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Review

Prediction of the type of milk and degree of ripening in cheeses by means of artificial neural networks with data concerning fatty acids and near infrared spectroscopy



Milton Carlos Soto-Barajas ^a, Ma Inmaculada González-Martín ^{a,*}, Javier Salvador-Esteban ^a, José Miguel Hernández-Hierro ^{a,1}, Vidal Moreno-Rodilla ^b, Ana Ma Vivar-Quintana ^c, Isabel Revilla ^c, Iris Lobos Ortega ^a, Raúl Morón-Sancho ^a, Belén Curto-Diego ^b

- a Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Ciencias Químicas, C/Plaza de la Merced s/n, 37008 Salamanca, Spain
- ^b Departamento de Informática y Automática, Facultad de Ciencias, C/Plaza de la Merced s/n, 37008 Salamanca, Spain
- c Área de Tecnología de los Alimentos, Escuela Politécnica Superior de Zamora, Avenida Requejo 33, 49022 Zamora, Spain

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ABSTRACT

The present study addresses the prediction of the time of ripening and type of mixtures of milk (cow's, ewe's and goat's) in cheeses of varying composition using artificial neural networks (ANN). To accomplish this aim, neural networks were designed using as input data the content of 19 fatty acids obtained with GC-FID of the cheese fat and scores obtained from principal component analysis (PCA) of NIR spectra. The best model of neuronal networks for the identification of the type of mixtures of milk was obtained using the information concerning the fatty acid concentration (80% of correct results in the training phase and 75% in the validation phase). Regarding the information of the near-infrared (NIR) spectra a neural network was designed. The aforesaid neural network predicted the ripening of cheeses with 100% accuracy in both training and in validation.

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1. Introduction

Cheese is currently one of the most widely consumed milk derivatives and is the one affected by the highest number of factors. The nutrient values of cheeses and their technological characteristics, such as the composition of the different fatty acids, are affected by numerous factors such as breed [1], season [2–4], stage of lactation [5–7], and diet [8–11]. Moreover, regarding diet, the geographical origin of the milk may have an influence due to seasonal variations and the changes in the forage composition of the animals' feed [12–14], the ripening time of the cheeses [15] and the source species (cow, ewe, and goat) [16].

Identification of the species that originally produced the milk represents a considerable problem for food analysts and law enforcement authorities, who must guarantee that milk and other dairy products are unadulterated and accurately labeled. In recent years, several analytical techniques for detecting mixtures of milk from different species have been developed. These include electrophoretic techniques [17,18], isoelectric focusing (IEF) [19] and capillary electrophoresis [20,21], and high-performance liquid chromatography (HPLC) [22,23], together with immunological methods, and more recently species-specific PCR [24,25] and near infrared spectroscopy (NIRS) [26].

Ensuring the traceability and quality of foods demands new developments in resolutive and/or fast analyses [27,28] that will provide data to satisfy consumer expectations and meet the legislative prescriptions regarding such products [29].

Artificial neural networks (ANN) combined with other analytical techniques have huge potential for the tracing of milk products. Thus, with gas-chromatography the data concerning mono- and sesquiterpenes have been used to determine the traceability of typical cheeses from Piamonte (Italy) [30] and to discriminate the geographic origin of Emmental cheese [31]. Additionally, ANNs have been used to classify pecorino cheeses based on their time of ripening and the manufacturing techniques employed, using data from electronic noses and analysis of volatile substances by GC–MS [32]. In products such as butter, the fatty

^{*} Corresponding author. Tel./fax: +34 23 294483.

E-mail address: inmaglez@usal.es (M.I. González-Martín).

¹ Current address: Food Colour & Quality Laboratory, Department of Nutrition & Food Science, Universidad de Sevilla, Facultad de Farmacia, 41012 Sevilla, Spain.

acid composition has been used together with ANN to predict seasonal variation [33]. In the aforementioned product, near-infrared (NIR) spectroscopy has been used to predict the dietary regime of the animals [34].

The aim of the study was to predict the ripening of cheeses and the types of milk mixtures (cow, ewe, and goat) with which they were elaborated, and to study the classification capacity of the developed models AANs were designed using as data the fatty acid composition of cheeses of varying compositions (cow, ewe, and goat) and the NIR spectra of those cheeses, were obtained with a remote reflectance fiber-optic probe.

