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Healthy reduced-fat Bologna sausages enriched in ALA and DHA and stabilized with *Melissa officinalis* extract

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ABSTRACT

Reduced-energy and reduced-fat Bologna products enriched with ω-3 polyunsaturated fatty acids were formulated by replacing the pork back-fat by an oil-in-water emulsion containing a mixture of linseed-algae oil stabilized with a lyophilized *Melissa officinalis* extract. Healthier composition and lipid profile was obtained: 85 kcal/100 g, 3.6% fat, 0.6 g ALA and 0.44 g DHA per 100 g of product and ω-6/ω-3 ratio of 0.4. Technological and sensory problems were not detected in the new formulations. Reformulation did not cause oxidation problems during 32 days of storage under refrigeration. The results suggest that it is possible to obtain reduced-fat Bologna-type sausages rich in ALA and DHA and stabilized with natural antioxidants, applying the appropriate technology without significant effects on the sensory quality, yielding interesting products from a nutritional point of view.

1. Introduction

Among the vegetable oils with a high content of α-linolenic acid (ALA), are those obtained from linseed (*Linum usitatissimum* L.), chia (*Salvia hispanica* L.) and perilla (*Perilla frutescens*) seeds, and linseed oil has been the most frequently used to improve the lipid profile of different foods. According to the Dietary Guidelines for Americans (2010), plant sources of ω-3 fatty acids cannot be considered as replacers for seafood-derived ω-3 polyunsaturated fatty acids (PUFAs), which are rich in docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), long chain ω-3 PUFAs. Health-related associations have established specific recommendations for DHA and EPA. Current recommendation from the American Heart Association includes consumption of DHA and EPA fatty acids of 1 g/day to reduce risk of cardiovascular diseases (Kris Etherton et al., 2012). Meanwhile, the European Food Safety Authority (EFSA) set Dietary Reference Values (DRV) of 250 mg for EPA plus DHA for adults (EFSA, 2009).

Algae oil has been described as one of the best natural sources of DHA, reaching in certain types of microalgae values up to 45% of total fatty acids (Anderson & Ma, 2009; Astiasarán & Ansorena, 2009; Mozaffarian & Wu, 2011). The use of algae oil from the micro-algaes *Ulkenia* sp. and *Schizochytrium* sp. as a novel food ingredient was recently regulated (European Commission, 2009a, 2009b). Different works have studied the successful incorporation of algae oil in products such as eggs (Sedoski, Beamer, Jaczynski, Partington, & Matak, 2012), surimi (Pietrowski, Tahergorabi, Matak, Tou, & Jaczynski, 2011), dry fermented-sausages (García-Íñiguez de Ciriano et al., 2010), yoghourt and milk (Chee et al., 2005).

Lipids are the bioactive components that have received most attention in the development of healthier meat products (Delgado-Pando, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, 2010, Delgado-Pando, Cofrades, Ruiz-Capillas, Teresa Solas, & Jiménez-Colmenero, 2010, Delgado-Pando et al., 2011). Decreasing the amount of fat or changing the characteristics of the lipid fraction has been attempted. However, the production of reduced-fat meat products may cause technological problems due to the fact that fat affects the flavour, palatability and texture of foods (Delgado-Pando, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, 2010; Delgado-Pando, Cofrades, Ruiz-Capillas, Teresa Solas et al., 2010, Horita, Morgano, Celeghini, & Pollonio, 2011; Hort & Cook, 2007). In order to solve this problem, different fat replacers have been tested, carrageenan providing good results for maintaining textural properties in different meat products (Ayadi, Kechaou, Makni, & Attia, 2009; Candogan & Kolsarici, 2003; Cierach, Modzelewska-Kapitula, & Szacilo, 2009; Kumar & Sharma, 2004). In fact, it is possible to develop Bologna-type sausages with very

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low amounts of pork back-fat using different emulsifiers (Omana, Pietrasik, & Betti, 2012; Sanjeewa, Wanasundara, Pietrasik, & Shand, 2010).

The lipid profile of cooked meat products can be modified by reformulation strategies (Jiménez-Colmenero et al., 2012; Trindade et al., 2011), including linseed oil, rich in α-linolenic acid (Berasategi et al., 2011; Delgado-Pando et al., 2012a, 2012b) in the formulations. However, to our knowledge, there are no studies where this enrichment is carried out with DHA and/or EPA.

In addition, the increase in long chain unsaturated fatty acids could increase lipid oxidation, which might cause sensory problems. It has demonstrated that it is possible to develop new stable high fat meat products rich in ω-3 PUFA by adding synthetic antioxidants such as butylhydroxyanisol (BHA) (Lee, Faustman, Djordjevic, Faraji, & Decker, 2006; Valencia, Ansorena, & Astiasarán, 2006a,b, 2007). However, public attitude towards these substances is not as positive as that for natural ones, which are well appreciated (García-Íñiguez de Ciriano et al., 2009; Johnston, Sepe, Miano, Brannan, & Alderton, 2005). In this sense, different methanolic, ethanolic and water extracts obtained from *Melissa officinalis* have shown antioxidant properties in vitro (Lopez et al., 2007; Zandi & Ahmadi, 2000), mainly due to the presence of flavonoids and hydroxycinnamic acid derivatives, known for their antioxidant capacity, with rosmarinic acid the major component (Dastmalchi et al., 2008). Some of these extracts efficiently controlled lipid oxidation in meat products with high fat contents (Berasategi et al., 2011; García-Íñiguez de Ciriano et al., 2010).

