

Analytical, Nutritional and Clinical Methods

## Fatty acid composition of cider obtained either by traditional or controlled fermentation

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### Abstract

A pilot-scale model for controlled fermentation in cider-making is described. This technology is compared with the conventional induction of alcoholic fermentation and spontaneous malolactic conversion on the basis of the cider's fatty acid profile. Controlled cider fermentations were carried out by means of sequential inoculation of *Saccharomyces cerevisiae* and *Leuconostoc oenos*. It was observed that there are significant differences in fatty acid composition depending on the fermentation process employed. The contents in fatty acids of ciders elaborated by a conventional process were higher than those of ciders obtained by controlled fermentation. The use of principal component analysis (PCA), linear discriminant analysis (LDA), soft independent modeling of class analogy (SIMCA) and partial least squares (PLS) in conjunction with the fatty acid composition allowed the authors to typify fermented apple products on the basis of the fermented technology. The most relevant variables for classification purposes were lauric and palmitic acids.

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### 1. Introduction

Nowadays, the fermentation process used to obtain cider in Asturias (Spain) is conducted by wild microflora, quality products only being obtained if the alcoholic and malolactic fermentations take place correctly. However, the process may be controlled by means of inoculation with selected yeast and bacterial strains so as to obtain cider with a correct and predictable composition (Cabrane, Mangas, & Blanco, 1996). Cider has a huge commercial, economic and social relevance in the region. Therefore, any study con-

cerned with improving production conditions is very important and useful.

As a result of the fermentative metabolism of yeast, certain organoleptic compounds are formed that have an effect on the quality of cider, such as alcohols, esters and other volatiles (Cabrane, Mangas, & Blanco, 1997). Obviously, adequate selection of raw materials and convenient methods of manufacture will determine the organoleptic characteristics of the cider. Among the aforementioned substances, fatty acids are known to play an important role in the sensory quality of food. They contribute to flavor as precursors of volatile compounds (Ratledge & Dickinson, 1995). Besides, the control of fatty acids might be of interest due to their effect on foam formation and stability (MacLeod, 1977; Prins, 1998), as the ability of cider to form foam is an important feature in terms of product attractiveness to the consumer.

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In this study, a model for controlled fermentation in cider-making is described. The model consists in inoculating a sterilized apple juice with a bi-strain system (*Saccharomyces cerevisiae* + *Leuconostoc oenos*). This technology is compared to the conventional induction of alcoholic fermentation and subsequent spontaneous malolactic fermentation, maturation and in-bottle conservation, showing how the use of selected starters for alcoholic and malolactic fermentations facilitates the obtainment of ciders possessing a correct and predictable composition.

The purpose of this research was to ascertain the influence of the fermentation technology on the fatty acid profile of cider and to differentiate between traditional fermentation and controlled fermentation using the fatty acid composition and chemometric techniques.

## 2. Materials and methods

### 2.1. Chemicals

Analytical grade standards of fatty acids were purchased from Sigma (St. Louis, USA). Solvents were supplied by Merck (Darmstadt, Germany), Romil (Cambridge, England) and Aldrich (Steinheim, Germany).

### 2.2. Samples

Two types of cider were used: cider produced by traditional means (T) and cider obtained by controlled fermentation (C). The apple juice used to make the ciders was prepared from a mixture of cider apples: sharp 30.7%, sweet 23.8%, and mildly-sharp 45.5%, all with different sensory properties, thus providing an overall acidic nature to the resulting juice. The varieties were classified according to the criteria described by Dapena (1998).

### 2.3. Sample preparation

Cider samples were prepared according to the method described by Blanco-Gomis, Mangas Alonso, Margolles Cabrales, and Arias Abrodo (2001). 50 mL of cider were extracted with 60 mL of a mixture of chloroform: methanol (2:1). The organic phase was evaporated to dryness. Esterification of fatty acids to methyl esters was carried out with  $\text{BF}_3$  in methanol. Fatty acid methyl esters were extracted with hexane.

