Roasted jackfruit seed as a potential substitute for chocolate aroma: obtainment, composition, olfactometry, and application

Fernanda Papa Spada

Thesis presented to obtain the degree of Doctor in Science. Area: Food Science and Technology

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This work is dedicated to my mother, Regina Célia for the dedication and love.
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Epigraph: Money makes rich men, knowledge is wise men and humility makes great men.

(Mahatma Gandhi)
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RESUMO

Semente de jaca torrada como um substitute potencial do aroma de chocolate: obtenção, composição, olfatometria e aplicação

As sementes de jaca são subaproveitadas em muitos países tropicais. Neste estudo, foi possível demonstrar que as sementes de jaca torrada possuem potencial para produzir aroma de chocolate e podem ser utilizada como um substituto do cacau em alimentos processados. Vinte e sete farinhas foram produzidas com a semente de jaca da variedade dura através de secagem (DJS), acidificação (AJS), ou fermentação (FJS) as sementes foram submetidas sob diferentes combinações de tempo e temperatura de torra. O aroma de chocolate quanto aos grupos de farinha foram avaliados sensorialmente por painel (n=162) e a metodologia superfície de resposta foi utilizada para identificar a melhor condição para produzir aroma de chocolate. As pirazinas foram instrumentalmente analisadas como marcadores do aroma de chocolate. Assim como, umidade, pH e cor também foram monitorados. O melhor aroma de chocolate foi produzido para as três farinhas de semente de jaca: DJS, AJS e FJS. Compostos voláteis e semi-voláteis foram avaliados utilizando GC-MS; GC-O e SPE-GC nesses três tratamentos as farinhas de sementes de jaca seus perfis foram comparados com o perfil do cacau em pó. Essas farinhas foram também avaliadas quanto sua solubilidade, molhabilidade, densidade aparente, viscosidade, preferência sensorial e intensidade do aroma de chocolate. O delineamento com ponto central foi utilizado para otimizar a solubilidade e o poder de absorção. As variáveis respostas foram temperatura da água e tempo de exposição da farinha. Devido a sua composição diferenciada quanto aos compostos voláteis, duas diferentes farinhas (DJS e FJS) foram aplicadas em seis formulações de cappuccino com 50, 75 e 100% de substituição do pó de cacau por farinha de semente de jaca. A aceitação dos cappuccinos pelos consumidores (n=126) e a análise quantitativa descritiva (ADQ) foram utilizadas para descrever as preparações. Propriedades físico-químicas das formulações de cappuccino também foram avaliadas. A maior concentração relativa de pirazinas foi formada em farinhas seca, acidificada e fermentada quando utilizado 156, 165 e 154°C, respectivamente. Claramente a fermentação é necessária para melhorar o aroma de chocolate da farinha de semente de jaca, foi possível selecionar a melhor condição de torração para cada tratamento quanto à percepção sensorial do aroma de chocolate. Essas condições ótimas foram encontradas como 171°C para 47 minutos para farinha seca; 180°C durante 40 minutos para as sementes acidificadas e 154°C durante 35 minutos para as farinhas fermentadas com alta solubilidade e molhabilidade em comparação com as demais farinhas. A viscosidade da farinha de semente de jaca foi baixa com alta solubilidade o que é desejável para o cacau em pó (CP). O aroma de chocolate foi mais intenso para FJS. Assim, as farinhas de semente de jaca tiveram propriedades tecnológicas e aroma de chocolate similar ou melhor que CP e de chocolate comercial (CC). Para as formulações de cappuccino 50 e 75% de pó de cacau foram substituídas por farinha de semente de jaca seca, e não houve mudança na aceitabilidade sensorial e nas propriedades tecnológicas A análise de componentes principais para ADQ explicou 90% da variância. A principal característica dos cappuccinos feitos com semente de jaca seca quanto ao aroma foram cappuccino, chocolate, canela e café e cappuccino e chocolate para o sabor. Assim, a farinha de semente de jaca seca é um substituto inovador do pó de cacau, este pode ser utilizado em
preparações alimentícias, consequentemente este resíduo agroindustrial pode ser incorporado como ingrediente comum na dieta humana.

Palavras-chave: Resíduo agroindustrial; Subproduto; Semente de jaca; GCO; Compostos voláteis; Desenvolvimento de produtos; Ingrediente
ABSTRACT

Roasted jackfruit seed as a potential substitute for chocolate aroma: obtainment, composition, olfactometry, and application

Jackfruit seeds are an under-utilized waste in many tropical countries. In this work, we demonstrated the potential of roasted jackfruit seeds to generate chocolate aroma for use as a cocoa substitute in foodstuffs. Twenty-seven different flours were produced from a hard pulp variety of jackfruit by drying (DJS), acidifying (AJS), or fermenting (FJS) the seeds prior to roasting under different time/temperature combinations. The chocolate aroma of groups of four flours were ranked by a sensory panel (n=162) and response surface methodology was used to identify optimum conditions for producing chocolate aroma. Pyrazines were analyzed instrumentally as markers of chocolate aroma, while moisture, pH, and color were also monitored. The best chocolate aroma was produced in three jackfruit seed flours: DJS, AJS, and FJS. Volatile and semi-volatile compound contents were evaluated by GC-MS, GC-O, and SPE-GC in these three jackfruit seed flours and their profiles were compared with the profile of cocoa powder. These flours were also evaluated for their solubility, swelling power, wettability, apparent density, viscosity, sensory preference, and intensity of chocolate aroma. The central composite design was used to optimize the solubility and swelling power. Water temperature and time to flour exposition were the response variables. Owing to their differing volatile compositions, two different flours (DJS and FJS) were applied in six cappuccino formulations with 50%, 75%, and 100% substitution of cocoa powder with jackfruit seed flours. The consumers acceptance of cappuccinos (n=126) and the quantitative descriptive analysis (QDA) were used to describe the preparations. Physicochemical properties in cappuccino formulations were also evaluated. The greatest relative concentration of pyrazines (p≤0.05) was formed in dry, acidified, and fermented flour when we used 156, 165, and 154°C, respectively. Clearly, fermentation is necessary to improve the chocolate aroma of jackfruit seeds, and it is possible to select the best roasting conditions for each treatment to optimize the sensory perception of chocolate aroma. These optimal treatment conditions were found to be 171°C for 47 min in DJS, 180°C for 40 min in AJS, and 154°C for 35 min in FJS. FJS had higher solubility and wettability than other flours. The viscosities of jackfruit seed flours were low with high solubility, properties that are desirable in cocoa powder (CP). Chocolate aroma was most intense for FJS. Therefore, jackfruit seed flours have technological properties and chocolate aroma similar to or better than CP and commercial chocolate (CC). For cappuccino formulations, 50% and 75% cocoa powder was replaced with dry jackfruit seed flour, and there was no change in sensory acceptability or technological properties. The principal component analysis of QDA explained 90% of variance. The primary characteristics of cappuccinos made with dry jackfruit seeds were cappuccino, chocolate, cinnamon, and coffee aromas, and cappuccino and chocolate tastes. Indeed, dry jackfruit seed flour is an innovative cocoa powder substitute; it could be used in food preparations, consequently utilizing this tropical fruit waste by incorporating it as an ingredient in a common product of the human diet.
Keywords: Agroindustrial waste; By-product; Jackfruit seeds; GCO; Volatile compounds; Product and development; Ingredient
1. INTRODUCTION

Jackfruit (Artocarpus heterophyllus Lam.) is a large tropical fruit (syncarp) which is abundant in South America, Asia, Africa, and Australia, and it is divided into two major variety groups: soft and hard pulp. Jackfruit is a fleshy compound fruit belonging to the Moraceae family. The fruit weight ranges from 2–36 kg and takes 3–6 months to reach maturity. Seeds represent 18–25% (db) of the fruit weight (Mahanta & Kalita, 2015). Each seed is covered with a golden-yellow fleshy aril and has a strong, sweet taste (Saxena, Bawa, & Raju, 2011). These seeds have high carbohydrate content 77%(db) and 0.7–2.2% lipid (Ocloo, Bansa, Boatin, Adom, & Agbemavor, 2010; Singh, Kumar, & Singh, 1991). Generally, the seeds are boiled, steamed, and roasted before eating, providing a cheap source of fiber (2% and 26% db for soluble and insoluble dietary fiber, respectively), protein, and minerals such as potassium, calcium, and sodium (Mahanta & Kalita, 2015; Ayala-Zavala et al., 2011). However, the majority of jackfruit seeds are thrown out as agroindustrial waste (John & Narasimham, 1993; Kee & Saw, 2010). In recent years, jackfruit seeds have gained the attention of researchers as an alternative source of starch and protein that can be industrially exploited (Madrigal-Aldana et al., 2011; Madruga et al., 2014; Saxena et al., 2011). However, the jackfruit is still underuse owing to seasonality, difficulty in logistics and conservation, and low consumption due to high intensity of taste and aroma, in addition to the association of jackfruit with poor communities. Thus, jackfruit is seldom added to other products. Jackfruit can develop an aroma similar to the aroma of cocoa upon fermentation.

Fermentation is an indispensable process for obtaining the sensorial characteristics of chocolate, since unfermented cocoa beans have no chocolate aroma. Briefly, cocoa fermentation increases the production of organic acids that are able to permeate the cell membranes of seeds, facilitating specific reactions. Pyrazines have been shown to contribute significantly to the unique flavor of roasted and toasted foods (Seitz, 1994) and are used to determine the quantity and quality of cocoa flavor (Farah, Zaibunnisa, & Misnawi, 2012). These compounds impart chocolate, cocoa, hazelnut, roasted, coffee, earth, and green aromas (Gu et al., 2013; Tran et al., 2015). However, as with cocoa, post-harvest treatments are likely to influence the quality of the aroma. All three stages of processing (fermentation, drying, and roasting) can influence the final pyrazine concentration. These products of
fermentation (amino acids and reducing sugars) are the precursors of pyrazines, which are formed during roasting from the Maillard reaction and are responsible for producing the characteristic chocolate aroma (Dimick & Hoskin, 1999; Siegmund, 2015).

In addition, roasting jackfruit seeds produced changes in their aroma sensory profile. Roasting jackfruit seeds using a proper combination of temperature and time facilitates the Maillard reaction that results in the characteristic cocoa flavor, and decreases undesirable odors, such as those from aliphatic acids. The main final volatile composition of jackfruit includes pyrazines, Strecker aldehydes, alcohols, esters, and furanes (Siegmund, 2015).

Aroma is key attribute in food development, but is a complex matrix; the knowledge of aroma and flavor is based on data from gas chromatography (GC) and mass spectra. In the last two decades, over 500 volatile compounds have been described in dry, fermented, and roasted cocoa beans (Stalcup 1993; Afoakawa 2010; Dimick; Hoskin, 1999). However, correlating sensory perception data with GC results is a difficult task. According to a survey of the literature, GC has been instrumental in advancing knowledge by measuring the potency of odor-active compounds, but it is necessary to detect and evaluate volatile compounds eluted from GC separation. This can be done with GC-olfactometry (GC-O) which is used to describe techniques with human assessors. GC-O has the potential to detect these compounds, measure the duration of odor activity, describe the quantity of the odor perceived, and quantify odor intensity (Delarunth, 2006). GC-O results are more reliable using intensity and frequency detection when expressed in modified frequency (San-Juan, Pet’ka, Cacho, Ferreira, & Escudero, 2010; Ubeda 2016). However, the direct correlation of the GC profile data of the aromatic fraction has been difficult (Dimick and Hoskin, 1999). Thus, it is necessary to clarify the diverse chemical compounds with GC-O analysis to verify each odor substance in the characteristic aroma.

Current competitiveness requires different resources with innovative applications, including in the food industry. Nowadays, cocoa is a noble commodity of high value, and the development of a chocolate replacer could be highly innovative because cocoa is a versatile raw material used in endless applications. In tropical regions, cocoa is traditionally fermented to produce chocolate flavor. There are several advantages of using fermentation in cocoa beans, such as the resulting increase in pyrazine concentration, since unfermented beans do not have a chocolate aroma. Recently, the price of cocoa has climbed, providing an
incentive to the food industry to find a cocoa substitute (Fadel, Abdel Mageed, Abdel Samad, & Lotfy, 2006). In addition, cocoa plants (*Theobroma cacao*) are vulnerable and highly sensitive to changes in climate, are susceptible to many typical diseases, and local farmers struggle to compete with international cocoa suppliers (Fairtrade Foundation, 2011; Oyekale, Bolaji, & Olowa, 2009). Global cocoa production is around 3.7 million tons and this is not expected to grow significantly in the next 10 years (FAO - Food and Agriculture, 2010); however, the demand is estimated to be 4.5 million tons by 2020 (Fairtrade Foundation, 2011). In this context, novel sources of chocolate aroma and flavor are required to meet this increased demand and provide alternative revenue streams for local farmers and communities in Brazil.

The dominant odor-active volatile compound present in roasted jackfruit seeds is pyrazine, similar to in fermented cocoa beans (Tran 2015 e Afoakwa 2010). This makes jackfruit seeds a potential candidate for chocolate aroma production. Jackfruit seeds are a great alternative starch source, thus having the potential for uses in food such as cakes, gum candies and fillings, and beauty products, with a low cost compared to other starchy ingredients (Madruga et al., 2014; Mahanta; Kalita, 2015). Functional properties of jackfruit seeds are also compatible to those of other starchy sources (Do, Vieira, Hargreaves, Mitchell, & Wolf, 2011; Madruga et al., 2014), or are better than corn in some applications (Rengsuttithi & Charoenrein, 2011). Therefore, studies have been conducted regarding the use of jackfruit seed starch in food preparations as a cookies, breads, and sweet products (Madrigal-Aldana et al., 2011; Mukprasirt & Sajjaanantakul, 2004; Santos et al., 2009).

Especially in chocolate milk beverages, instantaneity is a desirable characteristic (Dogan, Aslan, Aktar, & Goksel Sarac, 2016). However, it is expensive to improve instantaneity and solubility of cocoa powder, because cocoa is hydrophobic. Therefore, it would be advantageous to produce a natural chocolate replacer with similar or better functional properties, such as low viscosity and high solubility and wettability associated with high levels of starch.

Based on the natural innovation potential to produce pyrazines from jackfruit seeds, currently considered as fruit waste, and owing to the limited production of cocoa, this study aimed to determine the optimal method to obtain jackfruit seed flour with an intense chocolate aroma. We then evaluated the volatile compounds, sensory chocolate aroma, and
functional properties to apply these flours in foodstuffs. The specific objectives were to: determine the best combination of roasting temperature and time for dried, acidified, and fermented jackfruit seed; study the chemical and sensorial aromatic profiles of jackfruit seed flours; evaluate the functional properties of flours and compare them with those of chocolate and cocoa powder; and develop a hot beverage (cappuccino) using formulations containing jackfruit seed flours (with an aroma nearly similar to that of chocolate) as a substitute for the aroma of chocolate.

In order to summarize the objectives of this thesis, it is composed of four chapters. The first chapter presents the best conditions, mainly the combinations of time and temperature for roasting jackfruit seeds, to obtain the highest intensity of chocolate aroma in the three methods of production (dry - DJS, acidified - AJS and fermented - FJS) based on sensory perception of chocolate aroma and pyrazine concentration. Once the flours with the highest flavor intensity were obtained, the second chapter presents the qualitative and quantitative analysis of the volatile compounds present in flours obtained from each method (DJS, AJS, and FJS) as compared to the compounds in powdered cocoa. In this second step, SPE-MS, GC-MS, and GC-O were used. In the third chapter, the functional and sensorial properties of flours with the highest intensity of chocolate aroma were characterized and compared to the properties of chocolate and cocoa powder. In this way, characterization enabled the product to be used in foods where solubility is essential; hence, the fourth chapter presents the formulation of cappuccinos by replacing cocoa powder with 50%, 75%, and 100% dry and fermented jackfruit seed flours, because DJS and FJS showed different volatile composition and consequently, different sensory properties.

