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Differentiation of organic and non-organic ewe's cheeses using main mineral composition or near infrared spectroscopy coupled to chemometric tools: A comparative study

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ABSTRACT

Two independent methodologies were investigated to achieve the differentiation of ewes' cheeses from different systems of production (organic and non-organic). Eighty cheeses (40 organic and 40 non-organic) from two systems of production, two different breeds of ewe, different sizes, seasons (summer and winter) and ripening times up to 9 months were elaborated. Their mineral composition or the information provided by their spectra in the near infrared zone (NIR) coupled to chemometric tools were used in order to differentiate between organic and non-organic cheeses. Main mineral composition (Ca, K, Mg, Na and P) of cheeses and stepwise lineal discriminant analysis were used to develop a discriminant model. The results from canonical standardised coefficients indicated that the most important mineral was Mg (1.725) followed by P (0.764) and K (0.742). The percentage of correctly classified samples was 88% in internal validation and 90% in external validation, selecting Mg, K and P as variables.Spectral information in the NIR zone was used coupled to a discriminant analysis based on a regression by partial least squares in order to obtain a model which allowed a rate of samples correctly classified of 97% in internal validation and 85% in external validation.

1. Introduction

The term organic production is used under diverse denominations such as biological and ecological. All of them refer to the production of quality foodstuffs that respect the environment and conserve the fertility of the soils without using synthetic chemical products.

Differences between systems are based on the origin of the feed that the animals have and their conditions of husbandry. The existing legislation for the control of ecological production, biological or organic production is founded in the European norm (Council Regulation (EC) No 834/2007) [1].

The organic manufacture of diary products has increased in recent years due principally to the interest of public opinion in the safety of food, the welfare of animals and high environmental impact of systems of intensive farming. The dairy sector has had difficulties to compare organic and non-organic products. There are other implicit factors as well as the productive system such as breed, feed, seasonality and ripening that may influence on dairy products and make difficult the differentiation between organic and non-organic product [2,3]. The greatest differences between non-organic and organic systems have been found in the fat and fatty acids composition [4], since the composition of the fat in milk is fundamentally determined by feeding of animals [5]. Particularly, the relationship between the pasture and the content in *trans* octadecanoic acid C18:1 (TFA) [6–8], conjugated linoleic acid (CLA) and tocopherol have been investigated in previous studies [9–12].

Molkentin and Giesemann [12] have achieved the differentiation between organic and non-organic milk in diverse German cattle farms by the content of α-linoleic acid (C18:3ω3). They have completed the distinction between these production systems by analysis of stable isotopes ($\delta^{13}C$). Recently, it has been demonstrated that the concentration of phytanic and pristanic acid acids in cheeses and other diary products are greater in organic products than in non-organic ones [13]. Moreover, animal diet has been appeared to be the factor which has the most important effect on milk composition and its derivatives instead of production technology [14].

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Furthermore, mineral composition of non-organic diary products, especially in cheeses, depends on milk animal origin, geographical zone of production [15,16], seasonality [17,18] and process of storage and ripening [19,20] both in main elements such as Na, K, Ca and Mg and traces elements (Cu, Fe, Zn, Se. . .etc.). Differences between non-organic and organic milk have been found using macro and micro elements [21] but no references have been found for cheese.

Near infrared spectroscopy (NIRS) has been used for the discrimination of geographical origin of cows' milk products of protected origin in Italy [22] and Gruyere cheeses [23] and also in the determination of major components in butter [24], differentiation of cheeses produced with mixtures of cow's, ewes' and goats' milk among other parameters [25–27], sensory characteristics during ripening [28,29], measurement of colour or antioxidant capacity [30]. Nonetheless, no references have been found to NIRS technology for characterisation of organic cheeses.

