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Food Chemistry 91 (2005) 91–98
Food Chemistry
www.elsevier.com/locate/foodchem

Chemometric classification of Basque and French ciders based on their total polyphenol contents and CIELab parameters

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Received 29 March 2004; revised 20 May 2004; accepted 25 May 2004

Abstract

Total polyphenol contents, estimated by Folin–Ciocalteu method, and CIELab chromatic parameters were determined in Basque and French ciders with the aim of developing a classification system to confirm the authenticity of ciders. A preliminary study of data structure was performed by a multivariate data analysis using chemometric techniques such as cluster analysis and principal component analysis. Supervised pattern recognition methods, such as linear discriminant analysis, *K*-nearest neighbours (KNN), soft independent modelling of class analogy and multilayer feed-forward artificial neural networks (MLF-ANN), provided classification rules for the two categories based on the experimental data. KNN results for Basque ciders afforded an excellent performance in terms of recognition and prediction abilities (99%), providing a useful tool to detect genuine Basque ciders. Despite KNN and MLF-ANN giving the best results for French ciders, with a success rate of prediction ability around 91%, this would not be acceptable for authentication purposes.

Keywords: Polyphenol; Cider; Folin–Ciocalteu method; CIELab chromatic parameters; Chemometrics; Pattern recognition

1. Introduction

Cider characteristics are determined by its chemical composition. In this sense, polyphenols are involved in cider quality. The influence of polyphenols on the organoleptic properties of fruit juices and fermented beverages has been widely reported: thus, polyphenols contribute to the colour, bitterness and astringency of ciders (Lea & Drilleau, 2003), provide cider aroma (Beech & Carr, 1977) and can be responsible for haze formation during storage (Lea, 1990). Moreover, phenolic compounds are known to play an important role in the cidermaking processes, since they participate in the spontaneous clarification of ciders, and present an inhibiting effect on spoilage microorganisms, as well as, on clarification enzymes (Cowan, 1999; Sponholtz, 1993).

Abbreviations: CA, cluster analysis; KNN, K-nearest neighbours; LDA, linear discriminant analysis; MLF-ANN, multilayer feed-forward-artificial neural network; PCA, principal component analysis; RMSE, root medium square error; SD, standard deviation; SIMCA, soft independent modelling of class analogy.

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doi:10.1016/j.foodchem.2004.05.049

The Folin–Ciocalteu method is considered to be the most suitable method for estimating the total polyphenol content, and is widely used (Scalbert, 1992).

Colour measurement is an objective quality parameter that is used, among other applications, as a quality index for raw and processed food, for determinations of conformity of food quality to certain specifications, or for the evaluation of quality changes as a result of food processing, storage or other factors (Giese, 2000). Several methods have been developed for the analysis of colour, but the CIELab system is the one that nowadays presents a high acceptance since it is the most indicative of sensory perception. This system measures the degree of lightness (L^*), red/green chromaticity ($\pm a^*$) and yellow/blue chromaticity ($\pm b^*$). In the field of oenology, colour measurements have been widely used in order to evaluate wine quality (Ortiz, Herrero, Sánchez, Sarabia, & Iñiguez, 1995) and different ageing technologies (Miguel, Marín, Zamora de Alba, & Álvarez, 2001) and to characterize wines with a certified brand of origin (Meléndez, Ortíz, Sánchez, Sarabia, & Iñiguez, 1999).

