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Influence of storage conditions on the anthocyanin profile and colour of an innovative beverage elaborated by gluconic fermentation of strawberry

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ABSTRACT

Derived fruit products such as strawberry-based fermented beverages, increase the fruit's conservation period and offer new alternatives for the non-alcoholic market. The influence of storage time and temperature on the anthocyanin composition, antioxidant activity and colour of a fermented strawberry beverage were studied and indicated that 60-days is the half-life for the tested beverage. Twenty-three anthocyanin compounds were analysed by ultra high-performance liquid chromatography Orbitrap (UHPLC-MS/MS). This is the first time that pelargonidin 3-sambubioside, delphinidin 3-arabinoside, cyanidin 3-(6-acetyl)-glucoside and delphinidin 3-galactoside have been reported in any products derived from strawberry. Additionally, the accurate mass of the following anthocyanins were reported: catechin-(4–8)-pelargonidin 3-glucoside, afzelechin-pelargonidin 3-glucoside, pelargonidin dissacharide-(hexose + pentose)-acylated with acetic acid, 5-carboxypyranopelargonidin 3-glucoside, pelargonidin 3-arabinoside, pelargonidin 3-malonylglucoside, pelargonidin 3-(6-acetyl)-glucoside and pelargonidin 3-(6-succynil)-arabinoside. Functional aspects of this drink rely on its bioactive compounds and lack of glucose due to its transformation to gluconic acid which makes it suitable for diabetic consumers.

Abbreviations: UHPLC-MS/MS, ultra high-performance liquid chromatography; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; ORAC, oxygen radical absorbance capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl

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Chemical compounds: Pelargonidin 3-glucoside (PubChem CID: 443448); Pelargonidin 3-sambubioside (PubChem CID: 71627264); Pelargonidin 3-rutinoside (PubChem CID: 44256626); Pelargonidin 3-arabinoside (PubChem CID: 44256694); Pelargonidin 3- (6"-malonylglucoside) (PubChem CID: 45256635); Cyanidin 3-galactoside (PubChem CID: 44256700); Cyanidin 3-glucoside (PubChem CID: 92131208); Peonidin 3-glucoside (PubChem CID: 443654); Delphinidin 3-arabinoside (PubChem CID: 12137508); Delphinidin 3-glucoside (PubChem CID: 443650).

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1. Introduction

Strawberry [*Fragaria x ananassa* (*Rosaceae* Family)] is harvested in a very short period of time and is a very perishable product leading to the fruit rotting. The fruit has to be discarded if not sold, which entails substantial economic loss. Consequently, manufacturing derived products, such as fermented beverages using strawberries as a raw material, increases the conservation period and offers new sustainable and successful alternatives for the strawberry market, which drives economic profit.

Strawberry is a good source of nutrients, phytochemicals and fibre. The anthocyanins are among the principal bioactive compounds in strawberry (Basu, Nguyen, Betts, & Lyons, 2014). Additionally, these compounds are crucial for its colour quality. Several investigations have revealed antioxidant, anti-inflammatory, antihypertensive and anti-hyperlipidaemic or antiproliferative effects of strawberry anthocyanin compounds (Basu et al., 2014). Reduction on the risk of hypertension (8%) was observed when the consumption of strawberry anthocyanin was between 16 and 22 mg/day compared with a lower consumption (5–7 mg/day of anthocyanins) (Cassidy et al., 2011). Pure cyanidin 3-glucoside, induced endothelial nitric oxide synthase (eNOS) expression and increased nitric oxide (NO) release, which may help ameliorating endothelial dysfunction and maintain the blood pressure (Xu, Ikeda, & Yamori, 2004). Antioxidant and antiproliferative activities *in vitro* have been reported by Zhang, Seeram, Lee, Feng, and Heber (2008) when evaluating purified anthocyanin compounds in different human cancer cells (oral, colon and prostate).

It is well known that pelargonidin 3-glucoside is the major anthocyanin in strawberry (153–652 mg/kg fresh weight) followed by pelargonidin 3-rutinoside and other pelargonidin and cyanidin derivatives (Cerezo, Cuevas, Winterhalter, García-Parrilla, & Troncoso, 2010a; Lopes-da-Silva, Escribano-Bailon, Perez Alonso, Rivas-Gonzalo, & Santos-Buelga, 2007).