The aim of this work was to differentiate between ewe cheeses from organic and non-organic systems of production using two methods, on the one hand the mineral composition in Ca, K, Mg, Na and P coupled to lineal discriminant analysis and on the other hand the spectral information in the near infrared zone (NIR) coupled to the discriminant analysis based on the regression by partial least squares (DPLS).

2. Experimental

2.1. Samples of cheese

Cheese samples were made from bulk tank ewe's milk from flocks of two local breeds (Castellana and Churra) and two production systems (organic and non-organic), all of them from the same geographical area (Sayago, Zamora), in the North-West of the Central Spanish plateau.

Ewes constituted a Latin squares study (2×2) (i.e. 20 organic and 20 non-organic Castellana ewes, 20 organic and 20 non-organic Churra ewes). The organic ewes went to pasture (fresh oats *ad libitum*) and their diet was supplemented (maximum 30% of the ration, approximately 700 g) with a mixture certified by the "Organic Agriculture Council of Castilla y León (CAECYL)" which was composed of cereal seeds and leguminous plants. The non-organic ewes remained on a feedlot where they were allowed *ad libitum* access to the commercial mixture Unifeed which was composed of forage and concentrates (Cobadu S.A, Spain). The composition of the diets and their ingredients are shown in Table 1.

Two sizes of cheese (large and small) and milk collected from two seasons (winter and summer) were used to elaborate the cheeses which were allowed to ripen up to 9 months. Ripening was controlled at a temperature of 15 °C and 70% relative humidity. Cheeses were monitored over the 9 months using one piece each time. Eighty pieces of cheese were obtained (40 organic and 40 non-organic cheeses). The forty organic samples were composed by 20 Churra and 20 Castellana cheese samples. The same distribution was presented in the non-organic samples (i.e. 20 Churra and 20 Castellana cheese samples).

The whole group of cheeses (80 samples) were divided in two groups; the calibration set (60 samples) and the validation set (20 samples) in order to perform the chemometric analysis. All possible variations of the samples (i.e. production system, breed, season of collection of milk and ripening time) have been represented in both sets.

2.2. NIR spectroscopy

Cheese spectra were recorded with a Foss NIRSystem 5000 equipped with a fibre optic probe of remote reflectance, type 210/210 of 1.5 m (Ref no. R6539-A). The probe has a quartz window of 5 cm × 5 cm and the reflectance is measured in the IR zone between 1100 and 2000 nm. The spectra were recorded each 2 nm, performing 32 scans of the sample and of the reference. To minimise the errors the samples were analysed in triplicate. The software used was Win ISI 1.50. The records of the spectra were obtained by direct application of the probe on the slice of cheese, without treatment or manipulation of sample.

2.3. Mineral analysis

To obtain the mineral composition of cheese samples digestion by microwave was performed and afterwards determination by ICP-optic. The mineralization was carried out weighing 0.5 g of dry ground sample and introducing in a high pressure capsule. In a first stage 5 mL of HNO_3 c. were added and a potential of 300 W was applied for 5 min. Once the sample was cold 5 mL of HNO_3 c. and 1 mL of H_2O_2 30% were added and a potential of 300 W applied for 7 min. The sample was cooled to room temperature; the volume was brought up to 100 mL with distilled water and was maintained at 4 °C until its analysis.

A Jobin Yvon ICP OES model Ultima2 powered by a radiofrequency generator at 1100 W was used for elemental determination. The following conditions were used to carry out the elemental analysis: speed of the bomb: 20 rpm; plasma argon gas flow PL1: 12 L min^{-1}; pod gas flow G1: 0.2 L min^{-1}; nebulizer flow 1.0: L min^{-1}; nebulizer pressure 2.95 bars; with the use of an argon humidificator. The analytical lines used for the different elements were Ca: 317.9333 nm; K: 766.490 nm; Mg: 279.553 nm; Na: 589.592 nm; and P: 177.440 nm. The following range of the standards concentrations has been used for Ca, Na and P: 5–10–20 mg L^{-1}; for K and Mg: 0.25–0.50–1 mg L^{-1} (concentrations of the standard dissolution which in the equivalence in cheese

Table 1
Chemical composition and ingredients of ewes' diets according the type of rearing system.

