Stabilization of dry fermented sausages enriched in α-linolenic acid and Docosahexaenoic acid by a lyophilized antioxidant extract of *Melissa officinalis* L.

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Abstract— **OBJECTIVE A pre-emulsified mixture of linseed and algae oils (15/10) and stabilized with 686 ppm of a lyophilized water extract of *Melissa officinalis*, was successfully applied in dry fermented sausages to increase the ω-3 PUFA content (García-Íñiguez de Ciriano et al., 2010).The objective of this work was to evaluate the stability of the modified formulation (Modified) during the storage and to compare it to that of a traditional formulation (Control). EXPERIMENTAL DESIGN Control and Modified products were stored during 90 days at 4º C in aerobic conditions. Fatty acid profiles, TBARS and volatile compounds derived from oxidation were analyzed at 0, 30 days and 90 days of storage. RESULTS & CONCLUSION The fatty acid profiles did not significantly change along the storage period. The stabilizing effect of the natural antioxidants of *M. officinalis* could contribute to detect no losses of ω-3 PUFA in Modified (30 days: 2.13 g/100 g of product, 90 days: 2.33 g/100 g of product), whereas in Control products a slightly significant reduction was detected (30 days: 0.34 g/100 g of product 90 days: 0.29 g/100 g of product). After 90 days, the increases of TBARS and hexanal content were much higher in Control than in Modified (Control: 1.41 mg MDA/kg & 17915 ng dodecane/kg of dry matter; Modified: 0.48 mg MDA/kg & 2496 ng dodecane/kg of dry matter). In conclusion, the lyophilized water extract of *M. officinalis* protected high ω-3 PUFA of dry fermented sausages from oxidation along the storage time, guaranteeing the nutritional improvements achieved with the modified formulation.**

Index Terms— Dry fermented sausages; Oxidative stability; *Melissa officinalis* L.

I. INTRODUCTION

In the last years many authors have developed strategies to obtain new formulations of healthier meat products trying to improve their lipid fraction (Ansorena et al., 2004a & 2004b; Raes et al., 2004; Haak et al., 2006;Valencia et al., 2006a & 2007; Cáceres et al., 2008; García-Íñiguez de Ciriano et al., 2009 & 2010b; Berasategi et al., 2011). The lipid profile modification implies an increase of the poly-unsaturated (PUFA) fraction, specially of the ω-3 PUFA content, leading to a lower ω-6/ω-3 ratio, in order to achieve the current recommendations (1 to 4, WHO, 2003), and consequently, obtaining some beneficial effects as protective role against cardiovascular risk factors, inflammatory effects, sudden death, obesity, insulin-resistance and mental illness (Ruxton et al., 2004; Ross et al., 2007; Lee et al., 2004; Simopoulos et al., 2009; Okuyama, 2001; Myhrstad et al., 2011; Bei et al., 2011).

However, the inclusion of a higher PUFA content involves a higher lipid oxidation susceptibility, developing off-flavour volatile compounds deteriorating the quality, color and even the nutritional value of the products (Byrne et al., 2001; Rymer et al., 2005). Consequently, the acceptability of the meat and meat products could be compromised (Morrisey et al., 1998).

With the aim of preventing the oxidation of meat products, compounds with antioxidant activity should be included in the formulation, being an effective mean for delaying lipid oxidation (Alamed et al., 2006). Ansorena et al. (2004b) in a previous work used BHA+BHT as inhibitors of the lipid oxidation in dry fermented sausages enriched in ω-3 PUFA. In the last years, the use of natural sources of antioxidants against the development of rancidity in food and also, in the protection on the human health, are being studied as an interesting approach in food research (Gungaard et al., 2003; Gosslau et a., 2004; Naczk et al., 2006). Food industry has shown great interest on vegetable extracts from medicinal plants, spices, fruits and edible plants due to their technological and healthy roles. These products, in some cases, had shown a greater antioxidant efficacy than synthetic antioxidants, which is attributed to their content in antioxidant compounds such as polyphenols, lycopene and α-Tocopherol. (Khokhar et al., 2002; Yanishlieva et al., 2006, Calvo et al., 2008, García-Herreros et al., 2009; García-Íñiguez de Ciriano et al., 2009 & 2010a).

