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Characterisation of volatile compounds in an alcoholic beverage produced by whey fermentation

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ABSTRACT

An alcoholic beverage (35.4% v/v ethanol) was produced by distillation of the fermented broth obtained by continuous whey fermentation with a lactose-fermenting yeast *Kluyveromyces marxianus*. Forty volatile compounds were identified in this drink by gas chromatography. Higher alcohols were the most abundant group of volatile compounds present, with isoamyl, isobutyl, 1-propanol, and isopentyl alcohols being found in highest quantities (887, 542, 266, and 176 mg/l, respectively). Ethyl acetate had the highest concentration (138 mg/l) among the esters. Besides higher alcohols and esters, other components, including aldehydes, acids and terpenes were also identified in the whey spirit. Considering that the quality of an alcoholic beverage can be evaluated by the relation between isoamyl alcohol/2-methyl-1-propanol and 2-methyl-1-propanol/1-propanol, which have to be higher than unity, it was concluded that a novel spirit of acceptable organoleptic characteristics can be produced by cheese whey continuous fermentation with *K. marxianus*.

1. Introduction

Dairy industries generate significant liquid waste, of which, cheese whey is the most abundant. Whey, the liquid remaining after the precipitation and removal of milk casein during cheese-making, represents 85–95% of the milk volume (Siso, 1996) and its world production is estimated to be over 10^8 tons per year (Grba, Tomas, Stanzer, Vahcic, & Skrlin, 2002). Biological treatment of whey by conventional aerobic process is very expensive ≈ US$ 0.50/kg COD (chemical oxygen demand); (Ozmihci & Kargi, 2007) and most milk plants do not have proper treatment systems for its disposal. As a consequence, around 47% of the amount produced is disposed of in rivers or lakes, or loaded onto the land, causing serious pollution problems since whey is a heavy organic pollutant with high biochemical oxygen demand (40 g/l to 60 g/l) and COD (50 g/l to 80 g/l) (Athanasiadis, Paraskevopoulou, Blekas, & Kiosseoglou, 2004). When disposed of on land, it affects the physical and chemical structure of soil, decreasing crop yield; when released into water bodies, it reduces aquatic life by depleting the dissolved oxygen (Panesar, Kennedy, Gandhi, & Bunko, 2007).

Besides the environmental pollution aspect, dumping of whey constitutes a significant loss of potential food and energy, as whey retains about 55% of its total milk nutrients. The most abundant of these nutrients are lactose (45 g/l to 50 g/l), soluble proteins (6 g/l to 8 g/l), lipids (4 g/l to 5 g/l) and mineral salts (8–10% of dried extract). The mineral salts are comprised of NaCl and KCl (more than 50%), calcium salts (primarily phosphate) and others. Whey also contains appreciable quantities of lactic (0.5 g/l) and citric acids, non-protein nitrogen compounds (urea and uric acid) and B group vitamins (Panesar et al., 2007; Siso, 1996).

Availability of lactose and presence of other essential nutrients for the growth of microorganisms make whey a potential raw material for the production of different bio-products (Panesar et al., 2007). Recent research attempts have tried to develop technologies that employ whey as raw material to produce foods or chemicals of added value, and products such as single-cell proteins, lactic, citric and propionic acids, enzymes, glucose, methane, oligosaccharides, ethanol and others have been proposed (Athanasiadis et al., 2004; Djurić, Carić, Milanović, Tekić, & Panić, 2004; Koutinas et al., 2007).

The production of an alcoholic beverage by bioconversion of whey is an alternative of great interest for reuse of this industrial by-product. During the last two decades, various research efforts have been done on this theme, and yeasts like *Kluyveromyces fragilis* and *K. marxianus* have been proposed as suitable biocatalysts for this bioprocess (Koutinas et al., 2007). Although there are numerous literature reports about alcohol production from whey, most of them are based on the addition of fruit juices, such as mango, banana, pineapple, guava and strawberries, to whey (Kourkoutas et al., 2002). In addition, the scale-up of the process has been little explored and the development of a suitable large-scale procedure for effective utilisation of lactose is still necessary. Moreover,

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information regarding the volatile compounds presents in the distilled drink is scarce, since most of the studies are only concerned in increasing the ethanol yield during fermentation.

Distilled alcoholic beverages are characterised by the presence of volatile compounds (fusel alcohols, fatty acids, esters and others), which arise during fermentation, distillation and storage processes. Identification of these compounds is of major importance, not only to determine the flavor characteristics of the drink, but also to detect illicit spirits, and to identify anomalies that are indicative of inconsistent manufacturing practices (Fitzgerald, James, MacNamara, & Stack, 2000).

Based on these facts, the goal of the present study was to produce an alcoholic beverage by cheese whey fermentation in a large-scale reactor, using a continuous system, which is more advantageous than batch or fed-batch operations, since labour and cleaning costs are lower, equipment size is reduced; product quality is uniform, with high product yield in less time, increasing process productivity (Ozmihci & Kargi, 2007; Virkajärvi, Vainikka, Virtanen, & Home, 2002). The fermented broth was distilled and the volatile compounds present in the produced drink where identified. The organoleptic quality of this drink is discussed and compared with other alcoholic beverages.