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2. OPTIMISATION OF THE POST-HARVEST CONDITIONS TO PRODUCE CHOCOLATE AROMA FROM JACKFRUIT SEEDS

Abstract

Jackfruit seeds are an under-utilized waste in many tropical countries. In this work, we demonstrate the potential of roasted jackfruit seeds to develop chocolate aroma. Twenty-seven different roasted jackfruit seed flours were produced from local jackfruit by acidifying or fermenting the seeds prior to drying, and roasting under different time/temperature combinations. The chocolate aroma of groups of four flours were ranked by a sensory panel (n=162) and response surface methodology was used to identify optimum conditions. The results indicated a significant and positive influence of fermentation and acidification on the production of chocolate aroma. SPME/GC-MS of the flours showed that important aroma compounds such as 2,3-diethyl-5-methylpyrazine and 2-phenylethyl acetate were substantially higher in the fermented product, and that the more severe roasting conditions produced 2-3 times more 2,3-diethyl-5-methylpyrazine, but less 3-methylbutanal. Moisture, $a_w$, pH, luminosity and color were also monitored to ensure that these properties were similar to cocoa powder or cocoa substitutes.

Keywords: Jackfruit seeds; Chocolate aroma; Waste utilization; Sensory analysis; SPME/GC-MS

2.1. INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* Lam.) is a large tropical fruit which is abundant in South America, Asia, Africa and Australia. It is a fleshy compound fruit (syncarp) belonging to the *Moraceae* family and takes 3-6 months to reach ripeness. The fruit weight ranges from 2 to 36 kg and its seeds account for around 15-18 % of the total weight of the fruit (Mahanta & Kalita, 2015; Saxena et al., 2011) Generally the seeds are boiled, steamed and roasted before eating, providing a cheap source of fiber, protein and minerals. In many countries, including Brazil, jackfruit seeds are an under-utilized waste stream.

There are several publications reporting the use of waste jackfruit seeds to produce starch (Madruga et al., 2014; Mukprasirt & Sajjaanantakul, 2004; Rengsutthi & Charoenrein, 2011; Tulyathan, Tananuwong, Songjinda, & Jaiboorn, 2002), but there is little reported in the
literature on their potential to generate flavor. For the first time we found that after roasting, jackfruit seeds imparted an aroma similar to chocolate. Chocolate aroma has been well-characterized (Counet, Callemien, Ouwerx, & Collin, 2002; Schnermann & Schieberle, 1997) and a number of different aroma compounds have been found to contribute to the complex and characteristic aroma of chocolate. The most odor-active compounds in milk chocolate identified by Schnermann & Schieberle, (1997) include 3-methylbutanal, phenylacetaldehyde and 2,3-diethyl-5-methylpyrazine and, a few more in roasted cocoa beans (Counet et al., 2002). Some pyrazines have been shown to contribute significantly to the unique flavor of roast and toast foods (Seitz, 1994) and are used to determine the quantity and quality of cocoa flavor (Farah et al., 2012). They impart chocolate, cocoa, hazelnut, roasted, coffee, earth and green aromas (Gu et al., 2013; Tran et al., 2015). As with cocoa, the post-harvest pre-treatments and roasting of the jackfruit seeds are likely to influence the formation of these compounds and the quality of the aroma.

All three stages of the process (fermentation, drying and roasting) can have an influence on the final pyrazine concentration. During fermentation, enzymatic and microbial processes induce physical and chemical changes in seeds which result in browning reactions (Afoakwa, 2010). Some volatile compounds are formed at this stage, as well as free amino acids and sugars which are substrates for the subsequent flavor-forming reactions (Parker, 2015) which take place during roasting. The influence of fermentation parameters on the aroma of roasted cocoa beans is well understood and has been reviewed recently (Kongor et al., 2016). Kirchhoff, Biehl, & Crone (1989) demonstrated that chocolate aroma was correlated to proteolysis and the subsequent accumulation of free amino acids. The proteolytic enzymes such as endopeptidases and proteases are highly sensitive to pH, so pH control is important during cocoa fermentation to regulate the activity of different enzymes. These products of fermentation (amino acids and reducing sugars) are the precursors of pyrazines which are formed during roasting in the Maillard reaction (Ito & Mori, 2004; Kirchhoff et al., 1989; Koehler & Odell, 1970; Ledl & Schleicher, 1990).

Cocoa (Theobroma cacao) is a culture which is highly sensitive to changes in climate, is susceptible to many typical diseases and local farmers struggle to compete with international cocoa suppliers (Fairtrade Foundation, 2011; Oyekale et al., 2009). Global cocoa production is around 3.7 million tons and this is not expected to grow significantly in the next 10 years (FAO - Food and Agriculture, 2010.), however demand by 2020 is
estimated to be 4.5 million tons (Fairtrade Foundation, 2011). In this context, new sources of chocolate aroma and flavor are important to meet the increase in demand and provide alternative revenue streams for local farmers and communities in Brazil.

The aim of this work is to optimize the production of chocolate aroma from jackfruit seeds by treating them under conditions similar to those used in the cocoa process. Seeds were acidified or fermented before drying, and roasted under different time/temperature combinations. Sensory ranking tests were used to assess the chocolate aroma and key aroma compounds were analysed by SPME/GC-MS.

2.2. MATERIALS AND METHODS

2.2.1. Chemicals

Standards of 3-methylbutanal, phenylacetaldehyde, 2-phenylethyl acetate, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, 2,3,5-trimethylpyrazine, 2,3,5,6-tetramethylpyrazine, 2,3-diethyl-5-methylpyrazine, 1,2-dichlorobenzene and the alkane standards C<sub>6</sub>–C<sub>25</sub> were purchased from Sigma Aldrich Química, São Paulo, Brasil.

2.2.2. Jackfruit

Twenty five jackfruit of the hard pulp varieties were manually collected from one single tree, between October 2013 and January 2014, in the countryside of São Paulo, Brazil, selecting fruit of similar size (5 ± 1 kg) and ripeness, as indicated by the yellow color of the shell. Jackfruits were cleaned manually in flowing water, the seeds were removed and the pulp discarded. These seeds were submitted to one of three different treatments before roasting, producing either dried jackfruit seeds (DJS), acidified jackfruit seeds (AJS) or fermented jackfruit seeds (FJS). For each treatment, the seeds from 7-9 jackfruit were pooled, and treated and dried in four × 1.5 kg batches (3 kg batch for FJS) as described below. The dried seeds (50 g from each of the four batches) were roasted in 200 g portions. In total, 11 bags of roasted flour (200 g) were prepared for each of the three treatments.
2.2.3. Seed processing

For the dried jackfruit seed (DJS), the seeds were dried in an oven at 60 °C with air circulation. After 24 h, the spermoderms were manually removed, and the seeds remained for a further 24 h in the same oven at the same temperature.

For the acidified jackfruit seeds (AJS), treatment was carried out at ambient temperature (25 ± 3 °C). For each batch, the seeds (1.5 kg) were placed in polyethylene trays (28 x 42 x 7.5 cm) with 1% w/w acetic acid in potable water (3 kg). After five days the solution was removed and the seeds were dried using the same method as for DJS (2 × 24 h).

For the fermented jackfruit seeds (FJS), simulating what is done with cocoa, seeds (3 kg) were placed in polyethylene boxes (50 x 60 x 30 cm) to ferment with added jackfruit pulp (1.5 kg), perianth (0.52 kg), and banana leaves (0.1 kg) as a supply of yeast. For the first 6-7 days of fermentation, the boxes were closed to promote anaerobic fermentation but, for the remaining 7-8 days, the boxes were opened and the fermenting mass was rolled daily to promote oxidation. The seeds were removed and dried using the same method as for DJS (2 × 24 h). These processes are summarized in Figure 1A. The yield from each treatment was expressed as in equation 1.

\[
Yield(\%) = \frac{\text{weight of flour after drying}}{\text{weight of raw jackfruit seeds}} \times 100
\]

During acidification and fermentation, the ambient temperature and the temperature of the fermenting mass were measured every 24 h (AOAC Official Methods of Analysis, 1984) (AOAC Methods 13.010; 32.010; 32.016 and 32.017). For AJS, the pH of an aliquot of liquid extracted from the mass in the polyethylene boxes was measured directly. For the FJS mass, 10 g of the fermenting mass was added to 100 mL of distilled water. In both cases, the pH was measured using a pH meter with a glass electrode standardized at the experiment temperatures over the range from 7.0 to 4.0. For FJS, total titratable acidity (AOAC 945.08)(AOAC Official Methods of Analysis, 2010) was measured using 5 g fermenting mass, diluted 10 times and filtered. Proximate analysis was carried out according to (AOAC Official Methods of Analysis, 2010).
2.2.4. Roasting and Grinding

For each treatment, 11 batches of seeds (200 g) were roasted in a rotary electric oven (Probat® laboratory sample roaster, Emmerich am Rhein, Germany) with digital temperature control, using conditions defined by the response surface methodology. A central composite design was used for each treatment (Figure 1B). Two factors (roasting time and temperature) were each tested at five levels, with three repetitions of the central point totaling 11 samples. However, preliminary experiments showed it was necessary to select different roasting conditions for each treatment to avoid burning of the FJS yet achieve significant roasting in the AJS. The temperature ranged from 150 to 201°C ± 0.1°C and the roasting time from 33 to 47 min. The roasted seeds were then milled in a hammer mill to produce a “flour”. There was no heating of the sample during milling, minimizing loss of volatile compounds at this stage. Flours were packed under vacuum and stored without light at 8±1 °C.

2.2.5. Analysis of Flours

Water activity was determined from the temperature of the dew point (Aqualab®), moisture was determined by a standard gravimetric method, and color was measured instrumentally using a Minolta® colorimeter, with illuminant C, previously calibrated with a white surface (Y = 93.7, x = 0.3135 and y = 0.3195) based on the CIE-lab L,* a,* b,* scale. The pH was determined in triplicate using 2 g of flour added to distilled water (20 mL). The quality of the chocolate aroma was based on a sensory comparison and the relative concentration of selected aroma compounds was measured by GC-MS. The proximate composition was only carried out on flours with the highest sensory rankings.

2.2.6. Sensory analysis

All sensory evaluations were approved by the Ethics Committee of Human Research of the ESALQ/USP (COET/077/131).
2.2.6.1. Preliminary sensory tests

Preliminary tests were carried out to determine the optimum temperature and time for sample exposure prior to the panelists receiving the sample for assessment. In this preliminary assay using AJS flour, the samples were placed in a water bath for five different combinations of time (30, 60, 120 s) and temperature (25, 36.5, 48 °C) prior to sniffing by a small panel comprising 21 untrained members aged 18-40 years (76% women). Each panelist was asked to rank groups of three samples in increasing order according to the intensity of the chocolate aroma (Table 1). There was no significant difference between the conditions used to equilibrate the samples so the conditions were standardized at 40 °C for 120 s.

2.2.6.2. Sensorial ranking test

Ranking tests were used to determine the relative intensity of chocolate aroma in 11 samples for each treatment (DJS, AJS and FJS) using incomplete blocks (Figure 2). Each sample (3 g) was placed in an amber vial coded with a random three-digit number and, prior to sensory evaluation, the vial was heated for 120 s in a water bath at 40 °C, these conditions having been selected from the preliminary tests. Panelists received simultaneously four coded samples to rank in increasing order of intensity of chocolate aroma, from least (=1) to most (=4). Total ranking scores were used, thus the higher score representing the greater chocolate aroma. The data obtained from the panelists were collected and analyzed using Compusense five®. At the end of the session, the panelists were asked to describe different aromas they identified in each of the samples using their own free choice of descriptors.

2.2.6.3. Sensory experimental design

Untrained panelists (162) aged 18-54 years (60% women) were randomly divided into three equal groups of 54 – one group for each treatment (DJS, AJS and FJS). In order to reduce the number of comparisons to be assessed by the panel, a balanced, incomplete block, design of experiment was used (Cochran & Cox, 1950) to construct a second order model based on the 11 samples in the central composite design (Cochran & Cox, 1950). However, to minimize panelist fatigue, the central point was represented by a blend of the three central
points (reducing the number of samples to 9) and the three central points were assessed by the same panelists in a second sensory session.

In the first sensory session panelists received four samples in a balanced incomplete block (Figure 2). Each sample block consisted of 18 comparisons (9 samples each appearing 8 times). The sample block was repeated three times (for 54 panelists) and the parameters, as defined by Cochran & Cox, 1950, were T=9; k=4; r=8; B=18; L=3; E=84; Z=3.

In the second sensory session, the same panelists received three samples of the central point (0, 0) (Figure 2). These samples were delivered at the same time, in a randomized and balanced complete block (Cochran & Cox, 1950). Total ranking scores from the second session were transformed to be comparable to the first sensory session. Thus it was possible to assess the variation between the central points and validate the response surface for intensity of chocolate aroma.

### 2.2.7. Volatile analysis

Jackfruit flour (DJS, AJS and FJS) (3 g) was placed in a 20 mL SPME vial with 1µL of 1,2-dichlorobenzene in methanol (130.6 µg/mL) and vortexed for 2 min. After equilibration at 45 °C for 15 min, the triple phase fiber (65 µm PDMS/DVB/Carboxen from Supelco) was exposed (1cm) to the headspace above the sample for 55 min under magnetic agitation (635 rpm). These conditions had previously been optimized using surface response methodology.

The volatile compounds extracted by the fiber were analyzed by GC-MS using a Shimadzu® QP2010 GC-MS equipped with a RTX5MS column (30 m, 0.25 mm i.d., 0.25 µm film thickness). Volatile compounds were desorbed for 1 min at the a splitless injection at 200 °C. During desorption, the oven was maintained at 40 °C for a further 8 min, and then the temperature was raised at 4 °C/min to 200 °C, and then 10 °C/min to 280 °C totaling 56 min. MS was carried out using 70 eV electron impact, and m/z were monitored in the range 40 to 500, in scan mode. Helium was the carrier gas and the flow rate was 1 mL/min in constant flow. A series of n-alkanes C₆–C₂₀ was analyzed under the same conditions to obtain linear retention indices (LRIs) for comparison with authentic samples. All volatile compounds listed were identified by comparison of their mass spectrum and LRI with that of an authentic standard run under similar conditions. Each sample was analyzed three times.
Peak areas for 3-methylbutanal, phenylacetaldehyde and 2-phenylethyl acetate were measured using the total ion chromatogram. For the following compounds, the peak area was approximated using the area of a characteristic m/z which was multiplied by a factor calculated from the spectrum obtained from the authentic standard: 2-methylpyrazine m/z 94, factor 3; 2,5/6-dimethylpyrazine (coelute) m/z 108, factor 2.5; 2,3-dimethylpyrazine m/z 67, factor 4; trimethylpyrazine m/z 81, factor 14; tetramethylpyrazine m/z 54, factor 5; 2,3-diethyl-5-methyl-pyrazine m/z 121, factor 20. The approximate relative concentration of each compound was obtained by comparing the peak area against that of the internal standard (1,2-dichlorobenzene), using 1 as a response factor.

2.2.8. Statistical analysis and response surface methodology

The central composite design, Statistics® (2014), was selected for use. The two key responses were intensity of chocolate aroma, 3-methylbutanal and 2,3-diethyl-5-methylpyrazine concentration, although water activity, moisture, pH and color were also monitored. The two independent variables of the design, roasting time and temperature, were coded as x, and y, respectively. Equation 2 shows the quadratic polynomial model that was fitted to each response, where \( b_0, b_1, b_2, b_{11}, b_{12} \) and \( b_{22} \) are the regression coefficients; x and y are the values of the independent variables for roasting time (min) and temperature (°C) respectively.

\[
z = b_0 + b_1 x + b_2 y + b_{11} x^2 + b_{22} y^2 + b_{12} xy
\]

Eqn 2

The analysis of variance (ANOVA) tables were generated and regression coefficients of individual linear, quadratic and interaction terms were determined by using design expert software (Statistics®). The significances (p≤0.05) of all terms in the polynomial model were judged statistically by computing the F value. XLStat was used to carryout 2-way ANOVA on the volatile data and calculate Fisher’s least significant difference at p=0.05.
2.3. RESULTS AND DISCUSSION

2.3.1. Jackfruit seed processing

In cocoa beans, control of the fermentation process is required because unfermented beans develop little chocolate flavor, and excessive fermentation may also result in undesirable flavors when roasted (Dimick & Hoskin, 1999). Generally for cocoa, fermentation finish between 5 to 8 days, and the end point is determined by experience (Afoakwa, 2010; Beckett, 1999) based on reducing acid notes and maximizing chocolate flavor in the final roasted product (Beckett, 1999; Heemskerk, 1999; Jinap & Dimick, 1990; Rodriguez-Campos, Escalona-Buenda, Orozco-Avila, Lugo-Cervantes, & Jaramillo-Flores, 2011).