	Rearing system					
	Organic					Non-organic
Chemical composition[a]						
Dry matter (%)	69.14					89.52
Crude protein (%)	17.43					17.07
Ether extract (%)	4.89					6.93
Ash (%)	7.71					6.68
Feeding characteristics						
Fresh oats(*ad libitum*)		70%	Commercial Unifeed (*ad libitum*)			100%
Supplement		30%	Alfalfa	26%		
Alfalfa forage	35%		Barley	22%		
Peas	25%		Beetroot pulp	18%		
Oat	17%		Corn	12%		
Barley	13%		Soy	12%		
Sunflower seeds	10%					

were: Ca, Na and P: 1000–2000–4000 mg kg^{-1}; and K and Mg: 100–200–400 mg kg^{-1}). In those samples which the concentration was not found in its respective lineal margin were diluted with ultrapure water and were re-analysed until they were in the lineal response margin. The detection limits were: Ca: 10 mg kg^{-1}; K: 22 mg kg^{-1}; Mg: 10 mg kg^{-1}; Na: 36 mg kg^{-1}; P: 28 mg kg^{-1}.

The mineralization and the measurements by plasma ICP-optic were carried out in the Servicio de Análisis Químico of the Universidad de Salamanca (Spain). The results obtained for these elements in the 80 samples of cheese are presented in Table 2.

2.4. Chemometric techniques

The spectral information of the near infrared has been used to develop a discriminant model based on the regression by partial least squares (DPLS) developed in the WinISI 1.50 package, which is a qualitative supervised pattern recognition method [31]. This chemometric method assigns a new variable to each group and creates a temporal matrix and two new variables denominated dummy variables. The regression by partial least squares (PLS 2) was applied to that matrix and thus a discriminant model was obtained.

The classification of the cheeses with the data of the main mineral composition (i.e. Ca, K, Mg, Na and P) was carried out by lineal discriminant analysis with stepwise selection of variables [32,33] and the previously described mineral composition. These analyses were performed using the SPSS 13.0 for Windows package (SPSS, Inc., Chicago, IL).

Table 2
Main mineral composition of organic or non organic hard ewe cheeses obtained by ICP-optic.

Element (g kg^{-1} of cheese)	Organic (40 samples)	Non-organic (40 samples)
Ca	7.56^{a} ± 0.75 (6.29–9.31)	7.36^{a} ± 0.63 (5.93–8.86)
K	0.97^{a} ± 0.20 (0.61–1.40)	0.98^{a} ± 0.19 (0.57–1.40)
Mg	0.46^{b} ± 0.04 (0.39–0.57)	0.40^{a} ± 0.04 (0.31–0.49)
Na	9.32^{a} ± 3.01 (1.97–15.08)	9.69^{a} ± 3.31 (2.79–14.89)
P	5.24^{a} ± 0.54 (4.32–6.48)	5.12^{a} ± 0.40 (4.23–5.86)

Mean ± standard deviation of all samples corresponding to each group and range of concentration (in brackets). Statistical analysis: one-way ANOVA (α = 0.05). For each element different letters indicate statistical differences between organic and non-organic systems of production.

