Food Analytical Methods
https://doi.org/10.1007/s12161-020-01928-8

Study of Polyunsaturated Fatty Acids in Cheeses Using Near-Infrared Spectroscopy: Influence of Milk from Different Ruminant Species

Iris Lobos-Ortega[1,2] · Miriam Hernández-Jiménez[3] · María Inmaculada González-Martín[1] · José Miguel Hernández-Hierro[1,4] · Isabel Revilla[3] · Ana María Vivar-Quintana[3]

Received: 20 March 2020 / Accepted: 29 November 2020

Abstract

This study has examined the polyunsaturated fatty acid composition of 224 cheeses with variable percentages (0–100%) of milk from different species (cow, ewe's, and goat) during 6 months of ripening. We analyzed the concentration of ω3 (Σ C20:5 + C22:6 + C18:3), ω6 (ΣC20:4 + C18:2), Σ isomers of the conjugated linoleic acid (CLA), the total of polyunsaturated fatty acids (ω3 + ω6 + CLA), and the ω6/ω3 nutritional relationship of the cheeses. The importance of the animal species, the seasonality, the ripening time, and its influence on the composition of polyunsaturated fatty acids (PUFAs) has been studied. Concerning the species, sheep show a higher concentration of CLA levels and ω6. The seasonality affects above all the levels of CLA and ω3, while ripening only affects the CLA levels. The capacity of NIR technology to predict the concentration of PUFA in cheese by direct application to cheese slices was also assessed. The docosahexaenoic acid relationship (C22:6 ω-3) and the ω6/ω3 relationship presented the most accurate prediction equation (RSQ > 0.7).

Keywords Polyunsaturated fatty acids · NIRs · Cheese · Ewe's · Goat · Cow

Introduction

Consumers are increasingly interested in the composition of foods with a view to following a balanced and healthy diet. Nutritional recommendations indicate that in order to improve our diet, it is necessary to reduce our consumption of saturated fatty acids and increase that of polyunsaturated fatty acids (PUFAs) (Wood et al. 2003; Sierra et al. 2008). In this sense, the ingestion of PUFAs has been related to the regulation of different biological functions which range from the control of blood pressure to the development and operation of the brain and the nervous system (Wall et al. 2010). The beneficial effects of these fatty acids are based not only on their presence but also on their appropriate proportion. Thus, the ω-6/ω-3 PUFA ratio is an index frequently used to assess the nutritional value of the fat consumed by humans. The unbalanced dietary consumption of PUFA is detrimental to human health (Patterson et al. 2012).

In recent decades, conjugated linoleic acid (CLA) has been widely studied owing to its nutritional interest. CLA consists of a collection of positional and geometrical isomers of octadecadienoic acid including 28 positional and geometrical isomers, of which only cis-9, trans-11, trans-10, cis-12, trans-9, and trans-11 have been shown to have biological activities (Collomb et al. 2004). It is responsible for anti-carcinogenic properties, stimulates growth, prevents diabetes, aids the strengthening of the bones, leads to a decrease in LDL cholesterol and glucose in blood, and possesses antiteratogenic properties (Jung et al. 2006; Bondia-Pons et al. 2007; Silva-Hernández et al. 2007; Meluchová et al. 2008).

Milk and dairy products, mainly cheeses, are usually associated with high levels of long-chain saturated fatty acids, although they are also known for their important role in human nutrition and have recently been recognized as natural sources of oleic acid, medium- and short-chain fatty acids, and CLA (Partidário et al. 2008; Dawczynski et al. 2010). The fatty acids

✉ Ana María Vivar-Quintana
avivar@usal.es

[1] Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Chemistry, University of Salamanca, C/ Plaza de la Merced s/n, 37008 Salamanca, Spain

[2] Present address: National Institute for Agricultural Research, Remehue Research Centre, PO BOX 24-O, Osorno, Chile

[3] Food Technology Lab, Higher Polytechnic School of Zamora, University of Salamanca, Avenida Requejo 33, 49022 Zamora, Spain

[4] Present address: Food Color and Quality Lab. Dept. of Nutrition and Food Science, Faculty of Pharmacy, University of Sevilla, 41012 Sevilla, Spain

Published online: 06 January 2021

present in cheese are mainly palmitic acid (C16:00) and oleic acid (C18:1). Moreover, we find short-chain saturated fatty acids (C2:0-C10:00) medium-chain saturated fatty acids (C12:00 and C14:00), and polyunsaturated fatty acids: linolenic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid, and araquidonic acid. Within CLAs, the most prevalent isomer from a quantitative point of view is rumenic acid (9c, 11t C18:2). All these compounds are found in very variable amounts depending on the cheese type.

The fatty acid composition in cheese is primarily dependent on the FA content of the unprocessed raw milk (Nudda et al. 2005). In milk, the composition of the various fatty acids is affected by numerous factors, such as the period of lactation and the breed and age of the animal (Signorelli et al. 2008; Lurueña-Martínez et al. 2010). Nonetheless, it is the feeding that makes the proportion of unsaturated fatty acids vary greatly (Falchero et al. 2010). Moreover, in relation to the diet, the geographical origin also has a great influence owing to seasonal variations and the changes that occur in the composition of animals fed in the open air (Zlatanos et al. 2002).

Conventional methods used for the determination of PUFAs involve the extraction of fat with chemical solvents, the conversion of the FAs to their methyl esters, and their analysis by gas chromatography. This process makes the analysis of fatty acids in cheese expensive and time-consuming and generates hazardous waste (Fernández-Cabanás et al. 2011). Counting on a rapid and economical analytical technique that could be applied to unprocessed cheese samples would be of great interest to the food industry. In this sense, NIR spectroscopy can be a useful tool that is suitable for this objective. NIR technology has been successfully applied in order to calibrate the fatty acid content in ham (García-Olmo et al. 2000), flax seeds (Ribeiro et al. 2013), beef (Prieto et al. 2009), chicken breast (De Marchi et al. 2011), seeds of rapeseed (Kim et al. 2007), rabbit meat (Zomeño et al. 2012), and goat milk (Núñez-Sánchez et al. 2016).

The objective of this study is to assess the content of polyunsaturated fatty acids of nutritional interest and the influence of various factors (species, season, and maturation) in cheeses ripened for up to 6 months made from cow, goat, and ewe's milk in winter and summer. Traditional analytical techniques were applied initially, and the results obtained have been used to assess the NIRS capacity with remote reflectance fiber-optic probe in order to estimate polyunsaturated fatty acids in intact samples of cheese.

Materials and Methods

Samples and Cheese-Making Procedure

A total of 224 cheeses were produced and analyzed in this study. Cow, ewe's, and goat milk were used in different proportions, with percentages ranging from 0 to 100% as described in previous studies (González-Martín et al. 2007; Revilla et al. 2009). A total of 16 different formulations were used as shown in Table 1, and 7 cheeses were produced from each of them. Raw cow, goat, and ewe's milk were obtained directly from the producers in Zamora, Spain.