In this study, the fermentation process of 12 days was necessary, maybe because jackfruit seeds are bigger in comparison to cocoa beans and there is more substrate to ferment. During the fermentation it is important to kill the sprout at the beginning to ensure the success of the fermentation process and the formation of flavor compounds. Jinap and Dimick and Heemskerk et al. reported that a pH close to 4 would destroy the sprout; in jackfruit we found this value around day 3-4 of fermentation (Figure 3A) whereas the acidification process started at pH 3 and fluctuated between pH 3 and pH 4 (Figure 3B). In cocoa, samples are considered well fermented at pH > 4 although this varies with variety (Jinap & Dimick, 1990). In practice, an increase in pH of the seeds has been shown to improve chocolate flavor during fermentation and alkalization (Heemskerk, 1999; Rodriguez-Campos et al., 2011; Yousif & Alghzawi, 2000) reported that pH values lower than 4.5 in the seeds decreased the aromatic potential of the cocoa beans. So there is a balance between achieving a pH which is low enough to kill the sprout but high enough to form aroma compounds.

Although titratable acidity in FJS was very variable, the overall trend was for an increase as the pH dropped (Figure 3A and 3C). Rodriguez-Campos et al., (2011) reported similar results during cocoa fermentation with a correlation coefficient of -0.91 between pH and titratable acidity, and -0.86 for the correlation of the concentration of acetic and lactic acid with pH.

Acetic and lactic acid are present in the first and second stages of cocoa fermentation, when anaerobic yeasts and lactic acid bacteria are present, respectively. Towards the end of fermentation, when aeration increases, the acetic acid bacteria become more significant. They are responsible for converting alcohol to acetic acid, and since this
reaction is exothermic (Figure 3D), it is likely that they are also responsible for the increase in temperature of the fermenting jackfruit mass (Heemskerk, 1999). At the end of the jackfruit fermentation period (day 12), the temperature of the mass had risen from ambient to values near to 40 °C, similar to the rise during fermentation of cocoa beans although, in cocoa, temperatures can reach 45 °C (Jinap & Dimick, 1990). Figure 3 shows the pH, titratable acidity and temperature profile for FJS and pH for AJS.

For such a natural and variable process, these figures show that, with the exception of titratable acidity, these processes are fairly reproducible. In addition, it shows that jackfruit seeds can be fermented and dried under similar, albeit slightly longer, conditions to those applied to cocoa beans, resulting in a similar drop in pH which in cocoa results in the formation of aroma precursors.

### 2.3.1.1. Yield, pH, water activity (a\textsubscript{w}), moisture, luminosity (L*) and chroma (c*)

In terms of total mass, the yields of flour obtained from DJS, AJS and FJS were 48%, 45% and 40% respectively.

The pH of the roasted jackfruit seed flours were highest (pH > 5) in the flours which had been roasted at the highest temperature (independent of seeds processing) and the lowest pH (< 4.9) was found in general in the FJS flour (Table 2). These pH values are similar to those reported in traditionally fermented and roasted cocoa (4.75 to 5.19) (Heemskerk, 1999; Jinap & Dimick, 1990; Rodriguez-Campos et al., 2011). In other cocoa substitutes, Yousif & Alghzawi, (2000) found roasted carob powder to be pH 4.81 and Queiroz & Garcia, (2000) reported the pH of roasted cupuaçu flour as 4.77 - both similar to fermented and roasted jackfruit seeds (Table 2).

The pH of the flours can be fitted to a 3-dimensional surface as a function of time and temperature by using a combination of linear and quadratic terms, as well as an interaction term, to construct a polynomial equation. The correlation coefficient (r\textsuperscript{2}) indicates how well the data fit the model, and the p-value associated with each coefficient in the equation indicates the certainty with which this term influences the response (Table S1-supplementary). The correlation coefficient is good (r\textsuperscript{2} > 0.7) so it is possible to model and predict the pH of the flour from AJS and FJS as a function of time (x) and temperature (y) using equations 3 and 4 respectively. In FJS flour (Eqn 4), there was a linear and quadratic relationship with temperature (p = 0.006 and 0.03 respectively) and a linear correlation with
time (p = 0.04). For AJS flour we found significant linear effects with temperature (p = 0.01). However, for DJS flour, the final pH was relatively insensitive to changes in the roasting conditions and the model cannot be used predictively ($r^2$=0.6). The pH was on average higher in flours from DJS compared to AJS and FJS.

$$\text{pH}_{\text{AJS}} = 37.455 - 0.42557 x + 0.001036 x^2 + 0.000566 y^2 + 0.0019444 xy \quad (r^2 = 0.81) \quad \text{Eqn 3}$$

$$\text{pH}_{\text{FJS}} = 19.94 + 0.0899 x + 0.2223 y - 0.00159 x^2 + 0.000678 y^2 + 0.0002987 xy \quad (r^2 = 0.93) \quad \text{Eqn 4}$$

Generally the moisture was associated with water activity ($a_w$) in flours, and both tended to decrease as roasting conditions became more severe (Table 2). In FJS flours, the highest roast temperature (180 °C) for 40 min (0, 1.41) produced the lowest $a_w$ and the lowest moisture was obtained at 186-192 °C for a 35-40 min roast. In this study we found 2.3% moisture in flour from FJS at (0, 1.41) which was high compared to DJS and AJS flours roasted under similar conditions. By comparison, Yousif & Alghzawi, (2000) found 9.0 and 2.5% moisture for roast carob powder (150 °C for 60 min) and cocoa powder respectively, and Queiroz & Garcia, (2000) showed 3.0% moisture in roasted cupuaçu powder. The $a_w$ described for both these substitutes was around 0.4. Thus flours of jackfruit seeds have similar or lower moisture and $a_w$ in comparison to cocoa and other substitutes, which is important to restrict microbial growth in the flours and for application in other products. The surface response design allows use of equations 5, 6 and 7 (x= time and y= temperature) to predict the moisture in the flour of DJS, AJS and FJS ($r^2 > 0.7$); in all equations we could observe the significant linear effect of both roasting time and temperature in determining final moisture content (Table S1).

$$\text{Moisture}_{\text{DJS}} = 65.71 - 0.2845 x - 0.6427 y - 0.003497 x^2 + 0.001512 y^2 - 0.000383 xy \quad (r^2=0.97; \text{linear temperature effect } p=0.001) \quad \text{Eqn 5}.$$  

$$\text{Moisture}_{\text{AJS}} = 39.15 - 0.349499 x - 0.26448 y - 0.0067 x^2 + 0.0006943 y^2 - 0.0012498 xy \quad (r^2=0.92; \text{linear temperature effect } p=0.010) \quad \text{Eqn 6}.$$  

$$\text{Moisture}_{\text{FJS}} = 105.447 - 1.6209 x - 0.7213 y + 0.000926 x^2 + 0.001246 y^2 - 0.0050106 xy \quad (r^2=0.90; \text{linear temperature effect } p=0.020) \quad \text{Eqn 7}.$$
In contrast, where there was much greater variability in the responses, can only be predicted in DJS flour and only the linear term in temperature was significant (equation 8), and negative, showing that as the temperature increased, the aw decreased.

$$aw_{DJS} = -4.454 + 0.1165x + 0.03237y - 0.0011974x^2 - 0.000087113y^2 - 0.000127xy$$  
($r^2=0.75; \text{linear temperature effect } p=0.04$)  
Eqn 8.

Color in food is important because appearance can contribute to recognition, perception and enjoyment of the food. For substitutes, it is necessary to match the original product as closely as possible. In cocoa powder the luminosity ($L^*$) is low (near to black and brown), similar to the jackfruit flour which was produced from the high temperature roasts. $L^*$ tended to be lower (darker) in FJS compared to AJS flour. For chroma, the results were the reverse with high intensity color (larger chroma value) in the higher roasts, and the FJS flours having the least intense color, although there were few significant differences between roasting treatments. Luminosity results for fermented jackfruit seeds were similar to values in roasted cupuaçu. Cohen & Jackix, (2005) reported $L^*$ of 42 in cupuaçu liquor compared to values of 50-70 found in the jackfruit. Sacchetti et al., (2016) found $L^* = 21$ for roast cocoa beans (145 °C to 30 min) and Sengül, Fatih Ertugay, Sengül, & Yüksel, (2007) found $L^* = 19$. Only Gu et al., (2013) had slightly higher luminosity ($L^* = 41$) for roast cocoa (160 °C for 30 min). Therefore depending on the kind of product developed using jackfruit seed flour, it may be necessary to modify the color with other ingredients. It is possible to predict the luminosity and chroma of DJS flour using equations 9 and 10 ($x=$ time and $y=$ temperature, $r^2 > 0.7$). In both equations we observed a significant negative linear effect ($p \leq 0.05$) of roast temperature (i.e. as temperature increased, $L^*$ decreased and the product became darker), and, for DJS, roast time was also significant. For acidified and fermented flours we found no significant effect of roasting conditions ($r^2$ was 0.60 and 0.51 for chroma; and for luminosity 0.44 and 0.52 for AJS and FJS respectively).

$$L^*_{DJS} = 70.645 - 0.9291x - 0.2478y + 0.005709x^2 + 0.0000373y^2 + 0.0060888xy$$
($r^2=0.94; \text{linear temperature effect } p=0.007; \text{linear time effect } p=0.0002$)  
Eqn 9

$$\text{Chroma}_{DJS} = 19.39 + 0.27954x - 0.47968y - 0.008682x^2 - 0.00159939y^2 + 0.002884xy$$
($r^2=0.88; \text{linear temperature effect } p=0.03$)  
Eqn 10
2.3.2. Proximate composition of jackfruit seed flours

The proximate analysis was only carried out on the three best roast conditions determined by sensory score (Table 3). For DJS flours, where there was no significant difference between the samples in terms of sensory score, a sample with high pyrazine content and a high sensory score was selected. The different treatments produced different proximate composition. The moisture was smallest in AJS flours, maybe because during five days in acetic acid solution the seed had dehydrated. AJS and DJS were similar in proximate content. In FJS, the fermentation process results in the breakdown of carbohydrates and the release of CO₂. This is reflected in the proximate analysis where the remainder of the material is assumed to be carbohydrate. This is significantly lower in FJS (53%) compared to DJS (65%) and AJS (73%) respectively. The indirect consequence of this is a small increase in the % contribution from the other analytes.

Moisture, a_w, pH and color of the roasted jackfruit flours tended to vary with the time and temperature of the roasting conditions. However, the pH and moisture of the milled flours were similar to those of cocoa powder, and although the color was a bit pale (high L*), these flours have similar properties to cocoa, carob and cupuaçu powders, and could be used in similar products.

2.3.3. Sensory assessment of chocolate aroma of jackfruit flours

The response surfaces for the sensory ranking tests are shown in Figure 4 A-C and the data are shown in Table 4. The correlation coefficients for the 3D surface models for all three processes (dry, acidified and fermented) were ≥ 0.7. For DJS flours (Figure 4A), there was no significant difference between samples (p≤0.05) in the perception of sensory chocolate aroma, although the model showed a linear effect with temperature (p ≤ 0.03) suggesting that the higher temperature may increase slightly the chocolate aroma. For AJS flours, roasting at the temperature of the central point (180 °C) generated the greatest sensory perception of chocolate aroma (Table 4). The model showed a clear quadratic effect with temperature (p ≤ 0.02) shown in Figure 4B, which is also represented by a significant coefficient for y² (r² = 0.86) in the corresponding equation, indicating a decrease in chocolate aroma as the roasting conditions became more severe (and possibly over-cooked from a sensory perspective). However, the most sensory chocolate aroma was found in FJS flours. The sensory rankings of
chocolate aroma (SCA) were 72 for FJS (40 min to 150 °C) compared to 70 for AJS (40 min to 180° C) and the average of DJS was 60. Clearly, a fermentation or acidification process is necessary to produce chocolate aroma using jackfruit seeds, and it is possible to select the best roasting conditions for each treatment to optimize the sensory perception of chocolate aroma.

A range of descriptive terms were collected for the flours (Figure 5). All treatments were described with chocolate and coffee terms. In addition, sweet aroma attributes were used to describe DJS flours (honey, milk, etc.) suggesting a relatively mild processing treatment. Unfermented cocoa is very bitter and astringent with little apparent chocolate flavor (Beckett, 1999; Hashim, Selamat, Syed Muhammad, & Ali, 1998) whereas the unfermented jackfruit flour (from DJS) still had some chocolate aroma. For AJS flour, sweet aromas such as vanilla were similar to DJS flour, but other descriptors were used (e.g. earthy, rancid, acid, silage, fermented, green, etc.) which suggest that the chemical acidification process (rather than the natural fermentation process) may produce less desirable attributes which are not directly associate with food. However, FJS flour was described with fruity qualities (orange, passion fruit, cherry, jackfruit and guava). These aromas are likely to be related to fruity aldehydes, alcohols and esters which are products of the fermentation process. FJS flour was also described with caramel, soya, hazelnut and roast attributes suggesting a greater contribution from the Maillard reaction.

Overall, the sensory evaluation confirmed that a chocolate aroma can be generated from roasted jackfruit seeds, and demonstrated that it can be influenced by both the seed processing and the roasting conditions. The optimum chocolate aroma score was obtained under moderate roasting conditions when the seeds had been fermented in a process similar to that used for fermenting cocoa beans, or acidified with acetic acid prior to roasting. However, the last one was described by the panel with additional less desirable terms. The best conditions were not necessarily obtained from the most severe roasting conditions and, for AFS flour, there was a very clear optimum, after which there was a decrease in chocolate aroma as the roasting conditions became more severe.

![Image](image.png)

2.3.4. Volatile aroma compounds in jackfruit seed flours

Selection of aroma compounds was based on a survey of the literature (1997-2017), considering only those papers where the odor-active compounds in chocolate or other cocoa products had been established using GC-Olfactometry (Beckett, 1999; Counet et al., 2002;
From each paper, the 15-20 most important aroma compounds for chocolate or cocoa aroma were identified and collated, based on either their flavor dilution factors (FD), odor activity values (OAV) or frequency of detection. The results of the survey are shown in Table S2. Chocolate aroma is a complex mixture of 30-50 odor-active compounds, none of which imparts a recognisable chocolate note. Some are present at very low concentrations (e.g. 2-acetyl-1-pyrroline, 4-hydroxy-2,5-dimethyl-3(2H)-furanone), often below the detection threshold when using SPME. Others, although contributory, are reminiscent of aromas very different to that of chocolate (e.g. 3-methylbutanoic acid, 2-methyl-3-methyldithio) furan and 1-octen-3-one which impart cheesy, meaty and mushroom aromas respectively). In choosing just a few key compounds to monitor, our criteria were based on selecting those which had previously been identified as having high FD factors and high OAVs in chocolate or cocoa products, those which were relatively abundant, and those which had a relevant aroma. On this basis we selected 3-methylbutanal, one of the most abundant compounds and also one which at the appropriate dilution can be described as cocoa and malty. Phenylacetaldehyde and 2-phenylethyl acetate were selected as compounds which contribute the floral character to chocolate. 2,3-Diethyl-5-methylpyrazine and trimethylpyrazine were selected as compounds which contribute the nutty earthy character. The approximate relative contributions of these, plus four other pyrazines, are shown in Table 5.

The most obvious difference is the fact that in the FJS flours, all selected volatiles, except 3-methylbutanal, were present at significantly higher concentrations compared to the respective AJS and DJS flours, particularly the pyrazines (Figure S3), and 2-phenylethyl acetate which was 50 times higher across all conditions. Since these compounds are amongst those which have been shown most frequently to be associated with chocolate aroma (Table S2), and have also been shown to be amongst the most odor-active, it is highly likely that these compounds are responsible for the high sensory scores for chocolate aroma in FJS.

