant foods for human nutrition, 62*(4), 151-155.

López, V., Martin, S., Gomez-Serranillos, M., Carretero, M., Jager, A., & Calvo, M. (2009). Neuroprotective and Neurological Properties of *Melissa officinalis*. *Neurochemical research, 34*(11), 1955-1961.

López-López, I., Cofrades, S., Ruiz-Capillas, C., & Jimenez-Colmenero, F. (2009). Design and nutritional properties of potential functional frankfurters based on lipid formulation, added seaweed and low salt content. *Meat Science, 83*(2), 255-262.

Maqsood, S., & Benjakul, S. (2010). Comparative studies of four different phenolic compounds on in vitro antioxidative activity and the preventive effect on lipid oxidation of fish oil emulsion and fish mince. *Food Chemistry, 119*(1), 123-132.

Martín, D., Ruiz, J., Kivikari, R., & Puolanne, E. (2008). Partial replacement of pork fat by conjugated linoleic acid and/or olive oil in liver pates: Effect on physicochemical characteristics and oxidative stability. *Meat Science, 80*(2), 496-504.

Muguerza, E., Gimeno, O., Ansorena, D., & Astiasaran, I. (2004). New formulations for healthier dry fermented sausages: a review. *Trends in food science technology, 15*(9), 452-457.

Nowak, B., von Mueffling, T., Grotheer, J., Klein, G., & Watkinson, B. (2007). Energy content, sensory properties, and microbiological shelf life of German bologna-type sausages produced with citrate or phosphate and with inulin as fat replacer. *Journal of Food Science, 72*(9), S629-S638.

Pelser, W., Linssen, J., Legger, A., & Houben, J. (2007). Lipid oxidation in n-3 fatty acid enriched Dutch style fermented sausages. *Meat Science, 75*(1), 1-11.

Pereira, R., Fachinetto, R., Prestes, A., Puntel, R., da Silva, G., & Heinzmann, B. (2009). Antioxidant Effects of Different Extracts from *Melissa officinalis, Matricaria recutita* and *Cymbopogon citratus*. *Neurochemical research, 34*(5), 973-983.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology medicine, 26*(9-10), 1231-1237.

Roset, M. A. (2010). Actualidad en omega-3: recomendaciones, productos que lo consumen y dosis adecuadas. *Editorial del Boletin Informativo de la Sociedad Española de Nutrición, 56*, 1-2.

Sacchetti, G., Di Mattia, C., Pittia, P., & Martino, G. (2008). Application of a radical scavenging activity test to measure the total antioxidant activity of poultry meat. *Meat Science, 80*(4), 1081-1085.

Sasse, A., Colindres, P., & Brewer, M. (2009). Effect of Natural and Synthetic Antioxidants on the Oxidative Stability of Cooked, Frozen Pork Patties. *Journal of Food Science, 74*(1), S30-S35.

Sieri, S., Krogh, V., Ferrari, P., Berrino, F., Pala, V., Thiebaut, A.C.M., *et al.* (2008). Dietary fat and breast cancer risk in the European Prospective Investigation into Cancer and Nutrition. *American Journal of Clinical Nutrition, 88*(5), 1304-1312.

Simopoulos, A. (1997). Nutrition tid-bites - Essential fatty acids in health and chronic disease. *Food Reviews International, 13*(4), 623-631.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy, 56*(8), 365-379.

Siri-Tarino, P. W., Sun, Q., Hu, F. B., & Krauss, R. M. (2010). Saturated Fatty Acids and Risk of Coronary Heart Disease: Modulation by Replacement Nutrients. *Current atherosclerosis reports, 12*(6), 384-390.

UE REGULATION (CE). (2010). No 116/2010 of 9 February 2010 amending Regulation (EC) No 1924/2006 of the European Parliament and of the Council with regard to the list of nutrition claims. *Official Journal of the European Union, L(404)*, 16-18.

Ulbricht, T., & Southgate, D. (1991). Coronary heart-disease - 7 dietary factors. *Lancet, 338*(8773), 985-992.