In addition, the cheeses were produced from milk collected in summer (112 cheeses), and the process was later repeated with milk collected in winter (112 cheeses). The cheeses were also matured for 6 months, with one cheese being analyzed every month (0, 1, 2, 3, 4, 5, 6 months). In this way, we obtained samples produced from different mixtures of milk, from two different seasons, and with 7 degrees of maturation.

Fatty Acid Analysis

Lipids were extracted using the International Standard Method described in ISO 14156:2001. Fatty acids of all samples were methylated (Murrieta et al. 2003) and analyzed by gas chromatography (GC 6890 N, Agilent Technologies, USA) using a 100 m × 0.25 mm × 0.20 μm capillary column (SP-2560, Supelco, Inc., Bellefonte, PA, USA). The oven temperature program was 150 °C, increasing temperature by 1 °C/min until reaching 165 °C, then increasing by 0.20 °C/min until reaching 167 °C, and then finally increasing by 1.50 °C/min until reaching 225 °C, where it was maintained for 15 min. One microliter was injected into a chromatograph equipped with a split/splitless injector and an FID detector. The injector and detector temperatures were 250 °C.

Table 1 Composition of the cheeses produced

Cheese and no. of samples	% cow	% ewes	% goat
1 (7W-7S)*	100	0	0
2 (7W-7S)	0	100	0
3 (7W-7S)	75	25	0
4 (7W-7S)	50	50	0
5 (7W-7S)	25	75	0
6 (7W-7S)	0	0	100
7 (7W-7S)	25	0	75
8 (7W-7S)	50	0	50
9 (7W-7S)	75	0	25
10 (7W-7S)	0	25	75
11 (7W-7S)	0	50	50
12 (7W-7S)	0	75	25
13 (7W-7S)	33	33	33
14 (7W-7S)	10	45	45
15 (7W-7S)	45	10	45
16 (7W-7S)	45	45	10

*No. of cheeses produced from milk collected in winter (W) and in summer (S)

The carrier gas was helium at 1 ml/min and split (20:1). Samples were prepared separately in triplicate, and the average values were presented as the final results. The different fatty acids were identified by the retention time compared with the corresponding standards (C18:2, C18:3, C20:4, C20:5, and C22:6) and the four CLA isomers (CLA 9c,11t; CLA 10t,12c; CLA 9c,11c; and CLA 9t, 11t) (Larodan Fine Chemicals AB, Malmo, Sweden), and each compound was quantified in the corresponding calibration curve. The repeatability of the total procedure was examined by extracting and methylating three individual samples. The contents were calculated, and the relative standard deviation (RSD) was 3.60%, 2.06%, and 2.58% for ω6, ω3, and CLA, respectively.

NIR Spectroscopy

A Foss NIRSystem 5000 with a standard 1.5 m 210/7210 bundle fiber-optic probe was used (ref. no. R6539-A). The probe uses a remote reflectance system and a ceramic plate as a reference. The window is of quartz with a 5 × 5-cm surface area and measures reflectance in the IR zone close to 1100–2000 nm. Spectra were recorded at intervals of 2 nm, performing 32 scans for both the reference and samples. Recording of the NIR spectra was accomplished by direct application of the fiber-optic probe directly over a slice 1 cm thick from the central diameter of each cheese wheel with no prior treatment or manipulation. To minimize sampling error, all the samples were analyzed in triplicate. The spectra were measured using NIRS technology and a remote reflectance fiber-optic probe that was applied to the cheese samples. The software used was Win ISI 1.50 installed on a Hewlett–Packard Pentium III computer.

Statistical Analyses

In order to study the different factors (months of ripening, seasonality, and the % of cow milk, % of ewe's milk and % of goat milk), the SPSS (Statistical Package for the Social Sciences) was used for all the samples analyzed. The mean values of the quantitative variables were compared by analysis of variance (ANOVA), considering the differences to be significant when P was lower than 0.05. Pearson correlation coefficient tests with a significance test (two-tailed) were performed to study the correlation between the variables and the concentration of the fatty acids in the cheeses examined.

SIMCA-P software version 14.1 (Umetrics) was used to analyze the capacity of discrimination of the cheese, according to the milk used in its manufacture, from its composition in polyunsaturated fatty acids.

Calibrations were developed using WinISI II version 1.5 (Infrasoft International). The samples ($n = 224$) were divided into calibration ($n = 164$) and validation ($n = 60$) sets. All types of cheeses were represented in both the validation and calibration sets. The modified partial least squares (MPLS) regression method was used to obtain the NIR equations for all the parameters studied (Shenk and Westhaus 1995). Validation errors are combined into a standard error of cross-validation (SECV) (Davies and Williams 1996). The effects of scattering were removed using MSC (multiplicative scatter correction), SNV (Standard Normal Variate), or DT (DeTrend) (Dhanoa et al. 1995). Moreover, a number of mathematical treatments were tested in the development of the NIRS calibrations. The statistics used to select the best equations were RSQ (multiple correlation coefficients) and the standard error of cross-validation (SECV).

In addition, a discrimination of the samples was carried out based on the NIR spectral information. The discrimination method used was RMS X residual. A square root of the average squared (RMS) residual is calculated to provide the RMS X residual.

Results

Polyunsaturated Fatty Acids in Cheese

To study the influence of the different factors considered, the fatty acids were analyzed in groups. In this way, the sum of the acids ω3 (ΣC20:5 + C22:6 + C18:3) and ω6 (ΣC20:4 + C18:2), the sum of the CLA isomers, the total of polyunsaturated fatty acids determined (ω3 + ω6 + CLA), and the ω6/ω3 nutritional relationship were analyzed. The results obtained can be found in Table 2. The PUFAs found in the largest amounts in all the cheeses analyzed correspond to the ω6 series with an average of 30.92 mg/g of fat, which is much higher than those found in CLAs and the fatty acids of the ω3 series. The level of conjugated linoleic acid in cheese ranges from 1.07 to 14.66 mg/g fat. CLAs were quantified as the sum of 4 isomers. The isomers C18:2 9c11t and C18:2 10t12c were the most abundant and were present in the totality of the cheeses analyzed; the isomers C18:2 9c11c and 9t11t were detected only in some cheeses.

The factors included for study were the milk percentage of each species (cow, ewe's, and goat milk), i.e., the ratio of each species' milk to the other two used in the cheese which ranges from 0 to 100%, the season when the milk was collected (winter or summer), and the maturation time from 0 to 6 months of ripening.