In apple-derived products, colour is directly related to browning, playing an important role in the quality of the final product. In apple juices, browning is undesirable since it provides brown pigments that reduce the commercial value of juices. However, in ciders, a yellow–orange colour is beneficial from the point of view of their quality. Some works dealing with colour measurements for studying the species responsible for browning in apples, can be found in the literature (Amiot, Tacchini, Aubert, & Nicolas, 1992; Goupy et al., 1995; Oleszek, Lee, & Price, 1989). Several references allude to the fact that must and cider colours are mainly due to the oxidation products of polyphenols, polyphenoloxidase (PPO) activity and the polyphenolic profile being fundamental for colour development. First, PPO oxidises *o*-diphenols to *o*-quinones, that cause a series of coupled oxide-reduction reactions with other polyphenols, the end products of which are coloured pigments (colour range from yellow to brown). At this point, it is interesting to note that apple polyphenols are all colourless, except for flavonols, that are yellow, and anthocyanins, red, but they are generally minor compounds in apples. Mainly, hydroxycinnamic acids (caffeoylquinic acid) and catechins are the compounds involved in enzymatic oxidations by PPO (Janovitz-Klapp, Richard, Goupy, & Nicolas, 1990). Their *o*-quinones, with a yellow colour and very unstable (Rouet-Mayer, Ralambosoa, & Philippon, 1990), take part in coupled oxidation reaction with flavan-3-ols (Cheynier & Ricardo da Silva, 1991), dihydrochalcones (Oszmianski & Lee, 1991) or flavonols (Oleszek et al., 1989). These coupled reactions with hydroxycinnamic acid and flavan-3-ols can generate caffeicines (dimers of caffeic acid or caffeoylquinic acid: colourless or slightly yellow), dehydrodicatechins type A (yellow) and type B (colourless), type A procyanidins (colourless), heterodimers of caffeoylquinic acid and catechins or procyanidins (some of them coloured) (Sanoner, 2001). With dihydrochalcones, colourless, yellow or orange oxidation products can be obtained. Products arising from oxidation of catechins and dihydrochalcones were shown to account for about one-half of apple must colour (Lea, 1984). Flavonol oxidation products are lighter yellow than the flavonol of origin (Sanoner, 2001). Most apple polyphenol oxidation products conserve their polyphenolic structure, and some of them even present UV/Vis spectra similar to those of the native polyphenol (Jorgensen, Cornett, Justensen, Skibsted, & Dragsted, 1998). Browning degree depends on the degraded polyphenols; thus, some authors have established correlations between initial polyphenol concentrations or oxidised polyphenol concentrations and the chromatic parameters (L^*, a^*, b^*) in apple pulp (Goupy et al., 1995).

France, the United Kingdom and Spain are the main European producers of cider. In Spain, there exist two regions with ancient cidermaking traditions, Asturias and the Basque Country. In this paper, French and Basque ciders are studied, taking into account their total polyphenol content and their colour.

Cidermaking technologies used in the Basque Country and in France differ markedly, which is clearly reflected in the organoleptic properties of the final products. Sensory properties of ciders are due to their chemical compositions, that are very influenced by technologies. Differences between Basque and French cidermaking technologies were reported by Alonso-Salces et al. (2004).

Maceration, pressing, enzymatic clarification, centrifugation and filtration are considered to be the cidermaking steps mainly responsible for the difference between the polyphenolic compositions of Basque and French ciders. On the one hand, during maceration and the long pressings in the Basque tradition, an intensive enzymatic and chemical oxidation of polyphenols takes place. Therefore, the musts obtained show lower polyphenol contents. Moreover, must oxidation occurs to a greater extent when must is in contact with apple pulp (Lea et al., 2003).

Food authenticity is a relevant quality criterion at the present time; therefore, methods to guarantee it, based on chemical analysis and sophisticated data analysis procedures, are being demanded by food producers, consumers and regulatory bodies. Chemometric techniques are commonly used in order to develop systems for the determination of geographical origin or quality brand of foodstuffs and fraud detection (Ashurst & Dennis, 1996), which is of great interest from an economic point of view for the food industry. In this study, general parameter measurements, such as total polyphenol content and CIELab chromatic parameters, are used in or-

der to characterize ciders on the basis of their geographical origin, the Basque Country or France, and to develop a classification system to confirm the authenticity of ciders.

2. Experimental

2.1. Solvents and standard phenolics

Ethanol and glacial acetic acid of analytical quality were purchased from Biosolve Ltd. (The Netherlands). Folin–Ciocalteu reagent was provided by Merck (Germany). Deionized water was obtained with a Milli-Q water system (Millipore, Bedford, MA). (−)-Epicatechin was supplied by Sigma–Aldrich (France).

2.2. Samples

A total of 151 cider samples was analysed: 9 Basque ciders of 1998 vintage (18 samples), and 36 Basque ciders (65 samples) and 34 French ciders (68 samples) of 1999 vintage. For most ciders, two bottles of the same batch were sampled. Basque ciders were supplied by different cidermakers from Guipúzcoa and Vizcaya, and by the Experimental Orchard from Zalla that belongs to Diputación Foral de Bizkaia (Basque Government). French ciders were provided by cidermakers from Brittany and Normandy. These sets of ciders were considered to be representative of the ciders made in the Basque Country and France.