The objective of this study was to assess the effectiveness of a natural antioxidant (lyophilized extract of *M. officinalis*) as compared to a synthetic one (BHA) in reduced-fat Bologna formulations enriched in ALA and DHA.

2. Material and methods

2.1. Materials

Pork shoulder-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 algae oil (DHASCO®-S) was purchased from Martek Bioscience Corporation (Columbia, USA). BDRom Carne (a mixture of typical aromatic compounds) and the red colourant Carmin de Cochenille 50% (E-120) were obtained from BDF Natural Ingredients S.L. (Girona, Spain). Satiagel (a refined mixture of kappa and iota carrageenan) was donated by Cargill S.L.U. (Martorell, Barcelona, Spain). Curavi (a mixture of curing agents: sodium chloride, sodium nitrite (E-250), sodium nitrate (E-252) and sodium citrate (E-331)) and the rest of additives and spices used were kindly donated by ANVISA (Arganda del Rey, Madrid, Spain). All the chemical reagents were obtained from Sigma-Aldrich Chemical Co. (MO, USA).

2.2. Sausage formulation and processing

Three different formulations of reduced-fat (RF) Bologna-type sausages were manufactured in a pilot plant (Table 1). 5% pork back-fat was the only lipid source in low-fat control products (LF-Control) whereas the two modified batches substituted the pork back-fat by an oil-in-water (O/W) emulsion rich in ω-3 fatty acids. The emulsion was prepared as described by García-Íñiguez de Ciriano et al. (2010), using a mixture of linseed and algae oils (1:1). RF-control type was produced without the addition of extra antioxidants. According to previous experiments (Berasategi et al., 2011), addition of extra antioxidants is needed when cooked meat products are made with high PUFA fat sources. So, RF-BHA (200 ppm of BHA), and RF-melisa (200 ppm of *M. officinalis* lyophilized aqueous extract) were added.

All ingredients (including the emulsion, when used) were thoroughly minced in a chilled cutter for 1 min at low speed, and for 2 min at high speed until complete emulsification of the mixture was obtained. After the application of a vacuum process to exclude oxygen from the mixture (2 min), the batters were stuffed in 6 cm diameter water impermeable plastic casings. Sausages were cooked in a water bath at 80 °C for 1 h, until the core of the product reached 72 °C. Once heating was completed, the sausages were immediately cooled in a water bath for 2 h and stored under refrigeration (4 °C) until analysis. The experiment was carried out in triplicate.

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

Ingredients	RF-control	RF-BHA	RF-melisa
Pork meat (%)	55	55	55
Pork back-fat (%)	5	0	0
Ice (%)	40	40	40
Emulsion (%)	0	5	5
Linseed oil (%)		1.32	1.32
Algae oil (%)		1.32	1.32
Soy protein (%)		0.26	0.26
Water (%)		2.11	2.11
Iodized NaCl (g/kg)	26	26	26
Powdered milk (g/kg)	12	12	12
Garlic (g/kg)	2	2	2
Curavi[a] (g/kg)	3	3	3
Polyphosphates[b] (g/kg)	2	2	2
Sodium ascorbate (g/kg)	0.5	0.5	0.5
BDRom Carne (g/kg)	1	1	1
Monosodium glutamate (g/kg)	1	1	1
Carmin de Cochenille 50% (E-120) (g/kg)	0.1	0.1	0.1
Carrageenan (g/kg)	10	10	10
BHA (ppm)		200	
Melissa officinalis (ppm)			200

[a] Curavi: a mixture of curing agents: sodium chloride, sodium nitrite (E-250), sodium nitrate (E-252) and sodium citrate (E-331).
[b] Mixture of E-430i, E-454i and E-451i.

2.3. M. officinalis aqueous extract

2.3.1. Preparation of the extract

A lyophilized hydro-alcoholic extract of *M. officinalis* was prepared as described by Berasategi et al. (2011). A purification of this extract was done to increase its antioxidant capacity. Thus, the lyophilized hydro-alcoholic extract was washed with ethanol three times and the solid residue was redissolved in water. The aqueous extract was lyophilized with a freeze-dryer cryodo (Telstar, Barcelona, Spain), after freezing at −80 °C in a MDF-V5386S Ultra-Low-Temperature Freezer (Sanyo Electric Co., Ltd., Japan). The antioxidant capacity of the aqueous extract was evaluated using DPPH and ORAC methods.

2.3.2. Characterization of antioxidant capacity

2.3.2.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The DPPH assay was performed according to García-Íñiguez de Ciriano et al. (2010). Results were expressed as mg Trolox/g lyophilized extract of *M. officinalis*. Absorbance measurements were done in duplicate for each dilution of lyophilized extract.

2.3.2.2. Oxygen Radical Absorbance Capacity ($ORAC_{FL}$) Assay. $ORAC_{FL}$ assays were performed similarly to those described by Ou, Hampsch-Woodill, and Prior (2001). *M. officinalis* lyophilized (5 g) water extract was dissolved in 50 mL of phosphate buffer, 10 mM. A 0.5 M stock solution of Trolox was prepared in 10 mM phosphate buffer, and divided into 1 mL aliquots, which were stored at −20 °C until use. A new set of stock Trolox vials were taken from the freezer daily for the preparation of the calibration curve and the quality controls (12.5 and 50 μM). The phosphate buffer solution was used as blank, to dissolve the Trolox quality controls and to prepare the samples. To conduct the ORAC assay, 40 μL of the sample and 120 μL of the fluorescein solution (132.5