Food Research International 87 (2016) 161–167

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Formation of volatile compounds during cupuassu fermentation: Influence of pulp concentration

Simone de Nazaré Melo Ramos [a], Wolfgang Danzl [b], Gottfried Ziegleder [b], Priscilla Efraim [a,*]

[a] Faculty of Food Engineering, University of Campinas, Rua Monteiro Lobato, 80, Cidade Universitária Zeferino Vaz, Campinas, SP, Brazil. CEP, 13.083-862
[b] Fraunhofer Institute for Process Engineering and Packaging IVV, Giggenhauser Strasse 35, 85354 Freising, Germany

ARTICLE INFO

Article history:
Received 27 October 2015
Received in revised form 18 June 2016
Accepted 28 June 2016
Available online 29 June 2016

Keywords:
Cupuassu
Fermentation
Pulp
Cupulate
Volatile compounds

ABSTRACT

Cupuassu (*Theobroma grandiflorum* Schum) is a native fruit of the Amazon region and from its seeds fermentation, it is possible to obtain a product similar to chocolate, known as cupulate. The aim of this study was to evaluate the influence of the pulp concentration on the formation of volatile compounds during fermentation. Considering the high quantity of pulp that naturally involves the seeds (38%), which represents an obstacle for fermentation, two different fermentation experiments were conducted: with seeds totally (0%) and partially (15%) depulped. Seeds collected during and after fermentation, after drying (beans), after roasting and deshelling (nibs) and cupulates (obtained using the same methods of chocolate processing) were analyzed through identification and relative quantification by GC–MS in order to check the profile of volatile compounds formed. Results showed that the depulping implies in considerable reduction of important volatile compounds. A wider diversity of volatile compounds such as aldehydes, ketones and alcohols were found in the experiment with 15% pulp during fermentation. Tetramethylpyrazine was the unique pyrazine found in the cupulate samples that had significant difference ($p < 0.05$) among the samples in its concentration. Among the others compounds identified, it is important to emphasize the presence of compounds with fruity and floral notes, generally present in fine/flavor cocoa. This study demonstrated the importance of the pulp in the development of important compounds responsible for the desirable flavor.

1. Introduction

Cupuassu (*Theobroma grandiflorum* Schum) is an important native fruit of the Amazon forest found in many countries (Brazil, Venezuela, Ecuador, Costa Rica, Colombia, Guyana, Martinique, Costa Rica, Trinidad, Ghana, Sao Tome, Florida and Australia) (Venturieri, 1993; Martini & Tavares, 2005). The fruits have various shapes (oblong, oval, elliptical, obovoid or round), weighing between 200 g to 4000 g. Each fruit contains on average 38% of seeds (15–50 per fruit) involved by a mucilaginous pulp, which is the most abundant part of the fruit (39 to 43%) and the most important for commercial exploitation (Souza & Sousa, 2002).

The seeds have high quantity of fat, with an average content of 64.85% (Carvalho, García, & Wada, 2005) widely used in the cosmetic industry (Gondim, Thomazini, Cavalcante, & Souza, 2001). The seeds fermentation provides a product similar to chocolate, called "cupulate" (Cohen, Sousa, & Jackix, 2009). During this process, as occurs for example with cocoa, important chemical reactions are mediated by microorganisms that metabolize sugars and produce ethanol, a substrate used by acetic acid bacteria (AAB) for the production of acetic acid (Schwan & Wheals, 2004; Schwan, 1998). Citric acid is metabolized into lactic acid by lactic acid bacteria (LAB), which can be a negative factor by causing non-volatile acidity in the final product (Schwan & Wheals, 2004). However, selected LAB may be responsible for production of important compounds such as 3-methylbutanal (Ayad, Verheul, Engels, Wouters, & Smit, 2001), a substance with strong chocolate notes. This molecule is produced through transamination reaction of leucine followed by a decarboxylation step in the Strecker reaction (Counet, Callemien, Ouwerx, & Collin, 2002).