Valencia, I., Ansorena, D., & Astiasarán, I. (2007). Development of dry fermented sausages rich in docosahexaenoic acid with oil from the microalgae Schizochytrium sp.: Influence on nutritional properties, sensorial quality and oxidation stability. *Food Chemistry, 104*(3), 1087-1096.

Valencia, I., Ansorena, D., & Astiasarán, I. (2006). Nutritional and sensory properties of dry fermented sausages enriched with n-3 PUFAs. *Meat Science, 72*(4), 727-733.

Valencia, I., O'Grady, M., Ansorena, D., Astiasarán, I., & Kerry, J. (2008). Enhancement of the nutritional status and quality of fresh pork sausages following the addition of linseed oil, fish oil and natural antioxidants. *Meat Science, 80*(4), 1046-1054.

Wheatley, D. (2005). Medicinal plants for insomnia: a review of their pharmacology, efficacy and tolerability. *Journal of psychopharmacology, 19*(4), 414-421.

Wood, J., Richardson, R., Nute, G., Fisher, A., Campo, M., Kasapidou, E., Sheard, P., & Enser, M. (2004). Effects of fatty acids on meat quality: a review. *Meat Science, 66*(1), 21-32.

Wu, C., Duckett, S., Neel, J., Fontenot, J., & Clapham, W. (2008). Influence of finishing systems on hydrophilic and lipophilic oxygen radical absorbance capacity (ORAC) in beef. *Meat Science, 80*(3), 662-667.

Zandi, P., & Ahmadi, L. (2000). Antioxidant effect of plant extracts of Labiatae family. *Journal of food science and technology, 37*(4), 436-439.

Zhou, S., & Decker, E. (1999). Ability of carnosine and other skeletal muscle components to quench unsaturated aldehydic lipid oxidation products. *Journal of Agricultural and Food Chemistry, 47*(1), 51-55.

Table 1. Formulation of the four types of Bologna-type sausages.

INGREDIENTS	CONTROL	MODIFIED PRODUCTS		
		LINSEED	BHA	MELISSA
Pork meat (%)	55	55	55	55
Pork fat (%)	35	26.25	26.25	26.25
Ice (%)	10	10	10	10
Linseed oil (%)	0	8.75	8.75	8.75
Melissa (ppm)	0	0	0	965
BHA (ppm)	0	0	200	0
Iodized NaCl (g/kg)	26	26	26	26
Powdered milk (g/kg)	12	12	12	12
Garlic (g/kg)	3	3	3	3
Curavi[1] (g/kg)	3	3	3	3
Polyphosphates[2] (g/kg)	2	2	2	2
Sodium ascorbate (g/kg)	0.5	0.5	0.5	0.5
BDRom Carne (g/kg)	1	1	1	1
Monosodium glutamate (g/kg)	1	1	1	1
Carmin de Cochenille 50% (E-120) (g/kg)	0.1	0.1	0.1	0.1

