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Enrichment of ewe cheese with *Laminaceae* seed oil as a source of omega-3

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Abstract. In this study we evaluated the viability of using oil extracted from *Laminaceae* seeds with a hydraulic press at room temperature as a source of omega-3 enrichment for ewe cheeses. Two concentrations (1.5 and 2.5%; vol/vol) of seed oil prepared as emulsions stabilized with calcium caseinate were assayed in pressed sheep`s milk cheeses, and their physicochemical (fat, protein, dry matter and fattty acids), microbiological (total bacteria, lactic acid bacteria, enterobacteria, yeast and moulds) and organoleptic parameters were evaluated during 32 days of ripening. Sheep's milk coagulation parameters during cheese elaboration were not affected by the incorporation of the oil emulsion. The fortification with the emulsion had a positive impact on the cheese yield, obtaining 2% more in the cheeses containing 2.5% of vegetal oil. Similar results were observed in the fat, dry matter and omega-3 content, which increased proportionally to oil concentration. Moreover, total bacteria and lactic acid bacteria were not inhibited in the cheeses containing the highest oil concentration, confirming the hypothesis that oil addition would not interfere to the normal ripening process. We did not detect the presence of yeast, moulds or enterobacteria in any of the cheeses during ripening. Regarding the sensory analysis, the test "difference from control" showed that no significant differences were observed between the control and the enriched cheeses according to a semi-trained panel of judges.

Keywords. Sheep cheese – Omega-3 – Vegetable oil – *Laminaceae* seeds.

Fromage de lait de brebis enrichi avec de l'huile des graines de* Laminaceae *comme une source d'acides gras oméga-3

Résumé. *Dans cette étude, nous avons évalué la viabilité de l'utilisation de l'huile des graines de Laminaceae, extraite avec une presse hydraulique à température ambiante, pour enrichir le contenu d'acides gras oméga-3 dans un fromage au lait de brebis. Deux concentrations de l'huile (1,5 et 2,5%, vol / vol) ont été préparées sous forme d'émulsion stabilisées avec du caséinate de calcium et ajouté au fromage de brebis à pâte pressée. Les caractéristiques physico-chimiques (matières grasses, protéines, matières sèches et acides gras), microbiologiques (bactéries totales, bactéries lactiques, entérobactéries, levures et moisissures) et les paramètres organoleptiques ont été évalués pendant 32 jours d'affinage. Les paramètres de coagulation du lait de brebis pendant la fabrication du fromage n'ont pas été affectés par l'incorporation de l'émulsion. La fortification avec l'émulsion a eu un impact positif sur le fromage obtenant un rapport fromage/lait 2% plus haut dans les fromages contenant 2,5% de l'huile végétale. Un résultat similaire a été observé pour le contenu en matière grasse, matière sèche et la teneur en oméga-3 qui a augmenté proportionnellement à la concentration de l'huile. Entre autres, la croissance des bactéries totales et des bactéries lactiques n'ont pas été inhibées avec la concentration la plus élevée de l'huile, ce qui confirme l'hypothèse que l'addition de l'huile n'affecte pas l'affinage du fromage de lait de brebis à pâte pressée. La croissance de levure, moisissures et d'entérobactéries n'a pas été détecté dans aucun fromage. En ce qui concerne l'analyse sensorielle, des jurés semi-entrainés n'ont pas trouvé des différences sensorielles dans les fromages enrichis par rapport au fromage témoin selon le test « différence par rapport au contrôle ».*

Mots-clés. *Fromage de brebis – Omega 3 – Huile végétale.*

I – Introduction

In recent years, a wide range of omega-3-enriched dairy products has appeared on the market, due to the fact they provide extensive nutritional benefits for human health. Nevertheless, the main animal sources of unsaturated fatty acids are the oils of fish and shellfish (Ganesan *et al*., 2012). Likewise, the various products enriched with omega-3 are mainly milks or yoghurts, while the offer of cheeses is not frequent, especially for those made with sheep milk and enriched with these fatty acids (Dal Bello *et al*., 2015). In the previous studies that have been carried out in this field, as well as in the development of dairy products enriched with these essential fatty acids, it has been observed that there are hardly any studies on the enrichment of dairy products with vegetable sources such as nuts, seeds and vegetable oils (flaxseed, canola and soybean) that contain α-linolenic acid (ALA C18:3 n3). They have been used as a source of omega-3 (Ganesan *et al*., 2012), but in other foods than dairy. For these reasons, the objective of this work is to study the enrichment of a cheese with omega-3 plant origin (Oil from *Laminaceae* seeds, OLS), made with sheep milk.

II – Material and methods

1. Experimental design

Two different batches of pressed ewe milk cheese were made. Each one consisted in making 3 vats of cheese (30 L) from the same milk tank. These 3 vats included the control vat and 2 vats enriched with oil from *Laminaceae* seeds (1.5 and 2.5% vol/vol, respectively). Ten pieces (~ 0.5 kg) were taken from each vat and ripened for analysing at 8, 16, 24 and 32 d.