Table 5 shows the significant differences within each pre-treatment (AJS, FJS or DJS). 2-Way ANOVA showed that for most compounds, under all treatments, there was a highly significant difference between flours prepared at different temperatures. In some cases, the roasting time was also significant, and the interaction between the two was significant in some cases.

It is interesting, however, that the key aroma compounds behaved quite differently with roasting time and temperature. With all three pre-treatments (AFS, FJS and DJS), 3-
methylbutanal and phenylacetaldehyde showed a tendency to decrease as the more severe roasting conditions were employed. 3-Methylbutanal is both highly volatile and highly reactive: for example it readily undergoes aldol condensations with other aldehydes. Either or both of these may explain the decrease in concentration as the severity of the roasting process increased. This decrease in 3-methylbutanal may also contribute to the decrease in chocolate aroma which was observed particularly in AJS and also in FJS as the roasting conditions became more extreme.

The trends for 2-phenylethyl acetate were not clear or consistent, and within each pre-treatment group, the differences due to different time-temperature combinations were small or non-significant.

The 2,3-Diethyl-5-methylpyrazine, the most odor-active of the pyrazines identified in most chocolate and cocoa products, showed a tendency to increase with increasing severity of the roasting conditions, as is often the case for pyrazines. However, for trimethylpyrazine, another important compound in chocolate aroma, the trends were less clear, and in FJS it (and tetramethylpyrazine) tended to decrease with more severe conditions, although both tended to increase slightly in AJS and DJS. The dimethylpyrazines also tended to increase with increased roasting conditions in AJS and DJS, but did not vary much in FJS.

In AJS and DJS, as the roasting conditions became more severe, the 3-methylbutanal decreased whereas the 2,3-diethyl-5-methylpyrazine increased. Both being important for chocolate aroma, this is consistent with the sensory data which showed an optimum sensory chocolate aroma under moderate roasting conditions for AJS and DJS. In addition, the more severe conditions might also promote the formation of other pyrazines which at higher concentration would impart more roasted and burnt notes, as described in some DJS and FJS samples.

In FJS, most of the compounds were not sensitive to changes in roasting conditions, although 3-methylbutanal, phenylacetaldehyde and trimethylpyrazine tended to decrease. This is consistent with the sensory perception of chocolate aroma in FJS which showed a tendency to decrease as the roasting temperature increased.

2.3.5. Response surface methodology

The response surfaces for 2,3-diethyl-5-methylpyrazine are shown in Figures 6 A-C and the corresponding equations in Table S1. The most noticeable difference between the
treatments is the relative concentration of 2,3-diethyl-5-methylpyrazine in FJS flour which was approximately five and three times bigger than in flour from DJS and AJS respectively. Figure 6 clearly demonstrates the positive influence of time and temperature on the formation of this compound. However, in AJS and FJS flour, none of the coefficients relating to roast time or temperature had a significant impact on the response at p<0.05, either linear or quadratic, although they were significant at p<0.1. P-values were 0.07 and 0.09 respectively and positive, confirming the positive effect of temperature.

Direct comparison of the formation of 2,3-diethyl-5-methylpyrazine at the lowest and highest temperature (t= 40 min in all cases) showed that it was significantly higher in all three flours when the higher temperature was employed (Table 5). Furthermore, in DJS, four out of the six pyrazines monitored also showed a significant increase (all at p<0.001) and in AJS five out of six showed a significant increase (four of these at p<0.001). This is in agreement with many other studies (Farah et al., 2012; Hashim et al., 1998; Owusu, Petersen, & Heimdal, 2012) that show that pyrazine formation in general is greatly influenced by temperature. (Queiroz & Garcia, 2000) evaluated roasting time and temperature for cupuaçu seeds and concluded that increased time resulted in greater pyrazine formation and increased the scores for chocolate in the sensory profile. For cupuaçu, the best roasting conditions were 150°C for 42 min. For cocoa beans, Farah et al., (2012) reported an increase in the concentration of pyrazines, particularly tetramethylpyrazine, when they roasted beans at temperatures close to 160°C.

The Figure 6 (A, B and C) shows that the greatest relative concentration of 2,3-dimethyl-5-methylpyrazine was formed in dry, acidified and fermented flour when we used 171 or 186, 201 and 180°C, respectively. These temperatures are higher than those milder conditions (110 - 140 °C for 20 - 50 min) reported for cocoa by Jinap et al., (1998) or (Afoakwa, 2010) (120-150 °C for 5-120 min).

The response surfaces for 3-methylbutanal are shown in Figures 6 D-F. They clearly demonstrate that, opposite to 2,3-diethyl-5-methylpyrazine, high time and temperature are not the most favorable roasting conditions for the formation of 3-methylbutanal. The equation in Table S1 shows that the linear temperature coefficient in AJS is significant (p=0.03) and negative, indicating that the lower temperatures produce a greater response. For AJS and FJS, the lowest temperatures generated the most 3-methylbutanal, but in DJS, there was an optimum around the mid-point, consistent with the data presented in Table 5. Optimum
temperatures for 3-methylbutanal in DJS, AJS and FJS were 171, 165 and 154 °C, respectively, closer to those used for cocoa roasting.

The similarity of the optimum jackfruit roasting conditions, compared to cocoa, may be due to the fact that jackfruit seeds have a similar composition compared to cocoa beans, although dried jackfruit seeds have a lower lipid content (0.4% compared to dried cocoa beans which have range between 53 and 39%)(Afoakwa, 2010; Gu et al., 2013).

Whilst we have selected a few compounds as a marker of chocolate flavor, it is clear from these results that there are other factors involved, particularly those associated with the fermented product. Further work is currently being carried out to investigate more thoroughly the contribution from a wider range of volatile compounds.

Waste jackfruit seeds have been roasted to prepare a flour which has a chocolate aroma. Moisture, pH and color were similar to those of cocoa, and different aroma profiles were obtained by acidifying or fermenting the seeds prior to roasting under different time/temperature combinations. Optimum chocolate aroma scores were achieved when either fermentation or acidification was performed prior to roasting, and fermentation produced fewer off-notes. Utilization of this local waste stream can provide a new revenue stream for local farmers and boost local economies.

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Table 1. Results from preliminary ranking experiment using different pre-exposure conditions of the roasted flours prior to ranking

| Roasting conditions | Total ranking score for sensory chocolate | values with the same letter are not significantly different at p<0.05 |
|---------------------|------------------------------------------|---------------------------------------------------------------|
| Coded values        | Actual values                            |                                                               |
| x  y                | time (s)       | temperature (°C)         | aroma<sup>a</sup> |
| 1 -1                | 120            | 25                      | 13 a               |
| -1 -1               | 30             | 25                      | 15 a               |
| -1 1                | 30             | 48                      | 16 a               |
| 1 1                 | 120            | 48                      | 20 a               |
| 0 0                 | 60             | 36.5                    | 22 a               |
| 0 0                 | 60             | 36.5                    | 21 a               |
| 0 0                 | 60             | 36.5                    | 19 a               |

T=7; k=3; r=3; b=21; L=1; E=78 where T = number of samples; k = number of samples in each ranking test; r = number of times each sample was shown within each block; B= number of panelists in each block; L= number of times the samples were shown together; E = dependability of the analysis; Z= number times the block was repeated. Values with the same letter are not significantly different at p<0.05
Table 2. Mean ± standard error (n=3) pH, water activity, moisture L* and chroma* of the roasted jackfruit seed flours showing mean values.

| x (time) | y (temp) | pH   | aw     | moisture % | L*     | chroma |
|----------|----------|------|--------|------------|--------|--------|
| **Flour from dried jackfruit seeds (DJS)** |          |      |        |            |        |        |
| 0        | 1.41     | 5.4  | ± 0.04 | 33 ± 0.02  | 0.31 ± 0.04d | 1.0 ± 0.3d | 53 ± 3c | 33.6 ± 0.2a |
| 1        | 1        | 5.3  | ± 0.01 | 32 ± 0.02  | 0.32 ± 0.02d | 1.2 ± 0.4d | 55 ± 1de | 33.2 ± 0.2b |
| -1       | 1        | 5.3  | ± 0.02 | 32 ± 0.01  | 0.32 ± 0.01d | 1.3 ± 0.3d | 58 ± 3bcd | 32.1 ± 0.6bc |
| -1.41    | 0        | 5.3  | ± 0.02 | 37 ± 0.01  | 0.37 ± 0.01c | 3.7 ± 0.8b | 59 ± 3bcd | 32.1 ± 0.2bc |
| 1.41     | 0        | 5.4  | ± 0.02 | 31 ± 0.01  | 0.31 ± 0.01d | 2.0 ± 0.2ed | 59 ± 0.4bed | 33.3 ± 0.6b  |
| 0        | 0        | 5.2  | ± 0.01 | 38 ± 0.01  | 0.38 ± 0.01c | 3.4 ± 0.6be | 60 ± 2bedc | 32.9 ± 0.4b  |
| 0        | 0        | 5.3  | ± 0.01 | 42 ± 0.03  | 0.42 ± 0.03abc | 2.9 ± 0.5bc | 60 ± 3abcd | 33.2 ± 0.3b  |
| 0        | 0        | 5.2  | ± 0.01 | 43 ± 0.01 | 0.43 ± 0.01a | 3.2 ± 0.3be | 60 ± 2bedc | 32.5 ± 0.5abc |
| 1        | -1       | 5.2  | ± 0.01 | 44 ± 0.01 | 0.44 ± 0.01a | 5.8 ± 0.9b | 64 ± 2bc | 31.6 ± 0.5c |
| -1       | -1       | 5.4  | ± 0.00 | 40 ± 0.02  | 0.40 ± 0.02abc | 5.8 ± 0.8a | 66.0 ± 1.6a | 31.3 ± 0.6c  |
| 0        | -1.41    | 5.3  | ± 0.01 | 40 ± 0.01  | 0.40 ± 0.01bc | 6.4 ± 0.2a | 65.1 ± 1.5b | 31.2 ± 0.5c  |
| **Flour from acidified jackfruit seeds (AJS)** |          |      |        |            |        |        |
| 1.41     | 0        | 5.6  | ± 0.01 | 0.5 ± 0.1abc | 0.5 ± 0.4d | 0.5 ± 0.4d | 61.7 ± 0.9ed | 32.0 ± 0.8a |
| 1        | 1        | 5.6  | ± 0.01 | 0.50 ± 0.02a | 0.9 ± 0.9ed | 60.5 ± 1.6d | 31.5 ± 0.8b |
| -1       | 1        | 5.2  | ± 0.01 | 0.47 ± 0.01c | 1.6 ± 0.2bed | 65.3 ± 0.9bed | 30.2 ± 0.2b |
| 1.41     | 0        | 5.3  | ± 0.01 | 0.47 ± 0.01b | 1.9 ± 0.7bed | 66.1 ± 0.8be | 31.1 ± 0.2a |
| 1.41     | 0        | 5.2  | ± 0.01 | 0.48 ± 0.01abc | 2.4 ± 0.6abc | 64.7 ± 2.2bedc | 30.5 ± 0.7ab |
| 0        | 0        | 5.3  | ± 0.01 | 0.50 ± 0.01ab | 1.9 ± 0.7bed | 66.1 ± 1.4ab | 30.2 ± 0.5b |
| 0        | 0        | 5.2  | ± 0.01 | 0.47 ± 0.01bc | 2.0 ± 0.5bed | 65.3 ± 0.4bedc | 30.8 ± 0.5ab |
| 0        | 0        | 4.9  | ± 0.02 | 0.47 ± 0.01bc | 1.4 ± 0.7bed | 64.3 ± 3.0bedc | 31.1 ± 1.0a |
| 1        | -1       | 5.0  | ± 0.02 | 0.46 ± 0.01c | 2.6 ± 0.8ab | 63.2 ± 2.9abed | 31.2 ± 0.8a |
| -1       | -1       | 5.2  | ± 0.03 | 0.48 ± 0.01abc | 2.9 ± 0.2ab | 67.3 ± 1.9ab | 30.6 ± 0.8ab |
| 0        | -1.41    | 5.3  | ± 0.01 | 0.48 ± 0.01abc | 3.8 ± 0.2a | 69.3 ± 0.8a | 29.1 ± 0.2b |
| **Flour from fermented jackfruit seeds (FJS)** |          |      |        |            |        |        |
| 0        | 1.41     | 5.1  | ± 0.00 | 0.34 ± 0.01c | 2.3 ± 0.5f | 49.4 ± 1.9ab | 27.6 ± 0.5ab |
| 1        | 1        | 5.0  | ± 0.01 | 0.42 ± 0.01ab | 2.3 ± 0.1f | 50.1 ± 1.8ab | 27.8 ± 0.4ab |
| -1       | 1        | 4.8  | ± 0.01 | 0.39 ± 0.01ed | 2.5 ± 0.2f | 53.5 ± 1.3a | 28.1 ± 0.3ab |
| -1.41    | 0        | 4.7  | ± 0.01 | 0.38 ± 0.01d | 4.1 ± 0.5bed | 52.3 ± 3.5a | 28.9 ± 0.7a |
| 1.41     | 0        | 4.8  | ± 0.01d | 0.43 ± 0.01a | 3.6 ± 0.1ed | 53.7 ± 0.6a | 28.8 ± 0.4a |
| 0        | 0        | 4.8  | ± 0.02 | 0.39 ± 0.01ed | 3.3 ± 0.3def | 52.8 ± 1.9a | 27.7 ± 0.5ab |
| 0        | 0        | 4.7  | ± 0.00 | 0.40 ± 0.01bc | 3.0 ± 0.4ef | 44.5 ± 3.4b | 26.8 ± 0.8b |
| 0        | 0        | 4.8  | ± 0.01d | 0.37 ± 0.01d | 3.9 ± 0.5edc | 49.5 ± 2.1ab | 28.4 ± 0.4a |
| 1        | -1       | 4.7  | ± 0.01 | 0.41 ± 0.01be | 4.5 ± 0.1be | 51.5 ± 1.1a | 27.7 ± 0.3ab |
| -1       | -1       | 4.5  | ± 0.02 | 0.41 ± 0.01ab | 5.7 ± 0.2a | 51.3 ± 2.9a | 27.9 ± 0.4ab |
| 0        | -1.41    | 4.8  | ± 0.01d | 0.38 ± 0.01ed | 5.1 ± 0.4ab | 49.0 ± 2.9ab | 27.9 ± 0.4ab |

Within each column for each treatment, values with the same letter are not significantly different from each other (p≤0.05) using the Tukey test.
Table 3. Proximate composition (% ± standard error) of jackfruit flours roasted under the best conditions.

| flour | moisture | lipids | proteins | ash | fiber |
|-------|----------|--------|----------|-----|-------|
| DJS   | 5.88 ± 0.9 | 0.40 ± 0.05 a | 11.20 ± 0.7 b | 2.90 ± 0.02 b | 10.34 ± 0.05 b | 3.88 ± 0.010 a |
|       |          |        |          |     |       | 2.68 ± 0.003 |
| AJS   | 1.38 ± 0.7 b | 0.30 ± 0.05 b | 11.16 ± 0.5 b | 2.44 ± 0.12 c | 9.29 ± 0.01 c | b |
| FJS   | 5.10 ± 0.4 a | 0.50 ± 0.03 a | 14.82 ± 0.5 a | 4.70 ± 0.09 a | 18.9 ± 0.8 a | 3.34 ± 0.002 a |

aDJS = dried jackfruit seeds (47 min at 171 °C); AJS = acidified jackfruit seeds (40 min at 180 °C); FJS = fermented jackfruit seeds (40 min at 150 °C)

bMean (n=3), within each column, values with the same letter are not significantly different from each other (p ≤ 0.05) using the Tukey test.
Table 4. Total sensory chocolate aroma (SCA) ranking score for flour from DJS, AJS and FJS.