### 2.4. Strains of yeast and bacteria

The strains of *S. cerevisiae* and *L. oenos* belong to the SERIDA (Asturias, Spain) collection and were isolated from spontaneous industrial fermentations of cider.

### 2.5. Analytical procedure

Fatty acids (C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C16:1, C18:0, C18:1, C18:2) were determined according to the method described by Blanco-Gomis et al. (2001) by GC using a Unicam 610 gas chromatograph fitted with a flame ionization detector and an acidified polyethylene glycol capillary column MFE-1000 (50 m  $\times$  0.25 mm, 0.25  $\mu\text{m}$  film thickness). Nitrogen was used as carrier gas at 1.8 mL/min. The column was operated isothermally at 80  $^\circ\text{C}$  for 1 min and then programmed at 5  $^\circ\text{C}/\text{min}$  to 230  $^\circ\text{C}$ , and finally at 230  $^\circ\text{C}$  for 10 min. Injector and detector temperatures were maintained at 290  $^\circ\text{C}$  and 300  $^\circ\text{C}$ , respectively. Quantitative analysis was carried out using the internal standard method. Two internal standards were used: pelargonic (C9:0) and margaric (C17:0) acid. The validation of the proposed method included repeatability (RSD < 4%), reproducibility (RSD < 6%), detection limit (0.7–3.6 ng) and linearity studies ( $r > 0.999$ ). The accuracy of the proposed method was tested by spiking a sample of cider with fatty acid standards. The results obtained ranged between 92% and 105% (RSD < 6%), thus evidencing that the proposed method is suitable for the analysis of fatty acids in cider.

### 2.6. Experimental design

The apple mixture was processed in a pilot plant. The process included the following steps: milling with a hammer mill and slow pressing (4 days) with a batch mechanical press, 4000 liters of apple juice being obtained. 2000 liters were inoculated with a selected culture of *S. cerevisiae* (conventional induction), malolactic conversion being spontaneously conducted by wild lactic acid bacteria. The other 2000 liters were subjected to a clarification process by means of an  $\alpha$ -alumina crossflow microfilter (0.33  $\mu\text{m}$ ; 10  $\text{m}^2$  of filter area). The clarified apple juice was then sterilized by means of a polyvinylidene fluoride microfilter (0.22  $\mu\text{m}$ ). After that, the sterilized apple juice was sequentially inoculated with selected cultures of *S. cerevisiae* as a starter of alcoholic fermentation and *L. oenos* as a starter of malolactic fermentation. Lactic acid bacteria were inoculated when the density of cider was less than 1.020 g/L. The fermentation was conducted in stainless steel casks of 1000 liters of capacity and the experiments were carried out in duplicate. The analytical determination of fatty acids was carried out during four steps of the fermentation and maturation processes. All analyses were conducted in triplicate.

### 2.7. Data processing

The data were processed using the PARVUS statistical package (Forina, Leardi, Lanteri, & Armanino,

1998). A matrix was constructed with rows (16) representing ciders and columns (11) corresponding to fatty acids. Samples were categorized according to the fermentation technology employed, traditional (8, T) and controlled (8, C).

### 3. Results and discussion

#### 3.1. Fatty acids analysis

Table 1 shows the mean content in fatty acids of both types of cider. As can be seen, the contents in fatty acids of traditional cider are higher than those of ciders obtained by means of controlled fermentation. The results of ANOVA show that the fermentation technology has a significant influence on capric, lauric and palmitic acids. This result is important from the technological point of view, showing the influence of fermentation technology on the fatty acid content of cider and in consequence on its sensorial properties, mainly taste, flavor and foam.

Moreover, changes in the amount of fatty acids during the fermentation process were detected. A significant increase in caproic, caprylic and capric acids during the fermentation process in all types of samples (traditional and controlled) was found. At the same time, the amount of longer fatty acids in traditional fermentation decreased during process. The same evolution was observed in controlled fermentation, except for palmitic acid, in which a significant increase was observed.