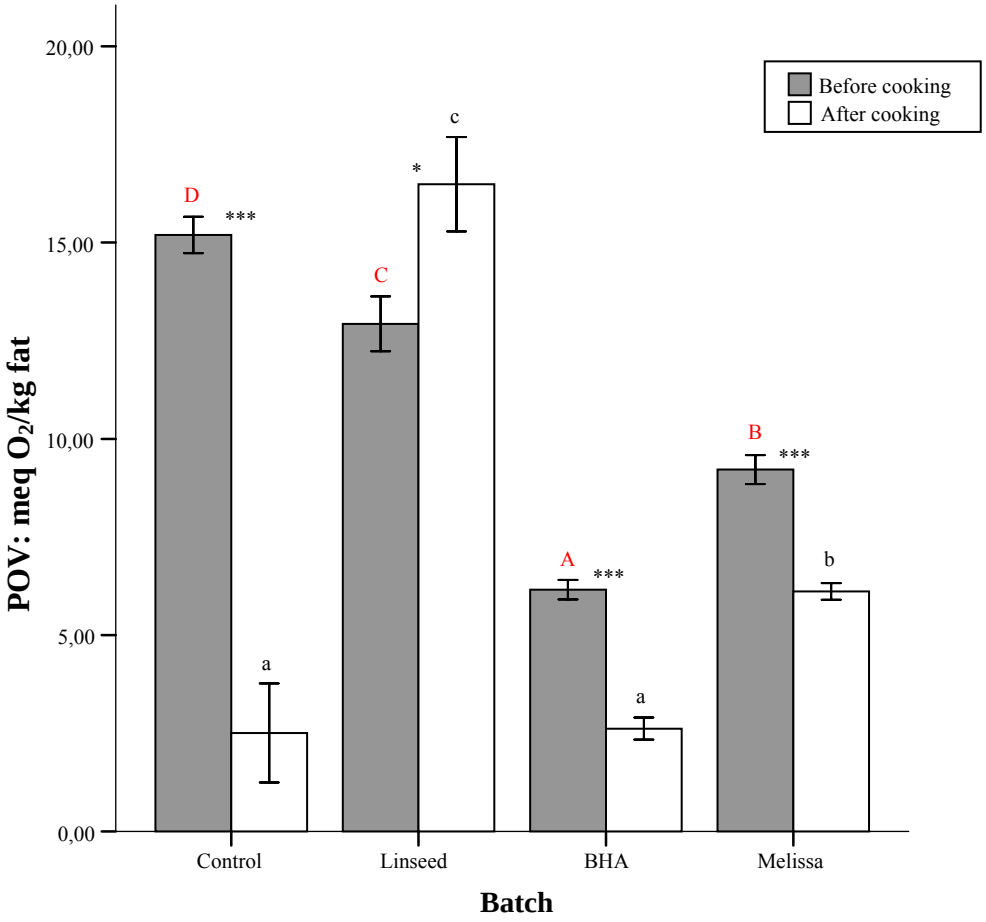
[1]*Curavi: a mixture of curing agents: NaCl, E-250, E-252 and antioxidant E-331.*
[2] *Mixture of E-430i, E-454i and E-451i.*

Table 2. Fatty acid composition of the four types of bologna-type sausages (g/100g fatty acids mean ± standard deviation).

