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A comparative study on aromatic profiles of strawberry vinegars obtained using different conditions in the production process

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ABSTRACT

Impact odorants in strawberry vinegars produced in different containers (glass, oak and cherry barrels) were determined by gas chromatography–olfactometry using modified frequency (*MF*) technique, and dynamic headspace gas chromatography–mass spectrometry. Aromatic profile of vinegar from strawberry cooked must was also studied. All strawberry vinegars retained certain impact odorants from strawberries: 3-nonen-2-one, (*E*,*E*)-2,4-decadienal, guaiacol, nerolidol, pantolactone + furaneol, eugenol, γ-dodecalactone and phenylacetic acid. Isovaleric acid, pantolactone + furaneol, *p*-vinylguaiacol, phenylacetic acid and vanillin were the most important aroma-active compounds in all vinegars. The strawberry cooked must vinegar accounted for the highest number of impact odorants. Wood barrels provided more aroma complexity than glass containers. Impact odorants with grassy characteristics were predominant in vinegar from glass containers, and those with sweet and fruity characteristics in vinegars from wood barrels. Principal component analysis indicated that the production process led to differences in the impact odorants.

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1. Introduction

Because of the high level of competitiveness in today's market, the success of a product requires the use of raw material that is different from traditionally used, and it needs the application of innovative production techniques. In Spain, vinegar is traditionally produced from grapes by means of double fermentation (alcoholic and acetous). There are several advantages of using fruits for vinegar production, such as their health and organoleptic properties. Thus, an important task within the production process is to maintain or even enhance these features.

The use of fruit surpluses is a good solution to save resources and provide extra income for the agricultural sector. Strawberries are grown in large quantities in Huelva (Spain), and every year, part of the crop is discarded for several reasons, including size, deformations or overproduction, which leads to surpluses. This fact, together with the qualities of this fruit, makes it a good candidate for fruit vinegar production.

Among the essential aspects to consider when developing a new food product is its aroma. The volatile composition of strawberry fruit has been extensively studied over the past 50 years, and more than 360 volatile compounds have been identified (Latrasse, 1991). These compounds comprise esters, which are qualitatively and quantitatively dominant, as well as furanones, sulphur compounds, lactones, alcohols and carbonyls (Zabetakis & Holden, 1997). However, not all volatile compounds contribute to the composition of the aroma and, therefore, gas chromatography–olfactometry (GC–O) analysis has become important for clarifying whether a volatile compound has an odour characteristic and for evaluating its contribution to the overall aroma (Fischer & Hammerschimdt, 1992). Among the methodologies used to analyse samples by GC–O, the modified frequency (*MF*) approach combines intensity and frequency detection methods and subsequently provides more reliable results (San-Juan, Pet'ka, Cacho, Ferreira, & Escudero, 2010).

A great deal of work has been done to elucidate the impact odorants in strawberries. Some key odorants in this fruit are furaneol, mesifuran, methyl butanoate, γ-decalactone, linalool and hexanoic acid (Du, Plotto, Baldwin, & Rouseff, 2011; Fukuhara, Li, Okamura, Nakahara, & Hayata, 2005).

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The key odorants of vinegar have been studied to a lesser extent. In red wine vinegar, Charles et al. (2000) found relevant aromas from diacetyl and acetoin and from acids, such as acetic, isovaleric and butyric acids, and alcohols, such as isoamyl alcohol and 2-phenylethanol. In sherry wine vinegar, the key odorants were ethyl acetate, diacetyl, butyl acetate, isoamyl acetate, ethyl octanoate, acetic acid, isovaleric acid and sotolon (Callejón, Morales, Silva Ferreira, & Troncoso, 2008). In addition, Aceña, Vera, Guasch, Busto, & Mestre, 2011 also recently described the compounds ethyl isobutyrate and ethyl *trans*-cinnamate as active odorants in this type of vinegar.

It is well known that when vinegars are aged in barrels, the overall aroma is enriched as the result of three important processes. Firstly, they are concentrated because water is lost through pores in the wood (Giudici, Lemmetti, & Mazza, 2015; Tesfaye, Morales, García-Parrilla, & Troncoso, 2002); secondly, the aromatic compounds are transferred from the wood; finally, new compounds, such as esters, are formed by chemical reactions (Morales, Benitez, & Troncoso, 2004). Notably, the use of barrels made from different types of wood produces differences in the aromatic composition of wine vinegar. This fact is related to the different porosity of wood as well as its peculiar chemical composition (Callejón, Torija, Mas, Morales, & Troncoso, 2010). The influence of container type on the production of vinegars from fruits other than grapes should be investigated.

The main objective of this work was to check the effects of different containers on the aroma profile of strawberry vinegars. In addition, we studied the use of two types of substrate, namely fruit puree and concentrated fruit puree. For these purposes, gas chroma tography–olfactometry (GC–O) and gas chromatography–mass spectrometry (GC–MS) analyses were performed.

2. Materials and methods

2.1. Chemicals

Dichloromethane and anhydrous sodium sulphate were purchased from Merck (Darmstadt, Germany), both of analytical quality.