#### 3.2. Statistical analysis

##### 3.2.1. Univariate analysis

Before the chemometric techniques were used to typify the ciders on the basis of the fermentation technology employed in their manufacture and according to their content in fatty acids, a univariate analysis was carried out to discover whether any of the fatty acids

by themselves would allow us to differentiate the two established categories. The highest Fisher weight computed corresponded to lauric acid. However, the use of the most discriminant variable did not allow us to distinguish both categories clearly, so multivariate analysis was needed.

##### 3.2.2. Cluster analysis

A selection of original variables was made in order to avoid collinearities between variables and to reduce noise in the constructed models. To do so, an exploratory cluster analysis of the variables was chosen. A Euclidean metric was used to determine similarities between variables and the Ward agglomerative method was employed to construct the dendrogram. As can be seen in Fig. 1, in which the dendrogram of variables is displayed, some groups of variables were detected. Following a selection criterion based on the Fisher weight, five fatty acids were removed from the data matrix. Thus, the new matrix was constructed with 16 observations and six variables (fatty acids: C8:0, C12:0, C15:0, C16:0, C18:1<sup>A9</sup> and C18:2<sup>A9,12</sup>).

##### 3.2.3. Principal components analysis

Once the selection of variables had been made, a principal component analysis (PCA) was carried out to detect the data structure and determine the relationships between samples and original variables. Three predictive components, which accounted for 87% of the variance, were computed with an internal procedure of validation, the full double-cross validation method, using five cancellation groups and 95% of the retained variance.

When the original variables were projected onto the factorial plane formed by the first and third components (retained variance 64%) (Fig. 2), a close connection was detected between lauric and palmitic acid and the first component (correlation coefficients,  $-0.55$  and  $-0.52$ , respectively), while caprylic, pentadecanoic and oleic acids were more correlated to the third component

Table 1  
Mean values and standard deviations of traditional and controlled cider

Fatty acid	Traditional		Controlled	
	mg/L	SD	mg/L	SD
Caproic (C6:0)	5.0	0.4	4.9	0.2
Caprylic (C8:0)	2.2	0.3	1.6	0.5
Capric (C10:0)	4.1	0.6	3.4	0.5
Lauric (C12:0)	0.7	0.1	0.47	0.05
Myristic (C14:0)	0.11	0.03	0.05	0.03
Pentadecanoic (C15:0)	0.11	0.05	0.09	0.02
Palmitic (C16:0)	0.73	0.01	0.5	0.1
Palmitoleic (C16:1)	0.10	0.03	0.03	0.03
Stearic (C18:0)	0.15	0.06	0.13	0.03
Oleic (C18:1)	0.07	0.08	0.07	0.01
Linoleic (C18:2)	0.08	0.03	0.02	0.02

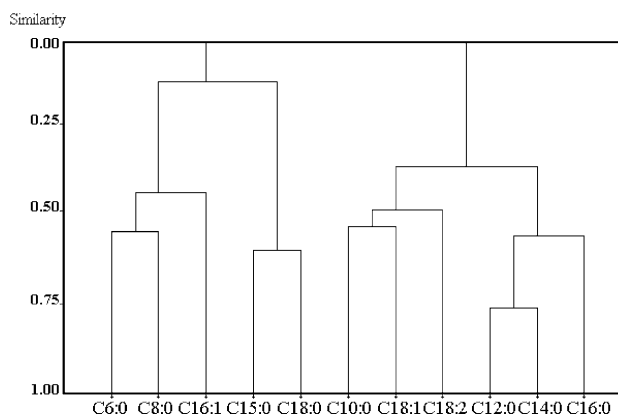


Fig. 1. Dendrogram of variables.