2. Materials and methods

2.1. Samples and cheese-making procedure

Raw cow's, goat's and ewe's milk was obtained directly from the producers in Zamora (Spain). 112 cheeses with different compositions were obtained, prepared with varying but known amounts of milk from cows, ewes and goats, with percentages ranging between 0% and 100%, as described in previous work [26,35]. They were made of milk collected directly from farms in winter. The samples were cylindrical, with an initial diameter of 10 cm and a thickness of 5 cm, and were monitored over 6 months (at 0.2, 1, 2, 3, 4, 5 and 6 months), using one of the pieces each time. The cheeses were then moved to a drying chamber, where temperature (15 °C) and relative humidity (70%) were controlled. Table 1 shows the composition of the cheeses analyzed, and the ripening time. From 112 samples, 92 were used to train the neural networks; then, the networks that provided the best prediction capacity were used in the external validation of 20 samples in order to demonstrate the classification capability of the models.

2.2. Fatty acid analysis

Lipids were extracted using the International Standard Method described in ISO 14156:2001. The fatty acids of all samples were methylated [36] and analyzed by gas chromatography (GC 6890 N, Agilent Technologies, USA), using a 100 m \times 0.25 mm \times 0.20 μm capillary column (SP-2560, Supelco, Inc., Bellefonte, PA, USA). The oven temperature program was 150 °C, with increasing temperature at 1 °C/min up to 165 °C, then increasing at 0.20 °C/min up to 167 °C and then increasing by 1.50 °C/min up to 225 °C, where it was maintained for 15 min. One microlitre was injected into the chromatograph, equipped with a split/splitless injector and an FID detector. The injector and detector temperatures were 250 °C. The carrier gas was helium at 1 ml/min and split (20:1). The 19 fatty acids were identified by their retention times comparing them with the corresponding standards and each compound was quantified by means of calibration curves (Table 2).

2.3. NIR spectroscopy

A Foss NIR System 5000 with a standard 1.5 m 210/210 bundle fiber-optic probe, Ref. no. R6539-A, was used. The probe employs a remote reflectance system and uses a ceramic plate as reference. The window is of quartz with a 5×5 cm surface area, measuring 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 (112 samples) was accomplished by direct application of the fiber-optic probe onto a 1-cm thick slice from the center of each cheese wheel with no prior treatment or manipulation (Fig. 1). To minimize sampling error, all the samples were analyzed in triplicate.

The software used was Win ISI 1.50 installed on a Hewlett–Packard Pentium III computer.

Table 1Characteristics of the samples of cheeses used in the determination of the fatty acid composition and NIR analysis.

Quality parameter	Category	Category			Binary code			
Ripening time in months	0.2				0	0	0	
	1.0				0	0	1	
	2.0				0	1	0	
	3.0				0	1	1	
	4.0				1	0	0	
	5.0				1	0	1	
	6.0				1	1	0	
	Cow	Ewe	Goat					
Percentage of milk in the mixture for each species	100	0	0	0	0	0	0	
	0	100	0	0	0	0	1	
	75	25	0	0	0	1	0	
	50	50	0	0	0	1	1	
	25	75	0	0	1	0	0	
	0	0	100	0	1	0	1	
	25	0	75	0	1	1	0	
	50	0	50	0	1	1	1	
	75	0	25	1	0	0	0	
	0	25	75	1	0	0	1	
	0	50	50	1	0	1	0	
	0	75	25	1	0	1	1	
	33	33	33	1	1	0	0	
	10	45	45	1	1	0	1	
	45	10	45	1	1	1	0	
	45	45	10	1	1	1	1	

2.4. ANN models

2.4.1. Building the network

A total of four types of networks was generated: two in which the fatty acid concentrations were used as input and the other two with NIR spectral inputs, and in both networks the type of mixture of milk and the ripening time were used as objectives (Table 3).