From Pearson's correlation coefficient, the relationship between the PUFA and the various factors (species, time of ripening, and seasonality) was studied (Table 3). It was observed that there is a negative correlation between the concentration of cow and goat milk used in making cheese and the contents of ω6, ω6/ω3, and PUFA. Owing to the fact that ω6 are mainly of fatty acid in all the samples analyzed, any change in this series of fatty acids affects both the ω6/ω3

Table 2 Minimum and maximum values of the PUFA (mg/g of fat) in the samples of cheese studied ($N = 14$ for each cheese composition)

Cheese composition (%)			PUFA (mg/g fat)[a]				
Cow	Ewe	Goat	ω3 Min-max	ω6 Min-max	Σ CLA Min-max	Σ PUFA Min-max	ω6/ω3 Min-max
100	0	0	1.83–4.49	11.27–34.93	2.42–11.53	15.99–52.60	5.92–8.70
0	100	0	2.68–4.50	20.65–49.56	4.77–10.59	28.17–64.21	7.56–11.03
75	25	0	1.51–6.18	9.32–56.74	2.46–14.66	13.29–77.32	6.16–9.84
50	50	0	2.04–5.33	17.03–52.96	1.07–12.25	20.16–71.22	8.33–10.03
25	75	0	3.20–5.27	32.79–55.24	2.79–12.83	42.80–72.98	9.85–11.51
0	0	100	2.50–6.77	13.43–38.80	3.24–12.82	20.39–58.74	4.52–9.18
25	0	75	2.17–5.20	10.86–38.95	3.52–8.70	16.85–49.20	4.62–7.98
50	0	50	2.35–5.18	16.15–34.64	2.85–9.77	22.44–47.78	5.02–7.92
75	0	25	2.01–4.22	9.35–33.90	2.91–10.74	14.57–48.70	4.65–8.63
0	25	75	1.39–5.32	8.05–44.19	1.86–10.74	11.33–60.32	5.80–9.07
0	50	50	2.29–4.79	21.02–40.73	3.97–9.85	27.33–55.19	7.92–10.97
0	75	25	2.78–4.84	23.73–45.61	5.02–11.11	31.86–61.66	8.13–11.49
33	33	33	2.74–5.93	21.50–47.18	2.59–11.65	28.97–65.38	7.54–8.61
10	45	45	3.27–4.75	28.10–39.26	2.73–9.88	38.13–54.16	7.68–9.13
45	10	45	2.43–5.63	17.57–41.46	2.91–10.89	23.99–57.47	6.37–8.70
45	45	10	3.19–4.60	29.62–42.48	1.69–9.17	38.49–56.33	7.94–9.85

[a] ω3 = (C20:5 + C22:6 + C18:3), ω6 = (C20:4 + C18:2), Σ CLA = (CLA 9c, 11t + CLA 10t, 12c + CLA 9c, 11c + CLA 9t, 11t), Σ PUFA = (ω3 + ω6 + CLA))

relationship and the PUFA content in the same way. The concentration of ewe's milk in cheeses shows a positive correlation in the levels of CLA, ω6, ω6/ω3, and PUFA.

The seasonal factor (winter-summer) presents a negative correlation with CLA and ω3; a decrease in the latter means that it presents a positive correlation with the correlation with ω6/ω3.

NIRS Prediction

To obtain the calibrations, an initial set of 164 cheese samples of varying compositions of cow, ewe's, and goat milk was used. Figure 1 shows the spectra of ten samples. Initially, a main component analysis was carried out (PCA). In all cases, the spectral variability explained exceeded 99%, and 9 principal components were required for C18:2, C22:6, and ω6 and 12 for ω6/ω3.

The calibration process was implemented with the spectra of the resulting samples and their chemical data. The statistical parameters of the calibration were obtained for each of the components after removing the samples for spectral (H criterion) or chemical reasons (T criterion). Anomalous spectra were detected by applying the Mahalanobis distance.

Calibrations were performed by modified partial least squares regression (MPLS). Using the $T > 2.5$ criterion, samples that were different from the population owing to the chemical criterion were removed from the set. On the basis of this chemical criterion, 4 samples were removed for C18:2, ω6; 14 for C22:6; and 9 for ω6/ω3. The best of the different mathematical treatments, concentration range, and standard deviation is shown in Table 4. Model assessments were performed by cross-validation. In this method, the set of calibration samples is divided into a series of subsets, in our case 4. Of these, 3 were taken for the calibration set and one for the

Table 3 Pearson correlation coefficients for species, maturation time, seasonality, and the concentration of the different groups of polyunsaturated fatty acids

Factor	ω3	ω6	CLA	ΣPUFA	ω6/ω3
Cow		− 0.195**		− 0.155*	− 0.237**
Ewe's		0.532**	0.243**	0.463**	0.772**
Goat		− 0.338**	− 0.217**	− 0.309**	− 0.535**
Maturation time			0.153*		
Season	− 0.209*		− 0.295**		0.225**

*Significant correlation at 0.05 level (bilateral); **significant correlation at 0.01 level (bilateral)

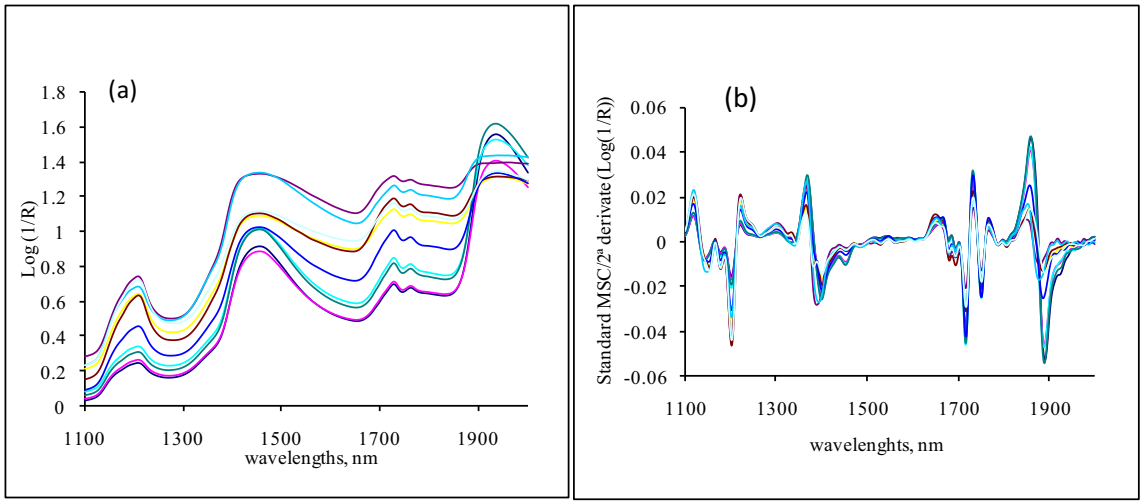

Fig. 1 **a** NIR spectra of 10 samples of cheese and **b** mathematical treatments (Standard MSC/2nd derivate) in the spectra

prediction set. The process was repeated as many times as there were sets in such a way that all passed through the calibration set and the prediction set. Using this process, we validated the models used and checked their prediction capacities. Figure 2 shows the correlation of the values obtained in the laboratory with those predicted by NIR with a fiber-optic probe. In Fig. 2, the similarity between the error of prediction (SEP) and the error of prediction corrected by the bias can also be seen.

The RSQ values obtained were acceptable for the content in ω6 (RSQ 0.58) and for the ω6/ω3 relationship (RSQ 0.76). For fatty acids individually, linoleic acid (C18:2 ω-6) (RSQ 0.58) and docosahexaenoic acid (C22:6 ω-3) (RSQ 0.75) obtained the most accurate prediction. The results obtained show the difficulties in calibrating fatty acids. Zomeño et al. (2012) found that the prediction models for PUFA (RSQ 0.83) were less accurate than for SFA (RSQ 0.96) and MUFA (RSQ 0.98), and there were no accurate predictions for ω3 FA (RSQ 0.50) in intramuscular rabbit fat. Similar results were found by Guy et al. (2011) in the prediction of fatty acids in intact no-ground meat lamb, with low coefficients of correlation for all PUFAs analyzed individually (R^2CV < 0.56) and for ω6 (R^2CV 0.37) and ω3 (R^2CV 0.35). Unsatisfactory prediction models were also found for PUFA (R^2CV 0.224) and CLA (R^2CV 0.586) in lamb meat (Sierra et al. 2008) and in frozen cheese (R^2CV < 0.80) (Lucas et al. 2008).