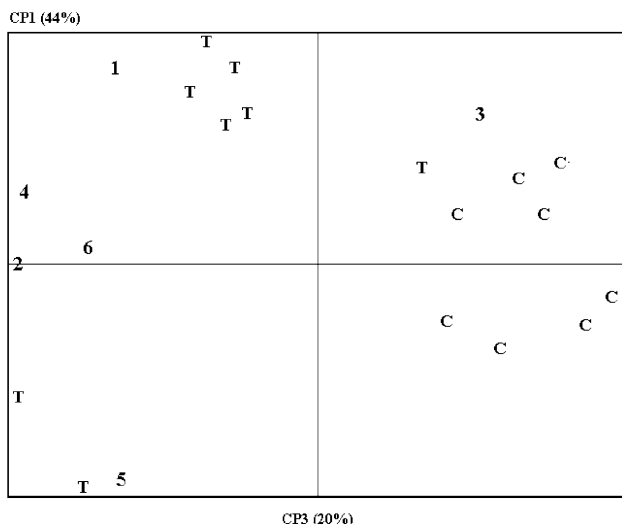


Fig. 2. Projection of samples and variables on factorial plane formed by first and third component. (T) Traditional, (C) controlled; 1: caprylic; 2: lauric; 3: pentadecanoic; 4: palmitic; 5: oleic; 6: linoleic acids.

(correlation coefficients, 0.58, 0.46, and  $-0.64$ , respectively). At the same time, the figure shows how 100% of the samples belonging to the controlled category were placed in the right corner of the plane.

### 3.2.4. Linear discriminant analysis

From the data structure visualized on the factorial plane, we computed and validated a decision rule by means of a linear discriminant analysis (LDA) in order to classify ciders according to their fatty acid content. Validation was carried out with a cross validation method using four cancellation groups. The matrix of prediction is shown in Table 2. As can be seen, hits were higher than 88% in all cases, so we may conclude that the mathematical rule constructed is sufficiently reliable and robust for classification purposes on the basis of fatty acid profile and the fermentation technology used.

A stepwise LDA technique was used to select the most relevant variables for classification purposes based on the minimization of Wilks' lambda together with an F test at a confidence level of 90%. The selected variables were lauric and palmitic acid. The Wilks' lambda value obtained was 0.19, which means that 81% of total variance is explained by within-group differences.

Table 2

Prediction matrix using an internal validation method with four cancellation groups (prediction hits, %)

True category	Assigned Category		Hits
	Traditional	Controlled	
Traditional	7	1	88
Controlled	0	8	100
Overall			98

### 3.2.5. Soft independent modeling of class analogy (SIMCA)

When the data matrix contains a reduced number of samples in relation to variables, a classification method such as SIMCA should be used. This chemometric technique presents interesting features, such as the principle of class modeling, computing a disjoint principal component model for each class. Three and four predictive components were computed for each class (traditional and controlled, respectively) using a SIMCA reduced model, which accounted for 92% and 99% of the variance, respectively. Fig. 3 shows a Coomans' diagram in which the two SIMCA boxes are displayed. Hits of classification were 100%, since all ciders belonging to the traditional class are placed above the diagonal, and all ciders belonging to the controlled class are placed below the diagonal. Sensitivities and specificities of both models are shown in Table 3.

The SIMCA models computed were sufficiently sensitive and specific; the traditional model accepted 12% of samples belonging to the controlled class, while the controlled class recognized its samples and did not accept any sample of the traditional category.