Ciders were degassed for 10 min in an ultrasonic bath and sampled as follows: for the determination of cider total polyphenol content (Folin–Ciocalteu assay), aliquots of 0.5 ml were sampled and stored at −20 °C prior to analysis. For performing CIELab chromatic measurements, ciders were previously centrifuged at 6000*g* for 15 min at 4 °C.

2.3. Analytical procedures

2.3.1. Total polyphenol content by Folin–Ciocalteu method

Estimation of the global polyphenol content in cider was performed by the Folin–Ciocalteu method adapted from Singleton and Rossi (1965). Cider aliquots (0.5 ml) were diluted 20-fold in ethanol–acetic acid 2.5% (10:90). Folin–Ciocalteu reagent (0.25 ml) was added to 0.5 ml of the diluted cider solution. The mixture was homogenised with a vortex and, after 3 min for allowing the reaction to take place, 1 ml of Na_2CO_3 (200 g/l) and 3.25 ml of ultrapure water were added and homogenised. Then, the mixture was incubated for 10 min at 70 °C. Once it had cooled down to room temperature, it was homogenised and its absorbance measured at 700 nm with a Uvikon 860 UV/Vis spectrophotometer (Milan, Italy) against a blank (0.5 ml ethanol–acetic acid 2.5% (10:90) plus reagents) in the reference cell. Quantification was achieved by reporting the absorbances in the calibration curve of (−)-epicatechin used as standard polyphenol.

2.3.2. CIELab chromatic parameters

Colour measurements were made on degassed and centrifuged cider aliquots, using a portable tristimulus colour analyzer Chromameter model CT100 (Minolta Camera Co. Ltd., Japan) and expressed in Commission Internationale d'Eclairage L^*, a^* and b^* colour-space co-ordinates.

2.4. Data analysis and chemometric procedures

The data matrix contained 151 different samples of cider (objects) and four variables measured on each object (total polyphenol content, L^*, a^*, b^*). Each cider sample was represented in a 4-dimensional space by a data vector which is an assembly of the four features. Data vectors belonging to the same category (Basque or French) were analysed using chemometric procedures that have been described in the literature (Latorre, Peña, García, & Herrero, 2000; Padín et al., 2001), namely cluster analysis (CA), principal component analysis (PCA), linear discriminant analysis (LDA), *K*-nearest neighbours (KNN), soft independent modelling of class analogy (SIMCA) and multilayer feed-forward neural networks (MLF-ANN). Statistical and chemometric data analyses were performed by means of the statistical software packages Statgraphics (1994–2000), Parvus (Forina, Lanteri, & Armanino, 2000) and SPSS (1989–1999).

3. Results and discussion

3.1. General

Total polyphenol contents by Folin–Ciocalteu assay (PPT) and colour parameters (L^*, a^*, b^*) of the ciders studied are summarised in Table 1. Total polyphenol content of Basque ciders (0.2–1.2 g EC/l) are, approximately, one-half of the content in French ciders (0.3–3.8 g EC/l). However, the concentration range of the total cider polyphenol contents determined by HPLC-DAD (Basque ciders: 24–331 mg/l and French ciders: 143–2488 mg/l) (Alonso-Salces et al., 2004) was wider than by Folin–Ciocalteu assay. The different results obtained by these two methologies for measuring total polyphenols could be explained by the strong polyphenol oxidation that takes place during Basque cidermaking procedures. Thus, the results of the Folin–Ciocalteu assay for Basque ciders were relatively higher, probably as a consequence of the contribution of interferences, e.g., by polyphenol oxidation products with free

Table 1
Total polyphenol content (g (−)-epicatechin/litre) by Folin–Ciocalteu method (PPT) and CIELab parameters (L^*, a^*, b^*) in Basque and French ciders[a]

	Basque ciders				French ciders			
	Mean	SD	Min	Max	Mean	SD	Min	Max
PPT	0.8	0.2	0.2	1.2	1.6	0.7	0.3	3.8
L^*	88	6	69	96	89	4	78	97
a^*	−3	2	−6	5	−2	2	−5	7
b^*	38	9	19	64	51	11	15	76

[a] SD, standard deviation; max, maximum; min, minimum.