Discussion

Polyunsaturated Fatty Acids in Cheese

The average value for ω6 coincides with the results found for commercial cow, ewe's, and goat cheeses by Prandini et al.

Table 4 Descriptors of NIR calibration with a coefficient coordinate (RSQ) exceeding 0.5

	C18:2	C22:6	ω6	ω6/ω3
N	152	142	152	148
Mathematical treatment	None 2,4,4,1	Standard MSC 2,4,4,1	None 2,4,4,1	Standard MSC 2,4,4,1
SD	9.87	0.02	9.91	1.47
Estimated (mg/g fat)	0.63–59.88	0.05–0.16	0.63–60.09	3.51–12.34
RSQ	0.58	0.75	0.58	0.76
SEC	6.36	0.01	6.39	0.72
SECV	8.40	0.01	8.41	0.94
No. fac PLS	10	10	10	10
Cross-validation groups	4	4	4	4
RPD	1.6	2.1	1.6	2.1

N number of samples, *MSC* multiplicative scatter correction, *SD* standard deviation, *RSQ* multiple correlation coefficients, *SEC* standard error of calibration, *SECV* standard error of cross-validation, *PLS* partial least squares, *RPD* ratio performance deviation

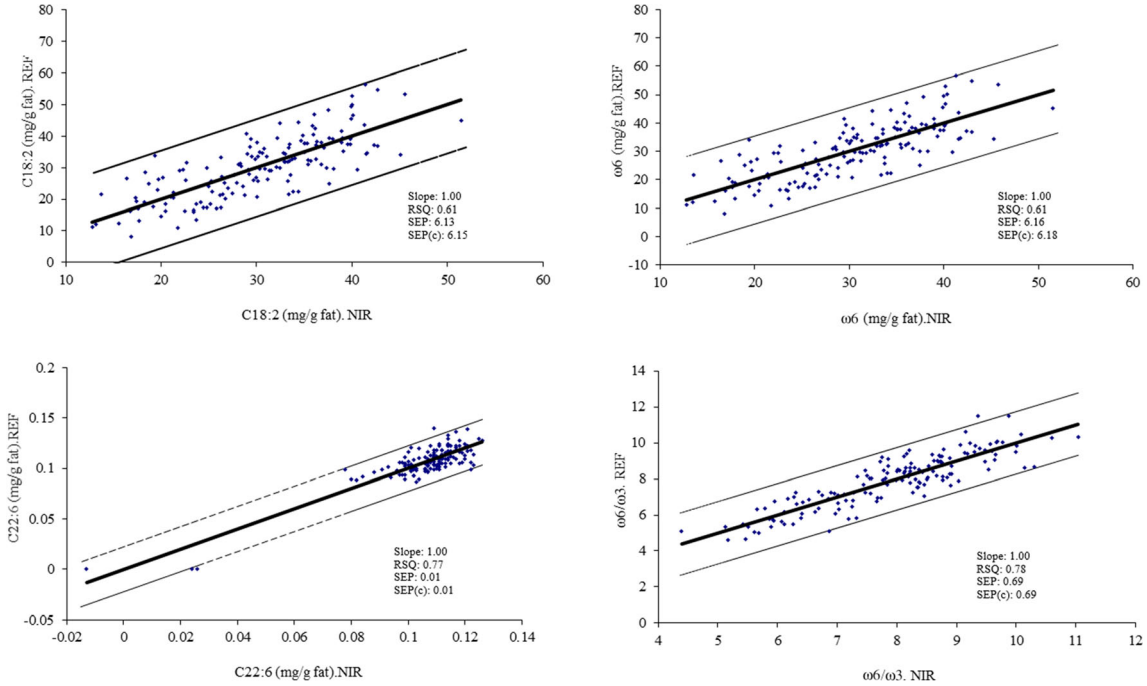

Fig. 2 Comparison of the reference values with the values predicted by means of the model MPLS in NIR for the determination of C18:2; C22:6, ω6, and ω6/ω3 in samples of cheese

(2011). Nonetheless, it is higher than those found by Revello-Chion et al. (2010) in cow cheeses ripened for 90 days, by Seçkin et al. (2005) in processed Turkish cheeses and by Lucas et al. (2008) for goat cheeses.

The level of conjugated linoleic acid in cheese coincides with that found by other authors in different types of cheeses (Luna et al. 2007; Prandini et al. 2007). The CLA content of the cheese mainly depends on the content present in the milk used (Bisig et al. 2007; Collomb et al. 2006) and the ruminant species of the milk used (Prandini et al. 2011). Rumenic acid (9c, 11t C18:2) is the major CLA in dairy products, representing 75–85% of the total CLAs in the fat in cow's milk (Fritsche and Steinhart 1998), 78–89% in ewe's milk, and 64% in goat's milk (Bouattour 2007).

With regard to ω3 acids, the average values found were lower than those found in the bibliography for cow, ewe's, and goat cheeses (Partidário et al. 2008; Zhang et al. 2006; Seçkin et al. 2005). ω3 acids such as EPA and DPA may be synthesized as from α-linolenic acid (ALA), although conversion occurs in very small amounts (Rubio-Rodríguez et al. 2010).

The mean value for the ΣPUFA coincides with the mean values found by Prandini et al. (2007, 2011) for cow, ewe's, and goat cheeses. The contents were much higher than those found by other authors in cheeses from ewes (Zhang et al. 2006), goats (Lucas et al. 2008), or cows (Revello-Chion et al. 2010).

The ω6/ω3 mean relationship presented a higher average value than that found by other authors (Partidário et al. 2008; Lurueña-Martínez et al. 2010), which shows that the samples of cheese analyzed have a high content of omega six fatty acids. The differences found could be due to the quantity of linolenic acid contributed by the diet. Recommendations for a proper diet mention a balance of ω-6:ω-3 of around 1–4:1 (Gómez Candela et al. 2011); these values are lower than those obtained in all the cheeses analyzed.

Influence of Species, Season, and Ripening on the PUFA Content

The differences due to the species can be explained by the difference of the regulation of the metabolism in the mammary gland, particularly in the process of the elongation of the fatty acids. These results coincide with the findings of Prandini et al. (2011) which showed that sheep cheeses were characterized by major levels of polyunsaturated fatty acids in comparison with cow cheeses and showed the highest mean values of CLA compared with cow and goat cheeses.

As far as the ripening factor is concerned, it is only correlated with the CLA content. The procedure used to analyze fatty acids in this study does not distinguish the fatty acids present in triglyceride form from those present in free form; for this reason, any changes that the cheeses may have undergone during ripening owing to the process of lipolysis are not analyzed. The increase observed may be related to the reasonable potential for production of CLA in culture media (Bisig et al. 2007). Different authors have studied the

possibility of increasing the CLA content in dairy products with microbial cultures (Alonso et al. 2003; Ogawa et al. 2005; Song et al. 2005).