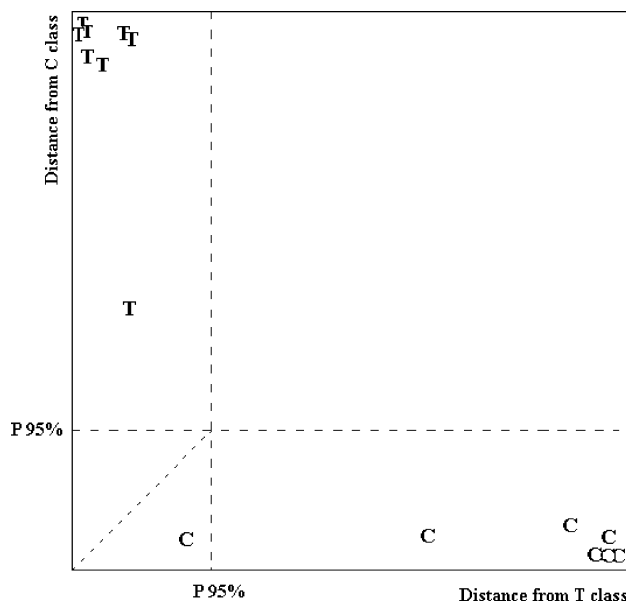


Fig. 3. SIMCA analysis. Coomans' plot; T, traditional class; C, controlled class.

Table 3

Sensitivity (%) and specificity (%) of traditional and controlled classes according to the SIMCA model

	Sensitivity	Specificity
Class		
Traditional	100	88
Controlled	100	100

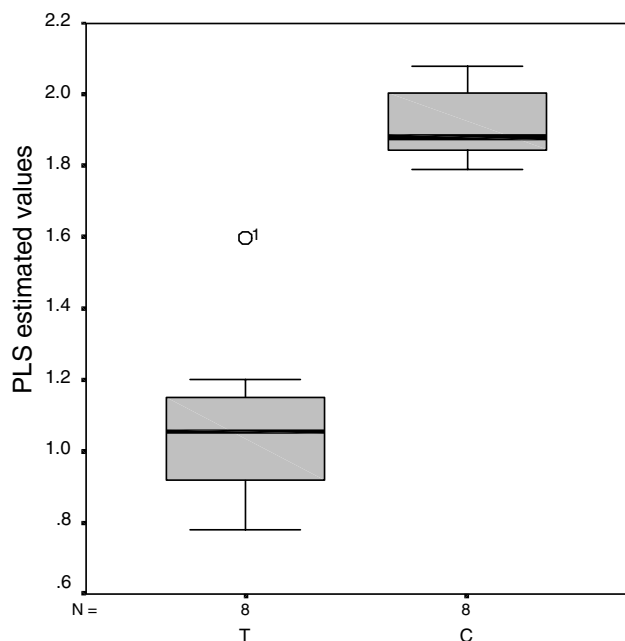


Fig. 4. Box-Whisker plots for PLS estimated value. T, Traditional class; C, controlled class.

### 3.2.6. Partial least squares (PLS)

The determination of the mathematical relationship between categories of ciders and fatty acids was carried out using PLS. The PLS method is especially recommended when the number of observations is small in relation to the number of variables.

We established a binary response (assigning value 1 to the traditional category and 2 to the controlled category), and then carried out multivariate regression between the new variable (criterion variable) and the fatty acids (predictor variables).

The model constructed using PLS regression consisted of one latent variable estimated by cross-validation with three deletion groups. The percentages of cross-validation explained variance and explained variance were 70% and 79%, respectively, and the calculated multiple linear correlation coefficient was 80%. Fig. 4 shows two Box-Whisker plots for each category using the PLS estimated value. As can be seen, the PLS technique enables correct discrimination of ciders based on the fermentation technology.

## 4. Conclusions

The use of multivariate techniques of data analysis allowed us to differentiate between cider produced by tra-

ditional fermentation and controlled fermentation. Univariate analysis using lauric acid (the most discriminant variable) did not allow us to separate the two categories satisfactorily.

PCA allowed three principal components to be established that account for 87% of variance, lauric and palmitic acid were linked to the first component and caprylic, pentadecanoic and oleic acid were closely related to the third component. The discriminant rule computed was robust and feasible for classification purposes, and SIMCA analysis allowed us to obtain models that are sufficiently sensitive and specific for differentiating traditional and controlled classes. The use of PLS provided an adequate model for the correct discrimination of ciders according to the fermentation technology used and on the basis of their fatty acid profile.

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