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Impact of gluconic fermentation of strawberry using acetic acid bacteria on amino acids and biogenic amines profile

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ABSTRACT

This paper studies the amino acid profile of beverages obtained through the fermentation of strawberry purée by a surface culture using three strains belonging to different acetic acid bacteria species (one of *Gluconobacter japonicus*, one of *Gluconobacter oxydans* and one of *Acetobacter malorum*). An HPLC–UV method involving diethyl ethoxymethylenemalonate (DEEMM) was adapted and validated. From the entire set of 21 amino acids, multiple linear regressions showed that glutamine, alanine, arginine, tryptophan, GABA and proline were significantly related to the fermentation process. Furthermore, linear discriminant analysis classified 100% of the samples correctly in accordance with the microorganism involved. *G. japonicus* consumed glucose most quickly and achieved the greatest decrease in amino acid concentration. None of the 8 biogenic amines were detected in the final products, which could serve as a safety guarantee for these strawberry gluconic fermentation beverages, in this regard.

1. Introduction

Strawberry (*Fragaria x ananassa* Duch.) is one of the most economically important fresh and processed fruits (Hancock, Sjulin, & Lobos, 2008) and a source of bioactives (Álvarez-Fernández, Hornedo-Ortega, Cerezo, Troncoso, & García-Parrilla, 2014a; Cerezo, Cuevas, Winterhalter, Garcia-Parrilla, & Troncoso, 2010; Stürtz, Cerezo, Cantos-Villar, & Garcia-Parrilla, 2011). Hence, there is a wide variety of processed strawberry products, such as purée, jams, juices, beverages, fruit preparations, etc. (Fügel, Carle, & Schieber, 2005; Hui et al., 2006). Recently, strawberry fermented products, such as wines and vinegars, have been produced as a good solution for using strawberry surpluses and as an alternative method for conserving this perishable fruit (Hidalgo, Torija, Mas, & Mateo, 2013; Ubeda et al., 2013).

Gluconic acid is abundantly available in grains, fruits and other foodstuffs, such as rice, meat, dairy products, honey and fermented products like wine and vinegar. It is a mild organic acid, which has applications in the food industry (Deppenmeier, Hoffmeister, & Prust, 2002; Ramachandran, Fontanille, Pandey, & Larroche, 2006; Singh & Kumar 2007). It is produced from glucose by different microorganisms, which include bacteria, yeast and some ectomycorrhizal fungus. Among them, some genera of the family *Acetobacteraceae*, such as *Gluconobacter*, are used industrially to produce gluconic acid (Deppenmeier & Ehrenreich, 2009; Ramachandran et al., 2006). There are several works that have studied gluconic acid fermentations. However, most of them are focused on biotechnology and its applications (Deppenmeier et al., 2002; Gupta, Singh, Qazi, & Kumar, 2001; Ramachandran, Fontanille, Pandey, & Larroche, 2006; Singh & Kumar, 2007) and reports focusing on the gluconic fermentation of fruits are scarce.

Acetic acid bacteria (AAB) can utilize a wide range of compounds as sources of nitrogen, from simple inorganic compounds to complex compounds, including amino acids (Merrick & Edwards, 1995). *Gluconobacter* strains are able to grow using ammonium ion as their sole source of nitrogen (Deppenmeier & Ehrenreich, 2009; Gupta et al., 2001). However, AAB have been shown to consume amino acids during the conversion of ethanol into acetic acid (Callejón, Troncoso, & Morales, 2010). Hence, free amino acids present in the medium could also be a good source of nitrogen for these bacteria, in addition to ammonium ions. The amino acid content of fruits and fruit derived products is studied since they contribute to the final aroma and taste, among other properties (Mandrioli, Mercolini, & Raggi, 2013).

Furthermore, some biogenic amines can be directly formed from amino acids by decarboxylation. These compounds can be formed and degraded during the normal metabolism of living organisms, although they have been quantified especially in fermented foods and beverages such as cheeses, dry fermented sausages or wine (Ancín-Azpilicueta, González-Marco, & Jiménez-Moreno, 2008; ten Brink, Damink, Joosten, & Huis In't Veld, 1990;

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Kirschbaum, Rebscher, & Brückner, 1999). High concentrations of biogenic amines in the final products could be due to poor quality raw materials, contamination, or to food processing and storage under unsuitable conditions (Önal, 2007; ten Brink, Damink, Joosten, & Huis In't Veld, 1990). Biogenic amines, in particular histamine and tyramine, can cause health problems when are present in foods in a high concentration (ten Brink, Damink, Joosten, & Huis In't Veld, 1990). These compounds could cause wide effects on consumer such as headache, inflammations, irritation, hypertension, and hypotension (Ancín-Azpilicueta et al., 2008; ten Brink, Damink, Joosten, & Huis In't Veld, 1990). The European legislation does not have a biogenic amines threshold, but the European Food Safety Authority (EFSA) has elaborated a scientific opinion on the risk associated with their formation in fermented products (European Food Safety Authority (EFSA) Panel on Biological Hazards (BIOHAZ), 2011).