phenolic hydroxyl groups (Singleton, Orthofer, & Lamuela-Raventós, 1999): caffeicines (Cilliers & Singleton, 1991), dehydrodicatechins (Guyot, Vercauteren, & Cheynier, 1996), procyanidins type A (Burger et al., 1990) and heterodimers of polyphenols (Richard-Forget, Amiot, Goupy, & Nicolas, 1995), to the measurement. This type of observation about Folin–Ciocalteu results not being in accordance with those obtained by HPLC analysis of individual polyphenols, has already been pointed out by other authors, and it justifies alluding to the non-enzymatic formation of browning intermediates (endiols and reductones) as responsible for the apparent total polyphenol increase (Spanos & Wrolstad, 1992). However, the Folin–Ciocalteu method is generally considered to be an appropriate assay for the total polyphenol content (Scalbert, 1992).

Lightness (L^*) values in both kind of ciders are similar, showing means for 88–89. In Fig. 1, histograms for L^* show that most frequent values of lightness in ciders are found in the range 85–95. Parameter a^* had negative values in most ciders, revealing a certain greenish tone in them, even though they were relatively small values. The mean a^*-value in Basque ciders was −3, which was smaller than that from French ciders, whose mean value was −2. Parameter b^* was the most discriminant variable between the two kinds of ciders, the mean values being 38 for Basque ciders, and 51 for French ones (Table 1). This difference is also observed in the histograms for b^* (Fig. 1): Basque ciders present the most frequent b^*-values in the range 30–45, whereas French ciders are between 45 and 60. The physical meaning of these positive b^*-values is related to cider yellow colour: the higher b^* is, the greater the yellow intensity will be. Thus, French ciders presented a stronger yellow colour than Basque ones, probably due to the higher acidities of Basque ciders (Cabranes, Moreno, & Mangas, 1991; Irastorza, 1988; Salih & Suárez-Díez, 1990). In this sense, it has been observed that more acidic pHs favour the

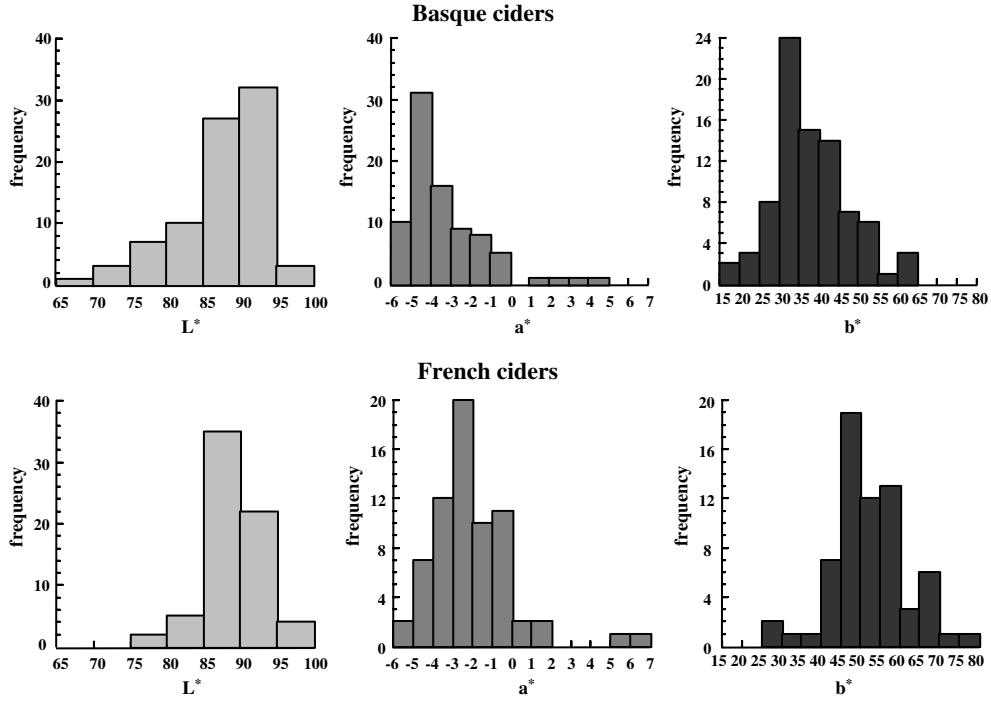

Fig. 1. Histograms obtained for CIELab parameters in Basque and French ciders.

formation of colourless oxidation products (Guyot, Cheynier, Souquet, Moutounet, & Drilleau, 1995). Moreover, the long macerating periods in the Basque procedure may favour the elimination of pigments resulting from oxidation, since these pigments and also tannins are largely retained on apple pulp (Lea, 1995).

