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Revalorization of strawberry surpluses by bio-transforming its glucose content into gluconic acid

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ABSTRACT

Modern societies produce massive surpluses of food, by-products and wastes that increase the interest for their revalorization. This work examines the use of a culture of *Gluconobacter japonicus* CECT 8443, without pH control, to convert selectively the glucose content of industrially pasteurized strawberry purée into gluconic acid for the development of new beverages. However, depending on the initial concentration of glucose, the microorganism could transform the acid formed into other compounds; for this reason, in this work the effect of initial sugar concentration on the preservation of the acid was investigated. The results show that the gluconic acid formed in strawberry purée containing no added sugars started to disappear after glucose depletion, but the acid concentration remained constant if sugar-enriched purée was used. The use of this industrial substrate resulted in the presence of yeasts and hence in some fructose uptake; however, the fructose consumption was negligible until after 20–30 h. The use of food by-products is an excellent opportunity not only to recover valuable compounds but for the development of new chemical and biotechnological approaches for their revalorization. This strategy should improve regional economies and contribute to a sustainable management of these underexploited resources.

1. Introduction

Efficiently producing, conditioning, distributing and using food continues to be one of the crucial problems in both developed and underdeveloped zones. About 1.3 billion tonnes of food is not used each year owing to farming, storage, transportation, processing, distribution or even consumption losses (Gustavsson et al., 2011).

Food by-products and wastes revalorization practices for the recovery/production of compounds of high interest are gaining much attention. This strategy should improve regional economies and contribute to a sustainable management of these underexploited resources (Galanakis and Schieber, 2014; Naziri et al., 2014; Perez-Jimenez and Viuda-Martos, 2015).

The problem of food surpluses is worse when the food is easily degradable. Such is the case, for example, with

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surpluses and residues from fruit and vegetable production processes, which account for about 50% of the global production of these products (Gustavsson et al., 2011). Although the situation varies geographically, developed countries typically generate fruit surpluses that cannot be sold for direct consumption and have a strongly adverse economic impact on their production zones. This is particularly so with strawberry, the first five world producers of which are China, United States, Mexico, Turkey and Spain (Faostat, 2015); the last country generates a surplus of about 20% of this fruit each year that is used mainly to obtain strawberry purée for various food production purposes.

The composition of strawberry purée, with a sugar content ranging from 30 to 200 g/L depending on its concentration grade, makes it a natural substrate with a high potential for obtaining various added-value bioproducts. One possible use of strawberry purée is for obtaining a gluconic acid based bio-oxidation product by converting the glucose into gluconic acid while preserving the initial content in fructose. Mixing with other fermentation products can be used to obtain new highly aromatic, refreshing non-alcoholic beverages sweetened by the fructose remaining in the medium (Cañete-Rodriguez et al., 2012, 2015, 2016; Sainz et al., 2012).

A search for microorganisms effecting this bioconversion led to acetic acid bacteria (Garcia-Garcia and Gullo, 2013; Raspor and Goranovic, 2008). Previous studies (Cañete-Rodriguez et al., 2015, 2016) showed that *Gluconobacter japonicus* CECT 8443 has a high potential for performing the sought transformation in a selective manner. The problem, however, is not limited to finding an appropriate microorganism; in fact, acetic acid bacteria can behave widely differently and lead to also different products depending on the particular cultivation medium and operating conditions. Also, preserving the sensory and antioxidant properties of the raw material requires avoiding sterilization prior to inoculation. As a result, fulfilment of the primary aim may be compromised by the presence of the natural micro flora of the fruit that includes mainly yeasts (Cañete-Rodriguez et al., 2015).

