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Evaluation of antioxidant activity and total phenols index in persimmon vinegars produced by different processes

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ABSTRACT

The total phenols index (TPI) and antioxidant activity of persimmon vinegars produced by different processes were evaluated. A novel extraction method was designed and optimised for this purpose with respect to the type and concentration of solvent and ultrasonication time. The best extraction conditions found were the use of 80% ethanol and 25 min of ultrasonication. Antioxidant capacity was determined by the oxygen-radical absorbance capacity of fluorescein (ORAC-FL) and 2,2′-diphenyl-1-pycrylhydrazyl (DPPH) free-radical assays. The antioxidant activities were the same in the fruit and the vinegar, except in the ORAC assay, which showed a significant decrease during the acetification process. The results showed that using the wild yeast strain native to the persimmon produced vinegars with higher antioxidant activity than that of an inoculated alcoholic fermentation. Finally, a comparison between our vinegars and other commercial examples was made. The TPI and antioxidant activity values of persimmon vinegars were always higher than those obtained from white and red-wine vinegars. The antioxidant activity and total phenols of the final product indicate that persimmon vinegar is a competitive product in the market.

1. Introduction

Currently, consumer interest in the health benefits of foods is increasingly important, motivating more research in this area in recent years. Furthermore, consumers are demanding value-added products with new characteristics; therefore, the purpose of many investigations has been to elaborate new products providing health benefits. The main raw materials used to obtain these new products are fruits and vegetables. Several studies have shown a negative correlation between the consumption of fruits and vegetables and risks for cardiovascular disease, cancer, inflammation or problems associated with ageing (Dillard & German, 2000; Garcia-Closas, Gonzalez, Agudo, & Riboli, 1999; Joseph et al., 1999; Prior & Cao, 2000; Steinmetz & Potter, 1996; Wargovich, 2000).

Each year a large fraction of every fruit harvested is discarded because their size is outside the standard range, deformations or overproduction. For this reason, we proposed a study of the utilisation surplus fruit for vinegar production. Persimmon was one of the fruits selected for this purpose; it is mainly consumed fresh and the processing industry is scarcely developed. Persimmon is widely consumed in China and traditionally used for medicinal purposes such as coughs, hypertension, dyspnoea, paralysis, burns and bleeding (Mowat, 1990). It has also been demonstrated to have an inhibitory effect on human lymphoid leukaemia cells (Achiwa, Hibasami, Katsuzaki, Imai, & Komiya, 1997), and in some persimmon varieties such as *Mopan* a positive effect on hypercholesterolemia has been reported (Gorinstein et al., 1998). It is assumed that these "nutraceutical" properties are due to the antioxidant components of this fruit, including phenolic compounds (Yokosawa & Okumura, 2007), vitamins and carotenoids.

There are many methods available for the evaluation of antioxidant activity; most are colorimetric assays, so it is necessary to have a sample or extract free of solid particles. Sometimes an extraction method is required due to sample consistency. The established techniques for the extraction of antioxidant compounds differ in some parameters such as the kind of solvent used, but the main objective of the extraction stage is always to recover as much of the bioactive fraction as possible with the highest efficiency (Spigno, Tramelli, & De Faveri, 2007a). Previous studies have reported the influence of several parameters (ultrasonication time, solvent type, temperature and percentage of extractant) in the extraction of phenolic molecules and antioxidant compounds in general (Alothman, Bhat, & Karim, 2009; Pinelo, Del Fabbro, Manzocco, Núñez, & Nicoli, 2005a; Spigno et al., 2007a).

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Table 1
Samples description.

Type of sample	Treatment/Fermentation	Sample codex
Puree	No treatment	K7Z1
Puree	Pectolytic enzymes and sulphur dioxide	K7Z2
Wine	From K7Z2 by spontaneous alcoholic fermentation	K7WE1-K7WE3
Wine	From K7Z2 by inoculated alcoholic fermentation	K7WI1-K7WI3
Vinegar	From K7WE made by spontaneous acetification	K7VE1-K7VE3
Vinegar	From K7WI made by spontaneous acetification	K7VI1-K7VI3

The aim of this work was the evaluation of the antioxidant activity and total phenols index of persimmon vinegar[1] at each production step in a double fermentation process (alcoholic and acetic); the effect of spontaneous versus inoculated alcoholic fermentation on these parameters was of special interest. For this purpose, an extraction method was designed in which the following variables were optimised: the kind of solvent, solvent-to-water ratio and ultrasonication time. Finally, the values obtained for our vinegars were compared with some commercial vinegars.

