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Employment of different processes for the production of strawberry vinegars: Effects on antioxidant activity, total phenols and monomeric anthocyanins

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ABSTRACT

The use of strawberry surpluses for the production of added value products seems to be a good solution choice to avoid the waste of this fruit. We produced strawberry vinegars through double fermentation (alcoholic and acetous) from three different harvests of *Fragaria x ananassa var. Camarosa.* The objective was to study the evolution of antioxidant activity, total phenols and monomeric anthocyanins during the vinegar production process. These parameters increased when sulphur dioxide and pectolytic enzymes were added to substrates. Inoculation with the *Saccharomyces cerevisiae* strain RP1 produced wines with half the anthocyanins with respect to the spontaneous fermentations. The use of wood barrels, particularly cherry wood barrels, had a positive effect on all the parameters determined. All measured parameters decreased during the double fermentation process. In general, the acetification stage led to a high loss of antioxidant compounds. Moreover, the production of these vinegars at a semi-pilot scale yielded final commodities with the best values for antioxidant activity, total phenols and monomeric anthocyanins comparing with the vinegars obtained in 2008 and 2009 harvest.

1. Introduction

Strawberries are a widely researched fruit for their nutritional and health benefits as well as their organoleptic properties. This fruit is rich in vitamins, minerals, fibre and phytochemicals. In addition, strawberries contain potentially bioactive compounds and are a great source of phenolic compounds such as flavonoids and phenolic acids (Aaby, Skrede, & Wrolstad, 2005; Määttä-Riihinen, Kamal-Eldin, & Törrönen, 2004; Seeram, Lee, Scheuller, & Heber, 2006). All of these phenolic compounds have been shown to prevent oxidative processes, particularly those caused by reactive oxygen species (ROS) (Aaby, Ekeberg, & Skrede, 2007; Cerezo, Cuevas, Winterhalter, Garcia-Parrilla, & Troncoso, 2010a). These compounds make strawberries a highly antioxidant fruit (Aaby et al., 2005; Wolfe et al., 2008) with potential health benefits. Among the numerous healthy properties described in the literature are anti-proliferative effects on cancer cells (Meyers, Watkins, Pritts, & Liu, 2003; Olsson, Andersson, Oredsson, Berglund, & Gustavsson, 2006) and the antioxidant and anti-inflammatory effects that have been shown to reduce cardiovascular disease risk factors in several prospective cohort studies (Hannum, 2004).

According to the latest data from the FAO (FAOStat, FAO, 2011), Spain is the second-largest strawberry producer in the world; a large portion of this production is harvested in Huelva (Andalucía). Every year, part of the crop is discarded for various reasons, including size or deformations of the berries, or overproduction which leads to surpluses. Because vinegar is generally an inexpensive product, its production requires low-cost raw materials, such as sub-standard fruit and seasonal agricultural surpluses (Solieri & Giudici, 2009). In addition, there is a growing demand for fruit vinegars, which are sold as a health food (Shau-mei & Chang, 2009). The use of strawberries of second quality, which are still suitable for human consumption, to production healthy vinegars with special organoleptic nuances may be a good method to reduce losses due to discarding the fruit.

For this purpose, we have produced strawberry vinegars using second-quality strawberries employing two-stage fermentation and assessed different conditions and treatments. The aim of this work was to evaluate the changes in the antioxidant activity (AA), total phenols index (TPI) and total monomeric anthocyanins (TA) during the production process of strawberry vinegar. In addition, an adequate extraction method to perform these determinations was designed.

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2. Materials and methods

2.1. Chemicals

The reagents acetone, methanol, Folin–Ciocalteu reagent, ethanol, di-potassium hydrogen phosphate (anhydrous), sodium di-hydrogen phosphate 1-hydrate, potassium chloride, sodium acetate and sodium carbonate (anhydrous) were purchased from Merck (Darmstadt, Germany). Fluorescein sodium and gallic acid were supplied from Fluka (Madrid, Spain). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH) and 2,2′-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. Samples

For the optimisation of the extraction process, we used strawberries (*Fragaria ananassa var. camarosa*) acquired at the market. The fruit was crushed in our laboratory, distributed into amber glass flasks and frozen at −20 °C.

For the production of the vinegars, we employed three different batches of strawberries (*Fragaria ananassa var. camarosa*) from the Huelva area (Spain), corresponding to three harvests: 2008, 2009 and 2010. The production processes were performed in the laboratories of the Dept of Biochemistry and Biotechnology, Faculty of Oenology, Univ Rovira i Virgili (Tarragona). In 2008 and 2009, the substrate employed were purees prepared in the laboratory using a beater. In 2010, we used a commercial puree provided by the Hudisa Company (Huelva). Sulphur dioxide (60 mg/L), sucrose and two types of pectolytic enzymes (Depectil extra-garde FCE® and Depectil clarification® from Martin Vialatte Oenologie, Epernay, France), both at a concentration of 15 mg/L, were added to the puree. After this point, the procedures were slightly different in each harvest.