3. Results and discussion

3.1. Discrimination by near infrared spectroscopy

Average and standard deviation (3 times amplified) spectrum of the whole group of organic cheeses (40 samples) and the whole group of non-organic cheeses (40 samples) in the NIR zone between 1100 and 2000 nm are presented in Fig. 1. Moreover, the absolute differences between the mean spectrum of the whole group of

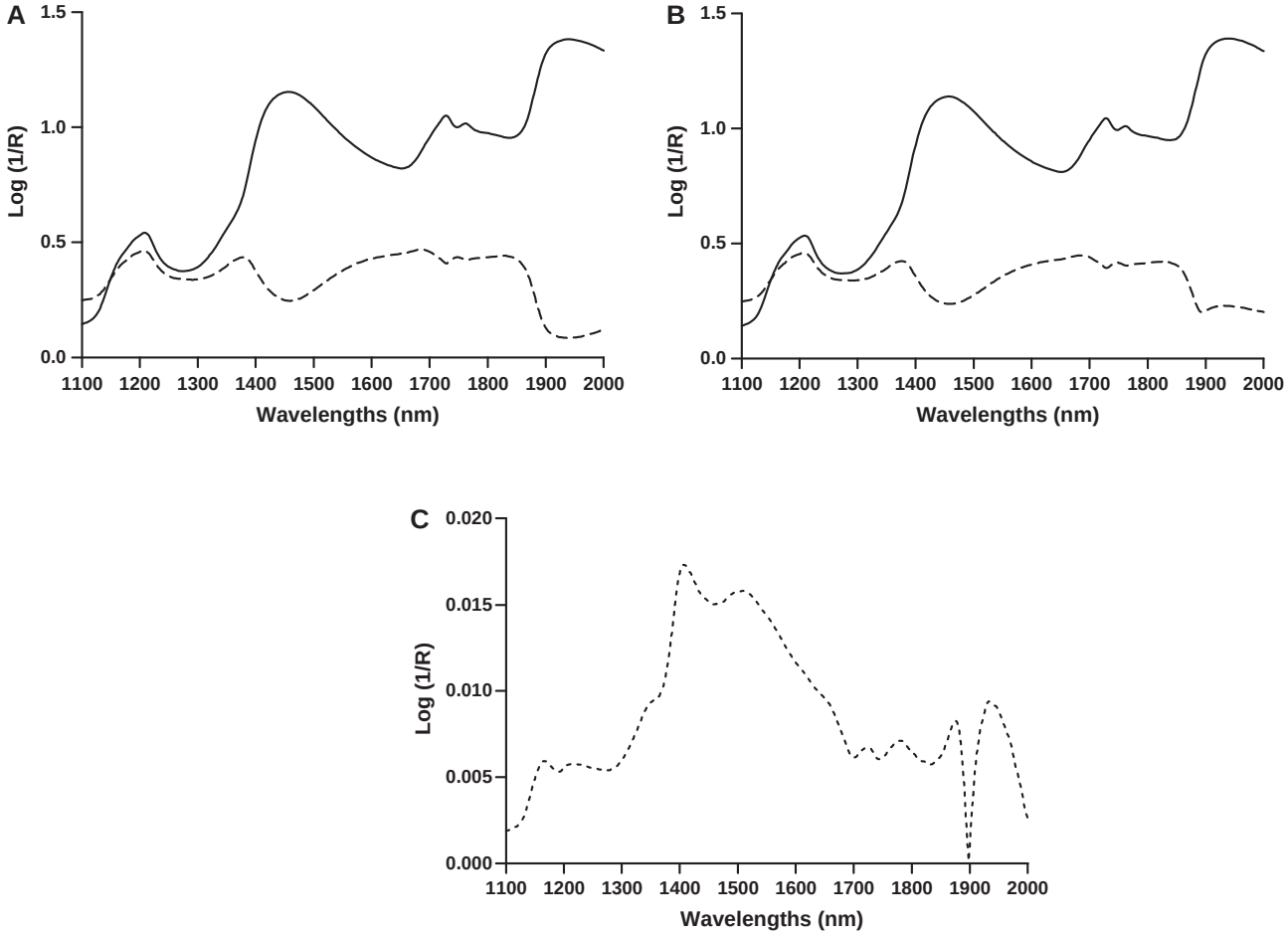

Fig. 1. Average (––) and standard deviation (—) (3 times amplified) spectrum of (A) the whole group of organic cheeses (40 samples) and (B) the whole group of non-organic cheeses (40 samples) in the NIR zone between 1100 and 2000 nm. (C) Absolute differences (—) between the mean spectrum of the whole group of organic cheeses and the mean spectrum of the whole group of non-organic cheese in the NIR zone between 1100 and 2000 nm.

