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Effects of gluconic and alcoholic fermentation on anthocyanin composition and antioxidant activity of beverages made from strawberry

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ABSTRACT

Strawberry is a very perishable fruit, well-known for being a source of bioactive compounds. The elaboration of the beverages by alcoholic and gluconic fermentation process has been explored as a worthy strategy for preventing food losses as well as preserving bioactive compounds with antioxidant properties.

To this end, this paper aims to characterize the anthocyanin composition of the resulting beverages and to evaluate their antioxidant properties with *in vitro* assays (ORAC, DPPH). Additionally, the protective effect against amyloid-β (Aβ) peptide toxicity in terms of Reactive Oxygen Species (ROS) production and PC12 cells viability was determined.

Eleven anthocyanin compounds were identified and quantified by UHPLC-DAD-MS. Pelargonidin 3-glucoside and its derivatives were the major compounds. Gluconic fermentation preserved anthocyanin composition being an advantage of this innovative process. Accordingly the values of antioxidant activity were higher for gluconic than alcoholic fermented beverages. Indeed, both of them increased cell viability (16–57% $p < 0.05$) and attenuate the oxidative stress triggered by Aβ (13–38% $p < 0.05$).

1. Introduction

Strawberry is an important fruit crop worldwide, especially for fresh consumption. An alternative for avoiding economic loss due to its perishable nature is the elaboration of derivatives products such as jams, yoghourts, products for biscuits or cakes and beverages made from strawberry purees.

Recently, different studies summarized the evidence for the health benefits of strawberry and other berry fruits (Basu, Nguyen, Betts, & Lyons, 2014; Giampieri et al., 2015). Indeed, strawberry is a good source of bioactive compounds. Furthermore, the antioxidant properties of strawberry have been attributed to its polyphenol and vitamin content, being ascorbic acid, ellagitanins and anthocyanins the greatest contributors to its antioxidant capacity (Aaby, Ekeberg, & Skrede, 2007; Manganaris, Goulas, Vicente, & Terry, 2014). Recently, strawberries were included among the 100 richest sources of dietary polyphenols and also listed in rankings of 89 foods and beverages that provide more than 1 mg of polyphenols per serving (Pérez-Jiménez, Neveu, Vos, & Scalbert, 2010). Anthocyanins are responsible for the red color of berry fruits, such as

Abbreviations: Aβ, amyloid-β; AAPH, 2,2′-diazo-bis-amidinepropane-dihydrochloride; DCFDA, 2′,7′-dichlorofluorescin diacetate; DPPH, 2,2-diphenyl-1-picrylhydrazyl; IC_{50}, Half Maximal Inhibitory Concentration; MTT, Thiazolyl Blue Tetrazolium Bromide; ORAC, Oxygen Radical Absorbance Capacity; ROS, Reactive Oxygen Species; UHPLC, Ultra High-Performance Liquid Chromatography.

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blueberries, blackberries and strawberries. It is well known that pelargonidin 3-glucoside is the major anthocyanin in strawberry (150–650 mg kg^{-1} of fresh weight) (García-Viguera, Zafrilla, & Tomás-Barberán, 1998; Lopes-da-Silva, Escribano-Bailón, Pérez Alonso, Rivas-Gonzalo, & Santos-Buelga, 2007). Indeed, there are crucial factors that influence significantly the stability of anthocyanin compounds such as process, time, and storage temperature (Clifford, 2000). Several efforts have been done to diminish the effect of process in the composition of products made from strawberry using different production systems and the employment of modified atmosphere in the storage (Fan et al., 2012; Oliveira et al., 2015).

Furthermore, the production of strawberry drinks is an innovative trend. In particular, the fermentation by *Gluconobacter japonicus* which transforms the glucose content of the fruit into gluconic acid to keep the fructose as sweetener (Cañete-Rodríguez et al., 2015). Additionally, alcoholic fermentation by *Saccharomyces cerevisiae*, is used to elaborate strawberry beverages (Hidalgo, Torija, Mas, & Mateo, 2013) The impact of gluconic fermentation has been evaluated in terms of amino acids and biogenic amines (Ordóñez et al., 2015). Besides, non-anthocyanin composition of these beverages (gluconic and alcoholic) has been described before showing their potential as a source of bioactive compounds (Álvarez-Fernández, Hornedo-Ortega, Cerezo, Troncoso, & García-Parrilla, 2014; Álvarez-Fernández, Cerezo, Cañete-Rodriguez, Troncoso, & García-Parrilla, 2015).

Among the healthy properties of strawberries, neuroprotective effects due to the anthocyanin content have been reported (Giampieri et al., 2015). Hence, recent studies show the effectiveness of anthocyanins against Aβ toxicity. Indeed, Badshah, Kim, and Kim (2015) demonstrated that an anthocyanin extract (cyanidin 3-glucoside, delphinidin 3-glucoside and petunidin 3-glucoside) of black soybean decreased the neuronal death in HT22 cells. Additionally, cyanidin 3-glucoside can inhibit $A\beta_{25-35}$ spontaneous aggregation into oligomers and their neurotoxicity in human neuronal SH-SY5Y cells (Tarozzi et al., 2010). To the best of our knowledge, the protective effect against Aβ peptide has not been explored neither with strawberry or its derivatives nor for pelargonidin and derivatives compounds. Therefore, our work intends to explore the hypothetical activity these compound and beverages may present.