Revello-Chion et al. (2010) found higher values in the family of the C18 (C 18:1 t11, C 18:2 ω6, C18:3 ω3, and CLA) in cheeses produced in summer compared with those made in winter, all of which is attributed to the available diet of the animals; grazing leads to a higher concentration of ω3, especially ALA, in cow's milk (Hauswirth et al. 2004). In the region where the milk used in this study has been collected, summers are very hot and dry, owing to which the animals are unable to feed on green pastures. This explains the lower concentration of ω3 found in summer cheeses. On the other hand, Nudda et al. (2003) found differences in the CLA content between sheep and goat milk only when the diet consisted mainly of pasture, which suggests that the species also conditions the fatty acid composition of the milk. The results of this study indicate a slight variation in the PUFA content with regard to the seasonality, presenting higher values in winter except for the ω6/ω3 relationship owing to the increase in the ω3 previously mentioned.

The capacity of discrimination of the cheese, according to the milk used in its production, was assessed by means of an orthogonal projection latent structure discriminant analysis (OPLS-DA). In order to do this, its composition in polyunsaturated fatty acids was used. Figure 3 shows the results obtained.

The ability to differentiate the type of milk used in the production of the cheeses was carried out from samples made from 100 or 75% of the milk considered in each case. The results obtained showed a percentage of correctly classified of 59.52% for cow cheese, 95.24% for ewe's cheese, and 78.57% for goat cheese. The best results obtained in the case of ewe's cheese may be related to a higher concentration of PUFAs in their milk, especially in cheeses made from summer milk. Similar results have been observed in butter where the PUFAs content of butter made from sheep's milk is higher than those found when using goat or cow milk (Sağdıç et al. 2004).

The discrimination of cheeses according to the seasonality can be seen in Fig. 3b. The results obtained show that the percentages of cheese correctly classified were 67.86% and 73.21% for cheeses made from winter and summer milk, respectively. One of the factors which most affect the levels of fatty acids in milk is diet. (Palmquist et al. 2005). Grazing pasture is frequently used as a feeding system for small ruminants, goats, and sheep, in Mediterranean countries. Feeding on grazing pasture has been studied as a means of improving the levels of polyunsaturated fatty acids (PUFAs), including CLA, in ruminants (Govari et al. 2020). However, the nutritional value of grazing pasture is affected by seasonality so that it is higher during the vegetation phase of pasture species. Pasture is therefore richer in PUFAs in spring than in winter (Cabiddu et al. 2005). Furthermore, in winter, a complementary feed (hay, silage, grain or a protein and mineral supplement) is necessary. PUFAs show their lowest values in January for both sheep and goat milk fat (Tsiplakou et al. 2006), which would explain why summer cheese gives the highest discrimination values.

The treatment applied did not discriminate between the cheeses according to the month of maturation on the basis of their PUFAs content.

NIRS Prediction

The difficulty in calibrating the PUFAs is related to the different reasons; on the one hand, these include the low concentration of PUFA present in the samples and the narrow range of variability of the PUFA in their NIRS absorption patterns (Windham content. On the other hand, these compounds are very similar & Morrison, 1998), and NIRS has a low capacity for detecting the double links present in PUFA (Sierra et al. 2008; Coppa et al. 2010; Zomeño et al. 2012). Although previous studies suggest that the prior grinding of the sample gives improved calibrations (Guy et al. 2011), the use of the remote reflectance fiber-optic probe without treating or manipulating the sample as in our study facilitates the use of this technique.

As far as the influence of the animal species, seasonality, and ripening time factors are concerned, it can be seen that the increase in the proportion of ewe's milk correlates with the sum of total polyunsaturated acids, the sum of ω6, the ω6/ω3 relationship, and the total CLA. Seasonality has an influence on winter cheeses, making them richer in CLA, and on the ω6/ω3 relationship. The ripening time of the cheeses slightly increases the ω3 fatty acids and CLA. The results found in the determination of the composition of fatty acids of nutritional interest show that NIR technology could be considered suitable for the estimating of C18:2, C22:6, ω6, and ω6/ω3 in unknown cheeses of variable composition (cow, ewe's, and goat) and with a ripening time of up to 6 months. However, changes in the registering of the samples need to be studied.

NIRS Discrimination

The discrimination models constructed using the NIRs spectra and various scatter/derivative pre-treatments are shown in Fig. 4.

The ability to classify the type of milk used in the production of the cheeses was carried out considering again samples of 100% or 75% of each milk type. The pre-treatment derivatives that gave the best classification results for cow and goat cheeses were SNV 2,4,4,1 and SNV-D 2,4,4,1.

The results obtained show that cow milk cheeses have the highest success rates and that cheeses made from ewe's milk

