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# Influence of the method of obtaining freeze-enriched juices and year of harvest on the chemical and sensory characteristics of Asturian ice ciders

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ABSTRACT

Ice cider is a special product made from apple juices enriched by freezing. In this paper, the method of obtaining the ice juices (cryo-extraction and exhaustion) and the year of harvest have been evaluated. For this purpose, a controlled raw apple mixture and an autochthonous *Saccharomyces bayanus* strain were used throughout the study. Both the enrichment system and the year of harvest significantly influenced the levels of total phenols, sucrose, malic acid, ethyl acetate and 2-phenylethanol. The ciders made by cryo-extraction presented the higher sugar/acidity and sugar/polyphenol ratios. These ciders were more fruity, less astringent and scored better for quality than those obtained by exhaustion. Additionally, a preliminary assay of juice enrichment by cryo-concentration is described. The corresponding ciders presented higher methanol and lower 2-phenylethanol contents than those obtained by the cryo-extraction and exhaustion methods.

## 1. Introduction

Ice cider is a special type of sweet cider first developed in Quebec during the early 90s that quickly gained international recognition (Leger, 2010). The design of high-valued beverages similar to those known as ice ciders is in the framework of the present product diversification policy carried out by the Asturian cider-making sector. Recently the Spanish legislation has established quality standards for Spanish ciders, which includes the definition of ice ciders (BOE, 2017). This regulation states, among other parameters, the minimum alcoholic degree (8% v/v) and residual sugar contents (100 g $L^{-1}$) required for the labeling of a product as an ice cider. Different technological factors influence the chemical and sensory properties of ice ciders, contributing to their excellence.

The making of this niche product starts with obtaining a high-sugar content juice by using cold temperatures. Two methods are commonly used. The first one, cryo-extraction, consists of freezing picked apples and pressing until the juice is squeezed from them; the second and most frequently used technique, cryo-concentration, involves the pressing of the fruit and the freezing of the resulting juice (Kirkey & Braden, 2014). Although some Canadian producers claim that the ice ciders obtained by cryo-extraction are often more complex and aromatic, no systematic studies have been reported to corroborate this assertion.

The enriched juice obtained is further inoculated with yeasts and fermented for some months. Due to the high sugar content of the ice juices, yeasts are subjected to large hyperosmotic stress so that, the selection of the most suitable strains is necessary to reach the desired alcoholic degree in the final product. Selected local yeast strains should contribute to avoid sluggish fermentations giving ciders their typical sensory characteristics. In this sense, the *Saccharomyces bayanus* species is present in fermenting cider habitats and its ability to ferment sugars at lower temperature, producing higher amounts of glycerol and less acetic acid than *Saccharomyces cerevisiae*, has been reported (Suárez Valles, Pando Bedriñana, Fernández Tascón, Querol Simón, & Rodríguez Madrera, 2007; López-Malo, Querol, & Guillamón, 2013; Pando Bedriñana, Querol Simón, & Suárez Valles, 2010; Bellon, Yang,

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Day, Inglis, & Chambers, 2015). Strains of *Saccharomyces bayanus* have recently been evaluated as starters in the making of ice ciders (Pando Bedriñana, Mangas Alonso, & Suárez Valles, 2017).

Regarding the raw material, a set of Asturian cider apple varieties belonging to the Protected Designation of Origin "Sidra de Asturias" (EC 2154/2005) has been evaluated for the production of ice juices through the assessment of their contents in sugars, acids and aromas (Picinelli Lobo et al., 2018). Moreover, taking into account the high prices that these ciders can reach, some research has been also devoted to find reliable physico-chemical indicators and analytical methods allowing the authentication of the ice ciders against other related apple derivatives which could not respect the ice cider regulation (Clément, Panneton, Bastien, & Fernandez, 2017).

The aim of this study was to assess the influence of the methods for obtaining the freeze-concentrated apple juices on the chemical composition and sensorial properties of the corresponding ice ciders. For this purpose, the same combination of apple mixture and autochthonous *S. bayanus* strain was processed by cryo-extraction in three consecutive years to evaluate the effect of the year of harvest. A second method referred to as exhaustion, closely linked to that one was also studied.

## 2. Materials and methods

### 2.1. Enrichment of the apple juices

Two extraction systems to obtain freeze-enriched apple juices (cryo-extraction and exhaustion) were applied. The raw material consisted of a mixture of five apple varieties (Verdialona, de la Riega, Raxao, Regona and Durona de Tresali) belonging to the Protected Designation of Origin "Sidra de Asturias". A total of 720 kg of apple harvested at their optimal point of ripening were used. In the cryo-extraction system, the apples were frozen at −20 °C and further pressed when they reached a temperature of −9 °C (measured inside the apple) by means of a mechanical press (Aton 800, from Zambelli Enotech, Italy), operating at $1.7 \times 10^4$ kPa and at an environmental temperature of 10 °C. The juice squeezed was collected so as to reach a final density value of 1.14241 g $mL^{-1}$ (33.2 °Brix). The juice fractions discarded from this operating procedure were mixed (1.07732 g $mL^{-1}$, 19.1 °Brix) and frozen at −20 °C. Subsequently, this juice was thawed at 4 °C and the enriched juice was collected so as to reach a final total soluble solid content of 32.9 °Brix (1.14105 g $mL^{-1}$). This system is called exhaustion. Both methods were evaluated during three consecutive harvests (2013–2015).