The aims of this paper are to (i) characterize the anthocyanin composition of fermented beverages elaborated from strawberry, (ii) to estimate the effect of alcoholic and gluconic fermentation on anthocyanin compounds, (ii) to evaluate their bioactive potential.

2. Material and methods

2.1. Samples

Hudisa Desarrollo Industrial S.A. (Lepe, Spain) provided strawberry purée. The process of elaboration of the strawberry mash is summarized as follows: the fruit is received, selected, cleaned and the stems and leaves are eliminated. After the strawberry has been mashed, an inactivation enzymatic (2 min, 55 ºC–65 °C) is performed, followed by a pasteurization process (3 min, >90 °C). Finally, the temperature is reduced to 5 °C. The mash is sieved to remove the seeds.

In this study, two harvests were analyzed (2012 and 2013). These purées were frozen (−20 °C) until fermentation was carried out. Alcoholic and gluconic fermentations were conducted in the Department of Inorganic Chemistry and Chemical Engineering of the University of Córdoba (Córdoba, Spain). Fermentation conditions were previously described (Álvarez-Fernández et al., 2014, 2015). Alcoholic fermentation was carried out with a *S. cerevisiae* (CET 13057 isolated from native strawberry yeast) used as a starter for the submerged fermentation process. The fermentation process was as follows: 3.6 L of strawberry purée were placed into the bioreactor and the conditions set (pH 3.32, 29 °C, 26.20 rad s^{-1}); the medium was saturated with oxygen only at the beginning of the fermentation process, before adding the inoculum (10% (w/v) glucose, 0.1% (w/v) $MgSO_4$, 0.2% (w/v) KH_2PO_4, 0.3% (w/v) $(NH_4)_2SO_4$, 0.4% (w/v) yeast extract and 0.36% (w/v) bacteriological peptone). The end of the fermentation process was established when the glucose had been totally consumed and final pH was 3.30.

For gluconic fermentation, 3 L of strawberry purée substrate were placed into the bioreactor and the conditions set (pH 3.24, 29 °C, 20% O_2 and 1250 g); after 10–20 min, 125 ml of inoculum of *G. japonicus* strain E1 were added (5% (w/v) glucose, 1% (w/v) bacteria extract and 2% (w/v) bacteriological peptone) and mixed for 20–30 min, then the initial sample was taken. The end of the fermentation process was established when the glucose had been totally consumed and final pH was 2.74.

Eight alcoholic fermentation (code A) experiments were performed: four with purées from the 2012 harvest (code 12) and four with those from the 2013 harvest (code 13). Additionally, six gluconic fermentation experiments (code G) were performed: four with purées from the 2012 harvest and two from the 2013 harvest. Samples were taken at the initial point of the fermentation experiment (I), the final point (F) and after pasteurization of the fermented product (FP). Pasteurization was carried out at 70–80 °C for 15 min. All samples were frozen until analysis. Table 1 displays the codes of the samples used in this study.

2.2. Chemicals and reagents

Amberlite XAD7HP, Dimethyl sulfoxide (DMSO), Dulbecco's modified Eagle's medium (DMEM)-Glutamax, Trypsine-EDTA, Thiazolyl Blue Tetrazolium Bromide (MTT), Phosphate Buffered Saline (PBS), L-glutamine, fetal horse serum and fetal bovine serum, streptomycin, 2′,7′-dichlorofluorescin diacetate (DCFH-DA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-diazo-bis-amidinepropane-dihydrochloride (AAPH), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic) were purchased from Sigma (Steinheim, Germany). Fluorescein sodium was obtained through Fluka (Steinheim, Germany).

VWR Chemicals (Llinars del Vallés, Barcelona) supplied methanol and acetic acid. Formic acid and acetonitrile were obtained by Fisher Chemical. Pelargonidin 3-glucoside was obtained from Chloride (Cromadex Inc., USA). Cells PC12-Adh were supplied by ATCC® CRL-1721.1™ (Manassas, USA) and Amyloid β-protein 25–35 ($A\beta_{25-35}$) by Synvec (Bordeaux, France).

Table 1
Sample codes.

Codes	Sample
A 12 I	Alcoholic, 2012 Harvest, Initial
A 12 F	Alcoholic, 2012 Harvest, Final
A 12 FP	Alcoholic, 2012 Harvest, Pasteurized
A 13I	Alcoholic, 2013 Harvest, Initial
A 13 F	Alcoholic, 2013 Harvest, Final
A 13 FP	Alcoholic, 2013 Harvest, Pasteurized
G 12 I	Gluconic, 2012 Harvest, Initial
G 12 F	Gluconic, 2012 Harvest, Final
G 12 FP	Gluconic, 2012 Harvest, Pasteurized
G 13 I	Gluconic, 2013 Harvest, Initial
G 13 F	Gluconic, 2013 Harvest, Final
G 13 FP	Gluconic, 2013 Harvest, Pasteurized