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

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

1. Introduction

Melissa officinalis is one of the most used medicinal plants. A mixture of *M. 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 et al. 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 et al. (2008) establishing the chemical composition and the *in vitro* antioxidative activity of a *M. 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, 2007). 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, Sun, Hu, & Krauss, 2010; Gonzalez & 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 et al., 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, et al., 2010; García-Íñiguez de Ciriano, Rehecho, et al., 2010). 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), and sodium citrate, have been proved to be efficient

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and needed for stabilizing meat products rich in long chain ω-3 PUFAs (Lee et al., 2006; Muguerza, Gimeno, Ansorena, & Astiasaran, 2004; Valencia, Ansorena, & Astiasarán, 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 et al., 2009; Sasse, Colindres, & Brewer, 2009). DeJong and 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 *M. officinalis* have been already pointed out (Zandi & Ahmadi, 2000; Ferreira, Proenca, Serralheiro, & Araujo, 2006; López et al. 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 et al., 2008).

García-Íñiguez de Ciriano, Larequi, et al. (2010) proved that a lyophilized aqueous extract of *M. 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, Rehecho, et al., 2010). Pereira et al. (2009) obtained lower amounts of phenolic compounds in ethanolic and methanolic extracts than in aqueous extracts from *M. officinalis*. However, it is well known that the different extraction conditions lead 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 et al., 2010).

Bologna-type sausages are subjected to a pasteurization process during processing, reaching T^a of 72–75 °C 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 et al. (2009) showed that heating process, 80 °C during 1 h, applied to cooked meat products (reaching 70 °C 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 assess 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 *M. 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).

2.1.1. Preparation of the lyophilized aqueous-ethanolic extract of M. officinalis

Aqueous-ethanolic extracts of *M. 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 min. 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 −80 °C in a MDF-V5386S Ultra-Low-Temperature Freezer (Sanyo Electric Co., Ltd., Japan). 23 g of lyophilized material was 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). Linseed oil emulsion was prepared as described in Valencia et al. (2007).

All ingredients were thoroughly minced in a chilled cutter for 1 min at low speed and 2 min at high speed until a complete emulsification of the mixture was obtained. After the application of a vacuum process to exclude oxygen from the mixture for 2 min, the batters were stuffed in 6 cm diameter water impermeable plastic casings. 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 (−20 °C) and kept under vacuum until analysis. 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 complete, the sausages were immediately cooled in a water bath for 2 h and stored frozen (−20 °C) under vacuum till analysis. The experiment was done in triplicate.

2.3. Chemical analysis

2.3.1. Characterization of antioxidant capacity of the lyophilized extract

2.3.1.1. Determination of Total phenolic content (TPC). TPC of the lyophilized aqueous-ethanolic extract of *M. officinalis* was determined spectrophotometrically following the Folin–Ciocalteu colorimetric method as described in Garcia-Hereros, Garcia-Iniguez de Ciriano, Astiasarán, and 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

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 emulsion (%)	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[a] (g/kg)	3	3	3	3
Polyphosphates[b] (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

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