The creation, training and simulation of the neural networks were performed on a personal computer (Intel Pentium IV, 3.0 GHz, 512 Mb RAM) using two different software tools. First, the *Java Neural Networks Simulator* (JavaNNS), is employed which is a graphical design tool for the specific task of the design and development of ANN. The second one was the *Neural Network Toolbox* (version 7.9.0) that works on the well-known mathematical tool MATLAB. The aim was to explore the advantages of these tools, which are widely used by the scientific community.

One of the most important tasks in ANNs is the consideration of architecture (number of layers, neurons quantity by layer and transference function at each layer). This design decision makes feasible or not the learning task. In this way, as the proposed learning task belongs to supervised paradigm (in such a way that there is an external expert knowledge that we want to reproduce), we proposean architecture of the multilayer perceptron (MLP) neural networks, made of three layers, for the four types of networks, with the following characteristics: input layer, when

Table 2Fatty acids used in the building of the artificial neural networks.

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	Lipid number (C:D)	Common name	Concentration (mg FA/g cheese)			SD of concentration (mg FA/g cheese)	
	c8:0	Caprylic acid	1.00	_	10.36	1.55	
	c9:0	Pelargonic acid	0.00	_	0.28	0.07	
	c10:0	Capric acid	3.56	_	27.92	4.97	
	c11:0	Undecylic acid	0.00	_	0.35	0.06	
	c12:0	Lauric acid	4.36	_	13.44	1.90	
	c13:0	Tridecylic acid	0.06	_	0.47	0.08	
	c14:1	Myristoleic acid	0.23	_	2.96	0.56	
	c14:0	Myristic acid	16.05	_	36.56	4.70	
	c15:0	Pentadecylic acid	0.32	_	3.40	0.61	
	c16:1	Palmitoleic acid	1.49	_	5.02	0.67	
	c16:0	Palmitic acid	44.45	_	107.36	13.70	
	c17:1	Heptadecenoic acid	0.27	_	2.15	0.21	
	c17:0	Margaric acid	0.61	_	3.67	0.33	
	c18:2	Linoleic acid	3.48	_	10.78	1.71	
	c18:1 cis	Oleic acid	24.45	_	58.30	7.93	
	c18:1 trans	Elaidic acid	2.07	_	39.73	3.95	
	c18:0	Stearic acid	14.27	_	47.49	7.38	
	c19:0	Nonadecylic acid	0.00	_	0.33	0.08	
	c20:0	Arachidic acid	0.00	_	0.72	0.14	

the concentrations of fatty acids 19 neurons were included from the concentration of each of them; when the input layer corresponded to the NIR spectra of the cheeses we originally started out from 451 neurons, but it is clear that with the available data it is impossible to perform the learning task, that is, the determination of weights value for this huge network. So it was necessary to consider the use of principal components analysis (PCA) a wellknown technique to reduce the problem of dimensionality with the minimum information lost; as result, only 4 neurons were used from the scores of the PCA analysis (with more than 99.7% of variability explained from 451 data of the NIR spectra 1100-2000 nm, every two nanometers). In the "intermediate" or hidden layer the number of neurons was varied as a function of the task to be performed. We assessed the working of the network using 5, 10, 15 and 20 neurons and selected the number that provided the highest number of successes, during the training phase of the network. In the output layer, the operator did not have any direct influence on it; the computing program constructed the layer automatically as a function of the classes. Here, the output layers contained 3 or 4 neurons, since the classes were defined by 7 or 16 categories expressed as 0–6 for the first one (ripening time) and from 0 to 16 for the following ones (mixtures of milk), in both cases using the binary code shown in Table 1.

Training. Once the structure of the artificial network had been established, training was begun running the retropropagation program; the Levenberg–Marquart algorithm was used. We then established the learning rate, expressed by the coefficient η in 0.0001 and the moment, μ =0.05. With this is possible to achieve a faster convergence of the network because the moment prevents the network from stopping when falling to a local limit and allows it to approach a global minimum [37]. The maximum number of periods was defined at 1,000,000 (referring to the number of times that the network selected and tested a new input value iteratively) [38]. Network training ceased when the condition of finalization had been met (in these networks, a median squared error of less

Table 3Number of neurons per layer of the artificial neural networks constructed for the prediction of the ripening times of the cheeses and the type of mixtures of the milk used in their elaboration.