| coded values | total ranking scores for sensory chocolate aroma<sup>a</sup> |
|--------------|------------------------------------------------------------|
| x (time)     | y (temp) DJS | AJS | FJS  |
| incomplete block | | | |
| 1            | 1          | -1  | 66<sup>b</sup> | 67<sup>ab</sup> | 68<sup>ab</sup> |
| 2            | -1         | -1  | 52<sup>a</sup> | 58<sup>ab</sup> | 66<sup>ab</sup> |
| 3            | -1         | 1   | 58<sup>a</sup> | 63<sup>ab</sup> | 60<sup>ab</sup> |
| 4            | 1          | 1   | 66<sup>a</sup> | 51<sup>ab</sup> | 51<sup>bc</sup> |
| 5            | 0          | 1.41| 51<sup>a</sup> | 42<sup>c</sup>  | 44<sup>c</sup>  |
| 6            | 0          | -1.41| 57<sup>a</sup> | 54<sup>bc</sup> | 72<sup>a</sup>  |
| 7            | 1.41       | 0   | 62<sup>a</sup> | 69<sup>a</sup>  | 61<sup>ab</sup> |
| 8            | -1.41      | 0   | 56<sup>a</sup> | 66<sup>ab</sup> | 55<sup>bc</sup> |
| blend        | 0          | 0   | 62<sup>a</sup> | 70<sup>a</sup>  | 63<sup>ab</sup> |
| complete block<sup>b</sup> | | | |
| 9            | 0          | 0   | 64<sup>k</sup> | 68<sup>k</sup>  | 71<sup>k</sup>  |
| 10           | 0          | 0   | 62<sup>k</sup> | 68<sup>k</sup>  | 65<sup>k</sup>  |
| 11           | 0          | 0   | 60<sup>k</sup> | 74<sup>k</sup>  | 53<sup>k</sup>  |

<sup>a</sup>DJS = dried jackfruit seeds; AJS = acidified jackfruit seeds; FJS - fermented jackfruit seeds. <sup>b</sup>Values are transformed for comparison with incomplete block. Within each column, means followed by the same letters are not significantly different (p ≤ 0.05).
Table 5. Approximate relative concentrations of selected volatiles in roasted jackfruit seed flours

| LRIa | compound IDb | roasting conditions | significancec | T | t | T×t |
|------|-------------|---------------------|----------------|---|---|-----|
|      |             | roasting temp       | 150 °C         | 156 °C | 156 °C | 171 °C | 171 °C | 171 °C | 171 °C | 186 °C | 186 °C | 192 °C |
|      |             | roasting time       | 40 min         | 35 min | 45 min | 33 min | 40 min | 40 min | 40 min | 47 min | 35 min | 45 min | 40 min |
| 657  | 3-methylbutanal | 6.0d6              | 7.1 bcd        | 8.1 abc | 8.8 ab | 8.1 abc | 9.7 a  | 6.5 cd  | 5.4 d  | 7.2 bcd | 5.6 d  | 3.4 e   |
| 827  | 2-methylpyrazine | 4.3 fg              | 3.3 g          | 4.6 fg  | 6.6 efg | 15 d   | 12 de  | 11 def  | 124 a  | 25 c   | 33 b   | 32 b   |
| 916  | 2,5/6-dimethylpyrazine | 39 e              | 54 e          | 66 de   | 101 cd  | 121 bc  | 124 bc | 96 cd   | 123 bc | 167 a | 185 a  | 155 ab |
| 922  | 2,3-dimethylpyrazine | 12 g               | 11 g          | 72 b    | 19 fg   | 30 def  | 34 d   | 21 efg  | 33 de  | 49 c   | 63 b   | 110 a  |
| 1008 | 2,3,5-trimethylpyrazine | 25 abc             | 23 abc        | 11 c    | 19 bc   | 25 abc  | 26 abc | 19 bc   | 44 ab  | 42 a   | 36 ab  | 35 ab  |
| 1058 | phenylacetaldehyde | 11.2a              | 8.9ab         | 9.7a    | 8.1 ab  | 6.6abc  | 7.8ab  | 10.6a   | 6.8abc | 6.8abc | 1.9c   | 3.4bc  |
| 1091 | 2,3,5,6-tetramethylpyrazine | 110 cde         | 92 cde        | 68 e    | 69 e    | 88 e   | 139 bcd| 110 cde | 91 de  | 180 ab | 210 a | 140 bc |
| 1157 | 2,3-diethyl-5-methylpyrazine | 1.5 fg            | 1.3 g         | 1.9 ef  | 1.9 ef  | 2.6 cd  | 3.2 b  | 2.2 de  | 4.3 a  | 3.9 a  | 4.3 a  | 3 bc   |
| 1263 | 2-phenylethyl acetate | 0.1 ab             | 0.1 ab        | 0.08 bc | 0.09 bc | 0.08 c  | 0.07 c | 0.08 bc | 0.12 a | 0.13 a | 0.12 a | 0.08 c |
|      |             | roasting temp       | 159 °C         | 165 °C | 165 °C | 180 °C | 180 °C | 180 °C | 180 °C | 195 °C | 195 °C | 201 °C |
|      |             | roasting time       | 40 min         | 35 min | 45 min | 33 min | 40 min | 40 min | 40 min | 47 min | 35 min | 45 min | 40 min |
| 657  | 3-methylbutanal | 11.2 bc             | 13.2 a         | 12.2 ab | 10.3 c  | 10.1 c  | 4.4 ef  | 7.5 d   | 7.8 d  | 5.2 e  | 4 f    | 2.3 g  |
| 827  | 2-methylpyrazine | 2.7 d               | 3.5 d         | 11.6 c  | 1.2 d   | 1.6 d   | 28 a   | 21 b    | 11 c   | 2.6 d  | 25 ab  | 29 a   |
| 916  | 2,5/6-dimethylpyrazine | 66 d               | 74 d          | 110 c   | 110 c   | 120 bc  | 146 a  | 137 ab  | 86 d   | 113 c  | 140 ab | 123 bc |
| 922  | 2,3-dimethylpyrazine | 37 g               | 126 a         | 58 f    | 59 f    | 68 ef   | 97 c   | 79 d    | 8.7 h  | 73 de  | 110 b  | 110 bc |
| 1008 | 2,3,5-trimethylpyrazine | 52 abc             | 65 a          | 28 cd   | 30 bcd  | 35 abc  | 44 abc  | 43 abc  | 18 d   | 45 abc | 59 abc | 65 ab |
| 1058 | phenylacetaldehyde | 10.7bc              | 11.8ab        | 10.8b   | 9.7brcd | 7.4cd   | 14.8a  | 7.1d    | 3.3e   | 3.4e   | 2.7e   | 0.9e   |
| 1091 | 2,3,5,6-tetramethylpyrazine | 28 cd              | 67 bc         | 38 bcd  | 44 bcd  | 59 bcd  | 57 bcd  | 79 bc   | 12 d   | 88 b   | 160 a  | 150 a  |
| 1157 | 2,3-diethyl-5-methylpyrazine | 1.2 f              | 1.1 f         | 1.8 ef  | 2.1 de  | 2.8 d   | 4.6 c   | 4.2 c   | 1.8 ef  | 4.9 bc | 7.8 a  | 5.4 b  | ** ns ** |
| 1263  | 2-phenylethyl acetate | 0.13  | 0.14  | 0.16cd | 0.14  | 0.17bcd | 0.15d  | 0.19abc | 0.07e  | 0.20ab | 0.21a  | 0.19abc | *** | *** | ns  |
|-------|-----------------------|-------|-------|--------|-------|---------|--------|--------|-------|--------|--------|--------|-----|-----|-----|
|       | **FERMENTED JACKFRUIT SEEDS (FJS)** |       |       |        |       |         |        |        |       |        |        |        |     |     |     |
|       | roasting temp         | 150 °C | 154 °C | 154 °C | 165 °C | 165 °C | 165 °C | 165 °C | 176 °C | 176 °C | 180 °C |       |     |     |     |
|       | roasting time         | 40 min | 35 min | 33 min | 40 min | 40 min | 40 min | 40 min | 47 min | 35 min | 45 min | 40 min |     |     |     |
| 657   | 3-methylbutanal       | 7 abcd | 11 a   | 9.8 ab | 9 abc  | 8.4 abc | 5.1 def | 6.8 abc | 4.3 ef | 5.9 cdef | 2.3 f  | 2.8 f  | *** | *  | ns  |
| 827   | 2-methylpyrazine      | 100 abc | 86 cd  | 123 ab | 86 bcd | 100 abc | 53 d   | 130 a  | 129 a  | 123 a  | 113 abc | 110 abc | ns  | ns  | ns  |
| 916   | 2.5/6-dimethylpyrazine| 375 ab  | 280 bc | 450 a  | 250 bc | 290 bc  | 234 c  | 312 bc | 266 bc | 278 bc | 215 c  | 240 c  |     |     |     |
| 922   | 2.3-dimethylpyrazine  | 487 ab  | 410 bc | 600 a  | 420 bc | 400 bc  | 421 bc | 513 ab | 510 ab | 511 ab | 402 bc | 320 c  | *   | ns  |   * |
| 1008  | 2.3,5-trimethylpyrazine| 560 abc | 480 bc | 690 a  | 380 bcd| 370 cd  | 110 e  | 130 e  | 98 e  | 210 de | 130 e  | 270 de | *** | ns  | ns  |
| 1058  | phenylacetaldehyde    | 430 ab  | 3840 bc| 5300 a | 4380 ab| 4070 b  | 3840 bc| 5320 a | 4190 b | 4200 b | 2700 d | 2830 cd | **  | ns  | **  |
| 1157  | 2,3-diethyl-5-methylpyrazine| 7.5 f  | 11 ef  | 14 bcd | 12 de  | 16 abc  | 13 cde | 18 a  | 16 ab  | 19 a  | 15 abc | *** | *  | ns  |
| 1263  | 2-phenylethyl acetate | 7.3 c  | 12 a   | 11 ab  | 7.6 c  | 7 cd    | 6.6 cd | 9.5 b  | 10.1 b | 7.6 c  | 6.3 cd | 5.5 b  | *** | *  | ns  |

*a* Linear retention index on RTX5MS column (30m), calculated from a linear equation between each pair of straight chain alkanes $C_6$-$C_{30}$.

*b* Identity: identity of all compounds confirmed by comparison of mass spectrum and LRI with that of the authentic standard run under similar conditions.

*c* Significance of differences between samples within one pre-treatment (AJS, FJS or DJS) - probability, obtained from 2-way ANOVA, that there is a difference between means; ns = no significant difference between means ($p>0.05$); * significant at the 5% level; ** significant at the 1% level; *** significant at the 0.1% level,
with respect to; $T =$ roasting temperature, $t =$ roasting time, $T×t$ interaction between roasting time and temperature.

*d* Mean ($n=3$) relative concentration (μg/kg) = peak area of compound × concentration of internal standard (ISTD) / peak area of ISTD, nd = not detected. Within each row, cells containing the same letter are not significantly different from each other at $p<0.05$. 

**Notes:**
- Use bold for table headers.
- Use italics for variable names.
- Use superscript for footnotes.
- Use subscripts for chemical formulas.
- Use asterisks (*) for significance levels.
- Use bold for emphasis.
- Use italics for compound names.
Figure 1A. Summary of jackfruit seed processing. DJS, AJS and FJS are dried, acidified and fermented jackfruit seeds respectively.

Figure 1B. Central composite design using two factors each at 5 levels; DJS, AJS and FJS are dried, acidified and fermented jackfruit seeds respectively.
Figure 2. Experimental design used for sensory ranking test where $T =$ number of samples; $k =$ number of samples in each ranking test; $r =$ number of times each sample was shown within each block; $B =$ number of panelists in each block; $L =$ number of times the samples were shown together; $E =$ dependability of the analysis; $Z =$ number times the block was repeated.
Figure 3. Variables followed during the processing of the seeds prior to roasting: A = pH during fermentation process; B = pH during acidification process; C = total titratable acidity (g/100g) during fermentation process and D = temperature (°C) during fermentation process; close = anaerobic 6-7 days; open = aerobic 7-8 days.
Figure 4. Response surfaces for roasted jackfruit seeds. A, B and C = total sensory chocolate aroma (SCA) ranking score for flour from DJS, AJS and FJS respectively.
Figure 5. Representation of aroma attributes used freely by the panelists to describe the roasted flours from fermented, dried and acidified jackfruit seed
Figure 6. Response surfaces for roasted jackfruit seeds. A, B and C = 2,3-diethyl-5-methylprazine for flour from DJS, AJS and FJS respectively. D, E and F = 3-methylbutanal from DJS, AJS and FJS respectively.
Table S1 Equations, coefficients, r² and p-value for all equations derived from the response surface methodology

| Trt. | Effect (p-value) | Time | Temperature | Equations |
|------|----------------|------|-------------|-----------|
|      |                | L    | Q           | L         | Q         | 1L by 2L | r²     |
| AJS  | aw             | 0.91 | 0.57        | 0.40      | 0.91      | 0.16     | 0.68   |
|      | Chroma         | 0.16 | 0.63        | 0.08      | 0.90      | 0.57     | 0.52   |
|      | L              | 0.11 | 0.57        | 0.02      | 0.60      | 0.70     | 0.60   |
|      | Moisture       | 0.27 | 0.38        | 0.02      | 0.41      | 0.65     | 0.93   |
|      | pH             | 0.56 | 0.72        | 0.16      | 0.18      | 0.19     | 0.81   |
|      | 2,3-diethyl-5-methylpyrazine | 0.55 | 0.49 | 0.07 | 0.73 | 0.97 | 0.80 |
|      | 3-Methyl-Butanal | 0.35 | 0.22 | 0.03 | 0.32 | 0.36 | 0.85 |
|      | SCA            | 0.09 | 0.81        | 0.02      | 0.43      | 0.85     | 0.86   |
| DJS  | aw             | 0.61 | 0.10        | 0.04      | 0.19      | 0.51     | 0.75   |
|      | Chroma         | 0.10 | 0.30        | 0.03      | 0.15      | 0.36     | 0.88   |
|      | L              | 0.01 | 0.87        | 0.0002    | 0.92      | 0.04     | 0.94   |
|      | Moisture       | 0.07 | 0.48        | 0.002     | 0.08      | 0.83     | 0.97   |
|      | pH             | 0.52 | 0.28        | 0.24      | 0.08      | 0.31     | 0.60   |
|      | 2,3-diethyl-5-methylpyrazine | 0.09 | 0.34 | 0.04 | 0.51 | 0.86 | 0.80 |
|      | 3-Methyl-Butanal | 0.68 | 0.78 | 0.35 | 0.17 | 0.50 | 0.68 |
|      | SCA            | 0.03 | 0.32        | 0.95      | 0.31      | 0.27     | 0.75   |
| FJS  | aw             | 0.13 | 0.15        | 0.18      | 0.34      | 0.40     | 0.68   |
|      | Chroma         | 0.82 | 0.26        | 0.89      | 0.93      | 0.97     | 0.52   |
|      | L              | 0.93 | 0.35        | 0.92      | 0.90      | 0.72     | 0.44   |
|      | Moisture       | 0.23 | 0.34        | 0.02      | 0.53      | 0.35     | 0.90   |
|      | pH             | 0.04 | 0.11        | 0.01      | 0.03      | 0.45     | 0.93   |
|      | 2,3-diethyl-5-methylpyrazine | 0.24 | 0.86 | 0.09 | 0.24 | 0.9 | 0.78 |
|      | 3-Methyl-Butanal | 0.13 | 0.66 | 0.06 | 0.53 | 0.54 | 0.80 |
|      | SCA            | 0.96 | 0.72        | 0.12      | 0.71      | 0.58     | 0.70   |