2.2. Samples

The strawberry vinegar samples analysed in this study were produced from second-grade-quality strawberries of the Camarosa variety that were harvested in 2009. For this purpose, a strawberry puree (SP) was inoculated with the isolated yeast strain *Saccharomyces cerevisiae* RP1. A total of four alcoholic fermentations were carried out, and the resulting strawberry wines were mixed and acetified in three different containers of 8 L capacity, that is, a glass vessel (GSV), and oak (OSV) and cherry wood barrels (CVS). Each container was filled with 5.5 L of wine and inoculated with acetic acid bacteria. Acetous fermentation was performed in duplicate. In order to increase the sucrose content, part of the strawberry puree was concentrated by heating in a water bath at 80 °C for 10 h., stirring frequently. Puree was concentrated 2.13 times. The resulting product was a cooked must. One litre of this must was subjected to alcoholic and acetous fermentation (CMSV) in a glass container by following the same procedure as the one used without concentration. Again, the alcoholic and acetous fermentations from cooked must were performed in duplicate. All fermentation processes were performed by surface culture.

Hence, in this study a total of 5 samples were analysed: the strawberry substrate (SV) and 4 different strawberry vinegars (GSV, OSV, CSV and CMSV). More details about production process may be found in Ubeda et al. (2011). The sample codes appear in brackets.

2.3. Gas chromatography–olfactometry

For the olfactometric analysis, representative extracts of the samples were obtained by liquid–liquid extraction method (Callejón, Morales, Troncoso, & Silva Ferreira, 2008). The procedure was as follows: to 50 mL of each sample, 5 g of anhydrous sodium sulphate were added and extracted twice with 5 mL of dichloromethane over the course of 5 min by using a magnetic stir bar. The two organic phases obtained were combined and dried over anhydrous sodium sulphate. After that, 2.5 mL of the organic phase were concentrated 5-fold under a nitrogen stream.

Analyses were conducted by using a Varian 3800 GC (Middelburg, The Netherlands) equipped with a flame ionisation detector (FID) and an OP275 olfactometer (GL Science Inc., Tokyo, Japan). Two microlitres of each extract were injected into a DB-WAX column (60 m × 0.25 mm; 0.22 μm film thickness) operating in splitless mode (J & W Scientific, Agilent Technologies Inc., Santa Clara, CA). The oven temperature program was as follows: 40 °C for 1 min, increasing to 220 °C at a rate of 2 °C/min and holding for 30 min. The column effluent was split 2:3 into an FID and a heated sniffing port. The injector and detector temperature were both 220 °C. The carrier gas was H_2 at a flow rate of 1 mL/min. The sensory panel was made up of three trained tasters, all of whom sniffed each sample twice and assigned an intensity level to each perceived odour, namely 1, 2 or 3. The results were expressed as the "modified frequency" (*MF*), which was calculated by using the formula $MF(\%) = [F(\%) \times I(\%)]^{1/2}$ proposed by Dravnieks (1985), in which *F* is frequency of occurrence and *I* intensity.

2.4. Dynamic headspace and gas chromatography–mass spectrometry (DHS–GC–MS)

The DHS sampling conditions were as follows: 5 mL of sample and 10 μL of 4-methyl-2-pentanol (IS) at 1045 mg/L were placed in a 20-mL headspace vial. The DHS sampling module conditions were as follows: the sample was incubated for 2 min at 30 °C and agitated at 250 rpm. The volatile compounds were purged with an N_2 flow of 80 mL/min for a total purge volume of 2100 mL. The analytes were trapped onto a Tenax TA adsorbent (Gerstel) set at 30 °C. A subsequent dry purge of the trap was performed at 30 °C with 750 mL of N_2 at 50 mL/min. The thermal desorption was then performed in solvent vent mode. The desorption temperature program was as follows: 40 °C for 0.5 min, ramped up at 100 °C/min to 230 °C and held for 5 min. The CIS-4 PTV injector with a Tenax TA inlet liner was held at 25 °C with liquid nitrogen for the total desorption time and then raised to 230 °C at 10 °C/s and held for 5 min. The solvent vent mode with a flow rate of 70 mL/min was used to transfer the sample to the analytical column.

Analyses were conducted by using an Agilent 6890 GC system coupled to an Agilent 5975 inert quadrupole mass spectrometer that was equipped with a Gerstel Dynamic Headspace unit, a Gerstel Thermo Desorption System (TDS2) and a Cooling Injector System CIS-4 PTV inlet (Gerstel, Müllheim an der Ruhr, Germany).

The analytical columns consisted of a CP-Wax 57CB column of 50 m × 0.25 mm and 0.20 μm film thickness (Varian, Middelburg, the Netherlands) and an HP5 column of 30.0 m × 0.25 mm and 0.25 μm film thickness (Agilent). The carrier gas was He at a flow rate of 1 mL/min. The oven temperature program was the same as the program employed for the GC–O. The quadrupole, source and transfer line temperatures were 150, 230 and 250 °C, respectively. Electron ionisation mass spectra data from *m*/*z* 29–350 were

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