	CONTROL[1]	LINSEED[1]	BHA[1]	MELISSA[1]	LS[2]
Caprilic C8:0	0.14 ± 0.00^{c}	0.14 ± 0.00^{b}	0.13 ± 0.00^{a}	0.13 ± 0.00^{a}	***
Capric C10:0	0.16 ± 0.00^{b}	0.16 ± 0.00^{c}	0.15 ± 0.00^{a}	0.16 ± 0.00^{b}	***
Lauric C12:0	0.07 ± 0.00^{a}	0.08 ± 0.00^{d}	0.07 ± 0.00^{b}	0.08 ± 0.00^{c}	***
Myristic C14:0	1.20 ± 0.01^{c}	1.08 ± 0.00^{b}	0.97 ± 0.01^{a}	1.07 ± 0.01^{b}	***
Palmitic C16:0	22.71 ± 0.06^{d}	19.95 ± 0.03^{c}	19.75 ± 0.01^{b}	19.67 ± 0.05^{a}	***
t-Palmitoleic C16:1t	0.27 ± 0.16	0.35 ± 0.00	0.34 ± 0.01	0.35 ± 0.02	ns
Palmitoleic C16:1	2.16 ± 0.01^{d}	2.05 ± 0.01^{c}	1.90 ± 0.01^{a}	1.95 ± 0.03^{b}	***
Stearic C18:0	11.30 ± 0.02^{d}	10.06 ± 0.05^{a}	10.74 ± 0.02^{c}	10.20 ± 0.03^{b}	***
Elaidic C18:1t	0.37 ± 0.00^{c}	0.31 ± 0.01^{a}	0.34 ± 0.01^{b}	0.35 ± 0.01^{b}	***
Oleic C18:1 (ω-9)	39.65 ± 0.06^{d}	35.46 ± 0.04^{a}	36.95 ± 0.05^{c}	36.18 ± 0.05^{b}	***
Vaccenic C18:1 (ω-7)	3.24 ± 0.02^{c}	2.78 ± 0.01^{b}	2.73 ± 0.01^{a}	2.81 ± 0.03^{b}	***
t-Linoleic C18:2t	0.14 ± 0.00^{c}	0.09 ± 0.00^{b}	0.08 ± 0.00^{a}	0.09 ± 0.00^{b}	***
c-t linoleic C18:1c.1t	0.16 ± 0.08^{b}	0.04 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	**
t-c linoleic C18:1t.1c	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	ns
Linoleic C18:2 (ω-6)	16.10 ± 0.03^{d}	16.77 ± 0.03^{b}	15.95 ± 0.02^{a}	16.54 ± 0.03^{c}	***
Arachidic C20:0	0.01 ± 0.04^{a}	0.04 ± 0.00^{a}	0.08 ± 0.00^{b}	0.07 ± 0.00^{b}	**
γ-linolenic C18:3 (ω-6)	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	ns
Eicosenoic C20:1 (ω-9)	0.70 ± 0.01^{b}	0.57 ± 0.00^{a}	0.70 ± 0.00^{b}	0.70 ± 0.01^{b}	***
α-linolenic C18:3 (ω-3)	0.95 ± 0.01^{a}	9.34 ± 0.01^{d}	8.33 ± 0.02^{b}	8.84 ± 0.02^{c}	***
Eicosadienoic C20:2 (ω -6)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	***
Behenic C22:0	0.08 ± 0.00^{a}	0.08 ± 0.00^{ab}	0.08 ± 0.00^{bc}	0.08 ± 0.00^{c}	**
Brasidic C20:1t	0.01 ± 0.00^{a}	0.01 ± 0.00^{c}	0.01 ± 0.00^{b}	0.01 ± 0.00^{ab}	***
Erucic C22:1	0.12 ± 0.00^{a}	0.11 ± 0.00^{a}	0.13 ± 0.00^{b}	0.13 ± 0.00^{b}	***
Eicosatrienoic C20:3 (ω-3)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	***
Arachidonic C20:4 (ω-6)	0.34 ± 0.04	0.38 ± 0.01	0.36 ± 0.00	0.41 ± 0.10	ns
SFA	35.66 ± 0.10^{c}	31.59 ± 0.08^{a}	31.98 ± 0.02^{b}	31.46 ± 0.08^{a}	***
MUFA	45.87 ± 0.07^{d}	40.98 ± 0.03^{a}	42.43 ± 0.06^{c}	41.76 ± 0.05^{b}	***
PUFA	17.43 ± 0.06^{a}	26.53 ± 0.05^{d}	24.68 ± 0.04^{b}	25.83 ± 0.09^{c}	***
ω-3	0.95 ± 0.01^{a}	9.34 ± 0.01^{d}	8.33 ± 0.02^{b}	8.84 ± 0.02^{c}	***
ω-6	16.48 ± 0.06^{b}	17.19 ± 0.04^{d}	16.35 ± 0.02^{a}	16.99 ± 0.10^{c}	***
ω-6/ω3	17.27 ± 0.14^{b}	1.84 ± 0.00^{a}	1.96 ± 0.00^{a}	1.92 ± 0.02^{a}	***
PUFA/SFA	0.49 ± 0.00^{a}	0.84 ± 0.00^{d}	0.77 ± 0.00^{b}	0.82 ± 0.00^{c}	***
PUFA+MUFA/SFA	1.78 ± 0.00^{a}	2.14 ± 0.01^{c}	2.10 ± 0.00^{b}	2.15 ± 0.01^{d}	***
trans	1.04 ± 0.20	0.91 ± 0.01	0.92 ± 0.01	0.95 ± 0.01	***
AI	0.38 ± 0.00^{d}	0.31 ± 0.00^{c}	0.31 ± 0.00^{b}	0.31 ± 0.00^{a}	***
TI	1.03 ± 0.00^{d}	0.54 ± 0.00^{a}	0.57 ± 0.00^{c}	0.55 ± 0.00^{b}	***