2. Preparation of vegetal oil emulsions

Oil was extracted from commercial organic *Laminaceae* seeds (OLS) with mean composition (g/100 g) of fat content of 31.1, protein of 21.2, SFA of 3.8 and omega-3 (α-linolenic acid) of 17.8. OLS was extracted with a hydraulic press (MECAMAQ Model DEVF 80, Spain) at the Vegetal Oil Extraction Pilot Plant of the UCLM (Albacete, Spain). Extraction conditions were performed at room temperature and 20 MPa pressure during 15 min. 0.22 µm-filtered vegetal oil emulsions were prepared according Stratulat *et al*. (2014) containing a dispersion of 70 g of calcium caseinate and 30 g of OLS. Emulsion was homogenised at 40ºC using an Ultra-Turrax T 25 Basic at 17,000 rpm for 2 min. The two OLS concentrations were used for milk enrichment.

3. Cheesemaking, milk and cheese analysis

Cheeses were manufactured at the Dairy Pilot Plant of the UCLM, using Manchega breed ewe milk. Cheese was elaborated according Licón *et al*. (2012) and ripened (8 ± 1°C and 85% RH conditions) during 32 d. Milk gross composition (protein, fat and total solids) was determined with an infrared spectrophotometer MilkoScanTM Minor Type 78100 (Foss Electric, Denmark). Cheeses dry matter and fat content were analysed through a near-infrared analyser FoodScan (Foss Electric). A pH meter Crison GPL 22 (Crison, Spain) was used for pH using a Crison 5232 probe. Fatty acids in lyophilized cheese samples (100 mg) were directly methylated with 2 mL of 0.5 M NaOCH3 at 50°C for 15 min, followed by 1 mL of 5% HCl in methanol at 50°C for 15 min (Bonanno *et al*., 2012). Fatty acid methyl esters (FAMEs) were recovered in hexane (1.5 mL). One microliter of each sample was injected into an Agilent 7820A gas chromatograph equipped with an Agilent 5975 mass spectrometer detector (Agilent Technologies Inc., USA). FAMEs were separated using a 60-m length, 0.25 mm i.d., 0.2 µm capillary column (HP-88; Agilent Technologies Inc., USA), using the following temperature program: 60°C for 2 min, increased at 3°C/min to 220°C and held for 5 min at this temperature, using helium at a flow rate of 1 mL/min (linear velocity of 25.9 cm/s) as the car-

rier gas. A fatty acid methyl ester hexane mix solution (Accustandard, USA) was used to identify and quantify 37 FAMEs. Total aerobic bacteria, lactic acid bacteria, enterobacteria and moulds and yeasts counts were determined according Licón *et al*. (2012). Counts were expressed as log cfu/g.

4. Sensory and statistical analysis

Sensory evaluation consisted of a Difference From Control test (DFC) carried out at 16 d of ripening. Forty-six untrained panelists between 18 and 61 year-old from the UCLM (Albacete, Spain) and familiar with the test format were selected for the study. Twenty-four out of the total were females and 22 were males. The scale used was a verbal and numeric category scale (0: not difference to 5: extremely different). Data obtained in the DFC test were analysed using ANOVA (two-factors, without replication) with samples and judges as factors. The Dunnett's Multiple Comparison Test was used to compare samples with the reference. An ANOVA was performed using the SPSS version 23.0 statistical package (SPSS Inc., Chicago, IL) to determine the effects of OLS concentration and ripening time on each parameter studied ($P < 0.05$). The Tukey test at a significance level of $P < 0.05$ was used to determine differences between means for concentrations and ripening days.

III – Results and discussion

The addition of the OLS emulsion to sheep milk resulted in significant increases ($P < 0.001$) in fat and dry matter (DM) contents (Fig. 1). Milk coagulation parameters during cheese elaboration were not affected by the incorporation of OLS emulsion reaching a pH of 5.3 in similar times (data not shown). A slight increase (2%) in cheese yield when 2.5% OLS emulsions were incorporated to milk was appreciated. Table 1 shows the physicochemical and microbial parameters of the studied cheeses at different ripening stages. There were no significant differences in pH (around 5.2) at any stage of ripening due to OLS addition. The fortification with the emulsion had a positive impact on fat content that increase almost proportionally, with increasing OLS ($P < 0.001$). This increase was observed also in the dry matter. The evolution of these parameters during ripening increased significantly ($P< 0.001$) and was similar for all types of cheeses. Regarding the effect of OLS on omega-3 level the concentration of α-linolenic acid (ALA C18:3n3) also increased proportionally to oil addition being 0.17, 2.8 and 4.31 mg/100 mg of cheese fat for control, 1.5% OLS and 2.5% OLS cheeses, respectively. These results suggest an effective inclusion in the curd of the assayed OLS, as already reported for cheeses fortified with other animal or vegetal omega-3 sources applied as emulsions (Calligaris *et al*., 2015; Stratulat *et al*., 2014).