2. Materials and methods

2.1. Cheese whey

Cheese whey (≈50 g/l lactose) obtained from a regional dairy industry (Quinta dos Ingleses, Caíde de Rei, Portugal), was centrifuged (2220 g for 20 min) to remove fines and cream, and pasteurised at 65 °C for 20 min, using a plate heat exchanger. The pasteurised and defatted cheese whey was applied to a 10,000 Da ultrafiltration unit to concentrate the proteins, giving a protein-rich fraction – the concentrate - and a low protein fraction – the permeate. The permeate, with a lactose concentration of 50 g/l, was kept at 4 °C in a holding tank before feeding to a 1000 l fermentation vessel.

2.2. Microorganism and cultivation conditions

The lactose-fermenting yeast strain *Kluyveromyces marxianus* ATCC 10022 was the microorganism used in the experiment. The yeast was maintained at 4 °C on slants containing a medium with the following composition: KH_2PO_4 5 g/l $(NH_4)_2SO_4$ 2 g/l, $MgSO_4 \cdot 7H_2O$ 0.4 g/l, yeast extract 1 g/l, and lactose 20 g/l.

2.3. Inoculum and continuous fermentation

For inoculum preparation, the yeast strain was transferred to 100 ml sterile whey in 500 ml Erlenmeyer flasks. The culture was grown overnight in a rotary shaker, at 30 °C and 120 rpm. In order to inoculate the 1000 l bioreactor, increasing volume inocula (inoculum volume was 1/5 to 1/10 of the volume of the inoculated vessel) were prepared in cheese whey, until a 100 l volume inoculum was obtained.

Continuous experiments were performed in a 1000 l stainless steel air-lift bioreactor with 700 l working volume. The bioreactor was filled with pasteurised and deproteinised cheese whey and inoculated with the above mentioned 100 l volume inocula and operated batchwise until residual sugar concentration was negligible. Continuous operation was started by feeding fresh pasteurised and deproteinised whey to the bottom of the reactor with a dilution rate of 0.2 h^{-1}. Pasteurized and deproteinized whey was kept at 4 °C to avoid any decomposition and was fed to the reactor under aseptic conditions using a centrifuge pump. During the experiment, the temperature was maintained at 30 °C and the pH was controlled at 4.0 by automatic addition of H_3PO_4. The system was aerated with filtered air at a rate of 0.1 vvm. The system was kept operating for two months.

2.4. Distillation

The fermented broth distillation was carried out in a stainless steel pot still of 500 l (Henrique Vieira & Filhos Lda, Aveiro, Portugal). The fermented broth was transferred to the vessel up to 3/4 of its capacity in order to be distilled; the distilled product, having an alcoholic grade of approximately 50%, was poured into glass bottles.

2.5. Analytical methods

Ethanol was quantified by high-performance liquid chromatography (HPLC), using a Jasco chromatograph equipped with a refractive index (RI) detector (Jasco 830-RI) and a Chrompack column (300 × 6.5 mm) at 80 °C, using 5 mM sulfuric acid as the eluent, at a flow rate of 0.3 ml/min and a sample volume of 20 μl.

Minor volatile constituents of the whey spirit were determined by extraction with dichloromethane and subsequent analysis of the extracts by gas chromatography–mass spectrometry (GC–MS) using a Varian 3400 chromatograph and an ion-trap mass spectrometer (Varian Saturn II). Samples of 1 μl were injected in a capillary column coated with CP-Wax 52 CB (50 m × 0.25 mm i.d., 0.2 μm film thickness; Chrompack). The temperature of the injector (SPI – septum-equipped programmable temperature) was programmed from 20 °C to 250 °C, at 180 °C/min. The oven temperature was held at 60 °C, for 5 min, then programmed to rise from 60 °C to 250 °C, at 3 °C/min, then held for 20 min at 250 °C and finally programmed to go from 250 °C to 255 °C at 1 °C/min. Helium at 103 kPa was used as carrier gas. The detector was set to electronic impact mode (70 eV), with an acquisition range from *m*/*z* 29 to *m*/*z* 360, and an acquisition rate of 610 ms per scan. Identification of volatiles was performed using the software Saturn version 5.2 (Varian), by comparing mass spectra and retention indices with those of pure standard compounds. All the compounds were quantified as 4-nonanol equivalents.

Major volatile constituents of the whey spirit were analysed directly, without any previous treatment, on a Chrompack CP-9000 gas chromatograph equipped with a Split/Splitless injector and a flame ionization detector (FID). A capillary column, coated with CP-Wax 57 CB (50 m × 0.25 mm i.d., 0.2 μm film thickness; Chrompack), was used. The temperature of the injector and detector were both set to 250 °C. The oven temperature was held at 60 °C, for 5 min, then programmed to rise from 60 °C to 220 °C, at 3 °C/min, and finally held at 10 min at 220 °C. Helium at 103 kPa was used as carrier gas, and the split vent was set to 13 ml/min. Before injection of 1 μl in the splitless mode (for 15 s), 4-nonanol was added to the sample (internal standard) to give a final concentration of 213.6 mg/l. Quantification of volatiles, as 4-nonanol equivalents, was performed with CP-Maître software version 2.5 (Chrompack), by comparing retention indices with those of pure standard compounds.

3. Results and discussion

It is known that alcoholic fermentation leads to a series of by-products in addition to ethanol, including carbonyl compounds, alcohols, esters, acids and acetals, all of them influencing the quality of the finished product. The composition and concentration levels of the by-products can vary widely. Some compounds appear in high concentrations (hundreds of mg/l), while a large