The result of the action of acetic acid bacteria depends largely on the prevailing metabolic pathway (Deppenmeier and Ehrenreich, 2009; Olijve and Kok, 1979a). The conversion of glucose into gluconic acid involves the nearly stoichiometric oxidation of the substrate on the outer surface of the cytoplasmic membrane via glucono-δ-lactone as intermediate product (King and Cheldelin, 1958). However, the resulting gluconic acid can undergo further conversion into keto-gluconates (Ano et al., 2011; Beschkov et al., 1995; Buse et al., 1992; Olijve and Kok, 1979a,b; Stubbs et al., 1940; Träger et al., 1992). In addition, glucose and gluconic acid can be used via the pentose phosphate and the Entner–Doudoroff pathways (Levering et al., 1988; Olijve and Kok, 1979a,b; Prust et al., 2005). The end result is strongly influenced by the pH and available concentration of glucose in the medium (Olijve and Kok, 1979a,b; Velizarov and Beschkov, 1994, 1998).

Thus, in this work, the effect of enriching strawberry purée with sugars on the preservation of the formed gluconic acid in the fermented end-product has been examined.

2. Material and methods

2.1. Raw material

Two different media were used as fermentation broths. One was strawberry purée (SP) industrially produced and pasteurized (92 °C for 90–120 s) by Hudisa Desarrollo Industrial, S.A. (Lepe, Spain) from fresh strawberries. The purée, containing approximately 34 g/L of sugars (roughly 50% glucose and 50% fructose) was stored at 0–4 °C prior to use. The other fermentation broth was enriched purée (ESP) obtained by enriching the previous broth with sugars up to a total concentration of 135–140 g/L (again 50% glucose and 50% fructose approximately).

2.2. Microorganism

G. japonicus CECT 8443 was selected for its high selectivity towards glucose relative to fructose (Cañete-Rodriguez et al., 2015, 2016; Navarro, 2011; Navarro et al., 2013; Sainz et al., 2016).

2.3. Preparation of inocula

G. japonicus was stored frozen at −18 °C in a 50:50 (v/v) water/glycerol mixture as cryoprotective agent. Prior to use, it was reactivated by thawing, and seeding in 125 mL of GYP liquid medium first [50 g/L glucose, 10 g/L yeast extract and 20 g/L bacteriological peptone] and tilted agar GY tubes [50 g/L glucose, 10 g/L yeast extract and 30 g/L agar].

As it is justified in Cañete-Rodriguez et al. (2015), the inoculum was prepared by seeding in 250 mL Erlenmeyer flasks containing 125 mL of GYP liquid medium that was previously autoclaved at 121 °C for 15 min. After shaking in an incubator at 29 °C and 150 rpm for 24 h, the medium was supplied with 125 mL of strawberry purée that was previously sterilized by autoclaving under identical conditions. After 24 h of additional incubation, the inoculum (the 250 mL of the final mixture) was ready for addition to the fermentation tank, which had previously been loaded with 3 L of pasteurized strawberry purée.

2.4. Determination of cell concentrations

Yeast cell concentrations were determined by direct counting under a microscope, using a Neubauer chamber as described elsewhere (Baena-Ruano et al., 2006). Bacterial cell concentrations were not determined since no accurate cell counting was possible owing to the characteristics of the purée (Cañete-Rodriguez et al., 2016).

2.5. Determination of sugars, gluconic acid and total acidity

Sugars and gluconic acid were quantified with the following enzyme kits from Megazyme®: K-GLUC 07/11 for glucose, K-FRUGL 12/12 for fructose and K-GATE 12/12 for gluconic acid. Additional information about the kits can be found at the Megazyme web site (Megazyme, 2016). Total acidity was measured by acid–base titration. Determinations were performed at least in triplicate and the resulting standard deviations are given in the figures.

2.6. Fermenter

Fermentation runs were conducted batchwise in a Biostat® 5 L bioreactor equipped with pH, agitation, dissolved oxygen and temperature controls. An average volume of 3 L, agitation at 500 rpm, a temperature of 29 °C and a dissolved oxygen at 20% air saturation were used in each run. The pH of the medium was allowed to evolve freely throughout. Since the