3.2. Univariante data analysis

Significant differences were observed in the PPT, L^*, a^* and b^* variables between Basque and French ciders. The Fisher index was calculated to establish the discriminant capacity of the variables one by one (Sharaf, Illman, & Kowalski, 1986). Fisher weights for PPT, a^* and b^* were 119, 16 and 63 ($p<0.001$), respectively, those presenting the best univariate discriminant capacities. In spite of this, Box–Whisker plots constructed for each variable showed an overlap between the ranges of values in the two classes for every feature: thus, none of the variables measured was able to discriminate between the Basque and French categories by itself. Therefore, a multivariate approach had to be evaluated.

3.3. Cluster analysis and principal component analysis

CA describes data structure, revealing the natural clusters that exist in a data set on the basis of the information provided by the measured variables. Owing to its unsupervised character, it is commonly applied before other multivariate techniques. In this study, CA was performed of the autoscaled data set, using the square of the Euclidean for calculating similarities between samples and Ward's hierarchical method for establishing clusters. The results achieved are presented as a dendogram (Fig. 2). Six natural groupings of ciders were found at a similarity level of 0.70. Clusters A and B were formed by Basque ciders and six French samples, in each cluster. Clusters F and C contained French ciders, and four and five Basque samples, respectively. Cluster E was made up of Basque ciders and a French one, and cluster D, only of French samples.

PCA was performed of the autoscaled data. This technique allowed us to reduce the number of variables. The first two principal components accounted for 88% of the total variability present in the data. Thus, a partial visualisation of data structure in a reduced dimension was achieved. The bidimensional plot of the sample scores in the space defined by the two first principal components shows a natural separation of Basque and French ciders (Fig. 3), even though a region where the two categories slightly overlap is observed. Those samples that in CA were included in clusters that mainly contained samples of the opposite class, are the samples found in the overlapping region in the bidimensional space. Hence, PCA results agreed with those obtained by CA, which suggests that the estimation of the total polyphenol content by Folin–Ciocalteu assay, together with chromatic measurements, could contain enough information to distinguish between Basque and French ciders.

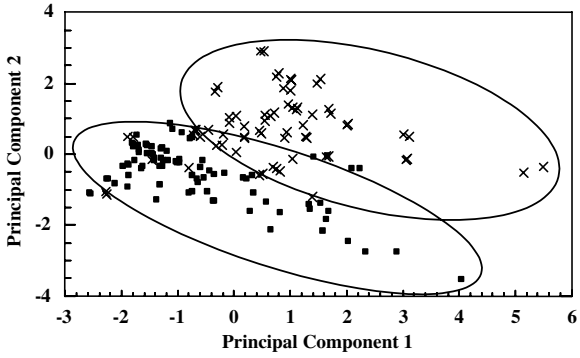

Fig. 3. Eigenvector projection of cider samples. Sample codes: ■, Basque ciders; ×, French ciders.

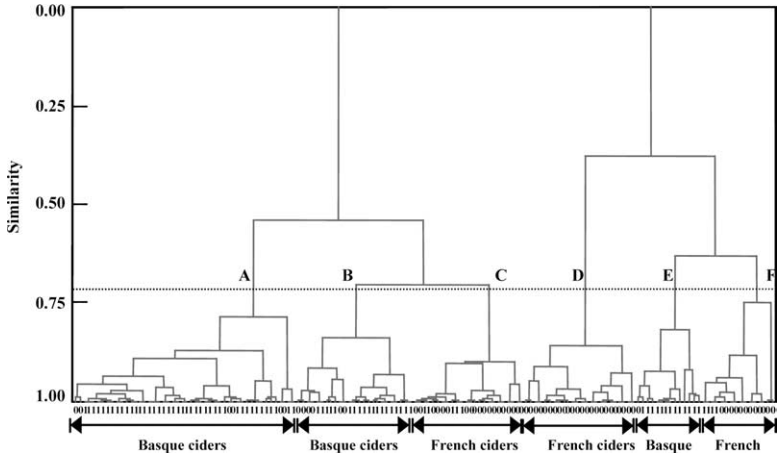

Fig. 2. Dendogram of cluster analysis. Sample codes: 0, French ciders; 1, Basque ciders.