Layer	Information provided	Number of neurons
Input	•	19
	Scores from principal components of NIR spectra	4
Hidde	en Hidden neurons	5-20
Outp	ıt Ripening time	4
	Type of mixture	3

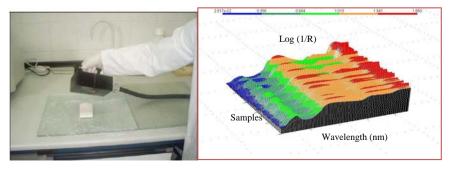


Fig. 1. Registration of cheese samples and their NIR spectra.

 Table 4

 Results from the neural networks for the determination of the ripening times of the cheese and the types of mixtures of milk used in their elaboration.

Layer	Neurons in the	Neurons in the hidden layer				
Input	Output	5	10	15	20	
Fatty acid concentration	Ripening time	6.30×10^{-1} 20^{a}	8.42×10^{-5} 60^{a}	2.30×10^{-7} 80^{a}	2.37×10^{-6} 80^{a}	
	Type of mixture	$\begin{array}{c} 2.80 \times 10^{-1} \\ 0^{a} \end{array}$	$6.94 \times 10^{-4} \\ 40^{a}$	1.30×10^{-9} 80^a	$4.15 \times 10^{-9} \\ 40^{a}$	
Scores from principal components of NIR spectra	Ripening time	9.0×10^{-3} 32^{a}	$4.48 \times 10^{-15} \\ 100^{a}$	$1.35 \times 10^{-16} \\ 100^{a}$	5.98×10^{-11} 100^a	
	Type of mixture	1.50×10^{-1} 2^a	$3.00 \times 10^{-1} \\ 20^{a}$	$5.00 \times 10^{-4} \\ 50^a$	$2.20 \times 10^{-1} \\ 10^{a}$	

Mean squared error.

than 0.005 or overshooting the maximum number of iterations established).

Validation. After the network had been trained (with 92 samples randomly selected) and the appropriate weight matrix for solving the problem of classification had been found, 20 samples (almost a 20% of all samples, percentage usually used in validation of artificial neuronal networks) were used in the validation set to identify the best networks, comparing the output values calculated by the network with real outputs and counting the successes.

3. Results

During the training phase of the network with a set of 92 samples, to select the most suitable type of networks, we performed tests of its architecture to study its efficiency with 5, 10, 15 and 20 neurons in the hidden layer (Table 3). When we used the concentration of fatty acids as the input data we observed that the mean squared error (MSE) decreased with the increase in the number of neurons in the hidden layer from 5 to 15 neurons. With 15 neurons the lowest error was obtained both in the prediction of the ripening time (MSE= 2.30×10^{-7}) and for the mixtures of milk (MSE= 1.30×10^{-9}). When spectral information was used as the input (the scores obtained from 4 principal components of the NIR spectra), the behavior was similar. With 15 neurons in the hidden layer, the lowest mean squared error in the prediction of the cheese ripening time was obtained (1.35×10^{-16}), and also for the determination of the type of mixture used to make the cheeses (5.0×10^{-4}) . When 20 neurons were used in the hidden layer, the mean squared error increased with respect to the results obtained with the networks with 15 neurons in the two quality parameters analyzed (ripening time and mixtures of milk). This kind of behavior may have been due to overtraining of the network and may have given rise to a loss of the generalization capacity, known as overfitting [39]. With a view to overcome this problem of overfitting, some authors [32] recommend networks with a quantity of hidden neurons between the total number in the input layer and the set of output neurons.

In the training phase, as can be seen in Table 4, with 15 neurons in the hidden layer the percentage of correct classifications for estimating the ripening times was 80% using the fatty acid composition data, with an efficiency of 100% when the data used were from spectral information. Regarding the percentage of correct classifications according to the type of milk mixtures (cow, ewe, and goat) with which the cheeses were elaborated,

this was 80% when the fatty acid concentrations were estimated and fell to 50% when the input data were the scores from principal components analysis of NIR spectra. It should be noted that for the identification of the type of mixture, even with the use of more principal components, it was not possible to improve the performance of the network. We consider that since the number of classes of the type of cheese mixtures was 16 it would be necessary to have more samples in the training group in order to obtain better results in the classification. In cases in which a classification of less than 100% was obtained the poorly classified samples did not correspond to any specific class, either regarding the ripening time or the type of milk mixtures, in both cases their distribution being completely random.