Table 3
Discrimination by near infrared spectroscopy. Classification results.

			Predicted group membership	
			Organic	Non-organic
Internal validation	Count	Organic	29.0	29
		Non organic	1.0	1.0
	%	Organic	96.6	3.3
		Non organic	3.3	96.6
External validation	Count	Organic	9.0	2.0
		Non organic	1.0	8.0
	%	Organic	90.0	20.0
		Non organic	10.0	80.0

organic cheeses and the mean spectrum of the whole group of non-organic cheese in the NIR zone between 1100 and 2000 nm were presented. Differences were observed in the NIR zone from 1300 to 1700 nm. This wavelength region is related to the first and second C–H_2, C–H_3 and O–H overtones. The raw spectral data and the discriminant analysis based on the regression by partial least squares (DPLS) were used and 15 PLS factors were fixed by cross-validation. Values of RSQ of 0.812 and SECV of 0.64 were obtained. Modelling of the groups was carried out using the NIR raw spectral data and two dummy variables, whose values were 0 or 1, thus the explicit algebraic models denominated DPLS were constructed. The DPLS developed model predicted dummy values for each sample and one unit was added. Then the samples were allocated according to the higher predicted dummy with a dummy variable breakpoint of 1.5. A predicted value of 2.0 is a perfect identification in this group, 1.0 is no identification, and 1.5 indicates the classification could go either way. Moreover, mistakes occurring in the internal and external validation. These mistakes were samples incorrectly classified whose higher dummy values did not correspond to the correct one. The obtained model allowed the correct classification of 97% of samples for the organic cheeses and 97% of the non-organic ones in internal validation. When the model is applied in external validation to 20 samples of cheeses not included in the calibration model, 90% of the samples were correctly classified in organic samples and 80% in non-organic samples (Table 3).

3.2. Discrimination by the mineral composition

The effect of organic and non-organic cheese was evaluated using one way ANOVA analysis. In this case, the main mineral raw data of the 80 samples composition was employed as dependent variables. Only in the case of Mg the effect of organic or non-organic cheese was significant, therefore this element might play an important role in the classification of cheese in organic or non-organic groups (Table 2).

The initial variables were the contents of Na, Ca, Mg, K and P of the 60 cheese samples that formed the calibration group, these samples were the same ones as those used in the section of NIR spectroscopy. Through the selection of variables the elements that finally served for the development of the lineal discriminant model were Mg, K and P. The lineal discriminant function was:

$$D = -6.854 + 44.111 \cdot \mathrm{Mg\ (g\ kg^{-1})} - 1.665 \cdot \mathrm{P(g\ kg^{-1})} - 3.616 \cdot \mathrm{K(g\ kg^{-1})} \quad (1)$$

Taking into account the values of the standardised canonical coefficients it can be seen that the most important variable was Mg (1.725) followed by P (0.764) and finally K (0.742).

The representation of the original values of the mineral composition of these three elements (in g kg^{-1}) allows the construction of a three-dimensional space where the cheeses belonging to each of the systems of production used in the study can be presented. The presentation of the 80 samples in the aforementioned space is shown in Fig. 2 where a good separation of the samples belonging to each of the classes can be observed.