The objective of this work was to evaluate the stability during storage of dry fermented sausages enriched in ω-3 PUFA by means of a pre-emulsified mixture of linseed and algae oils and stabilized by a lyophilized water extract of *Melissa officinalis* L.

II. MATHERIAL & METHODS

Experimental design

686 ppm of a lyophilized water extract of *Melissa officinalis* L. were used for controlling the lipid oxidation of a pre-emulsified mixture of linseed and algae oils (15/10). According to the procedure described by García-Íñiguez de Ciriano et al. (2010b) this emulsion was used as a partial substitute of 25 % of the pork back fat in dry fermented sausages (Modified). A batch prepared according to a traditional formulation was used as Control (Control).

Control and Modified products were stored during 90 days at 4 ºC in aerobic conditions. Total fatty acid profiles, TBARS and volatile compounds derived from oxidation were analyzed at 0 (Final product), 30 days and 90 days of storage.

Determination of the total fatty acid profile

The method of Folch et al. (1957) was used for the extraction of lipids. Fatty acids were determined in the lipid extract by gas chromatography, previous methylation with boron trifluoride/methanol (AOAC, 2002). The quantification of fatty acids was based on the internal standard method, using heptadecanoic acid methyl ester (Sigma, St. Louis, MO, USA).

Determination of TBARS value

TBARS value was determined according to Tarladgis et al. (1960) with modifications by Tarladgis et al. (1964). Results are shown in mg malonaldehyde (MDA)/kg sample (ppm).

Volatile compounds from lipid oxidation

A Likens-Nickerson extraction followed by a GC-MS detection and quantification was carried out according to the method described by Ansorena et al. (1998). The results were expressed as nanograms of dodecane (Internal Standard) per gram of dry matter.

Statistical analysis

The experimental data were submitted to an ANOVA analysis at a 5 % level of significance, testing the differences among the different storage times with the Tuckey b post hoc test. The differences between batches were analyzed by a Student t test being the signification $p<0.05$(*), $p<0.01$(**), or $p<0.001$(***) (SPSS 15.0).

III. RESULTS & DISCUSSION

The enrichment of dry fermented sausages with linseed and algae oils increased their content on α-linolenic acid (ALA) and docosahexaenoic acid (DHA) in dry fermented sausages (García-Íñiguez de Ciriano et al., 2010b). The changes in the lipid profile of these products were analyzed during their storage period (table 1). After 30 days of storage, no significant differences were found for ALA compared to Final product in Modified batches, whereas a slight reduction was noticed after 90 days (1.6 %). Valencia et al. (2006b) did not find significant differences of ALA in dry fermented sausages enriched in linseed and stabilized with synthetic antioxidants (BHA+BHT) when they were stored aerobically from 2 to 5 month in aerobic condition. Besides, in the present work, DHA remained constant in the Modified batches (1.99±0.01 g/100 g of FAME) along the storage. Also, the total ω-6 content was not modified during the storage. In consequence, the interesting ω-6/ω-3 ratio observed in the final product (1.96), was kept below 2 until the end of storage, and significantly lower than that observed for Control products (mean =16). The different natural susceptibility of SFA and PUFA to oxidation might induce changes of the ratio PUFA/SFA along storage. However, this ratio remained constant as well, pointing to the high stability of the new formulation.

Results expressed on basis on 100 g product confirmed again the stability of the PUFA fraction in Modified products (figure 1). Even slight increases of ω-3, ALA and DHA were detected after 90 days compared to 30 days of storage, which could be attributed both to the maintenance of the fatty acids un-oxidized and to a slight loss of moisture during the aerobic conditions of storage.

In summary, the nutritional improvement of the new formulation reported by García-Íñiguez de Ciriano et al. (2010b) could be guaranteed along the self-life of the products, due to the adequate preservation of these compounds by the protective role of *M. officinalis*.