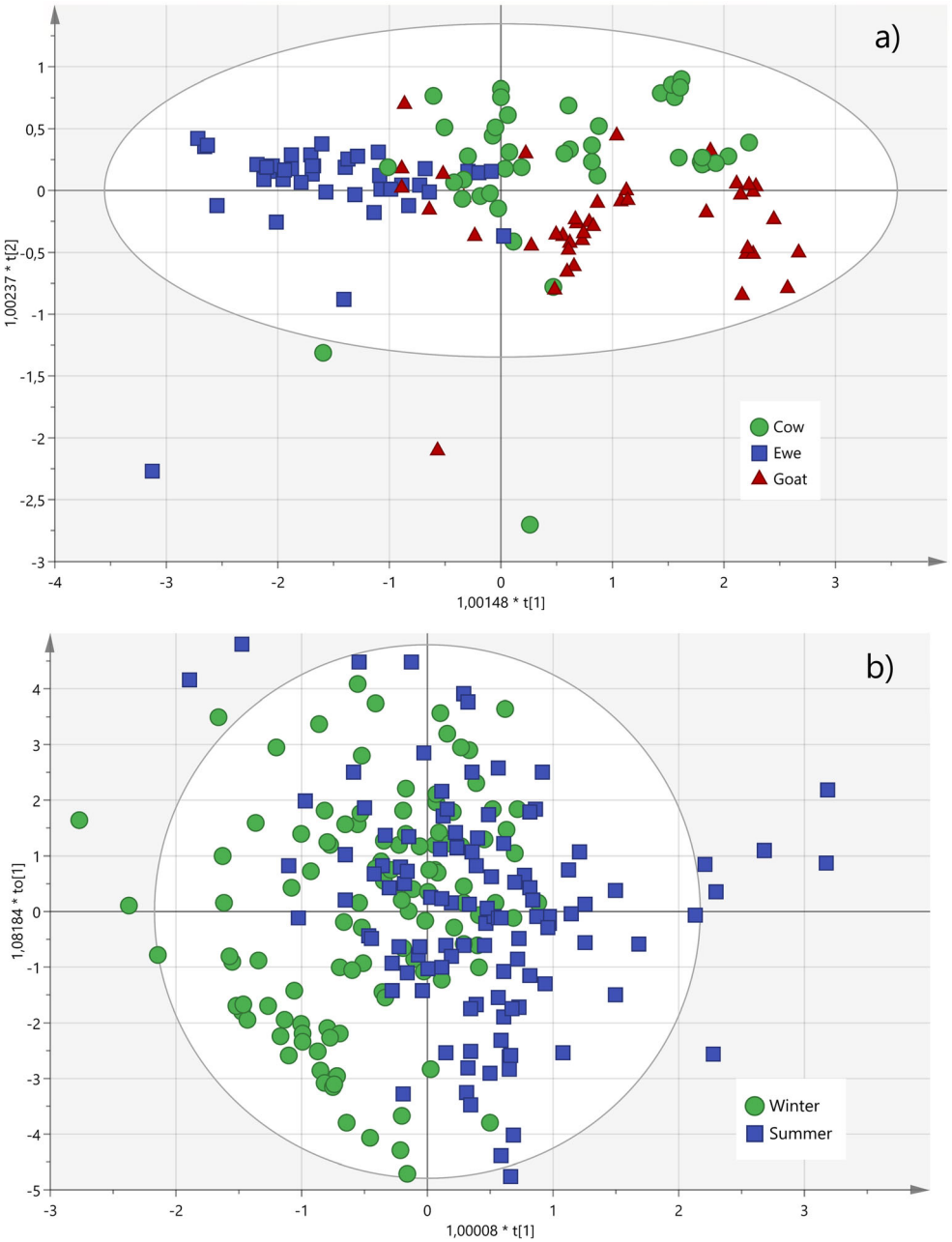

Fig. 3 Discrimination of cheeses according to polyunsaturated fatty acids, according to the type of milk used. (**a**) and the seasonality of the milk collected (**b**)

were correctly assessed with a 92.11% success rate, irrespective of the pre-treatment derivative studied in both cases (Fig. 4a). Goat cheese obtained the lowest classification probability scores, which are classified incorrectly and always misidentified as cow cheese. The discrimination capacity of the NIR spectra shows better results than those obtained in the discrimination from their PUFA content as described in "Influence of species, season and ripening on the PUFA content". In all cases, the percentages of correctly classified samples were higher.

The discrimination of cheeses according to the seasonality can be seen in Fig. 4b. Cheeses made from milk collected in winter can be discriminated with a success rate of over 95% by any of the pre-treatment derivatives studied. The success rate decreases when classifying cheeses made from summer milk. These differences are probably due to the feeding of the animals in both seasons as has been mentioned. Furthermore, in the case of seasonality, the results obtained from the NIR spectra are better than those observed when using PUFA's levels.

The discrimination capacity of the month of maturation of the cheese from the NIR spectra was also analyzed. The treatments detrend 2,4,4,1 and None 2,4,4,1 allowed the prediction of the month of maturation of the cheese with a success rate of over 93%. Only one sample was misclassified in the 0 month of maturation in cheeses made from milk collected in winter

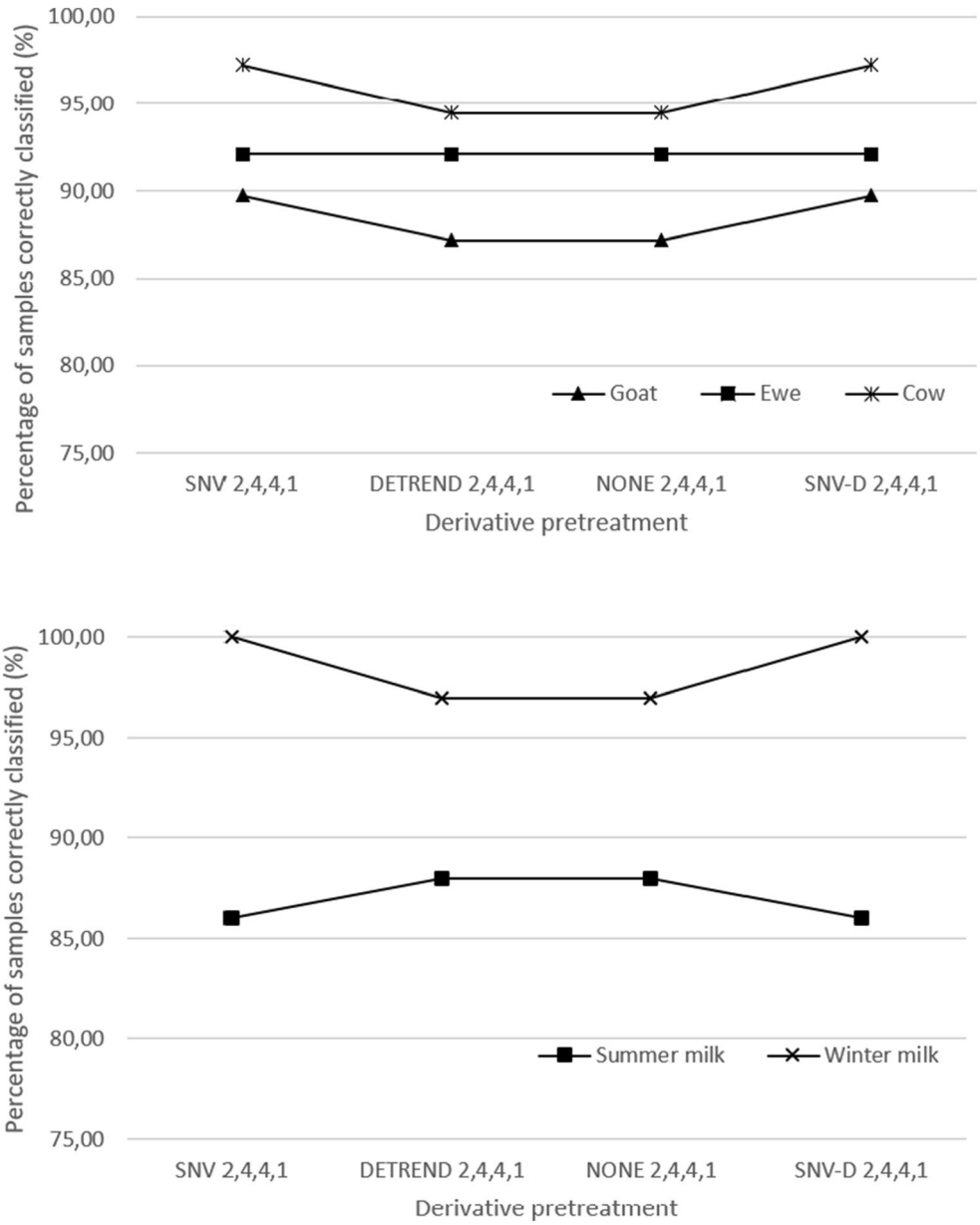

Fig. 4 Percentage of samples correctly classified, from registered NIR spectra, by different data pre-treatments: discrimination is according to the type of milk (**a**) and the seasonality (**b**). *SNV (standard normal variate), DETREND (detrending), NONE (no scatter correction) and SNV-D (standard normal variate followed of detrending)

and in the 4th month of maturation in those made from milk collected in summer.