Trt = Treatment; DJS = dried jackfruit seeds; AJS = acidified jackfruit seeds; FJS - fermented jackfruit seeds. 1L by 2L = Interaction with linear effects.
| Sample method | Cocoa | Cocoa Powder | Dark Chocolate | Milk Chocolate | Cocoa Liquor | Milk Chocolate | Cocoa Powder | Chocolate |
|---------------|-------|--------------|----------------|----------------|--------------|----------------|--------------|-----------|
| Strecker aldehydes | | | | | | | | |
| (2)3-methylbutanal | y | y | y | y | y | y | y | y |
| Phenylacetaldehyde | y | y | y | y | y | y | y | y |
| 2-methylpropanal | | | | | | | | |
| Benzaldehyde | | | | | | | | |
| 2-methylbutanal | | | | | | | | |
| Carboxylic acids | | | | | | | | |
| (2)3-methylbutanoic acid | y | y | y | y | y | y | y | y |
| Acetic acid | y | y | y | y | y | y | y | y |
| Phenylacetic acid | y | y | | | | | | |
| Butanoic acid | y | y | | | | | | |
| Methylpropanoic acid | | | | | | | | |
| Hexanoic acid | | | | | | | | y |
| Pyrroles | | | | | | | | |
| 2-Acetyl-1-pyrroline | y | y | y | y | y | y | | |
| 2-Acetylpyrrole | y | | | | | | | |
| Pyrazines | | | | | | | | |
| 2,3-Diethyl-5-methylpyrazine | y | y | y | y | y | y | | |
| Trimethylpyrazine | y | y | y | y | | | | y |
| 2-ethyl-3,5-dimethylpyrazine | y | y | | | | | | |
| 2-ethyl-3,6-dimethylpyrazine | y | y | | | | | | |
| 2-isobutyl-3-methoxypyrazine | | | | | | | | |
| Tetramethylpyrazine | | | | | | | | y |
| 2,5-Dimethylpyrazine | | | | | | | | y |

Table S2. Summary of odor-active compounds found in cocoa and chocolate products 1997 - 2017
| Compound                                      | y | y | y | y | y |
|-----------------------------------------------|---|---|---|---|---|
| 2-Ethyl-5,6-dimethylpyrazine                  |   |   |   |   |   |
| Sugar derivatives (and vanillin)              |   |   |   |   |   |
| 4-hydroxy-2,5-dimethyl-3(2H)-furanone         | y | y | y | y | y |
| 3-hydroxy-4,5-dimethyl-2(5H)-furanone         | y | y |   |   |   |
| Vanillin                                      | y | y | y |   |   |
| Maltol                                        | y |   |   |   |   |
| Sulfur compounds                              |   |   |   |   |   |
| Dimethyl trisulfide                           | y | y | y | y | y |
| 2-Methyl-3-(methylthio)furan                  | y |   |   |   |   |
| Dimethyl disulfide                            |   |   |   | y |   |
| Esters                                        |   |   |   |   |   |
| 2-Phenylethyl acetate                         | y | y | y | y |   |
| Ethyl cinnamate                               | y |   |   |   |   |
| Ethyl pentanoate                              | y | y |   |   |   |
| Ethyl hexanoate                               | y | y | y |   |   |
| Ethyl heptanoate                              | y | y |   | y |   |
| Ethyl dodecanoate                             | y | y |   | y |   |
| Ethyl 2-methylbutanoate                       | y | y |   | y |   |
| Ethyl 2-methylpropanoate                      | y | y |   | y |   |
| Alcohols                                      |   |   |   |   |   |
| Phenylethanol                                  | y | y | y | y | y |
| 2-Heptanol                                     | y |   |   |   |   |
| Benzyl acetate                                | y |   |   |   |   |
| Lipid derived aldehydes and ketones           |   |   |   |   |   |
| 1-Octen-3-one                                 | y | y | y |   |   |
| Hexanal                                       | y | y | y | y | y |
| (Z)-2-nonenal                                  | y | y | y | y | y |
| (E)-2-nonenal                                  | y | y | y | y | y |
| (E,Z)-2,6-nonenadienal                        | y | y | y |   | y |
| (E,E)-2,4-nonenadienal                        | y | y | y | y | y |
| (E,E)-2,4-decaadienal                         | y | y | y | y | y |
| 2-Nonanone                                     | y |   |   |   |   |
| Nonanal                                       | y |   |   |   |   |
| lactones                  |     |     |     |     |
|--------------------------|-----|-----|-----|-----|
| d-octenolactone          |     |     |     | y   |
| g-decalactone            |     |     |     | y   |
| d-decalactone            |     |     |     | y   |
| d-decenolactone          |     |     |     | y   |
| g-octalactone            |     |     |     | y   |

| Miscellaneous            |     |     |     |     |
|--------------------------|-----|-----|-----|-----|
| 2-methoxyphenol          |     |     |     | y   |
| Linalool                 |     |     |     | y   |
| Acetophenone             |     |     |     |     |
| Methylacetophenone       |     |     |     | y   |
| 3-methylindole           |     |     |     | y   |
| 4-methylphenol           |     |     |     |     |
| Butanediols              |     |     |     |     |
| 2,3-butanedione          |     |     |     | y   |

For each paper, the key aroma compounds were identified (y) using either the flavour dilution factor (FD), odor activity values (OAV) or the frequency of detection when several assessors were used (freq). For FD and freq, the cut off point was selected so that the top 15-20 compounds were considered, for OAV the cut off was OAV>1. The relative contribution of each is not indicated here, but was taken into account when selecting the volatiles to analyze.
Figure S3. Response surfaces for roasted jackfruit seeds: Y, W and Z total pyrazine peak area (Py) for flour from DJS, AJS and FJS respectively.
3. CHARACTERISATION OF THE AROMA COMPONUDS IN ROASTED JACKFRUIT SEEDS

Abstract

Jackfruit is an exotic fruit and few studies have reported their properties and characterized their volatile fraction. The seeds of fruit are generally discarded as a waste in some tropical regions around the world. This chapter aimed to characterize, the aroma compounds present in the flours prepared from roasted jackfruit seeds and compare three different pre-treatments (dring, acidification and fermentation) to a typical Brazilian cocoa powder. In total, about 130 compounds were reported in jackfruit seed flours. The pyrazines production was intense when the seeds were fermented, and this result suggests a considerable synthesis resulting from high amino acids concentration linkage to Maillard reactions and Strecker degradations. Some important compounds present in cocoa were increased in fermented sample and the active aromas were detected with more intensity. The aroma intensity of fermented jackfruit seeds flows was some times higher that of a typical cocoa powder.

Keywords: Volatile compounds; GCO; SPE; Pyrazines

3.1. INTRODUCTION

Jackfruit (Artocarpus heterophyllus Lam.) is a syncarp native to India, that grows in tropical regions mainly in Brazil, Bangladesh, Indonesia, Malaysia, Nigeria, Sri Lanka, and Philippines. It is composed of pulp and seeds (Gohain Barua & Boruah, 2004), seeds represents nearest 18% the fruit weight (Madrigal-Aldana et al., 2011). Generally, jackfruit is eaten raw and processed (canned juice and leather), and the latter are eaten after boiling, steaming and roasting (Madrigal-Aldana et al., 2011; Saxena; Bawa; Raju, 2011). However, most of the seeds are rejected as an agroindustrial waste (John & Narasimham, 1993; Kee & Saw, 2010).

Jackfruit is an exotic fruit, thus presenting an increasing of economic importance in tropical regions. Desrite this fact, only few studies have reported their properties and characterized their volatile fraction (Maia, Andrade, & Zoghbi, 2004; Nazaruddin, Seng, Hassan, & Said, 2006). Aroma is a key attribute in food development, but is considered a
complex matrix. In the last two decades over 500 volatile compounds for cocoa powder was described (Afoakwa, 2010). Furthermore the direct correlation of the gas chromatographic profile data of the aroma fraction has been arduous (Dimick & Hoskin, 1999). Nowadays cocoa is a noble commodity of high value, since it is a versatile raw material for endless applications. Thus, the development of natural replacer for chocolate could be a very innovative proposal.

Recently, Spada et al., (2017) chapter 1, showed that when jackfruit seeds are roasted, they impart a desirable chocolate aroma. Three different post-harvest processes were investigated. In the seeds, and although they all produced a chocolate-like aroma, seeds that were fermented in a similar way to the traditional cocoa fermentation process were ranked with higher notes in terms of sensory chocolate aroma. The aim of this study was to characterize the aroma compounds of flours prepared from the roasted jackfruit seeds and compare three different pre-treatments: i) where the seeds were simply drying and roasting, ii) where the seeds were acidified for five days prior to drying and roasting and iii) where the seeds were fermented using jackfruit pulp and banana leaves as a source of yeast. In addition, the roasted jackfruit flours were compared to a typical Brazilian chocolate.

3.2. MATERIALS AND METHODS

3.2.1. Chemicals

For SPE extraction, HPLC grade methanol was purchased from Merck Ltd. and methyl acetate, sodium sulphate and HPLC grade water from Fisher Scientific (Loughborough, UK). The 3-chlorophenol was purchased from Sigma–Aldrich Co. Ltd. For 1,2-dichlorobenzene (DCB) in methanol (130.6 µg/ml) and the alkane standards C$_6$–C$_{25}$ (100 µg/ml in diethyl ether) were obtained from Sigma–Aldrich Co. Ltd.

The following compounds were obtained from Sigma Aldrich, Poole, U.K.: 2-acetylpyrazine; 2-methoxy-3-methylpyrazine; 2,3,5,6-tetramethylpyrazine; furfuryl methyl disulfide; 2,4 heptadienal; 2,4 hexadienal; dihydro-beta-ionone; Methyl-2-methyl-3furfuryl disulfide; (E,E)-2,6 nonadienal; (E,E)-2,4 nonadienal; 2,3-butanediol; dimethyl disulfide; ethyl 2-methylbutanoate; ethyl 3-methylbutanoate; furfuryl acetate; R-(+) limonene; S-(−)Limonene; methyl hexanoate; benzeneacetic acid, ethyl ester; guaiacol; benzenepropanol;
2-ethyl-3methoxypyrazine; 2-isobutyl-3-methoxypyrazine; trimethylpyrazine; 2-ethyl-5(6)-methylpyrazine; 2-isobutyl-3-methylpyrazine; 2-methyl-3-propylpyrazine; 2-acetylpKyle; indole; 2-methoxy-4-vinylphenol, ethyldimethylpyrazines and 2,3-diethylpyrazine.

The 2,3-dimethylpyrazine; 2,3-diethyl-5-methylpyrazine; 2-ethyl-3,5(6)-dimethylpyrazine; 2-ethyl-3-methylpyrazine and metilpyrazine were obtained from Oxford Organics, Hartelpoole, U.K. Finally, 2-sec-butyl-3-methoxypyrazine and 2-isopropryl-3-methoxypyrazine from TCL and butyrolactone from Mane.

The unlab pyrazines used were: 2,5 (6) dimethyl-pyrazine; 3,5 dimethyl-2-methylpyrazine; 2-ethyl-5-methylpyrazine; 2ethyl-3,5-dimethylpyrazine; 2,5-diethyl-3methylpyrazine and 2-ethyl-3-ethyl-5-methylpyrazine; ethylpyrazine; 2-ethyl-3-methylpyrazine; 2-methyl-3-propylpyrazine; acetylpKyle Sigma Aldrich, Poole, U.K.

Sodium phosphate monobasic (Na₂H₂PO₄2H₂O) and Dibasic sodium (Na₂HPO₄2H₂O). Glucose, glicine, leucine, isoleucine and valine. All other chemicals were standard laboratory chemicals.

### 3.2.2. Jackfruit, seeds and procedures (dry, acidified or fermented)

The preparation of the roasted jackfruit seed flours were described in details in the study of Spada et al., (2017), chapter 1. Briefly, ripe jackfruits (Artocarpus heterophyllus Lam.) of the hard pulp variety were collected from one single tree from Nov 2014 – Jan 2015, and three different treatments were applied to their seeds. For dried jackfruit seeds (DJS), they were dried for 24 h in an oven at 60 °C with air circulation. After that time the spermoderms were manually removed and the seeds returned to the oven for a further 24 h at 60 °C. Portions of 200 g of dryied seeds were roasted in a rotary electric oven (Probat® laboratory sample roaster, Emmerich am Rhein, Germany) for 47 min at 171 °C. For acidified jackfruit seeds (AJS), the seeds were placed in polypropylene trays with a solution of 1% acetic acid for 5 days at 25 ± 3 °C, after that, the solution was removed and the acidified seeds were dried as for DJS and roasted at 180 °C for 40 min. For fermented jackfruit seeds (FJS), the seeds, pulp and banana leaves were placed in a closed bucket for three days to encourage anaerobic fermentation and alcohol production. For the next five days the bucket was opened and the fermentating mass was manually turned over daily to promote alcohol
oxidation and the acetic acid production. After eight days, the pulp and banana leaves were removed and the fermented seeds were dried as for DJS and roasted at 154 °C for 35 min. Finally, seeds portions (200 g) were ground to produce a flour using a hammer mill and stored at 4 °C without light.

Chocolate. A Brazilian cocoa powder (Cargill) which was fermented, not alkalized, and roasted was used for comparison.

3.2.3. Analysis of amino acids

Samples (100 uL) were derivatized using the EZ-Faast (Supelco, U.K.), method based in (Elmore, Koutsidis, Dodson, Mottram, & Wedzicha, 2005; Methven, Tsoukka, Oruna-Concha, Parker, & Mottram, 2007) and analysed by GC-MS. The free amino acid content of the jackfruit seeds flours was measured using the EZ-Faast amino acid derivatization technique for GC-MS (Phenomenex, Torrance, CA). The sample was measured into a 7mL vial. Hydrochloric acid (0.01 M) was added (5 mL) to the vial, and the sample was stirred for 15 min at room temperature. After stirring, the sample was allowed to settle for 45 min. An aliquot of supernatant (2 mL) was then centrifuged at 7200g for 30 min. One hundred microliters of the centrifuged supernatant was then derivatized. The EZ-Faast amino acid analysis kit was used to prepare derivatized amino acids for analysis by GC-MS. The preparation of a sample for GC-MS began with the addition of 20 nmol of norvaline internal standard, followed by a solid-phase extraction and then a two-step derivatization. The derivatized amino acids were extracted into isooctane/chloroform and analyzed in electron impact mode at 70 eV using the Clarus 500 GC-MS system. An aliquot of the derivatized amino acid solution was injected at 250 °C in split mode (5:1) onto a 10 m × 0.25 mm Zebron ZB-AAA capillary column. The oven temperature was 110 °C for 1 min, then increased at 30 °C/min to 320 °C, and held at 320 °C for 2 min. The transfer line was held at 320 °C, and the carrier gas flow rate was kept constant throughout the run at 1.1 mL/min. The ion source was maintained at 220 °C. Samples and standards were analyzed in triplicate. Standards of 19 nonbasic amino acids (Ala, -Ala, Aaba, Gaba, Asp, Glu, Gly, His, Ile, Leu, Lys, Met, Orn, Phe, Pro, Ser, Thr, Val) in 0.1 M hydrochloric acid and 3 basic amino acids (Asn, Gln, and Try) in water were prepared. A calibration curve was plotted for each amino acid, and the
gradient of this curve was used to calculate the amount of each amino acid in the three foods and the cakes prepared from them. A specific mass spectral fragment ion was chosen for quantification of each amino acid. The area of this ion in the peak of each amino acid was measured relative to the area of the m/z 158 ion of norvaline.

3.2.4. Extraction of volatiles by solid-phase-microextraction (SPME)

Extraction was based on the method reported by Spada et al (2017) Chapter 1. For the roasted jackfruit seed, flour (3.0 ± 0.1 g) was added to 6 mL of Milli-Q water (1.18µS/cm) in a 20 mL vial. For chocolate samples, a slightly different ratio was used because on cocoa powder the proportion used to jackfruit produced a solid chocolate (1.0 ± 0.1 g) was added to 6 mL water. For quantification method I, 1,2-dichlorobenzene in methanol (10 µL of 130.6µL/mL) was added as the internal standard. For specific quantification of pyrazines method II, 2,5-Dimethyl-d6-pyrazine; 3,5-Dimethyl-2-methyl-d3-pyrazine; 2-Ethyl-d5-5-methylpyrazine; 2-Ethyl-d5-3,5-dimethylpyrazine; 2,5-Diethyl-3-methyl-d3-pyrazine and 2-Ethyl-d5-3-ethyl-5-methylpyrazine were used by standard. After equilibration at 45 °C for 15 min, the fiber (a 50/30 µm divinylbenzene/carboxen on polydimethylsiloxane fiber, Supelco®, U.K.) was exposed to the headspace above the sample for 55 min, under magnetic agitation (635 rpm). These extraction conditions had previously been optimized and validated using surface response methodology.