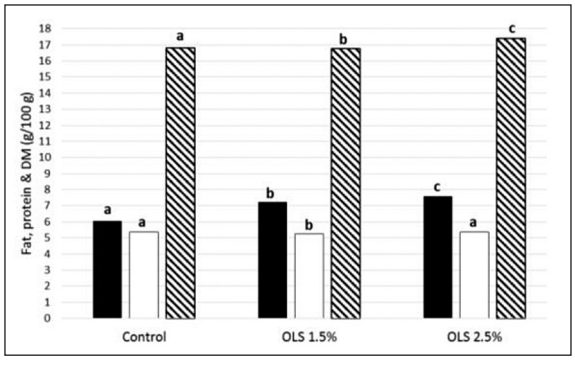

Fig. 1. Effect of OLS addition on milk parameters.
[a-c] Means with different superscripts differ ($P < 0.05$).

No significant differences were found between control and OLS enriched cheeses total bacteria, or lactic acid bacteria (Table 1). There was not growth of yeast and moulds and enterobacteria in none of the cheese during ripening (data not shown). As Bermúdez-Aguirre & Barbosa-Cánovas (2011) and Dal Bello *et al*. (2015) have described for cheeses fortified with different sources of omega-3, the inclusion of the OLS has not negative effects on microbial development during ripening.

Although mean values in the DFC test of samples showed a slight preference for control cheeses (1.5) with respect to the other two (1.8 for 1.5% OLS and 2.12 for 2.5% OLS cheeses), the semi-trained judges did not appreciate significant differences (P > 0.05) between the control and the enriched cheeses. In addition, Bermúdez-Aguirre & Barbosa-Cánovas (2011) reported good scores for cheeses fortified with flaxseed oil. These results demonstrate that fortification levels of up to 2.5% OLS may be applied to pressed sheep`s milk cheeses without negatively affecting shelf-life or consumer acceptation.

Table 1. Effect of OLS addition on cheese parameters (mean ± sd)

		Ripening time (d)				
Parameter	**Cheese**	**8**	**16**	**24**	**32**	***P*-value**
DM	Control	58.50 ± 0.03^{ax}	59.75 ± 0.15^{bx}	61.38 ± 0.34^{cx}	62.32 ± 0.04^{dx}	***
	OLS 1.5%	59.67 ± 0.15^{ay}	60.73 ± 0.41^{by}	62.10 ± 0.07^{cy}	63.65 ± 0.02^{dy}	***
	OLS 2.5%	59.76 ± 0.20^{ay}	61.74 ± 0.08^{bz}	63.07 ± 0.02^{cz}	64.33 ± 0.03^{dz}	***
	P-value[1]	***	***	***	***	
Fat	Control	29.27 ± 0.21^{ax}	29.45 ± 0.03^{ax}	30.02 ± 0.01^{bx}	30.22 ± 0.01^{bx}	***
	OLS 1.5%	30.25 ± 0.03^{ay}	30.76 ± 0.02^{by}	31.28 ± 0.08^{cy}	31.47 ± 0.04^{dy}	***
	OLS 2.5%	31.19 ± 0.05^{az}	31.57 ± 0.03^{bz}	32.22 ± 0.13^{cz}	32.87 ± 0.01^{dz}	***
	P-value	***	***	***	***	
Total bacteria	Control	9.53 ± 0.14	9.40 ± 0.33	9.17 ± 0.08	9.23 ± 0.00	NS
	OLS 1.5%	9.55 ± 0.20^{a}	9.13 ± 0.06^{b}	9.30 ± 0.04^{ab}	9.39 ± 0.16^{ab}	**
	OLS 2.5%	9.47 ± 0.12^{a}	9.07 ± 0.02^{b}	9.19 ± 0.12^{b}	9.23 ± 0.04^{b}	***
	P-value	NS	NS	NS	NS	
Lactic acid bacteria	Control	9.45 ± 0.17^{a}	9.35 ± 0.10^{ab}	9.14 ± 0.09^{bx}	9.32 ± 0.03^{ab}	*
(M17 media)	OLS 1.5%	9.28 ± 0.17^{a}	9.20 ± 0.09^{a}	9.69 ± 0.03^{by}	9.33 ± 0.26^{a}	**
	OLS 2.5%	9.35 ± 0.13	9.28 ± 0.07	9.34 ± 0.19^{x}	9.15 ± 0.15	NS
	P-value	NS	NS	***	NS	

$^{x-z}$ Means within a column with different superscripts differ ($P < 0.05$).

$^{a-d}$ Means within a row with different superscripts differ ($P < 0.05$).

[1] Significance differences are indicated as follows: *$P < 0.05$, **$P < 0.01$ & *** $P< 0.001$.

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