Recently, delphinidin 3-glucoside, peonidin 3-glucoside, and cyanidin 3-galactoside were identified in strawberry for the first time (Cerezo et al., 2010a). Other minor anthocyanin compounds, such as 5-carboxypyranopelargonidin 3-glucoside, pelargonidin acylated derivatives and pelargonidin linked to flavanol, were also present in strawberry (Fossen, Rayyan, & Andersen, 2004; Lopes-da-Silva, de Pascual-Teresa, Rivas-Gonzalo, & Santos-Buelga, 2002; Lopes-da-Silva et al., 2007).

Different types of processes are used to obtain more appropriate and attractive strawberry derivative products. The anthocyanin composition of processed products from berries such as liqueurs, juices, nectar, purée, condiments and jams has been studied only in terms of total anthocyanins (Da Silva, Lajolo, & Genovese, 2007; Klopotek, Otto, & Böhm, 2005; Sokół-Lętowska et al., 2014; Ubeda et al., 2013). However, there are no previous studies involving characterization of anthocyanin profile of strawberry fermented products, being restricted to non-anthocyanin composition (Álvarez-Fernández, Hornedo-Ortega, Cerezo, Troncoso, & García-Parrilla, 2014).

Process, time, and storage temperature are crucial factors that influence significantly the stability of anthocyanin compounds (Clifford, 2000). Tiwari, O'Donnel, Patras, Brunton, and Cullen (2009) observed greater stability of pelargonidin 3-glucoside and antioxidant activity at 4 °C compared to 20 °C in strawberry juice. Furthermore, anthocyanin compounds in liqueurs made from red fruits have been preserved for 3 months at 15 °C (Sokół-Lętowska et al., 2014). Therefore, the stability of anthocyanin compounds in each food matrix might be different. Indeed alcoholic and acetic fermentation exert a different impact on anthocyanin profile. Effects of alcoholic fermentation have been extensively studied. During red wine fermentation, the monomeric anthocyanins undergo several reactions and associations leading to the formation of anthocyanin-derived pigments. These reactions include self-association and co-pigmentation, such as the formation of polymeric anthocyanins with flavan-3-ols and proanthocyanidins, as well as the formation of new pigments, such as pyranoanthocyanins (Brouillard, Chassaing, & Fougerousse, 2003; Jackson, 2008; Wrolstad, Durst, & Lee, 2005). Acetification process of red wine also modifies anthocyanin composition, increasing vitisin-type and ethyl-linked compounds and decreasing the monomeric anthocyanins (Cerezo, Cuevas, Winterhalter, Garcia-Parrilla, & Troncoso, 2010b). However, the impact to anthocyanin composition of the gluconic fermented matrix products has not been studied.

These beverages represent an innovative trend as they lack glucose and therefore can be consumed by diabetics who can benefit from the health effects of anthocyanin.

The aim of this study was to examine the effect of storage time and temperature on the bioactive compounds (anthocyanins), antioxidant activity and colour, as this sensory parameters influence consumer acceptability of a strawberry beverage obtained through gluconic fermentation. UHPLC coupled with a hybrid mass spectrometer, which combined a linear trap quadrupole (LQT) and an Orbitrap mass analyser, was used to identify and quantify the anthocyanin compounds. Finally, the antioxidant activity of the extracts was evaluated using oxygen radical absorbance capacity (ORAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) essays; their colour parameters were also studied.

2. Materials and methods

2.1. Chemicals and materials

Amberlite XAD7HP, 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 by Fluka (Steinheim, Germany). Acetonitrile was obtained from Merck (Darmstadt, Germany) and formic acid was obtained from Panreac (Barcelona, Spain).

Pelargonidin 3-glucoside, cyanidin 3-glucoside, delphinidin 3-glucoside and peonidin 3-glucoside were purchased from Chromadex Inc. (Irvine, CA, USA).

2.2. Gluconic fermented strawberry beverage

The strawberry beverage was obtained by gluconic fermentation previously described (Álvarez-Fernández et al., 2014). The process is detailed below: strawberry purées were the