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Analytical differentiation of cider inoculated with yeast (*Saccharomyces cerevisiae*) isolated from Asturian (Spain) apple juice

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Abstract

This paper reports the influence of fermentation conditions (temperature and yeast strain) on the chemical composition of cider. The cider was analysed for the non-volatile acids, polyalcohols, residual sugars and major volatile compounds. The application of principal components analysis enables the ciders to be differentiated on the basis of the two factors considered. The first principal component achieved the separation according to the type of strain and the second principal component separates the samples according to the fermentation temperature. The variables that carry most weight on first component were ethyl acetate, acetaldehyde and isobutanol. On second component the variables more relevant were: acetoin and 2,3-butanediol.

Keywords: Cider; Chemical composition; Volatile compound; Yeast; Temperature

1. Introduction

The advantages and drawbacks of fermentations brought about by pure strains of microorganisms and by indigenous microflora have been debated since the 1950s (Beech, Burroughs, & Codner, 1951; Rankine, 1968; Berry, 1995).

In spontaneous fermentations, a large diversity of microorganisms participate, including oxidative and fermentative yeasts, homo and hetero-fermentative lactic acid bacteria, and acetic bacteria (Beech, 1972; Heard & Fleet, 1986; Cabranes, Moreno, & Mangas, 1990). The main argument in favour of these fermentations is that they lend a greater typicality and organoleptic complexity to the fermented products (Beech & Davenport, 1970; Moreno, Millán, Ortega, & Medina, 1991; Le Queré & Drilleau, 1993; Lea, 1995; Rérolles, 1995). The main drawbacks reported are variability in the product quality and the risk of anomalous fermentations (Beech & Carr, 1977; Splittstoesser, 1982).

The use of pure cultures of yeasts, generally in the form of active dry yeast, provide a useful tool for standardising the product (Kraus, Reed, & Villettaz, 1983; Barre & Vezinhet, 1984; Fleet & Heard, 1993). Indigenous yeasts selected in particular regions is a solution towards ensuring adequate control of the alcoholic fermentation and preserving the positive contribution of the indigenous yeasts (Riberau-Gayon, 1985; Mafart, 1986; Martini & Martini, 1990).

In Asturias Region (Spain), it is common practice in the production of cider for both the alcoholic fermentation and the malolactic fermentation to rely on the indigenous flora. Studies into the population dynamics of cider fermentation have demonstrated the following predominant species: *Kloeckera apiculata*, *Saccharomyces cerevisiae*, *Oenococcus oeni* and *Acetobacter aceti* (Cabranes, Mangas, & Blanco, 1997). Monitoring during several years spontaneous fermentations in cider

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cellars and in the pilot plant at our Research Centre led to the isolation, selection and physiological and biochemical characterisation of autochthonous microorganisms (Cabranes, 1994).

Moreover, the identification and characterisation of yeast strains are relevant processes in order to guarantee the quality of alcoholic beverages. Different strategies, based on the detection of DNA polymorphism have been used for ecological research attempting to study intraspecific diversity of indigenous microbiotica during production of alcoholic beverages. Oligonucleotide primers are complementary to intron splice sites used to produce PCR fingerprints and display polymorphisms between isolates of the same yeast species (De Barros Lopes, Soden, Henschke, & Landridge, 1996; De Barros Lopes, Soden, Martens, Henschke, & Landridge, 1998). A PCR-based method using intron splice site primers has been developed for identification of cider yeast strains (Pando, 2002).

The aim of this study was to examine the chemical composition of ciders produced using different yeast strains. All of the small-fermentations were carried out using the same non-sterilised must with the aim of intervening as little as possible with the natural flora.

2. Material and methods

2.1. Yeast strains

Three strains of cider yeasts that belong to the collection of pure cultures of the SERIDA (referred to as: SSA, SSB, SSC) as well as a commercial dry wine yeast Uvaferm PM (Lallemand., France) were used. DNA extraction and genetic strain identification was affected according to the method described in Pando (2002). The method was based upon the PCR using oligonucleotide primer that is complementary to intron splice sites. PCR was performed in 25 μl with 1 μl primer LA2 (10 pmol/l) with the sequence CGTGCAGGTGTTAGTA, 5 μl DNA template (10 ng/μl), 2.5 μl dNTPs (2 mmol/l), 1.25 μl $MgCl_2$ (4.7 g/l), 1.25 μl DMSO and 0.5 μl Taq polymerase (5 U) (Bioline Ltd, UK). Products of amplification reaction were resolved on agarose gel stained with ethidium bromide and visualised under UV light. The gels were photographed and scanned to produce a computer image. PCR profiles were analysed by the software Gene Tools (Bio Image System, UK). The polymorphisms between strain and the amplified bands are given in Table 1.

The inocula were prepared under sterilised conditions on a YPD medium (10 g/l yeast extract, 20 g/l glucose, 20 g/l peptone) at 30 °C under continuous agitation for 18 h.

2.2. Small-scale fermentations

The must to be fermented was obtained in automatic hydraulic Bucher–Guyer press from a mixture of cider apples provided by the cellar *El Gaitero S.A*. Its chemical and microbiological characteristics are shown in Table 2.

The assays were carried out at the authors' experimental cellar in 1-l capacity Erlenmeyer flasks. All the fermentations were carried out in duplicate, each of the replicates being dealt with independently. The must was inoculated with 20 ml of each of the pure cultures. Sugar consumption (measured as decrease in density) was used as the criteria to measure the evolution of the fermentation. At the end of the fermentation process, when the density decreased below 1000 g/l, the experimental units were stored at 4 °C for 16 h to facilitate the separation of solids in suspension. Finally, the ciders obtained were filtered through hydrophilic cotton and frozen (−20 °C) until further analysis. The must was fermented at 18 °C with selected cider yeasts (SSA, SSB,

Table 2
Chemical and microbiological composition of the apple juice used for fermentation experiments

Parameter	
Relative density (20 °C/20 °C)	1069
Total acidity (g sulphuric acid/l)	1.98
PH	4.19
Total polyphenols (g tannic acid/l)	1.09
Fermentable nitrogen (FAN) (mg/l)	112
Total yeast count (cells/ml)	6.55×10^7
Lactic acid bacteria count (cells/ml)	2.94×10^5
Acetic acid bacteria count (cells/ml)	1.48×10^5

Table 1
PCR profiles obtained using the primer LA2 in the yeast strain differentiation

Physiological race of strains[a]	Size of amplified bands (Kbp)										Source
S. cerevisiae r. *cerevisiae* SSA	2.63	2.31	1.80	1.31	1.07	0.91	0.80	0.49			Cidermaking isolate. (Asturias) Spain
S. cerevisiae r. *uvarum* SSB	3.36	2.84	2.23	1.41	1.31	1.07	0.93	0.56	0.48	0.30	Cidermaking isolate. (Asturias) Spain
S. cerevisiae r. *cerevisiae* SSC	2.23	1.77	1.30	1.18	1.06	0.91	0.78	0.56	0.48		Cidermaking isolate. (Asturias) Spain
S. cerevisiae r. *bayanus* UVA-PM	4.33	3.45	2.57	2.05	1.85	1.70	1.59	1.46	1.35	0.85	Dry wine yeast. Lallemand

[a]Identification of the physiological race was based on the criterion of Kreger-Van Rij (1984).