Conclusion

As far as the influence of the animal species, seasonality, and ripening time factors are concerned, it can be seen that the increase in the proportion of ewe's milk correlates with the sum of total polyunsaturated acids, the sum of ω6, the ω6/ω3 relationship, and the total CLA. Seasonality has an influence on winter cheeses, making them richer in CLA, and on the ω6/ω3 relationship. The ripening time of the cheeses slightly increases the ω3 fatty acids and CLA. The results found in the determination of the composition of fatty acids of nutritional interest show that NIR technology could be considered suitable for the estimating of C18:2, C22:6, ω6, and ω6/ω3 in unknown cheeses of variable composition (cow, ewe's, and goat) and with a ripening time of up to 6 months. In order to improve the predictive capacity of NIRS for the polyunsaturated fatty acids present in cheese, other methods of sample preparation prior to recording their spectra could be tested, such as grinding the sample.

Authors' Contributions Writing—original draft preparation: Iris Lobos-Ortega. Formal analysis: Miriam Hernández Jiménez. Methodology: Maria Inmaculada González-Martín. Validation: José Miguel Hernández-Hierro. Conceptualization, writing—reviewing and editing: Isabel Revilla. Investigation, writing—reviewing and editing: Ana María Vivar-Quintana.

Data Availability Not applicable.

Compliance with Ethical Standards

Conflict of Interest Iris Lobos-Ortega declares that she has no conflict of interest. Miriam Hernández Jimenez declares that she has no conflict of interest. María Inmaculada González-Martín declares that she has no conflict of interest. José Miguel Hernández-Hierro declares that he has no conflict of interest. Isabel Revilla declares that she has no conflict of interest. Ana María Vivar-Quintana declares that she has no conflict of interest.

Code Availability Not applicable.

Informed Consent Not applicable.

References

Alonso L, Cuesta EP, Gilliland SE (2003) Production of free conjugated linoleic acid by Lactobacillus acidophilus and Lactobacillus casei of human intestinal origin. J Dairy Sci 86:1941–1946

Bisig W, Eberhard P, Collomb M, Rehberger B (2007) Influence of processing on the fatty acid composition and the content of conjugated linoleic acid in organic and conventional dairy products – a review. Lait 87:1–19

Bondia-Pons I, Moltó-Puigmartí C, Castellote AI, López-Sabater MC (2007) Determination of conjugated linoleic acid in human plasma by fast gas chromatography. Journal Chromatography A 1157:422–429

Bouattour MA (2007) Efectos de la utilización de diferentes fuentes de grasa vegetal para incrementar el ácido linoleico conjugado en leche de pequeños rumiantes e interacción con enzimas fibrolíticas PhD Thesis, University Autónoma of Barcelona, Spain

Cabiddu A, Decandia M, Addis M, Piredda G, Pirisi A, Molle G (2005) Managing Mediterranean pastures in order to enhance the level of beneficial fatty acids in sheep milk. Small Rumin Res 59:169–180

Collomb M, Sollberger H, Butikofer U, Sieber R, Stoll W, Schaeren W (2004) Impact of a basal diet of hay and fodder beet supplemented with rapeseed, linseed and sunflowerseed on the fatty acid composition of milk fat. Int Dairy J 14:549–559

Collomb MA, Schimd A, Sieber R, Wechsler D, Ryhänen EL (2006) Conjugated linoleic acids in milk fat: variation and physiological effects. Int Dairy J 16:1347–1361

Coppa M, Ferlay A, Leroux C, Jestin M, Chilliard Y, Martin B, Andueza D (2010) Prediction of milk fatty acid composition by near infrared reflectance spectroscopy (NIRS). Int Dairy J 20:182–189

Davies AMC, Williams P (1996) Near infrared spectroscopy. The future waves. In: Proceedings of the Seventh International Conference on Near Infrared Spectroscopy. Chichester, West Sussex, UK, pp 1-742

Dawczynski D, Martin L, Wagner A, Jahreis G (2010) n -3 LC-PUFA-enriched dairy products are able to reduce cardiovascular risk factors: double-blind, cross-over study. Clin Nutr 29:592–599

De Marchi M, Riovanto M, Penasa M, Cassandro M (2011) At line prediction of fatty acid profile in chicken breast using near infrared spectroscopy. Meat Sci 90:653–657

Dhanoa MS, Lister SJ, Barnes RJ (1995) On the scales associated with nearinfrared reflectance difference spectra. Appl Spectrosc 49(6): 765–772

Falchero L, Lombardi G, Gorlier A, Lonati M, Odoardi M, Cavallero A (2010) Variation in fatty acid composition of milk and cheese from cows grazed on two alpine pastures. Dairy Sci Technol 90:657–672

Fernández-Cabanás VMF, Polvillo O, Rodríguez-Acuña R, Botella B, Horcada A (2011) Rapid determination of the fatty acid profile in pork dry-cured sausages by NIR spectroscopy. Food Chem 124: 373–378

Fritsche J, Steinhart H (1998) Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. Z Lebensm Unters Forsch 206:77–82

García-Olmo J, De Pedro Sanz EJ, Garrido Baro A, Jimenez A, Salas J, Santolalla M (2000). Fatty acids analysis of iberian pig fat by near infrared spectroscopy (NIRS). In: Almeida JA, Tirapicos Nunes J (eds) Tradition and innovation in Mediterranean pig production. Zaragoza: CIHEAM, 2000. (Options Méditerranéennes: Série A. Séminaires Méditerranéens; n. 41), pp 191-195

Gómez Candela C, Bermejo López ML, Loria Kohen V (2011) Importance of a balanced omega 6/omega 3 ratio for the maintenance of health: nutritional recommendations. Nutr Hosp 26:323–329

González-Martín I, Hernández-Hierro JM, Morón-Sancho R, Salvador-Esteban J, Vivar-Quintana A, Revilla I (2007) Determination of the percentage of milk (cow's, ewe's and goat's) in cheeses with different ripening times using near infrared spectroscopy technology and a remote reflectance fibre-optic probe. Anal Chim Acta 604:191–196

Govari M, Lliadis S, Papageorgiou D, Fletouris D (2020) Seasonal changes in fatty acid and conjugated linoleic acid contents of ovine milk and Kefalotyric cheese during ripening. Int Dairy J 109:104775

Guy F, Prache A, Thomas D, Bauchart D, Anduez D (2011) Prediction of lamb mat fatty acid composition using near-infared reflectance spectroscopy (NIRS). Food Chem 127:1280–1286

Hauswirth C, Scheeder MRL, Beer JH (2004) High omega-3 fatty acid content in alpine cheese: the basis for an alpine paradox. Circulation 109(1):103–107

Jung MY, Kim GB, Jang ES, Jung YK, Park SY, Lee BH (2006) Technical note, improved extraction method with hexane for gas chromatographic analysis of conjugated linoleic acids. J Dairy Sci 89:90–94

Kim KS, Park SH, Choung MG, Jang YS (2007) Use of near-infrared spectroscopy for estimating fatty acid composition in intact seeds of rapeseed. J Crop Sci Biotechnol 10:15–20