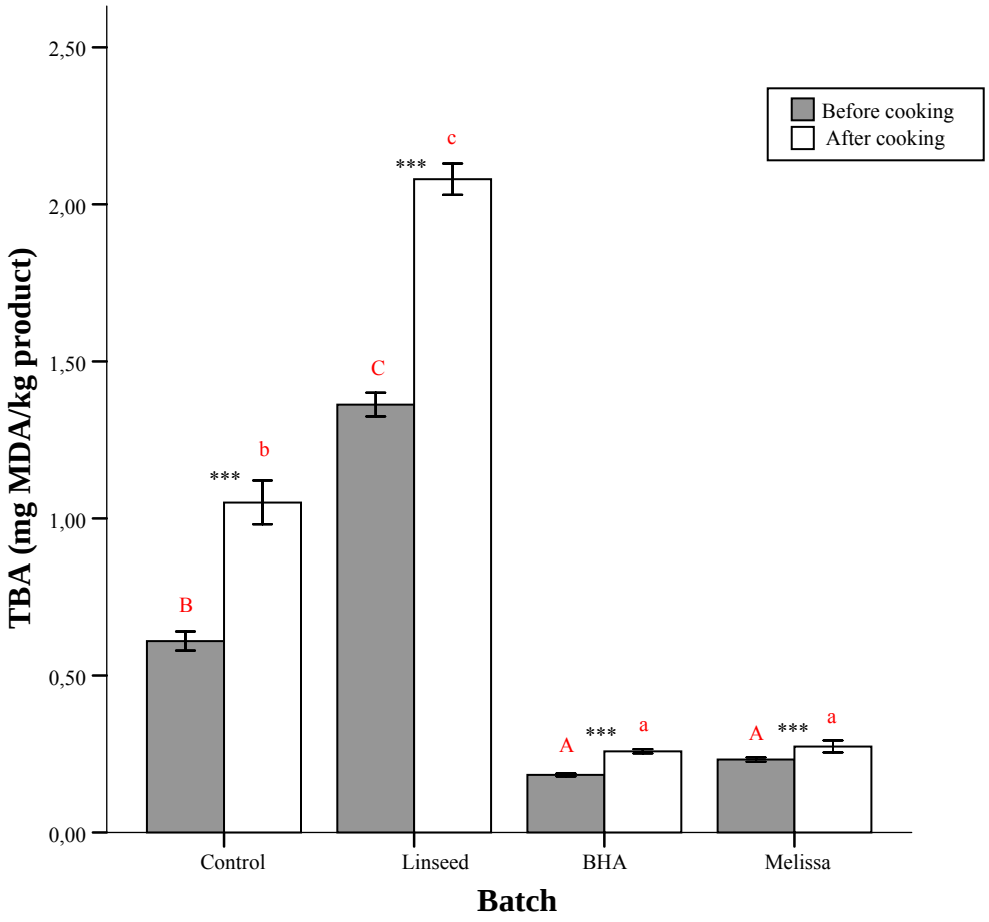
[1]*Different letters denote significant differences among samples ($p<0.05$).* [2]*LS (level of significance of the ANOVA): ns (not significant) $p > 0.05$; ** $p < 0.01$; *** $p < 0.001$. AI: atherogenic index; TI: thrombogenic index.*

Figure 1. Peroxide values (POV) before and after the cooking process of the four types of Bologna-type sausages (meq O_2/kg fat).