Although the modified formulation resulted in a healthier product along the time, it is greatly important to evaluate the stability problems related to the potential lipid oxidation that might overcome along the storage time. Results obtained from TBARS analysis did not show relevant oxidation problems in the tested experimental conditions (table 2). During the first 30 days, Modified batches showed higher values than Control batches. After 90 days of storage, the TBARS was only 0.48 ppm in Modified batches but reached 1.4 ppm in Control batches. Zanardi et al. (2002) reported mean values of 0.34 ppm in traditional dry fermented sausages after 60 days with a protective atmosphere, considering these values as acceptable in this type of products. In the present work, although under aerobic storage, the Modified batches showed after of 90 days acceptable values of TBARS, pointing out the protective activity of *Melissa officinalis* on these new formulations. Other vegetal sources of phenolic compounds as rapeseed and pine bark showed also an effective inhibitor effect of the formation of lipid oxidation products in cooked pork meat patties (Vuorela et al., 2005).

Following a similar trend, the volatile compounds related to the oxidation process showed an increase during the storage time for both products, which was more intense in Control products. Hexanal, the typical oxidation marker of linoleic acid, is considered a good indicator of the oxidation level (Shahidi et al., 1994). Hexanal only showed significant increases for both type of batches during the last 60 days of storage, being this increment much higher than in Modified batches. A similar fact occurred in dry fermented sausages enriched in linseed oil elaborated by Valencia et al. (2006b), where the hexanal content, after of 2 and 5 month of aerobic storage increased much less than in the traditional formulation. Regarding the total volatile compounds, a similar behavior was observed. Heptanal, octanal and nonanal showed along the time higher increases in the Control batch than in Modified. High hexanal values an other aldehydes with an important increase of TBARS value suggested that at 90th day the Control batch underwent significant oxidation process that could led the generation of compounds associated to rancid taste and odors (Ansorena et al., 2004a). No peaks of t,t-2,4-Heptadienal, t,t-2,4-Nonadienal, and low peaks of 2-Octenal and 2,4-Decadienal were detected in Modified batches. All these compounds are strongly related with the sensorial aspects, due to their off odour and low threshold (Frankel, 1998). In general, in the Modified products a slower rate formation of volatile lipid oxidation derived compound was detected, suggesting that the lyophilized water extract of *Melissa officinalis* performed a protect role along the storage time necessary to prolong the self life of the product.

In conclusion, the lyophilized water extract of *M. officinalis* protected ω-3 PUFA and long chain ω-3 PUFA of dry fermented sausages from oxidation along the storage time, guaranteeing the nutritional improvements achieved with the new formulation.

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Table 1.- Fatty acids, fatty acid fractions and important ratios obtained for Control and Modified dry fermented sausages along their aerobic storage at 4 ºC (g/100 g of FAME).