2. Materials and methods

2.1. Samples

In this work we have employed three different persimmon (*Diospyros kaki var. Sharoni*) batches. Persimmons were harvested at commercial ripeness in November, 2007. This variety belongs to the group of non astringent persimmon. Batch 1 and batch 2, were acquired in the market and employed for the extraction process optimization. The batch 3, provided by Agromedina company, was used for the vinegar production. The elaboration process was performed in the laboratories of the Department of Biochemistry and Biotechnology (Faculty of Enology, University Rovira i Virgili, Tarragona), according to the following procedure: ~50 kg of persimmon fruit was crushed with a beater to obtain 45 L of puree. 60 g/L of sulphur dioxide were added to avoid undesirable microbial growth. Additionally, two pectolytic enzymes were incorporated: Depectil extra-garde FCE® for volatiles release and Depectil clarification® to help clarify the product (Martin Vialatte Oenologie, Epernay, France), both at a concentration of 15 mg/L. This puree was then distributed into six glass vessels, with 6 L of sample in each. Three of these vessels were inoculated with the enological yeast QA23 at the concentration of 2×10^6 cells/mL and a spontaneous alcoholic fermentation was allowed to occur in the other three vessels. The resulting wines were acetified by a spontaneous process to produce the persimmon vinegars. At each fermentation stage, samples were taken (Table 1). Samples were stored in 30-mL amber glass flasks at −20 °C until analysis.

For solvent and percentage selection we used puree prepared in our laboratory from persimmon batch 1 and for the ultrasonic extraction time selection we have employed puree from persimmon batch 2.

2.2. Chemicals

The reagents acetone, methanol, Folin-Ciocalteu reagent, ethanol, anhydrous dipotassium hydrogen phosphate, sodium dihydrogen phosphate monohydrate, potassium chloride, sodium acetate and anhydrous sodium carbonate were provided by Merck (Darmstadt, Germany). Fluorescein sodium and gallic acid were supplied by 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) free-radical were purchased from Sigma–Aldrich (Steinheim, Germany).

2.3. Sample-extraction process

Due to the different consistencies of the samples studied, it was necessary to establish an extraction system for the determination of total phenols index and antioxidant activity. To design the extraction method, we modified the procedures proposed by Gorinstein et al. (1999) and Chen, Fan, Yue, Wu, and Li (2008). Optimisation of the most influential parameters in the extraction method was required; the parameters optimised were type of solvent (acetone, methanol or ethanol), percentage of solvent (50%, 80% or 100%) and ultrasonic extraction time (15, 25, 35 or 50 min). The selection of the best extraction parameters was made by taking into consideration the maximum values obtained in each assay as well as economy of time and solvent use. The extraction conditions are shown in Fig. 1.

2.4. Antioxidant-activity assays

2.4.1. Oxygen-radical absorbance-capacity assay (ORAC-FL)

ORAC-FL was performed in a black 96-well microplate (BD Falcon, BD Biosciences, UK), following the method described by Dávalos, Gómez-Cordovés, and Bartolomé (2004) with some modifications. This assay was realised with a Multidetection plate reader (Synergy HT, Vermont, USA). Previously, fluorescein (60 nM) and appropriate dilutions of the samples were prepared along with solutions of different Trolox concentrations (0.5, 2, 3.5, 5, 6.5, 8,

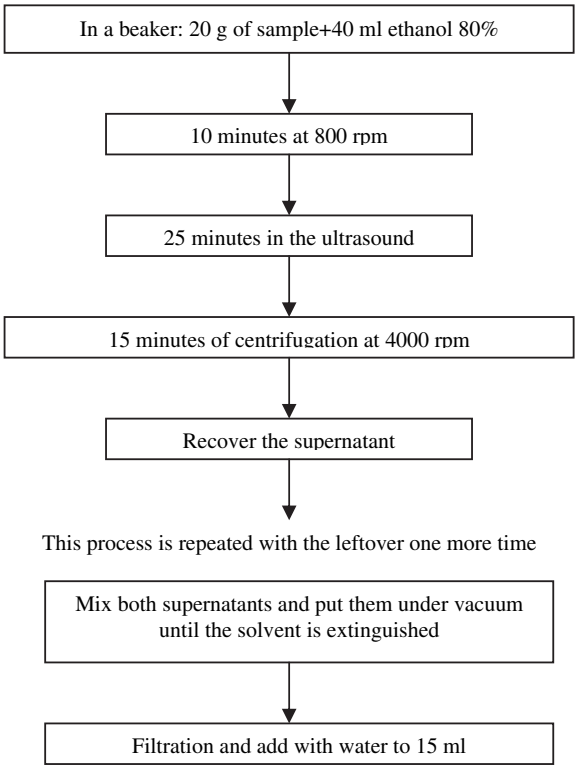

Fig. 1. Extraction process.

[1] Footnotes: Given the acidic nature of these products and the lack of a suitable alternative term, we decided to refer to these products as vinegars throughout the text, despite the fact that according to Spanish regulations some of these products are not sufficiently acidic to be classified as vinegars.