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Metabolite profiling and volatiles of pineapple wine and vinegar obtained from pineapple waste

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ABSTRACT

Vinegar is an inexpensive commodity, and economic considerations require that a relatively low-cost raw material be used for its production. An investigation into the use of a new, alternative substrate – pineapple waste – is described. This approach enables the utilization of the pineapple's (*Ananas comosus*) peels and core, which are usually discarded during the processing or consumption of the fruit. Using physical and enzymatic treatments, the waste was saccharified, and the resulting substrate was fermented with *Saccharomyces cerevisiae* for 7–10 days under aerobic conditions at 25 °C. This resulted in an alcohol yield of approximately 7%. The alcoholic medium was then used as a seed broth for acetic fermentation using *Acetobacter aceti* as the inoculum for approximately 30 days at 32 °C to obtain 5% acetic acid. Samples were analyzed at the beginning and end of the acetification cycle to assess the volatile and fixed compounds by GC–MS and UHPLC-QTOF-MS. The metabolomic analysis indicated that L-lysine, mellein, and gallic acid were significantly more concentrated in the pineapple vinegar than in the original wine. Higher alcohols, aldehydes, and ketones characterized the aroma of the final pineapple vinegar, whilst off-flavors were significantly reduced relative to the initial wine.

This study is the first to highlight the metabolite profile of fruit vinegar with a slight floral aroma profile derived from pineapple waste. The potential to efficiently reduce the post-harvest losses of pineapple fruits by re-using them for products with added food values is also demonstrated.

1. Introduction

The high demand of wine and vinegar, as subsequent derived product, has provided an opportunity for value-added product development. Nowadays, the wine market is turning towards diversification by means of the development of original, novel, and enriched new products through innovative formulas, technologies, and alternative raw materials. Simultaneously, in recent years the problem of food waste has attracted considerable interest from food producers, processors, retailers, and consumers alike. Food waste is considered not only a sustainability problem related to food security, but also an economic problem since it directly impacts the profitability of the whole food supply chain. Therefore, to effectively manage food waste, there needs to be awareness of the benefits of postharvest waste utilization by farmers, food processors, and government agencies (Ghosh, Fawcett, Sharma, & Poinern, 2016).

Vinegar production is low in costs, because inexpensive raw materials such as by-products from food processing, fruit waste, substandard fruit and agricultural surpluses are used generally (Solieri & Giudici, 2009). The waste may contain valuable substances such as pigments, sugars, organic acids, flavors, and bioactive compounds such as antioxidants, enzymes, antimicrobial compounds, and fibers that could be thus applied to bioprocesses in order to generate products with higher added value (Ghosh et al., 2016). Confirming this, fruit or vegetable vinegars with distinctive sensory characteristics have appeared in the marketplace and consumed in large amounts (Shahidi, McDonald, Chandrasekara, & Zhong, 2008). Different raw materials, pepper leaves, strawberry, persimmon, blueberry, and sweet potato have been successfully applied to produce vinegars (Cejudo-Bastante, Durán-Guerrero, Natera-Marín, Castro-Mejías, & García-Barroso, 2013), and physiological and biochemical properties were demonstrated (Solieri & Giudici, 2009). These effects of vinegar might be due to bioactive substances such as amino acids, organic acids or

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phenolic compounds derived from its raw materials. Moreover, these compounds can be produced and/or increased as a result of overall vinegar fermentation process (Solieri & Giudici, 2009), where phenolic compounds are transformed into new antioxidative molecules (Shahidi et al., 2008). Additionally, vinegar owes much of its sensory character to its aroma, being one of the most crucial criteria impacting on consumer acceptance and quality, and influenced by the raw materials used, the compounds formed during the fermentation process, and the fermentation type used (Morales, Tesfaye, Garcia-Parrilla, Casas, & Troncoso, 2002; Ubeda et al., 2012).

In the context of metabolomics-based approaches, recent research has shown that, in addition to its well-known antibacterial and cleaning agent activity, vinegar confers considerable health benefits and medical remedy (Shimoji et al., 2002). Actually, the combination of more traditional analytical approaches with metabolomics has provided the tools needed to assess the multiple aspects, such as food composition, aroma, flavor, and food properties that have a substantial effect on food quality and consumers acceptance (Castro-Puyana & Herrero, 2013).

In this paper, we examine how pineapple waste, a relatively low-cost material, can productively be used as input raw material for a prepared food product, such as vinegar. The aim of this study was to investigate whether this process was suitable for vinegar production and to profile chemical compounds in the wine and vinegar to obtain information regarding the vinegar's quality.

2. Materials and methods

2.1. Pineapple waste samples

Pineapples, *Ananas comosus* (L.) Merr. (*Bromeliaceae*), were purchased from the supermarket in Italy. Each pineapple was maintained at 22 ± 2 °C prior to experimentation. After being washed, the waste (peel and core) was separated from the edible pulp and the crown. To produce a homogeneous mixture, the pineapple waste was arranged and crushed (Roda, De Faveri, Giacosa, Dordoni, & Lambri, 2016). Pineapple waste samples (500 g) were stored in a freezer (−18 °C) prior to use. The initial sugar content was approximately 22 g per kg of fresh weight (g/kg_{fw}) in the pineapple waste samples (Roda, De Faveri, Dordoni, & Lambri, 2014).