Several techniques have been developed for analyzing amino acids and biogenic amines in foods (Callejón et al. 2010; Hernández-Orte, Ibarz, Cacho, & Ferreira, 2003; Önal, 2007; Peña-Gallego, Hernández-Orte, Cacho, & Ferreira, 2012). Nevertheless, the analytical technique most frequently employed for the determination of amino acids and biogenic amines is HPLC with C18 reverse-phase columns (Peña-Gallego et al., 2012). This method is less time-consuming than other techniques and the instrumentation used is simple (Hernández-Orte et al., 2003). The direct detection of amino acids by HPLC yields matrix interferences (Callejón et al., 2008) and biogenic amines do not have good absorption properties in the visible, ultraviolet or fluorescence wavelength ranges (Peña-Gallego, Hernández-Orte, Cacho, & Ferreira, 2009). For these reasons, the determination of these compounds requires a chemical derivatization to improve detection limits and to avoid matrix interference (Callejón et al., 2010; Gómez-Alonso, Hermosín-Gutiérrez, & García-Romero, 2007; Peña-Gallego et al., 2012). The reagents most widely used are 2,2-dihydroxy-1,3-indanedione (Ninhydrin), dansyl chloride (DnsCl), dabsyl Chloride (DbsCl), phenylisothiocyanate (PITC), o-phthaldialdehyde (OPA), 6-aminoquinolyl N-hydroxysuccinimidyl carbamate (AQC) and diethyl ethoxymethylenemalonate (DEEMM), among others (Callejón et al., 2010; Peña-Gallego et al., 2012). Some of these techniques have been able to determine amino acids and biogenic amines simultaneously, such as the method proposed by Gómez-Alonso et al. (2007), which used DEEMM as the derivatization agent to increase the specific absorbance of the analytes, followed by reverse phase HPLC and UV–vis photodiode array detection. This method has been proposed for wines and beers.

The aims of this study were: (a) to adapt an analytical method to determine the profile of amino acids, biogenic amines and ammonium ion in different gluconic acid fermented products and in the starting substrate (strawberry purée) by HPLC using DEEMM as the derivatization agent; (b) to study the differences in amino acid consumption by the different AAB strains employed; (c) to verify whether the fermented products can be discriminated or grouped according to the strain that performed the fermentation, taking the amino acid profiles as variables, and (d) to check whether these fermented beverages are safe for human consumption by determining the concentrations of biogenic amines.

2. Materials and methods

2.1. Reagents and standards

Most of the amino acid standards were purchased from Fluka (Buchs, Switzerland). The aspartic acid, glutamic acid, histidine, alanine, lysine, γ-aminobutiric acid (GABA), biogenic amines, ammonium sulphate, diethyl ethoxymethylenemalonate, acetic acid glacial, boric acid, 2-aminoadipic acid (internal standard) and sodium azide were supplied by Sigma–Aldrich (Steinheim, Germany). The glycine, ornithine, methanol (HPLC grade) and acetonitrile (HPLC grade) were acquired from Merck (Darmstadt, Germany). The sodium acetate and sodium hydroxide were obtained from Panreac (Castellar del Vallès, Barcelona). The ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

The stock standard solutions were prepared individually by dissolving the pure compounds in HCl 0.1 N. The calibration solutions were prepared by diluting the stock standard solutions with water.

2.2. Samples

We analyzed samples of the fermentation of strawberry purée using a surface cultures of acetic acid bacteria (AAB), supplied by HUDISA, S.A. (Lepe, Spain). These gluconic fermentations were conducted in the laboratories of the Biochemistry and Biotechnology Department (Facultat d'Enologia, Universitat Rovira i Virgili. Tarragona, Spain). These fermentations were carried out with different AAB strains: one of *Acetobacter malorum* (3 samples), one of *Gluconobacter oxydans* (3 samples) and one of *Gluconobacter japonicus* (2 samples). The initial substrate used for these processes was also studied. AAB were grown in a GY medium (1% yeast extract and 5% glucose) and incubated at 28 °C with stirring. The fermentation substrate consisted of mixing 90% strawberry purée with 10% rectified concentrated must (Concentrados Pallejà, Riudoms, Spain). For each fermentation, 500 mL of the substrate were inoculated with 2×10^6 cell/mL of the AAB strains in a 1 L Erlenmeyer flask. Fermentations were performed at 28 °C with a stirring speed of 128 rpm. In all cases the fermentations were considered finished after 10 days of fermentation, when more than 90% of the initial glucose had been consumed. Only *G. japonicus* strain practically exhausted glucose after the 10 days. All samples were frozen immediately after sampling. Table 1 displays the sample codes and their concentrations in glucose, fructose and gluconic acid.

2.3. Sample preparation

First, 2 mL of sample were centrifuged at 6000 rpm for 15 min (Eppendorf centrifuge 5415R, Hamburg, Germany). The

Table 1
Samples codes and glucose, fructose and gluconic acid concentration in strawberry purée and gluconic fermented.

Samples		Codex	Glucose (g/L)	Fructose (g/L)	Gluconic acid (g/L)
Strawberry purée		*SP*	62	62	–
Strawberry gluconic acid fermentation beverages (SGFB)	*Acetobacter malorum* (SGFAM)	*SGFAM1*	4.74	51.72	49.91
		SGFAM2	6.68	54.69	45.73
		SGFAM3	2.25	51.33	47.78
	Gluconobacter oxydans (SGFGO)	*SGFGO1*	4.74	56.96	51.95
		SGFGO2	4.12	50.47	46.16
		SGFGO3	2.57	54.22	49.48
	Gluconobacter japonicus (SGFGJ)	*SGFGJ1*	0.93	50.94	52.38
		SGFGJ2	0.78	47.49	47.95