		Final Product	LS	30 days	LS	90 days	LS
α-linolenic C18:3(ω-3)	Control	0.92 ± 0.01^{b}	***	0.94 ± 0.02^{c}	***	0.85 ± 0.01^{a}	***
	Modified	4.82 ± 0.01^{b}		$4.75 \pm 0.08^{a,b}$		4.67 ± 0.01^{a}	
Docosahexaenoic C22:6(ω-3)	Control	0.05 ± 0.00^{b}	***	0.06 ± 0.00^{a}	***	0.05 ± 0.00^{b}	***
	Modified	2.00 ± 0.07^{a}		1.98 ± 0.08^{a}		1.98 ± 0.02^{a}	
SFA	Control	36.75 ± 0.18^{b}	***	36.50 ± 0.02^{a}	***	36.92 ± 0.03^{b}	***
	Modified	35.05 ± 0.04^{b}		34.94 ± 0.09^{a}		34.88 ± 0.02^{a}	
MUFA	Control	$45.41 \pm 0.25^{a,b}$	ns	45.25 ± 0.31^{a}	**	45.80 ± 0.05^{b}	***
	Modified	43.68 ± 0.07^{a}		43.95 ± 0.06^{b}		44.05 ± 0.02^{b}	
PUFA	Control	16.74 ± 0.06^{b}	***	17.16 ± 0.27^{c}	***	16.21 ± 0.02^{a}	***
	Modified	20.29 ± 0.04^{b}		$20.14 \pm 0.14^{a,b}$		20.10 ± 0.03^{a}	
ω-3	Control	1.00 ± 0.01^{b}	***	1.04 ± 0.02^{c}	***	0.92 ± 0.01^{a}	***
	Modified	6.86 ± 0.07^{b}		$6.78 \pm 0.08^{a,b}$		6.71 ± 0.02^{a}	
ω-6	Control	15.74 ± 0.05^{b}	***	16.12 ± 0.25^{c}	***	15.29 ± 0.02^{a}	***
	Modified	13.43 ± 0.03^{a}		13.36 ± 0.09^{a}		13.39 ± 0.01^{a}	
ω-6/ω-3	Control	15.69 ± 0.15^{a}	***	15.45 ± 0.14^{a}	***	16.60 ± 0.14^{b}	***
	Modified	1.96 ± 0.02^{a}		$1.97 \pm 0.02^{a,b}$		2.00 ± 0.01^{b}	
PUFA/SFA	Control	0.46 ± 0.00^{b}	***	0.47 ± 0.01^{c}	***	0.44 ± 0.00^{a}	***
	Modified	0.58 ± 0.00^{a}		0.58 ± 0.01^{a}		0.58 ± 0.00^{a}	
PUFA+MUFA /SFA	Control	1.69 ± 0.01^{b}	***	1.71 ± 0.00^{b}	***	1.68 ± 0.00^{a}	***
	Modified	1.83 ± 0.00^{a}		1.83 ± 0.01^{b}		1.84 ± 0.00^{b}	
trans	Control	0.99 ± 0.02^{a}	**	0.98 ± 0.02^{a}	***	0.98 ± 0.03^{a}	***
	Modified	0.85 ± 0.04^{a}		0.83 ± 0.01^{a}		0.84 ± 0.01^{a}	

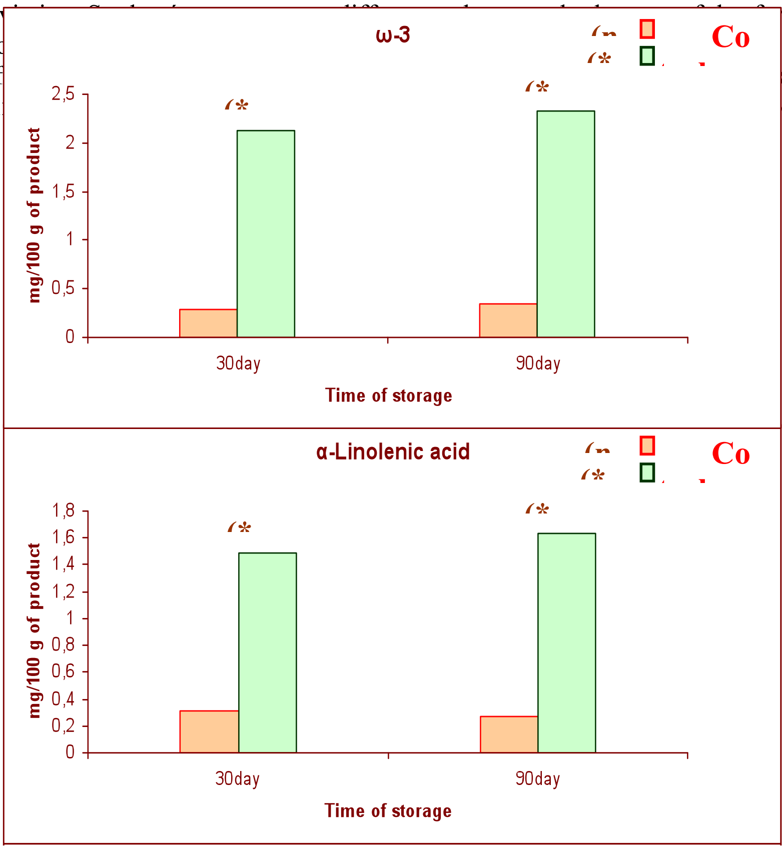
Results are expressed in g/100 g of FAME as mean ± standard deviation. Student´t test compare differences between both types of dry fermen sausages. LS (level of significance): ns (not significant); p > 0.05; * p < 0.05; **p < 0.01; ***; p < 0.001. Different letters in the same row den significant differences (p<0.05) among the different times according to an ANOVA test and Tuckey b post hoc test.