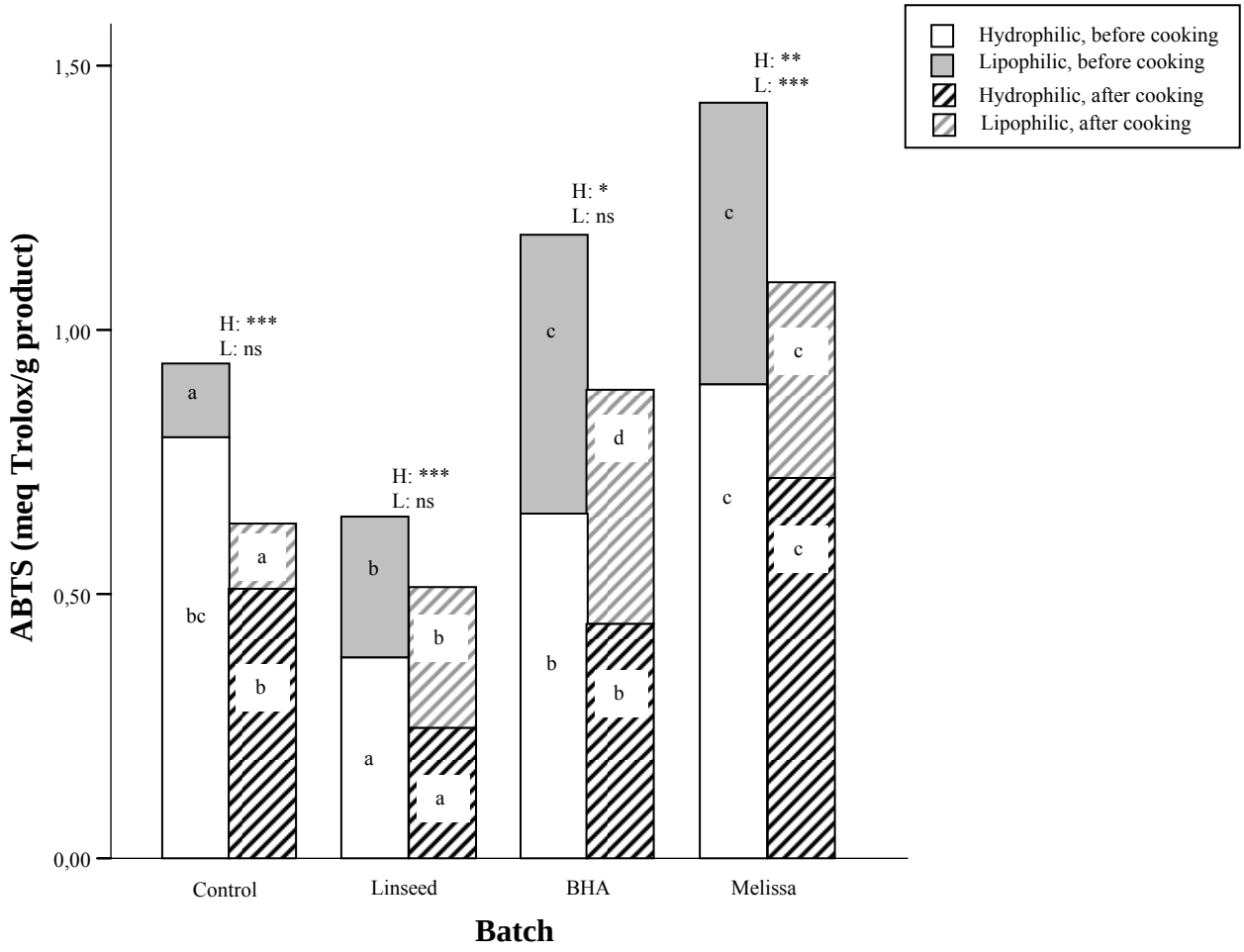
*Standard deviation bars are indicated (n = 3). Level of significance for the Student t test that compare products before and after cooking: * p < 0.05; *** p < 0.001. Different capital letters denote significant differences among batches before cooking and different lowercase letters denote significant differences among batches after cooking (p<0.05).*

Figure 2. TBARs values before and after the cooking process of the four types of Bologna-type sausages (mg malondialdehyde/kg product).