3.4. Supervised pattern recognition methods

Several different supervised pattern recognition techniques, such as LDA, KNN, SIMCA and a MLF neural network, were applied to the autoscaled data matrix, conformed by 151 cider samples and four variables, in order to characterise ciders in the established categories, Basque and French. A cross-validation of the classification rules proposed by these techniques was performed by dividing the complete data set into a training set and an evaluation set. Samples were assigned randomly to a training set, consisting of 75% of them, and the test set, composed of the remaining 25% samples. Such a division allows a sufficient number of samples in the training set and a representative number of members among the test set. The same process was repeated four times with different constitutions of both sets, to ensure that all the samples had a possibility of being included in the evaluation set at least once. The different pattern recognition techniques were applied to the four training-test sets obtained. The reliability of the classification models achieved was studied in terms of recognition ability (percentage of the members of the training set correctly classified) and prediction ability (percentage of the members of the test set correctly classified by using the rules developed in the training step).

KNN was performed using the inverse square of the Euclidean distance as the criterion for calculating the distance between samples, and the number of neighbours (K) was selected after studying the success in classification with different K values when applying this technique to a training set with all the samples. K-values assayed were 3, 5, 7, 9 and 11. For $K \leqslant 5$, three samples were misclassified by the model; for $K > 5$, the number of samples wrongly classified increased with K value. Hence, $K = 5$ was selected.

WinNN32 MLF-ANN (1993–1996) was applied to predict the category of ciders (Basque or French) on the basis of an input pattern consisting on the autoscaled data matrix. Some empirical preliminary trials were performed to determine an adequate MLF structure. The neural architecture that achieved better results was a MLF with three layers: an input layer with four neurons, one hidden layer with five neurons and an output layer consisting of a neuron with binary output (Table 2). Networks with simpler structures (4-3-1 or 4-2-1) did not manage to learn. The target output was assigned as 0 for French ciders and 1 for Basque ones, and a sigmoidal function $f(x) = 1/(1 + [\exp(-x)])$ was used as a transfer function. The neural network was trained by means of an algorithm that combined the use of an adaptative learning rate parameter (ALRP) (η) and a momentum (μ) which have been described previously (Padín et al., 2001). The initial values of the weights associated with the connections between neurons were selected randomly in the range -3 to 3. The number of maximum of epochs was 2000 and the initial values of η and μ were 0.2 and 0.5, respectively; the target error was 0.1. Validation of the MLF-ANN classification rule was performed by a cross-validation in 10 steps, the evaluation set containing 25% of the samples.

Table 2
MLF-ANN architectures assayed and their prediction abilities

MLF-ANN architecture	Prediction ability (%)	RMSE[a]
4-5-1	91.4	0.060
4-7-1	90.2	0.060
4-9-1	90.2	0.059
4-5-2	90.8	0.064

[a] RMSE, root mean square error.

Table 3 shows the recognition and prediction abilities afforded with each multivariate technique. KNN achieved excellent results for Basque ciders, with recognition and prediction percentages of 98.8%. Recognition and prediction abilities of this technique for French ciders were lower, and similar to those attained by LDA, about 90%. These inappropriate LDA results could be expected, taking into account the LDA assumption that categories must be linearly separated. In this case, they are not, as Fig. 3 shows. According to these data, KNN provides an adequate classification system to authenticate Basque samples, because the

Table 3
Classification results for the supervised pattern recognition techniques applied to cider samples

Technique	Class	Recognition ability (%)	Prediction ability (%)
LDA	French	90.2	88.2
	Basque	91.3	91.0
KNN ($K = 5$); inverse squared Euclidean distance	French	92.2	91.2
	Basque	98.8	98.8
SIMCA; normal range; $\alpha = 0.05$	French	90.7	88.2
	Basque	77.0	70.0
MLF-ANN ($4 \times 5 \times 1$); $\eta = 0.2$; $\mu = 0.5$; sigmoidal transfer function	French	100.0	91.5
	Basque	100.0	91.3

probability of a genuine Basque cider being classified as French is very low. The MLF-ANN classification rule gives very good results with a complete recalling performance (100%) for both categories. However, the prediction abilities implied that some ciders from both categories (9%) were misclassified; hence, certain genuine Basque and French ciders could be considered as false. The network results could be less successful than expected owing to the complex distribution of the samples in the multidimensional input space, presenting groups and subgroups with samples of different classes (Figs. 2 and 3).