3.2.5. GC-MS analysis of SPME extracts.

The volatile compounds extracted by SPME were analyzed on an Agilent HP5890 Series II chromatograph equipped with a ZB-wax column (Phenomenex® 30 m x 0.25 mm x 0.25 µm film thickness) and a 5972 MS detector. The injector and detector temperatures were maintained at 250 °C. During desorption, the oven was held at 40°C. The oven was maintained at 40 °C for a further 1 min and then the temperature was raised at 5 °C/min to 250 °C. MS was carried out using 70 eV electron impact, and m/z were monitored in the range 33 to 500, in scan mode. Helium was the carrier gas and the flow rate was 1 mL/min in constant flow. A series of n-alkanes C6–C30 was analyzed under the same conditions to
obtain linear retention indices (LRIs) for comparison with authentic samples, reaction mixtures, and in a few cases, the literature values. Each sample was analyzed three times. Identities were confirmed by running the jackfruit flours and the reaction mixtures on an FFAP column and on a DB5 column.

### 3.2.6. Approximate quantification of SPME extracts using DCB.

For all compounds, the peak area was approximated using the area of a characteristic m/z which was multiplied by a factor calculated from the spectrum obtained from the authentic standard when possible or, for a few compounds, from the library spectrum. For all compounds, except the pyrazines, the approximate relative concentration was obtained by comparing the peak area against that of the internal standard (1,2-dichlorobenzene), using 1 as a response factor.

### 3.2.7. Extraction by solid phase extraction (SPE)

Jackfruit seed flour or chocolate powder (10 g ± 0.1) was mixed with 35 mL µlQ® water (1.18µS/cm) and the internal standard 3-chlorophenol (100 µl of 1 mg/mL in 10% methanol/water) was added. The mix was stirred at 40 °C for 40 min and then centrifuged in polypropylene conical VWR centrifuge tubes at 9.500 rpm (g) for 20 min at 4 °C in a Sigma Laborzentrifugen (3K10 Howe, U.K.) centrifuge. The supernatant was filtered and used for solid-phase extraction (SPE). PolymericStrata–33µX™ reverse phase 500 mg/12 mL Giga tubes (Phenomenex U.K.) were conditioned with methanol (10 mL), followed by 10 mL of HPLC water. The flour extracts were loaded, using a slight vacuum (100 mbar), ensuring that the sorbent was never allowed to dry out. After the passage of the sample, the sorbent was washed twice with 10 mL of HPLC grade water and dried under vacuum for 1 hour. The semivolatile compounds were eluted with 10 mL of methylacetate and collected in glass tubes. The solution was dried with Na₂SO₄ and the volumes of the samples were adjusted to approximately 1 mL by evaporation under a gentle stream of nitrogen.
3.2.8. GC-MS analysis of SPE extracts

An aliquot of 1µL of the extract (dried, acidified and fermented) was introduced onto the column using a splitless injection and analyzed using the same conditions as for the SPME extract. The approximate quantification of SPE extracts was performed using 3-chlorophenol. The method was identical to the method I, except that internal standard was the 3-chlorophenol. The quantity of selected compounds was approximated from the GC peak areas, by comparison with the peak area of the 3-chlorophenol standard, using a response factor of 1.

3.2.9. GC-O analysis

The SPME device was inserted into the injection port of an Agilent HP5890 Series II ODO 2 (SGE) GC–O system equipped with a ZB-wax column (Phenomenex® 30 m x 0.25 mm x 0.25 µm film thickness). The outlet was split 1:1 between a flame ionisation detector and a sniffing port. The contents of the SPME fibre were desorbed for 3 min in a split/splitless injection port, in splitless mode, onto five small loops (5 cm diameter) of the column in a coil, which were cooled in solid carbon dioxide, contained within a 250 mL beaker. The injector and detector temperatures were maintained at 250 °C. During desorption, the oven was held at 40°C. After desorption, the solid carbon dioxide was removed from the oven. The oven was maintained at 40 °C for a further 1 min and then the temperature was raised at 5 °C/min to 250 °C, and held for 5 min. Helium was the carrier gas and the flow rate was 2.5 ml/min. Four assessors were used for the detection and verbal description of the odor active components of extracts. Each odor was scored on a seven-point line scale (2-8) where 3=weak, 5=medium and 7=strong. The n-Alkanes C_6–C_{30} were analyzed under the same conditions to obtain linear retention index (LRI) values for comparison with the GC–MS data.

3.2.10. Reactions mixtures.

Glucose (0.1 mol L^{-1}), glycine (0.1 mol L^{-1}) and a second amino acid (0.5 mol L^{-1}) in phosphate buffer pH 9 (50 mL) were placed in a 100 mL Duran bottle and heated in an
autoclave (Maker U.K.) at 125 °C for 30 min. The amino acids were either valine (RM1), isoleucine (RM2) or leucine (RM3).

3.2.11. Statistics

The XLStat was used to perform ANOVA on the four samples and Fisher’s least square difference (LSD) was calculated at p<0.05 by Tukey test.

3.3. RESULTS AND DISCUSSION

3.3.1. The influence of different pre-treatments on the amino acid content

The table 1 shows that using a fermentation process similar to that used for cocoa products, an increase in free amino acids was found, particularly glycine, valine, alanine, leucine and isoleucine, but interestingly not phenylalanine. This is also true when the seeds were treated with acid, where a raise was observed in most cases, but usually with a slightly less increase compared to that found in fermented seed flour.

Fermentation and acidification processes improved the free amino acids concentration (Table 1), Maillard reaction is an important event for in flavour development, especially during in roasting, where free amino acids, peptides and reducing sugars all participate (Rohan and Stewart, 1967). During roasting the hydrophobic amino acids released by proteinase activities during fermentation, are important precursors, of the Maillard reaction. Reducing sugars, fructose and glucose, which are derived from sucrose hydrolysis are also key precursors for the Maillard reaction.

3.3.2. Identification of volatile compounds

About 130 compounds were reported in the jackfruit seed flours. Of these, of 77 had their identity confirmed by comparison with an authentic standards run under similar conditions. Sixteen of them were identified by comparison with the literature data, but in most cases these compounds were isomers of those that had been identified or pairs of
isomers where the order of elution was not determined. A further 21 pyrazines were compared with those generated in reaction mixtures that contained glucose, glycine and a third amino acid, providing evidence of the nature of the side chains (Table 1).

3.3.3. Identification of Pyrazines

Pyrazines are formed from the reaction between two amino ketones that are derived from highly reactive dicarbonyl compounds such as glyoxal and methylglyoxal. These intermediates are formed from the breakdown of glucose, initiated by an amino acid, in a complex reaction known as the Maillard reaction. They can be formed from glucose and glycine alone, and in this case, pyrazine, methylpyrazine and diethylpyrazines are the major products. However, side chains can also be introduced onto the pyrazine ring by incorporation of an aldehyde, which can react with a dihydropyrazine in a mechanism described by as the “x+x+y” mechanism where x refers to the α-dicarbonyl or hydroxycarbonyl (2-amino carbonyl species) and y to an aldehyde, and there is evidence of this occurring in model systems (Yaylayan, 2003) and in real foods (Methven et al., 2007). In Maillard reaction these aldehydes can be generated from the Strecker degradation of the corresponding amino acid, thus, the Strecker aldehyde 2-methylpropanal will be generated by incorporation of valine into a glucose/glycine system, and the incorporation of this into the pyrazine molecule will produce pyrazines with a 2-methylpropyl substituent. Likewise isoleucine will produce 2-methylbutyl substituents via 2-methylbutanal and leucine will produce 3-methylbutyl substituents. Thus, for many of the unknown pyrazines, we have additional information about the precursors, which when considered along with the mass spectrum and the relative positioning on both DB5 and DBWax columns, provides more certainty to the identification.

Pyrazine, methylpyrazine, and the C2 pyrazines (ethylpyrazine and dimethylpyrazines) were all confirmed against authentic standards. The C3 pyrazines (propylpyrazine, ethylmethylpyrazines and trimethylpyrazines) were all confirmed against authentic standards, except the order of elution of 2-ethyl-5-methylpyrazine and 2-ethyl-6-methylpyrazine, with was determined from the literature (Hwang, Hartman, Rosen, & Ho, 1993) since as the authentic standard was supplied as a mixture. 2-Methyl-6-propylpyrazine
was identified by comparison with a pair of isomers, with the 2-methyl-5-propylpyrazine being found 10 LRI units later.

The C4 pyrazines tetramethylpyrazine and 2,3-diethylpyrazine were compared against authentic standards. The 2,5 dimethyl-3-ethylpyrazine and 3,5 dimethyl-2-ethylpyrazine were compared against a mixture of these isomers and the order was assumed from the literature, since they may coelute with the third isomer 2,3-dimethyl-5-ethylpyrazine.

Two of the C5 diethylmethylpyrazines were compared to authentic standards (2,3-diethyl-5-methylpyrazine and 3,5-diethyl-2-methylpyrazine) and a compound coeluting with the former was tentatively identified as the third isomer 2,5-diethyl-3-methylpyrazine. Three isomers with a strong m/z of 108 were found, which were also found in RM1 that contained valine. The identity of 2-methyl-3-isobutylpyrazine was confirmed by comparison with a standard synthesized in our lab (Elmore, Parker, Halford, Muttucumaru, & Mottram, 2008) and the two peaks with similar spectra were tentatively identified as the 2-methyl-5/6-isobutylpyrazine isomers.

The C6 isomers with a strong m/z of 108 were a mixture of methyl-2-methylbutylpyrazines and methyl-3-methylbutylpyrazines (6 isomers), which were distinguished through and the mass spectra and the RM2 and RM3.

3.3.4. Influence of different pretreatments on volatile profile

3.3.4.1. Strecker aldehydes

The Strecker aldehydes are key compounds for chocolate or cocoa aroma Spada et al., (2017) (chapter 1, Supplementary table 2) reported that, particularly 3-methylbutanal, imparts a cocoa aroma. This compound was significantly higher in chocolate compared to the three jackfruit seed flours, as was phenylacetaldehyde which gives a honey rose note, also important for chocolate aroma. However, despite the fact that leucine, the precursor for 3-methylbutanal, was found two fold higher in FJS and AJS compared to DJS in unroasted flours, the same trend was not observed in roasted flours, suggesting that it is not the free amino acid leucine that is limiting the formation of this key compound. There was very little difference in terms of Strecker aldehydes amoung the jackfruit seed flours. However,
Strecker aldehydes are reactive, and participate in further reactions such as aldol condensation, pyrazine formation and oxidation. Oxidation to the corresponding 3-methylbutanoic acid provides the precursor for the 3-methylbutanoate esters.

### 3.3.4.2. Aldol condensates

The aldol condensate removes many of the short-chain aldehydes generated in intermediate stages of Maillard reaction, particularly the Strecker aldehydes, which are responsible for colour improvement and specific flavors, in cocoa (Crafack et al., 2014), cocoa substitutes (Fadel, Abdel Mageed, Abdel Samad, & Lotfy, 2006) and cooked potato (J. S. Elmore et al., 2010; J. Stephen Elmore et al., 2008). In this study, ad1, ad2, ad3 and ad4, which are all derived from 3-methylbutanal/leucine, were all significantly higher in chocolate, and this is consistent with the fact that 3-methylbutanal was also significantly higher in chocolate. Aldol condensates ad3 and ad4 were also found in RM3. The increase of the two aldol condensates derived from phenylacetaldehyde (2-phenyl-2-butenal and 5-methyl-2-phenyl-2-hexenal) in chocolate was less substantial, despite the fact that phenylacetaldehyde was five times higher in chocolate compared to jackfruit seed flours. 5-Methyl-2-phenyl-2-hexenal has a strong chocolate-cocoa flavor and it is also reported as being a key constituent of chocolate aroma (Crafack et al., 2014; Fadel et al., 2006). The 5-methyl-2-phenyl-2-hexenal is produced from phenylacetaldehyde and isovaleraldehyde in an aldol condensation reaction, potentialized in pH between seven and eight (Lindsay, 1985).

### 3.3.4.3. Pyrazines

Most pyrazines are generated during thermal processing of food, at temperatures > 100 °C (J. K. Parker, 2015). Pyrazines are nitrogen-containing heterocyclic intermediates of the Maillard reaction, and they are predominantly formed through Strecker degradation reactions between amino acids and α-dicarbonyls (Guerra & Yaylayan, 2010). In cocoa pyrazines and ester are the two major groups of volatile compounds (Jinap et al., 1998). Trimethylpyrazine is one of the main components of cocoa aroma, and is responsible for the nutty, roasted, and chocolate flavor notes (Afoakwa, 2010; Liu et al., 2015; Owusu, Petersen,
Rodriguez-Campos (2011) identified tetramethylpyrazine and trimethylpyrazine in cocoa processes. Also 2,3 diethyl-5methylpyrazine; 2ethyl-3,5-dimethylpyrazine; 2 ethyl-3,6-dimethyl pyrazine; 2-isobutyl-3-methylpyrazine and 2,5(6)-dimethylpyrazine were describe as important compounds to improve chocolate aroma in cocoa samples (Bonvehí, 2005; Frauendorfer & Schieberle, 2008; Liu et al., 2015; Owusu et al., 2012; Schnermann & Schieberle, 1997).

Every one of these pyrazines was also found in roasted jackfruit seeds flours. Tetramethylpyrazine was found in higher concentration in fermented and roasted jackfruit seeds in comparison to chocolate, dried, and acidified jackfruit seeds flours. The content of 2-Ethyl-3,5-dimethylpyrazine and 2 isobutyl-3-methylpyrazine found in fermented samples were more similar to that found in chocolate, because in dried and acidified seeds these concentrations were higher levels. However, 2-ethyl-3,6-dimethylpyrazine; and 2,5(6) dimethyl pyrazine were lower in fermented flours in comparison to chocolate, and dried and acidified jackfruit seeds. Maybe, jackfruit seed fermentation could be improved using microorganisms specifics to cocoa fermentations, for exemple *Kluyveromyces marxianus e Saccharomyces cerevisiae*.

1st group: C2 substituted pyrazines MWt 108. There was a tendency for these pyrazines (dimethylpyrazines and ethylpyrazine) to be higher in the AJS and DJS flours compared to FJS. These are relatively “simple” pyrazines, which in the most cases derive their substituents from the reducing sugars that are involved in the Maillard reaction (x + x mechanism, Low). The participation of other aldehydes is minimal (Mei, Parker, & Mottram, 2007). Since FJS flour had more precursors than the AJS or DJS, this drop in pyrazines may be a reflect on of the less severe processing conditions applied during the roasting of FJS.

2nd group: C3 substituted pyrazines MWt 122. Similar to those in group 1, the ethylmethylpyrazines and propylpyrazine were all significantly lower in FJS compared to the other three flours. However, there were no significant differences in trimethylpyrazine among the samples, suggesting that trimethylpyrazine levels are not driven by time and temperature, and other events may be limiting its formation.

3rd group: C4 substituted pyrazines MWt 136. The trends in these C4 pyrazines were all different reflecting different formation pathways and / or precursors. This group of pyrazines, especially the trisubstituted ones, are often related to chocolate aroma and of
these, 2-ethyl-3,5-dimethylpyrazine is the most often reported. There was no significant difference between the jackfruit flours for this compound. Tetramethylpyrazine had a major concentration in fermented seeds, despite the fact that this sample was subjected to milder thermal process.

4th group. C4 and C5 pyrazines MWt 120 or 134. These pyrazines with an unsaturated substituent, such as many of those in groups 2 and 3, were significantly lower in FJS. The proposed pathways for their formation do not involve additional aldehydes, and the carbon skeleton is likely to be derived from the reducing sugars. They are not generally reported to be important in chocolate aroma.