Table 2.- Parameters related with the lipid oxidation in the dry fermented sausages.

		Final Product	LS	30 days	LS	90 days	LS
TBARS	Control	0.09 ± 0.01[a]	**	0.10 ± 0.01[a]	***	1.41 ± 0.10[b]	*
	Modified	0.11 ± 0.01[a]		0.25 ± 0.01[b]		0.48 ± 0.01[c]	
Hexanal	Control	1369.51 ± 122.31[a]	*	1924.72 ± 744.11[a]	ns	17915.40 ± 569.87[b]	***
	Modified	813.55 ± 365.75[a]		1476.20 ± 396.13[a]		2496.07 ± 435.96[b]	
Heptanal	Control	65.32 ± 11.19[a]	ns	184.72 ± 27.84[b]	ns	978.26 ± 58.50[b]	***
	Modified	114.08 ± 35.58[a]		187.34 ± 41.72[b]		197.89 ± 11.56[b]	
t2,-heptenal	Control	30.86 ± 5.88[a]	**	168.79 ± 22.19[a]	***	1753.15 ± 307.33[b]	**
	Modified	<0.01[a]		<0.01[a]		122.04 ± 18.69[b]	
2-pentil amilofuran	Control	360.14 ± 34.15[a]	ns	776.60 ± 303.19[b]	ns	4512.55 ± 259.05[c]	***
	Modified	459.47 ± 102.70[a]		778.36 ± 313.11[a]		673.43 ± 48.66[a]	
Octanal	Control	127.35 ± 3.37[a]	ns	282.87 ± 67.53[a]	ns	1376.74 ± 200.96[b]	***
	Modified	181.56 ± 57.17[a]		259.91 ± 43.01[a]		237.65 ± 17.46[a]	
t.t 2-4 heptadienal	Control	4.78 ± 5.53[a]	***	<0.01[a]	ns	238.94 ± 39.63[b]	***
	Modified	n.d.		n.d.		n.d.	
2-Octenal	Control	48.04 ± 6.41[a]	ns	121.28 ± 31.83[a]	ns	2061.42 ± 130.85[b]	***
	Modified	54.54 ± 25.02[b]		78.62 ± 14.96[b]		<0.01[a]	
Nonanal	Control	506.41 ± 122.10[a]	***	1218.75 ± 100.80[b]	*	3037.76 ± 196.58[c]	***
	Modified	1484.74 ± 91.02[a]		2015.49 ± 392.51[a]		1484.33 ± 286.45[a]	
tt,2,4-Nonadienal	Control	12.11 ± 2.47[a]	**	20.78 ± 5.76[a]	**	280.79 ± 58.07[b]	**
	Modified	n.d.		n.d.		n.d.	
tt, 2,4-Decadienal	Control	13.31 ± 4.77[a]	*	99.48 ± 21.79[a]	**	2632.31 ± 452.69[b]	***
	Modified	<0.01[a]		35.97 ± 1.11[a,b]		105.77 ± 82.37[b]	
2,4-Decadienal	Control	74.22 ± 33.62	*	508.75 ± 94.55[a]	**	11944.91 ± 2424.12[b]	**
	Modified	n.d.[a]		168.65 ± 6.00[a,b]		436.81 ± 324.35[b]	
Σ Volatile	Control	2612.05 ± 94.17[a]	ns	5306.74 ± 1390.34[a]	ns	46732.22 ± 4517.29[b]	***
	Modified	3107.95 ± 606.37[a]		5000.53 ± 1178.09[a,b]		6979.08 ± 2805.43[b]	

TBARS are expressed in mg MDA/kg and the volatile compounds in ng dodecane/g dry matter, both as mean ± standard de mented sausages. LS (level of significance): ns (nc rent letters in the same row denote significant dif t and Tuckey b post hoc test.

Figu f product).



Two Student´t tests compare differences between 30 day *vs* 90 day for Control and Modified batches respectively ([a]) and Control *vs* Modified for 30days and 90day, respectively ([b]). Level of significance: ns (not significant); $p > 0.05$; * $p < 0.05$; **$p < 0.01$; ***; $p < 0.001$.