2.2. Preparation of pineapple must

Twenty samples of pineapple waste (500 g) were added to distilled water at 1:2 ratios and placed into 1000-mL Pyrex bottles, which were sealed with screw caps. The samples were then subjected to high-pressure physical pre-treatment in an autoclave (ATV211, Chimicacentro, Recanati, MC, Italy) at 121 °C and 205.24 kPa for 15 min (Roda et al., 2016). At the end of the pre-treatment, the samples were cooled to 50 ± 2 °C and subjected to enzymatic hydrolysis (Roda et al., 2016), using cellulase (from *Aspergillus niger*, 0.8 enzyme units/mg solid, Sigma C1184-25KU, Sigma-Aldrich, St. Louis, MO, USA), hemicellulase (from *Aspergillus niger*, 1.5 enzyme units/mg solid, using a β-galactose dehydrogenase system and the locust bean gum as a substrate, Sigma H2125150KU, Sigma-Aldrich, St. Louis, MO, USA), and pectinase (>200 enzyme units PL/g, EnartisZym Quick, Enartis, Novara, Italy). For sucrose hydrolysis, invertase (enzyme activity >300 U/mg) from Baker's yeast, *Saccharomyces cerevisiae*, was procured from Sigma-Aldrich (USA). The liquid fraction (the pineapple must) was separated from the residual solid waste by manual pressing.

An optimal total soluble solid level in pineapple waste juice is required to produce vinegar with a high acetic degree. After enzymatic hydrolysis, the liquid fraction was concentrated up to 13 °Brix under a vacuum (−80 kPa) at 56 °C using a rotavapor (Buchi Rotavapor R-3, Labortechnik AG, Switzerland) prior to alcoholic fermentation (Roda et al., in press).

2.3. Processing for the production of pineapple vinegar

This process was based on two consecutive fermentation steps that occurred after saccharification (Fig. 1). The first step was an alcoholic fermentation at 25 °C using a yeast strain, and the second step was an acetic fermentation at 32 °C using an acetic bacterial strain.

2.4. Alcoholic fermentation of the pineapple must

The yeast culture used for alcoholic fermentation was purchased by Esseco srl (San Martino Trecate, NO – Italy). The active dry yeast strain of *Saccharomyces cerevisiae* EnartisFerm TT was rehydrated in a sanitized beaker at a 1:10 ratio with water and at a temperature of approximately 40 ± 5 °C. After hydration, the aqueous dispersion was allowed to stand for 10 min. The agitation was resumed, and hydration continued for another 10 min.

Triplicate samples of 200 mL of the pineapple must were placed into 500-mL sterile Erlenmeyer conical flasks, plugged with cotton wool and inoculated with the rehydrated yeast using initial *Saccharomyces cerevisiae* inoculums of 1.45×10^7 cell/mL (corresponding to a 0.25 g/L dose). The inoculated samples were supplemented with 0.4 g/L of nutrients. A Supervit fermentation activator (Esseco Srl, San Martino Trecate, NO – Italy) that contained ammonium sulfate (61.8%), ammonium phosphate dibasic (33%), E501 potassium bicarbonate (5%), and thiamine hydrochloride (vitamin B1) (0.2%) were used along with an amino regulator of fermentation (C.E.A.V.A. Srl, Castel San Giovanni, PC – Italy) that contained pure cell walls with a high levostimoline content and condensed mannoproteins. The mixtures were incubated at 25 °C for 72–96 h.

2.5. Acetic fermentation of the pineapple wine

The acetic bacterial culture that was used for acetic fermentation was supplied by Microbiologics Inc., (200 Cooper Avenue North, St. Cloud, Minnesota, USA, 56303) as a lyophilized pellet of *Acetobacter aceti* (0511 K, ATCC® 15973™* KWIK-STIK). A single QC microorganism *Acetobacter aceti* strain in a lyophilized pellet was rehydrated in the hydrating fluid reservoir, inoculated onto a primary culture plate containing nonselective agar medium (TSA) using a sterile swab, and incubated at 26 °C for 48 h. The *Acetobacter aceti* was subsequently inoculated onto a secondary culture plate, containing Chocolate Agar Base as the maintenance medium, and incubated at 35 °C; growth was observed within 48 to 72 h.

After the incubation, 25 mL of the broth containing 10^6 cfu/mL was collected for use as the inoculum for acetic fermentation. Fermentation was commenced by adding 100 mL of the previously fermented juice to the 25-mL acetic bacterial suspension of 10^6 cfu/mL. The mixture was incubated at 32 °C for 30 days.

2.6. Analysis of ethanol, acetic acid, acetaldehyde, and pH

Ethanol was measured in wine according to OIV (2010) and in vinegar by using enzymatic procedures (Ethanol Assay Kit – MAK076, Sigma-Aldrich, St. Louis, MO, USA). The acetic acid content was determined by titrating 1 mL of the sample with 0.1 N NaOH, using phenolphthalein as an indicator. The acidity of the vinegar was expressed as the degree of acetic acid and reported as the mass (g) of pure acid per 100 g of vinegar (Lotong, Malapan, & Boongorsrang, 1989). Acetaldehyde was measured both in the wine and in the vinegar using the Acetaldehyde Assay