When SIMCA was applied, similar results to LDA were secured for the French category; however, lower percentages of recognition and prediction abilities were obtained by the Basque model, (77% and 70%, respectively). In the Coomans plot (Fig. 4), the distance from the model for the Basque class was plotted against that from the model for the French class. In most of the French samples, there is a great distance from the Basque model, however, the samples from the Basque category present shorter distances from the French model. These results were studied in terms of sensitivity (percentage of objects belonging to the category which are correctly identified by the mathematical model) and specificity (percentage of objects foreign to the category which are classified as foreign) (Meléndez et al., 1999). In practice, the results achieved by SIMCA meant that the French model accepted 90% of its samples and also 68% of Basque ones, whereas the Basque model recognised 91% of Basque ciders and 26% of French ones. Whence, the probabilities that SIMCA models would give wrong results were considerable. Thus, this technique is inadequate for authenticating the mentioned types of ciders.

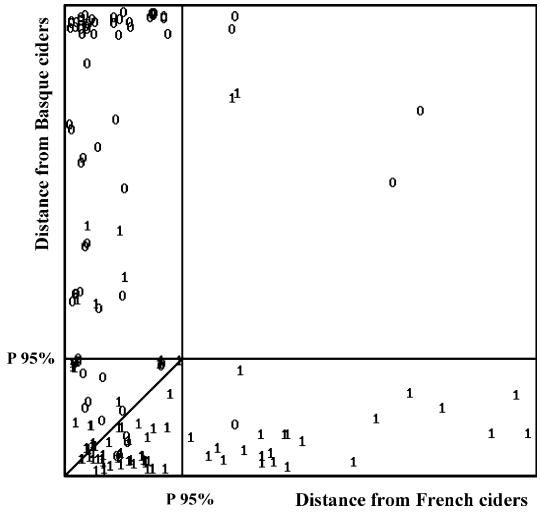

Fig. 4. Coomans plot for the squared SIMCA distances. Codes: 0, French ciders; 1, Basque ciders.

4. Conclusion

From the results obtained by the different supervised pattern recognition techniques applied, it is concluded that general parameter measurements in ciders, such as total polyphenol content estimated by Folin–Ciocalteu method and the CIELab chromatic parameters (L^*, a^*, b^*), jointly with certain chemometric techniques, are able to distinguish between Basque and French ciders with percentages of correct classification of about 90%. Moreover, KNN is a very effective technique for identifying Basque ciders by analysing these four features (recognition and prediction abilities of 98.8%). Thus, total polyphenol content and colour measurements data processed by KNN could be proposed as an authentication system for French ciders, in order to detect Basque ciders passed off as French, since there exists a low probability of a genuine Basque cider being classified. However, on the other hand, if the aim is to guarantee that a cider is genuinely Basque, the measurement of the mentioned general parameters in ciders and chemometrics does not achieve convenient results for authentication purposes. Therefore, it is necessary to resort to a more precise analysis of cider polyphenolic profile for achieving a proper authentication system that assures such a level of certainty in the prediction, which is reported in another work by Alonso-Salces et al. (2004).

The interest of this paper lies in the fact that measurements of total polyphenol contents (Folin–Ciocalteu assay) and CIELab parameters on cider samples have the advantage of being less time-consuming and less expensive than the determination of cider polyphenolic profiles by HPLC-DAD. Hence, depending on the aim pursued, measurements of the mentioned general parameters may be sufficient or not for cider authentication.

Acknowledgments

The authors express their gratitude to Diputación Foral de Bizkaia, as well as, French and Basque cidermakers for providing cider samples. Rosa M[a] Alonso-Salces wishes to thank Gobierno Vasco/Eusko Jaurlaritza for a Ph.D. Grant. This research was supported by Gobierno Vasco/Eusko Jaurlaritza (projects PI-1997-19 and PI-1999-106).

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