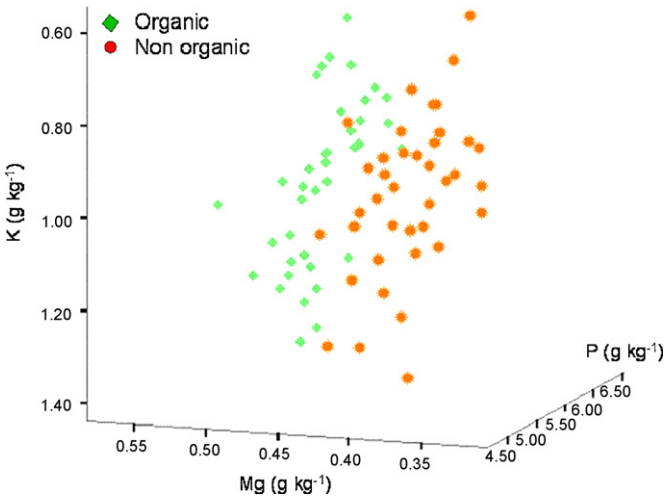

Fig. 2. Representation of cheese samples in the space defined by Mg, P and K concentrations (g kg^{-1}).

Table 4
Discrimination by the mineral composition. Classification results.

			Predicted group membership	
			Organic	Non organic
Internal validation	Count	Organic	26.0	4.0
		Non organic	3.0	27.0
	%	Organic	86.7	13.3
		Non organic	10.0	90.0
External validation	Count	Organic	9.0	1.0
		Non organic	1.0	9.0
	%	Organic	90.0	10.0
		Non organic	10.0	90.0

This plot shows that the differentiation is marked by the axis belonging to the content of Mg, the element that greatly contributes to the discriminant model generated. The obtained discriminant model (Eq. (1)) was applied using the contents of Mg, P and K. The samples were allocated according to the predicted discriminant score with a cut off value of 0. A positive discriminant score indicated that this sample belonged to the organic samples group and a negative discriminant score indicted that this sample belonged to the non-organic group. Moreover, mistakes occurring in the internal and external validation. These mistakes were samples incorrectly classified. The percentage of samples correctly classified in internal validation was 86.7% for organic cheeses and 90% for non-organic. In the case of external validation a value of 90% for both organic and non-organic cheeses was reached as indicated in Table 4.

The results demonstrate that the mineral composition together with chemometric tools can discriminate between ewes' cheeses produced organic or non-organic. The most important element in the created model was Mg, which could be related to the quantity of green pasture consumed by the animals that supply the milk for the production of organic cheeses, since Mg forms part of the chlorophyll molecule present in these pastures.

4. Conclusion

Either of the two proposed methods presents an adequate potential for the discrimination between ewes' cheeses made from milk of organic or non-organic origin. The advantages of NIR spectroscopy are the lack of sample preparation and shorter analysis time than the main mineral composition methodology. In view of the results of classification, in the external validation it is observed that the percentages of samples correctly classified are similar in both methodologies. The samples which are not correctly classified by each of the methods do not coincide. Although the results obtained are good and very promising, it would be necessary to expand the study to different geographical zones and also to different breeds of ewes with the objective of obtaining a model with greater applicability, in those circumstances a fusion of data from both techniques could be considered, since the information obtained by both methods could be complementary.