From what may be deduced on considering the results of the network training shown in Table 4, regardless of whichever of the two input parameters were used (concentration of fatty acids or the scores from principal components of the NIR spectra) with 15 neurons in the hidden layer, it was possible to obtain lower values of the mean squared error and higher percentages of correct classification with respect to the quality parameters of the samples of cheeses evaluated (ripening time or the mixtures of milk with which the cheeses were made).

After completing the design of the networks with the best capacity during training (a higher number of correctly classified and the lowest mean squared error), we validated the design with a set of 20 samples that were not used during the training. Fig. 2 shows the percentage of correctly classified samples, using 15 neurons in the hidden layer (selected according to the training results). From the validation of these networks with unknown samples, it was deduced that when the fatty acid concentration is used as input it is possible to correctly identify 80% of the cheese samples according to their ripening time and to correctly predict 75% of the type of milk mixture used (cow, ewe, and goat) in an unknown cheese. When we used the scores from four principal components (99.7% of variability explained) of the NIR spectra as input data, the artificial neural network classified 100% of the samples of unknown cheeses correctly in the case of the determination of the ripening time. However, it only classified the type of mixture of milk correctly for 50% of the samples, but we must bear in mind that should be classified into 16 groups with different percentages of cow's, sheep's and goat's milk (Table 1). Nevertheless, the network is not a finished product, and it can be improved through a harder process of training. However we demonstrate the validity of the technique.

Using only raw information from NIR spectra (without applying ANN) was not possible to find differences among the 16 groups of

^a Percentage of correct classification.

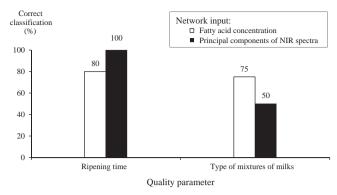


Fig. 2. Results of the validation of the neural networks.

the mixtures of milk (cow, sheep, goat) shown in Table 1. Neither is it possible to know the ripening time (from 0.2 to 6 months) using only the spectral information. The results of this article show that, with the use of ANN and NIR spectra (4 scores of principal components from NIR spectra as input data, which explain 99.7% of spectral variability), the neuronal network was able to classify 100% of the unknown cheese samples for the ripening time. However, it classified correctly 50% of the cheese samples among 16 groups belonging to the different mixtures of milk.

Regarding the identification of the ripening time of the cheese samples the NIR spectra are more efficient that the composition of fatty acids because they give us more information about the samples. In the first place, NIR spectra are recorded between 1100 and 2000 nm and for each sample we have information every 2 nm, that is 451 data of the $\log 1/R$, where R is the reflectance. In the second place, a measure of reflectance is obtained directly when the probe is placed onto the cheese sample, without any manipulation and that introduces a least error in the chemical measurement of the fatty acids. In this last technique the profile of fatty acids depends on the following: (1) in the lipidic extraction, the temperature of extraction affects mainly the minority fatty acids giving rise to a possible thermal degradation of the fatty compounds; (2) incomplete elimination of the solvents [40,41]; (3) the mechanical grinding (needed to carry out the extraction) could produce lipolysis on the sample and (4) as an the analytical tool, gas chromatography could affect the fatty acids content (when using highly polar capillary columns, they would not be able to separate minor isomers [42].

From the results above, it may be deduced that the characteristics of the prediction models constructed on the basis of the artificial neural networks reveal a greater relationship between the type of milk used in the elaboration of the cheeses and the fatty acid composition, and between the time of ripening and the NIR spectral information.

4. Conclusions

The use of artificial neural networks offers a useful tool for predicting the ripening time of cheeses and the determination of the mixtures of different milk (cow, ewe, and goat) used in their elaboration using the chemical data concerning their fatty acid concentration or the data from the principal components of their NIR spectra.

Nonetheless, a comprehensive study using more samples should be made in order to evaluate not only other milk percentages, but also other factors such as different production areas and different elaboration procedures for the complete development of the aforesaid artificial neural network.

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