The seeds contain a wide range of essential and nonessential amino acids. When the seeds are fermented and roasted they generally increase the concentration of the hydrophobic amino acids (alanine, tyrosine, leucine, and phenylalanine) (Mattietto, 2001). The latter together with reducing sugars are transformed into important flavor compounds through non-enzymatic reactions (Maillard reaction) (Rizzi & Bunke, 1998; Biehl & Ziegleder, 2003).

After the fermentation, the beans are dried, which is an important step that also impacts the quality of the final product since metabolic reactions that contribute to the formation of flavor still occur (Hii, Law, Suzannah, Misnawi, & Cloke, 2009). Later, the beans are roasted in order to form flavor compounds, to eliminate the volatile compounds that give acidity, bitterness and astringency of the product and facilitate

* Corresponding author.
E-mail address: efraim@fea.unicamp.br (P. Efraim).

http://dx.doi.org/10.1016/j.foodres.2016.06.025
0963-9969/© 2016 Elsevier Ltd. All rights reserved.

shell detachment (Afoakwa, Paterson, Fowler, & Ryan, 2008). In this step, the condensation of carbonyl groups occurs with free α-amino acids (Strecker reaction) resulting in the formation of aldehydes and other important flavor compounds to produce chocolate, such as pyrazines, pyridines, pyrrols, and oxazoles (Counet et al., 2002; Martins, Jongen, & Boekel, 2001; Oberparleiter & Ziegleder, 1997; Rizzi, 2008; Ziegleder, 2009).

The processing of obtaining cupulate is similar to that used for chocolate (Afoakwa et al., 2008; Schwan & Wheals, 2004) and include mixing, refining, conching, tempering, moulding, demoulding and packaging. During the conching step other chemical reactions occur resulting in the formation of important flavor compounds (Counet et al., 2002). However, the main aroma improvement during conching seems to arise from a change in the flavor distribution within the chocolate matrix, because sugar surfaces are increasingly coated by flavor substances and lipids (Danzl & Ziegleder, 2014; Ziegleder, Balimann, Mikle, & Zaki, 2003). Following, tempering is performed to form stable crystals that enhance the product uniformity (Cohen, Luccas, & Jackix, 2004).

Regarding cocoa seeds, their chemical compounds are not only formed during fermentation but are also originated from the pulp (Kadow, Bohlmann, Phillips, & Lieberei, 2013), being demonstrated that compounds related to fruity and floral aromas in the products from cocoa beans were originated in the pulp. These compounds are present in fine chocolate and absent in bulk chocolate (Sukha, Butler, Umaharan, & Boult, 2008). These findings support the role of pulp on the formation of flavor compounds. Alcohols such as linalool, 3-methyl-2-buten-1-ol, 3-methylbutanol, and ethanol have been found in cupuassu (Quijano & Pino, 2007). Most of these are important flavor compounds identified in cocoa beans extracts (Ziegleder, 1991).

Although in the composition of the cupuassu pulp there are flavor-essential compounds, depulping is a normal practice otherwise the fermentation would not occur. The abundance of pulp becomes a hindrance, since the absence of liquefaction prevents the environment aeration. As a result, the development of acetic acid bacteria is hindered and temperature increase doesn't occur. The latter is important to the loss of seed germination capacity and increase of cell wall permeability, for producing flavor precursors compounds, as occurs with cocoa (Fowler, 2009).

Despite the use of cupuassu for cupulate production being incipient, previous findings of sensory analysis showed that it could be a potential product because its satisfactory acceptance (Cohen et al., 2004; Lopes, Pezoa-García, & Amaya-Farfán, 2008; Nazaré, Barbosa, & Viêgas, 1990). Thus, the aim of this study was to determine the profile of the volatile compounds formed during the fermentation in totally depulped (0% of pulp) and partially depulped (15% of pulp) cupuassu seeds, also in dried beans, roasted beans (nibs) and cupulates. Besides identifying important compounds, the results will show if the total depulping can impact the final flavor negatively.