A third method for juice enrichment, cryo-concentration, was also tested in the 2013 harvest. In this case, 450 kg of the same mixture of apples were pressed by the above mentioned press, the juice obtained (1.05736 g $mL^{-1}$, 14.2 °Brix) was frozen at −20 °C and finally thawed at 4 °C to reach a juice with a final total soluble solid contents of 37.9 °Brix (1.16617 g $mL^{-1}$).

### 2.2. Cultivation conditions of yeast

The production of the *S. bayanus* strain referred to as C6, belonging to the SERIDA collection of pure culture (Asturias, Spain), was carried out in a 2-L stirred tank bioreactor (Biostat B plus, Sartorius). The batch medium consisted of sterile apple juice (4 °Brix) supplemented with silicone antifoam liquid (0.1%) to avoid foaming. The essays were carried out at 30 °C for 22 h with agitation at 300 rpm. Aerobic conditions were obtained by providing the batch medium with 3.5 L $min^{-1}$ of air. During batch cultivation, 2 M NaOH and 1 M HCl were used to control pH at 5.5. The yeast cells were separated from the batch medium by using a 0.45 μm-hydrosart cassette (0.1 $m^2$, Sartorius), fitted into a cross-flow filtration apparatus (Sartoflow Slice 200, Sartorius). The cells were concentrated from 2 L to 0.2 L and washed twice with purified (Milli-Q A10 system, from Merck Millipore, Madrid, Spain) and sterilized water. The yeast cream obtained ($10^{10}$ cfu $mL^{-1}$) was stored at 4 °C until use.

### 2.3. Fermentation

All the fermentations were performed in duplicate at 14 °C in 16 L glass containers equipped with an air-lock filled with sulfur dioxide (1.8 g $L^{-1}$). The juices (15 L) were inoculated with 10 g of yeast cream. A stepwise acclimatization was carried out by adding double volumes of juice every 30 min. At the end of the fermentation process, the ice ciders were preserved at 4 °C for 72 h to facilitate the removal of fermentation lees. Then, the ice ciders were racked-off, clarified by the addition of bentonite (10 g $hL^{-1}$, 10 days, 4 °C), stabilized (60 mg $L^{-1}$ sulfur dioxide), filtered (1.2 μm polypropylene and 0.45 μm polyvinylidene fluoride membrane filters, Merck-Millipore) and bottled in dark glass 375 mL bottles. The final ciders were kept at 15 °C until their analytical and sensory analysis at three months.

### 2.4. Analysis of the raw and fermenting apple juices

#### 2.4.1. Microbiological counting

Samples of the enriched juices and ice ciders at different stages of the cider-making process were aseptically taken. At each sampling, several 1:10 (v/v) dilutions were performed in a Ringeŕs solution, spread by triplicate onto agar mediums and incubated according to the following conditions to count the populations of yeasts and bacteria:

*Yeasts*: WL Nutrient Agar (Microkit, Spain) supplemented with 25 mg/L penicillin G potassium salt (Calbiochem, UK) and 100 mg/L streptomycin sulfate (Acofarma, Spain). Plates were incubated at 30 °C for 5 days.

*Lactic Bacteria*: ZMA medium (apple juice concentrate diluted at 10 °Brix, 0.4% yeast extract, 0.1% peptone, 0.1% tryptone, 0.05%, ammonium phosphate tribasic, 0.05% potassium phosphate dibasic, 0.05% di-sodium hydrogen phosphate anhydrous, 0.1% polysorbate 80 and 3% agar) supplemented with 50 mg/L pimaricin (Calbiochem, UK). Plates were incubated in anaerobic jars at 30 °C for 5 days.

*Acetic bacteria*: basal medium (apple juice concentrate diluted at 10 °Brix, 1% yeast extract and 3% agar, pH = 4.5) supplemented with 25 mg/L penicillin G potassium salt and 50 mg/L pimaricin (Calbiochem, UK). Plates were incubated at 30 °C for 3 days.

#### 2.4.2. Implantation capacity

The implantation of the inoculated strain was evaluated 12 days after inoculation in all of the experimental units by mitochondrial DNA-Restriction Fragment Length Polymorphism (mtDNA-RFLP) according to the method reported elsewhere (López, Querol, Ramón, & Fernández-Espinar, 2001). A sample of cider was taken and handled as described in Section 2.4.1. Then, 10 yeast colonies were randomly isolated from WL Nutrient agar. These yeasts together with the *S. bayanus* strain referred to as C6 used as starter were grown overnight in a GPY medium (0.5% peptone, 0.5% yeast extract and 4% glucose) at 30 °C. Cells were spun down in a microcentrifuge and the total DNA was extracted according to the method described by Querol, Barrio, Huerta, and Ramón (1992). Yeast DNA was digested overnight at 37 °C with the *Hinf*I restriction enzyme (Roche, Mannheim, Germany) and the fragments were separated in 1% agarose gels in 1X TAE (Tris-acetic acid-EDTA) buffer at 100 V, using a Power-Pac 300 (Bio-Rad, Hercules, CA, USA). Gels were stained with ethidium bromide, and the DNA fragments were visualized under UV light and scanned by means of a camera charge-coupled device (CCD) Gene Genius (Syngene, Cambridge, UK). The restriction fragments were visually compared and additionally their sizes were measured as base pairs in comparison with a λ DNA standard (Roche, Mannheim, Germany) digested with the *Pst* I restriction enzyme (Roche, Mannheim, Germany) using a Gene Tools gel Analysis Software (Syngene, Cambridge, UK).