Lucas A, Andueza D, Ferlay A, Martin B (2008) Prediction of fatty acid composition of fresh and freeze-dried cheeses by visible-near-infrared reflectance spectroscopy. Int Dairy J 18:595–604

Luna P, Juárez M, De La Fuente MA (2007) Conjugated linoleic acid content and isomer distribution during ripening in three varieties of cheeses protected with designation of origin. Food Chem 103:1465–1472

Lurueña-Martínez MA, Palacios C, Vivar-Quintana AM, Revilla I (2010) Effect of the addition of calcium soap to ewes' diet on fatty acid composition of ewe milk and subcutaneous fat of suckling lambs reared on ewe milk. Meat Sci 84:677–683

Meluchová B, Blasco J, Kubinec R, Gorova R, Dubravská J, Marguetín M, Soják L (2008) Seasonal variations in fatty acid composition of pasture forage plants and CLA content in ewe milk fat. Small Rumin Res 78:56–65

Murrieta CM, Hess BM, Rule DC (2003) Comparison of acidic and alkaline catalysts for preparation of fatty acid methyl esters from ovine muscle with emphasis on conjugated linoleic acid. Meat Sci 65:523–529

Nudda A, Mele M, Battacone G, Usai MG, Macciotta NPP (2003) Comparison of conjugated linoleic acid (CLA) content in milk of ewes and goats with the same dietary regimen. Ital J Anim Sci 2: 515–517

Nudda A, McGuire MA, Battacone G, Pulina G (2005) Seasonal variation in conjugated linoleic acid and vaccenic acid in milk fat of sheep and its transfer to cheese and ricotta. J Dairy Sci 88:1311–1319

Núñez-Sánchez N, Martínez-Marín AL, Polvillo O, Fernández-Cabanás VM, Carrizosa J, Urrutia B, Serradilla JM (2016) Near infrared

spectroscopy (NIRS) for the determination of the milk fat fatty acid profile of goats. Food Chem 190:244–252

Ogawa J, Kishino S, Ando A, Sugimoto S, Mihara K, Shimizu S (2005) Production of conjugated fatty acids by lactic acid bacteria. J Biosci Bioeng 100(4):355–364

Palmquist DL, Lock AL, Shingfield KJ, Bauman DE (2005) Biosynthesis of conjugated linoleic acid in ruminants and human. Adv Nutr Res 50:179–214

Partidário AM, Ribeiro JCS, Prates JAM (2008) Fatty acid composition and nutritional value of fat in three PDO ewe's milk Portuguese cheeses. Dairy Sci Technol 88:683–694

Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C (2012) Health implications of high dietary Omega-6 polyunsaturated fatty acids. Journal of Nutrition and Metabolism 539426

Prandini A, Sigolo S, Tansini G, Brogna N, Piva G (2007) Different level of conjugated linoleic acid (CLA) in dairy products from Italy. J Food Compos Anal 20:472–479

Prandini A, Sigolo S, Piva G (2011) A comparative study of fatty acid composition and CLA concentration in commercial cheeses. J Food Compos Anal 24:55–61

Prieto N, Roehe R, Lavín P, Batten G, Andrés S (2009) Application of near infrared reflectance spectroscopy to predict meat and meat quality: a review. Meat Sci 83:175–186

Revello-Chion A, Tabacco E, Giaccone D, Peiretti PG, Battelli G, Borreani G (2010) Variation of fatty acid and terpene profiles in mountain milk and "Toma piemontese" cheese as affected by diet composition in different seasons. Food Chem 121:393–399

Revilla I, González-Martín I, Hernández-Hierro JM, Vivar-Quintana AM, González-Pérez C, Lurueña-Martínez MA (2009) Texture evaluation in cheeses by NIRS technology employing a fibre-optic probe. J Food Eng 92:24–28

Ribeiro LF, Peralta-Zamora PG, Sales Maia BHLN, Pereira Ramos L, Pereira-Netto AB (2013) Prediction of linolenic and linoleic fatty acids content in flax seeds and flax seeds flours through the use of infrared reflectance spectroscopy and multivariate calibration. Food Res Int 51:848–854

Rubio-Rodríguez N, Beltrán S, Jaime J, Diego SM, Sanz MT, Carballido JR (2010) Production of omega-3 polyunsaturated fatty acid concentrates, a review. Innovative Food Sci Emerg Technol 11:1–10

Sağdıç O, Dönmez M, Demirci M (2004) Comparison of characteristics and fatty acid profiles of traditional Turkish yayik butters produced from goats', ewes' or cows' milk. Food Control 15:485–490

Seçkin AK, Gursoyb O, Kinikb O, Akbulut N (2005) Conjugated linoleic acid (CLA) concentration, fatty acid composition and cholesterol content of some Turkish dairy products. LWT Food Sci Technol 38:909–915

Shenk JS, Westhaus MO (1995) Analysis of agriculture and food products by near infrared reflectance spectroscopy. Monograph NIR systems Infrasoft International, NIR Systems USA, Silver Spring Inc

Sierra V, Aldai N, Castro P, Osoro K, Coto-Montes A, Oliva M (2008) Prediction of the fatty acid composition of beef by near infrared transmittance spectroscopy. Meat Sci 78:249–255

Signorelli F, Contarini G, Annicchiarico G, Napolitano F, Orrù L, Catillo G, Haenlein GHW, Moioli B (2008) Breed differences in sheep milk fatty acid profiles. Opportunities for sustainable use of animal genetic sources. Small Rumin Res 78:24–28

Silva-Hernández ER, Suárez-Jácome MM, Herrera-Lee RG, Nakano T, Ozimek L, Guzman IV (2007) Alto contenido de ácido linoleico conjugado (CLA) en leche y productos derivados al incorporar semillas de girasol a la dieta vacuna Implicaciones sobre el riesgo trombo/aterogenico. Arch Latinoam Nutr 57(2):173–178

Song YS, Kang SW, Oh DK, Rho YT, Hong SI, Kim SW (2005) Bioconversion of linoleic acid to conjugated linoleic acid by Bifidobacterium breve. Biotechnol Bioprocess Eng 10:357–361

Tsiplakou KE, Mountzouris C, Zervas G (2006) Concentration of conjugated linoleic acid in grazing sheep and goat milk fat. Livest Sci 103: 274–284

Wall R, Ross RP, Fitzgerald GF, Stanton C (2010) Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. Nutr Rev 68:280–289

Wood JD, Richardson RI, Nute GR, Fisher AV, Campo MM, Kasapidou E, Sheard PR, Enser M (2003) Effects of fatty acids on meat quality: a review. Meat Sci 66:21–32

Zhang RH, Mustafa AF, Zhao X (2006) Effects of feeding oilseeds rich in linoleic and linolenic fatty acids to lactating ewes on cheese yield and on fatty acid composition of milk and cheese. Anim Feed Sci Technol 127:220–233

Zlatanos S, Laskaridis K, Feist C, Sagredos A (2002) CLA content and fatty acid composition of Greek feta and hard cheeses. Food Chem 78:471–477

Zomeño C, Juste V, Hernández P (2012) Applications of NIRS for predicting fatty acids in intramuscular fat of rabbit. Meat Sci 91: 155–159