2.2.1. 2008 harvest

One portion of the strawberry puree was pressed to study the effect of two types of starting substrates (semi-solid and liquid) (Table 1). Six glass containers were filled with 6 L of fruit substrate (four purees and two liquids). Half of the containers of each type of substrate were inoculated with the yeast *Saccharomyces cerevisiae* QA23 at a concentration of 2×10^6 cells/ml, and spontaneous alcoholic fermentation was allowed to occur in the other half. All wines were spontaneously acetified keeping it in the same containers. Two final treatments were tested in vinegars: pasteurization or centrifugation. The average acetic degrees in the 2008 strawberry vinegars were 4.8.

2.2.2. 2009 harvest

For the vinegar production in 2009, eight glass vessels were filled with 6 L of strawberry puree each. Half of these vessels were inoculated with the yeast strain *S. cerevisiae* RP1, isolated during the 2008 spontaneous alcoholic fermentation, and spontaneous alcoholic fermentation was allowed to occur in the other half. All of the wines obtained from the inoculated alcoholic fermentation were mixed and dispensed in three different types of containers: a glass vessel and oak or cherry wood barrels. Samples were then inoculated with a strain of acetic acid bacteria isolated from the 2008 acetification. Wines from the spontaneous alcoholic fermentation were processed in the same way and left to acetify spontaneously. The vinegars obtained were pasteurised. Inoculated vinegars from the 2009 harvest reached an acetic degree of 5.5 (glass container), 6.6 (oak barrel) and 6.3 (cherry barrel).

A portion of the puree from the 2009 strawberries was concentrated by heating in a water bath at 80 °C during 10 h, to test another method of increasing the sugar content; the resulting product was a cooked must (Table 1). The sucrose final concentration was 140 g/L. One litre of this substrate was fermented by a spontaneous process and 1 L was inoculated with the RP1 strain of yeast. The inoculated wines (IWs) were acetified with the same acetic acid bacteria isolated in 2008, and the spontaneous wines (SWs) were left to acetify spontaneously.

2.2.3. 2010 harvest

In this harvest, the pectolytic enzymes added were Rohapect® (12 mg/hL) and the pH was adjusted to 3.5 with 2 g/L $CaCO_3$. In this case, 45 L of puree were fermented in a stainless steel container on a semi-pilot scale, after inoculation with *S. cerevisiae* RP1. The acetous fermentation was performed in a cherry wood barrel. The vinegar had an acetic degree of 6.3.

All vinegars from 2009 to 2010 harvest were pasteurized as final treatment.

Forty-one samples, taken throughout these production processes, were analysed. The codes and characteristics of the samples are shown in Table 1. In addition, five commercial vinegars were also

Table 1
Samples description.

Harvest	Treatment	Puree Sample	Treatment	Sample substrate	Alcoholic fermentation (time)	Wine Sample	Acetification (time)	Treatment or Recipient	Vinegar sample
2008	Crushed	F8P1	SO_2 Pectolytic enzymes Sucrose (50 g/L)	F8P2	Inoculated (4 days)	F8WI1–F8WI4	Spontaneous (2 months)	Centrifugation	F8VIC1–F8SVIC2
								Pasteurization	F8SVIP1–F8SVIP2
					Spontaneous (5 days)	F8WE1–F8WE4		Centrifugation	F8SVEC1–F8SVEC2
								Pasteurization	F8SVEP1–F8SVEP2
	–	F8P2	Pressing	F8L	Inoculated (4 days)	F8LWI	–	–	–
					Spontaneous (5 days)	F8LWE			
2009	Crushed	F9P1	SO_2 Pectolytic enzymes Sucrose (75 g/L)	F9P2	Inoculated (5 days)	F9WI1–F9WI4	Inoculated (2 months)	glass vessel	F9SVIG
								oak barrel	F9SVIO
								cherry barrel	F9SVIX
					Spontaneous (8 days)	F9WE1–F9WE4	Spontaneous (2 months)	glass vessel	–
								oak barrel	–
								cherry barrel	–
			Heating Concentrated	F9MC	Inoculated (7 days)	F9MCWI1–F9MCWI2	Inoculated (5 months)	glass vessel	F9MCVI1–F9MCVI2
					Spontaneous (7 days)	F9MCWE1–F9MCWE2	Spontaneous (2.5 months)	glass vessel	F9MCVE1–F9MCVE2
2010	Crushed	F10P1	SO_2 Pectolytic enzymes Sucrose (65 g/L) $CaCO_3$	F10P2	Inoculated (4 days)	F10WI	Inoculated (1.5 months)	cherry barrel	F10VI