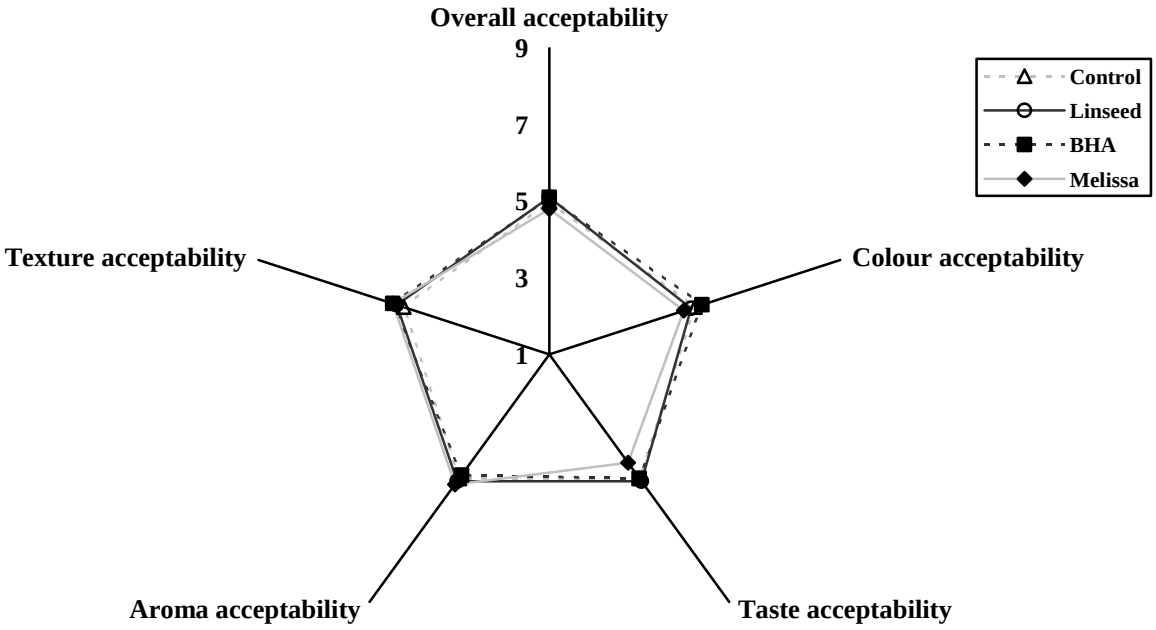
*Standard deviation bars are indicated (n = 3). Level of significance for the Student t test that compare products before and after cooking: ***; p < 0.001. Different capital letters denote significant differences among batches before cooking and different lowercase letters denote significant differences among batches after cooking (p<0.05).*

Figure 3. ABTS before and after the cooking process of the four types of Bologna-type sausages (meq Trolox/g product).



*Within each legend condition different letters denote significant differences among batches. Level of significance for the Student t test compare products before and after cooking (H: hydrophilic samples; L: lipophilic samples).: ns (not significant) p > 0.05; * p < 0.05; **p < 0.01; *** p < 0.001.*

Figure 4. Results of the hedonic sensorial analysis test.



Control samples are given a 5 point score for every attribute. Modified samples are scored by panellists between 1 and 9.

FIGURE CAPTIONS

Figure 1. Peroxide values (POV) before and after the cooking process of the four types of Bologna-type sausages (meq O_2/kg fat).

Figure 2. TBARs before and after the cooking process of the four types of Bologna-type sausages (mg malondialdehyde/kg product).

Figure 3. ABTS before and after the cooking process of the four types of Bologna-type sausages (meq Trolox/g product).

Figure 4. Results of the hedonic sensorial analysis test.

TABLE CAPTIONS

Table 1. Formulation of the four types of Bologna-type sausages.

Table 2. Fatty acid composition of the four types of Bologna-type sausages (g/100g fatty acids. mean ± standard deviation).