2. Material and methods

2.1. Cupuassu seeds fermentation

In order to establish the pulp concentrations that would be studied, preliminary experiments of fermentation with 0, 20, 30, 40 and 100% of pulp in 4 kg batches were conducted (Ramos, 2015). The experiment with 20% of pulp presented the best results (maximum temperature of the mass temperature: up to 38 °C), thus demonstrating that this pulp concentration would be already high, due to the delay in the increase of mass temperature even after five days of fermentation. Therefore, fermentation with 7.5 and 15% were conducted and the latter showed satisfactory results in the sensory analysis (data not shown).

Approximately 1 t of ripe and healthy fruits were collected in a single day or up to three days after being dropped at the PERI farm, in the city of Presidente Figueiredo, Amazonas state - Brazil. Then, they were completely and partially depulped to obtain concentration with 0 and 15% of pulp, respectively.

Polystyrene boxes were used to perform the fermentation, triplicated in 8 kg batches each. Generally, seed fermentation is carried out in wooden boxes. However, polystyrene boxes were used to maintain the elevated temperature considering the small amount of sample used in each experiment. The periods of fermentation for the experiments with 0% pulp and 15% pulp (of 60 and 108 h respectively) were determined by the temperature drop and increase of the mass pH (>7.0) for both experiments and respective triplicate (data not showed). These events determined stopping the fermentation to prevent the formation of undesirable compounds.

2.2. Drying

Due to the rainy weather in the period, the beans were naturally dried (solar) during 24 h and artificially dried (oven with air circulation MARCONI MA 035/5/10P São Paulo, BRASIL at 40 °C) for 5–7 days until they reached 6–8% moisture. In Brazil, for cocoa and cupuassu fermentation is common the combination of natural (solar) and artificial (mechanical dryers) for drying because of the high rainfall and relative humidity. Studies demonstrated that mixed drying methods (natural and artificial) or only natural are recommended for the production of raw material of good chemical quality (Zahouli, Guehi, Fae, & Nemlin, 2010).

2.3. Nibs and cupulates processing

The results of physical and chemical analysis performed with dried beans demonstrated that there no difference between their triplicates, neither during fermentation and nor drying (data not shown). Therefore, the triplicates were pooled into one. The fermented and dried beans were cleaned, classified and roasted at 120 °C/120 min in 4.0 kg batches in a rotary electric oven (JAF INOX São Roque, Brazil) and then broken to obtain the nibs in a compact equipment (prototype test/JAF INOX, Brazil). The nibs were ground in a blender, homogenized in planetary mixer (Kitchen Aid - Artisan Model 5KSM150, EUA) and then refined in a three cylinders refiner (PILLON, Brazil), internally cooled with water at 15 °C to obtain powder of particle size smaller than 26 μm.

The cupulates were produced according to the following formulation: 48.6% cupuassu mass, 50% of refined sugar (Glaçucar Union), 1.4% cupuassu butter (extracted from other cupuassu mass through hydraulic press), 0.4% soya lecithin (CH Solec - the Solae Company) and 0.4% polyglycerol polyricinoleate (Grindsted Super - Danisco). The conching step was performed in a jacketed mixer (INCO, Brazil) at 70 °C for 12 h. The tempering was performed manually in granite surface in a controlled temperature room (20.0 ± 1.0 °C). Then, the samples were placed in PVC bar molds, subjected to cooling and demolding according to Cohen et al. (2004).

2.4. Analysis of volatile compounds

The extraction of volatile compounds in seeds (collected during fermentation), dried beans, nibs and cupulates was performed in duplicate according to Ziegleder et al. (2003) and Ziegleder and Biehl (1988).

2.4.1. Sample preparation and fractioned steam distillation

The samples were initially ground in a mortar and then in a mini processor. In a distillation tube, 20 g of pulverized sample were weighed and 20 mL of distilled water were added and then homogenized. The tube was attached to a distillation apparatus (Antona apparatus, Greiner & Gassner, Munich). Fig. 1 shows the schematic model explaining the principle of extraction of MHE (Multiple Headspace Extraction) of volatile compounds in a fractional form.