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References

[1] Council Regulation (EC) No 834/2007 of 28 June 2007, Official Journal of the European Union L189 (2007) 1–23.
[2] A. Sundrum, Livest. Prod. Sci. 67 (2001) 207–215.
[3] T. Sundberg, B. Berglund, L. Rydhmer, E. Strandberg, Livest. Sci. 126 (2009) 176–182.
[4] W. Bisig, P. Eberhard, M. Collomb, Lait 87 (2007) 1–19.
[5] D. Precht, Milchwirtsch Kiel Forschungsber 46 (1994) 65–79.
[6] P. Bergamo, E. Fedele, L. Iannibelli, G. Marzillo, Food Chem. 82 (2003) 625–631.
[7] G. Jahreis, J. Fritsche, H. Steinhart, Fett/Lipid 98 (1996) 356–359.
[8] D. Precht, J. Molkentin, Milchwiss.-Milk Sci. Int. 52 (1997) 564–568.
[9] C. Stanton, F. Jawless, G. Kjelmer, J. Food Sci. 62 (1997) 1083–1086.
[10] M.L. Kelly, E.S. Kolver, D.E. Bauman, M.E. Van Amburgh, L.D. Muller, J. Dairy Sci. 81 (1998) 1630–1636.
[11] T.R. Dhiman, G.R. Anand, L.D. Satter, M.W. Pariza, J. Dairy Sci. 82 (1999) 2146–2156.
[12] J. Molkentin, A. Giesemann, Anal. Bioanal. Chem. 388 (2007) 297–305.
[13] W. Vetter, M. Schroder, Food Chem. 119 (2010) 746–752.
[14] A. Prandini, S. Sigolo, G. Piva, J. Dairy Res. 76 (2009) 278–282.
[15] B. Prieto, I. Franco, J.M. Fresno, A. Bernardo, J. Carballo, Int. Dairy J. 10 (2000) 159–167.
[16] D. Samarzija, N. Antunac, M. Pecina, B. Mioc, D. Havranek, I. Pavlovic, Milchwiss Milk Sci. Int. 60 (2005) 158–161.
[17] M.I. Herrera Garcia, P. Peláez Puerto, M. Fresno Baquero, E. Rodriguez Rodriguez, J. Darías Martin, C. Díaz Romero, Int. Dairy J. 16 (2006) 182–185.
[18] Y.W. Park, Small Ruminant Res. 37 (2000) 115–124.
[19] T.G. Bontinis, H. Mallatou, E. Alichanidis, A. Kakouri, J. Samelis, Int. J Dairy Technol. 61 (2008) 229–236.
[20] A.J. Cichoscki, E. Valduga, A.T. Valduga, M.E. Tornadijo, J.M. Fresno, Food Control 13 (2002) 329–336.
[21] M. Gabryszuk, K. Sloniewski, T. Sakowski, Anim. Sci. Pap. Rep. 26 (2008) 199–209.
[22] M.A. Brescia, M. Monfreda, A. Buccolieri, C. Carrino, Food Chem. 89 (2005) 139–147.
[23] R. Karoui, G. Mazerolles, J.O. Bosset, J. Baerdemaeker, E. Dufour, Food Chem. 105 (2007) 847–854.
[24] D. Brennan, J. Alderman, L. Sattler, B. O'Connor, C. O'Mathuna, Measurement 33 (2003) 67–74.
[25] M.I. González-Martín, J.M. Hernández Hierro, R. Morón-Sancho, J. Salvador-Esteban, A.M. Vivar-Quintana, I. Revilla, Anal. Chim. Acta 604 (2007) 191–196.
[26] M.I. González-Martín, J.M. Hernández Hierro, I. Revilla, A.M. Vivar-Quintana, C. González-Pérez, Food Chem. 114 (2009) 1564–1569.
[27] M.I. González-Martín, J.M. Hernández-Hierro, I. Revilla, A. Vivar-Quintana, I. Lobos Ortega, Food Chem. 127 (2011) 147–152.
[28] M.I. González-Martín, P. Severiano-Pérez, I. Revilla, A.M. Vivar-Quintana, J.M. Hernández-Hierro, C. González-Pérez, I.A. Lobos-Ortega, Food Chem. 127 (2011) 256–263.
[29] G. Downey, E. Sheehan, C. Delahunty, D. O'Callaghan, T. Guinee, V. Howard, Int. Dairy J. 15 (2005) 701–709.
[30] A. Lucas, D. Andueza, E. Rock, B. Martin, J. Agric. Food Chem. 56 (2008) 6801–6808.
[31] L. Stahle, S. Wold, J. Chemometr. 1 (1987) 185–196.
[32] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics Part A , Elsevier Science Inc., 1997.
[33] B.G.M. Vandeginste, D.L. Massart, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics: Part B , Elsevier Science Inc., 1998.