5th group: C5 substituted MWt 150. 2,3-diethyl-5-methylpyrazine and 2,5-diethyl-3-methylpyrazine belong to the group of pyrazines that are consistently lower in FJS and seem to be controlled by the roasting conditions. Those with other trends across the set, tend to be those where one of the substituent is derived from an aldehyde (x + x + y mechanism Low) and the formation pathways are different and dependent upon the presence of the aldehydes. Another set of C5 pyrazines are the methyl-2-methylpropylpyrazines, with a substituent derived from valine. They were all significantly higher in DJS and AJS, lower in FJS, possibly due to the application of low thermal process, which are even and very lower temperatures in the in chocolate production. However, these compounds are not amongst those reported to be contributing to chocolate aroma.

6th group: C6 substituted MWt 164. There are many pyrazines in this group, since they can be dimethyldiethylpyrazines, dimethylpyrazines with an extra 2-methylpropyl substituent (or ethyl equivalents), or they can be methylpyrazines with either a 2-methylbutyl or a 3-methylbutyl substituent. Those in the first group are not fully characterised and have only been identified very tentatively. However, with the use of the reaction mixtures RM1, RM2 and RM3, we have been able to assign the identities of the others with more confidence. However, the overall picture for the aldehyde-substituted pyrazines is very clear and all of them were significantly higher in DJS and AFS, and FJS much lower in chocolate and due to low roasting conditions. These are not major compounds of chocolate, and seem to be typical of jackfruit seed flours.

7th Group: C7 MWt 178. Again this is a big group of pyrazines covering those that are dimethylpyrazines with a methylbutyl substituent (or corresponding ethyl equivalents).
We have tentatively identified them with RM2 and RM3. The trends are similar to group 6, and it is clear that these compounds are typical of roasted jackfruit seeds.

### 3.3.4.4. Pyrroles

Pyrroles are found in most cooked foods, being typically associated with roasted, cooked and burnt flavours generated in the Maillard reaction. 5-Methyl-1H-pyrrole-2-carboxaldehyde and 2-acetylpyrrole were not significantly (p≤0.05) different between samples, and the latter was the major pyrrole in all samples.

Several specifics volatiles are produce by specific microorganisms in Brazilian conditions. For example, *Bacillus cereus* in cocoa fermentation, under specific growth conditions: temperature (35°C), nitrogen sources (proline and glutamic acid); and glucose (Adams & Kimpe, 2006). During FJS production the temperature was near 35°C at the end of the fermentation process (Spada et al., 2017) Chapter 1, and glutamine and high percentage of glucose were present in the jackfruit pulp utilized during jackfruit seeds fermentation (Saxena et al., 2011). The odors identified for the 2-acetylpyrrole are those of chocolate and hazelnut, which that culminate with the sensory perception of sweet chocolate (Aprotosoaie, Luca, & Miron, 2016).

The 1-(3-methylbutyl) pyrrole was found in the same concentration in DJS, AJS and cocoa powder. The others pyrroles were more concentrated in DJS and AJS. Elmore et al., (2008) found that 1-(3-methylbutyl), 1-(2-methylbutyl) pyrrole and 1-(2-methylpropyl) pyrrole were responsible for aroma in cooked wheat. These pyrroles derive their substituent from the respective amino acid, but unlike the pyrazines, they are also present in chocolate.

### 3.3.4.5. Maillard-derived furans

The Maillard derived furans showed small changes among the four flours. Bonvehí, (2005) reported for the 2-acetylfuran an almond aroma; for 2-acetylfuran a sweet, balsamic, and a slightly coffee aroma for 5-methylfurfural sweet caramel and chocolate aroma. Thus, these compounds are relevant to provide the characteristic chocolate flavour. Dried and acidified jackfruit seed flours had similar (p≤0.05) concentrations for all Maillard-derived furans: 2-acetylfuran and 2-furanmethanol had equal concentration (p≤0.05) for all flours.
Ito & Mori, (2004) and Ledl & Schleicher, (1990) described the influence of heating and pH in Maillard-derived furans. For 2-furanmethanol, the optimal pH was six. The pH of the flours was between 4.7 and 5.6, and probably this fact was responsible for the same concentration of 2-furanmethanol indifferent jackfruit flours. The yield of this compound decreased at higher pH whereas pyrazine formation was remarkably increased (Ito & Mori, 2004). The any others Maillard-derived furans related in this study could be supply off-odor-volatile (Aprotosoaie et al., 2016).

3.3.4.6. Sugar-derived compounds

These are Maillard reaction derived compounds and important to impart chocolate aroma. They tended to be higher in jackfruit seed flours, but were found in qualities below the threshold of the GC-MS, however furaneol was detected by GC-O.

3.3.4.7. Lipid-derived aldehydes

Jackfruit seeds are very low in fat compared to cocoa beans (Spada et al., 2017) Chapter 1, and in general, very few lipid-derived volatiles were detected in the jackfruit seeds. All lipid-derived aldehydes were greater in dried jackfruit flour. Hexanal is formed from the oxidation of w-6 fatty acids, and can also be thermally derived from 2,4-decadienal (Zamora, Navarro, Aguilar, & Hidalgo, 2015), they found that the best conditions to produce hexanal using 2,4-decadienal was at 120°C and pH between 6 and 8. The highest roasting time and temperature used for DJS and AJS may also increase thermal oxidation and could be responsible for the higher hexanal levels in these two flours compared to FJS and chocolate. Generally, lipid-derived aldehydes are associated with floral, fruity, green beans and cut-grass descriptions depending on the concentration (J. Stephen Elmore et al., 2008; Prescott & Monteleone, 2015) In chocolate, the key lipid-derived compounds are the lactones (Spada et al., 2017) Chapter 1 in supplementary table 2 and they impart sweet creamy, coconut and peach aromas particularly in milk chocolate. Lactones were not found in jackfruit seed flours.
3.3.4.8. Lipid derived furans

Furans are generated during drying and roasting processes (up to 100°C) in cocoa beans, via Maillard reaction or lipid oxidation. Some parameters such as moderate temperatures and high humidity levels favor the formation of these compounds (Rodriguez-Campos et al., 2011). For 2-pentylfuran the yield is just with lipid oxidation, the diene radical generated by the cleavage of 9-hydroxy linoleic acid may react with oxygen to produce vinyl hydroperoxide, which undergoes cyclisation via the alkoxy radical (Martin, Muriel, Antequera, Perez-Palacios, & Ruiz, 2008). The concentration of 2-pentylfuran was four and five times greatest in dried and acidified jackfruit seed flours, respectively, in comparison to cocoa powder. Whereas in fermented seed the concentration was just two times higher. The 2-pentylfuran is not common related to cocoa, buttery, green beans and pungent aroma are associated with 2-pentylfuran. However, it was related in Brazilian varieties of cocoa, in jackfruit seed flours the concentrations still smaller (64% average) in comparison Menezes et al., (2016).

3.3.4.9. Lactones

Lactones are usually characteristics compounds in fresh exotic fruits or in peaches. They are associated with sweet, creamy, flowery and fruity aroma. In this study butyrolactone was found in high concentration in cocoa powder; however that compound has not been previously identified in this product.

3.3.4.10. Esters

Esters are another family of volatile compounds that are important in chocolate flavour (Spada et al., 2017) (chapter 1 Supplementary table 2). Esters are fundamental for most of the fruit aromas; the ethyl group in ester is typical in ripe fruits, and in chocolate, this flavour represents fruity, floral and honey odors (Aprotosoaie et al., 2016). In this study esters were more concentrated in fermented roasted jackfruit seed flour and this may be attributed to the yeast metabolism. Basically there are two main factors for the rate of ester formation: acetyl-coA and fusel alcohol (2-and-3methylbutanols) concentration; as well as
the activity of enzymes activity. Levels of fatty acid, nitrogen and oxygen, in addition to the temperature are important, because all of them directly influenced ester synthesis by changing the levels of acetyl-coA. High levels of oxygen benefits the growing of yeast, thus the consumer of acetyl-coA by the yeast reduce ester formation; other limited factor is the formation of a high concentration of alcohols (Verstrepen et al., 2003). Furthermore, the increase in alcohols on fermented products will promote ester formation during roasting.

The concentration of ethyl acetate and ethyl butanoate in FJS flour were not different in comparison to cocoa powder. Although the ethyl (2 and 3) methylbutanoate were not found in cocoa powder by GCMS, they were detected by GC-O in all flours. Ethyl acetate and ethyl butanoate are responsible for the aroma of fruits (pineapple, apple and banana). Ethyl 2 and 3-methylbutanoate are more odour active and also characterized by fruity flavor (Afoakwa, 2010; Aprotosoaie et al., 2016; Jinap, Dimick, & Hollender, 1995; Rodriguez-Campos et al., 2011). Thus fermented jackfruit seed flour could improve the quality of chocolate aroma provided by the esters. The 2-phenylethyl acetate is important for chocolate aroma (Spada et al., 2017) Chapter 1, and it was significantly higher in FJS compared to chocolate, which had a significantly higher amount than AJS or DJS. In addition, C5 (amyl) acetates, associated with off-flavors (Bonvehi, 2005), were not found in jackfruit seeds flours.

3.3.4.11. Alcohols

The high production of alcohols could be explained by the fermentation. In this study, the alcohols a2 ,a3 and a4 were all significantly higher in fermented products (FJS and chocolate), particularly the phenylethanol which has been reported to be important in chocolate aroma (chapter 1; suplementary table 2; phenylethanol). In this study ethanol (a1) was not found in chocolate. In cocoa beans the high production of alcohols is a result of the sugar fermentation occurring in the pulp. Schwan & Wheals, (2004) reported 2,3-butanediol and 2-phenylethanol as the responsible compounds for high quality in cocoa products. Aculey et al., (2010) and Frauendorfer & Schieberle, (2008) indicated phenylethanol as a typical flowery and candy aroma in the sensory profiling of chocolate. Therefore, that could explain the chocolate flavour produced by roasted jackfruit seeds, particularly FJS.
3.3.4.12. Phenols

Important compounds in many foods, they impart smoky spicy notes. Ethylguaiacol and p-vinylguaiacol were significant higher in jackfruit seeds whereas guaiacol was higher in the fermented product. Acid compounds were also high in FJS as might be expected due to the fermentation process.

In summary the Strecker aldehydes and aldol condensates were found in jackfruit seeds at levels similar to those found in chocolate, although phenylacetaldehyde was much higher in chocolate. The pyrazine levels were very high in jackfruit seeds compared to chocolate, although the use of the less severe roasting conditions in FJS brought the levels in line with those of chocolate. In addition to those pyrazines already reported in chocolate, jackfruit seeds contain more pyrazines than it has ever been reported in cocoa, coffee, nuts etc. Pyrazines tended to have a substituent derived from a Strecker aldehyde, and were much higher in AJS and DJS compared to the other samples. The esters also tended to be much higher in the jackfruit compared to chocolate, particularly in FJS where the fermentation step would have provided additional alcohols for the formation of esters.

3.3.5. Amino acids and GCO-oflactometry

3.3.5.1. Aminoacids and pyrazines production

Leucine, isoleucine and valine with in the presence of glucose and glycine, produce pyrazines when submitted to similar temperatures to that used during the roasting jackfruit seeds. The amino acids precursors of flavor in cocoa are leucine, valine, alanine, isoleucine and phenylalanine (Kongor et al., 2016). All of them were found in jackfruit seeds (Table 1). But they were more concentrate in fermented and acidified samples. Leucine promoted the higher pyrazine production. In cocoa leucine and glucose yield aroma notes described as “sweet chocolate”; threonine, glutamine and glucose give “chocolate” notes when heated to 100°C, and valine and glucose heated to 180°C give a note described as “penetrating chocolate” (Dimick & Hoskin, 1999). Aspartic acid is the most referenced aminoacid to precursor of the cocoa flavour (Janek, Niewienda, 2016). In Jackfruit seeds aspartic acid was reduced in acidified and fermented seeds in comparison to the dried ones. That fact
indicates that biotransformation in fermented samples was higher. Fermentation and acidification improved the concentration of this aminoacid (Table 1).

All amino acids with a 3-methylbutyl sidechain are derived from 3-methylbutanal from the Strecker degradation of leucine. All with 2-methylbutyl sidechain are from 2-methylbutanal from all with 2-methylpropyl sidechains are from 2-methylpropanal depending on valine concentration. Aspartic acid is the most referenced responsible to cocoa flavour (Janek, Niewienda, 2016). In Jackfruit seeds it was reduced in acidified and fermented in comparison to dry seeds. That fact indicates the biotransformation in fermented samples was higher, because we found more chocolate aroma and also more valine concentration.

The most aromatic pyrazines (3-ethyl-2-methylbutyl pyrazine; 2-(2/3-methylbutyl)-3-methylpyrazine; 3-methylbutylpyrazine; trimethylpyrazine and 2-methylpropyl pyrazine) received the right notes when fermented and acidified (Table 2). They were described with green house, earthy, coffee, peanuts, soup, mushroom, green herbs and flowers aroma. Other five important aroma and compounds were honey, violets, fly spray and rose, all provided by the phenyl acetaldehyde, the aroma intensity was the same to jackfruit seeds and cocoa powder. The other one was 3-methylbutanoic, in this case Parmesan cheese aroma was similar to dried, acidified jackfruit seeds and cocoa powder; thus during the fermentation process there was an increase in that compound (Table 3).

In fermented we found more intensity aroma compounds in 3-ethyl-2-(methylbutyl) pyrazine and trimethylpyrazine. This is also found at odor-active compounds in cocoa and chocolate products to (Frauendorfer & Schieberle, 2008; Liu et al., 2015; Owusu et al., 2012). In fermented jackfruit seeds flours the intensity aroma of trimethylpyrazine “mushroom, earthy, odd cooked remains, peanuts” was 75% great in comparison to cocoa powder. Still in fermented samples the 3-methylbutanoic is a compound very important in cocoa samples (Frauendorfer & Schieberle, 2008; Liu et al., 2015; Owusu et al., 2012; Schnermann & Schieberle, 1997) 3-methylbutanoic is responsible to “parmesan cheese”, in jackfruit seeds flour this aroma was 30% more intense rather than cocoa powder.

The phenylacetaldehyde that gives a “honey, violets, fly spray and rose aroma”. It is important aroma in cocoa samples by (Frauendorfer & Schieberle, 2008; Liu et al., 2015; Owusu et al., 2012; Schnermann & Schieberle, 1997) and Bonvehi (2005) and it showed
similar aroma intensity to cocoa powder with dry, acidified and fermented jackfruit seeds flours. Fermented seeds had also “green” aroma provide by the methylpyrazine and 2,5 dimethyl pyrazine, which were higher or similar to cocoa powder.

Dried and acidified samples were similar amounts, thus the amino acid increase was not the main influence to improve the volatile intensity. Fermentative process is a complex reaction with the presence of microorganisms. Another reason for the different aroma intensity could be related to the roasting temperatures used in fermented seeds, since it was 20°C lower compared to that used in dried and acidified seeds. The use of high roasting temperatures in fermented samples may have resulted in sample burning.

3.4. CONCLUSION

This study characterized, for the first time, the aroma compounds present in roasted jackfruit seeds flours, this study was observed different profiles when the seeds were submitted to acidification and manly fermentation, typical to ester and aldehydes yield. The pyrazines production was also intense when the seeds were fermented, and these results suggest a considerable synthesis due to high amino acids concentration linkage to Maillard reactions and Strecker degradations. Some important compounds present in cocoa were increased in fermented sample and the active aromas were detected with more intensity. The aroma intensity was higher in fermented flour than typical cocoa powder.

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Table 1. Amino acids concentration (µmolg⁻¹) in jackfruit seeds.

|                        | Dry     | Acidified | Fermented |
|------------------------|---------|-----------|-----------|
| Alanine                | 10.5 ± 0.2c | 11.1 ± 0.2b | 13.8 ± 0.2a |
| Alpha-amino butiric acid | 0.1 ± 0.0a | 0.1 ± 0.0ab | 0 ± 0b      |
| Asparagine             | 40.8 ± 1.8a | 65.2 ± 4.3b | 58.7 ± 6.3a* |
| Aspartic acid          | 12.8 ± 0.2a | 10.3 ± 0.6b | 9.8 ± 0.8b  |
| GABA                   | 11.7 ± 0.6c | 14.2 ± 0.8b | 16.3 ± 0.6a* |
| Glutamine              | 2.2 ± 0.3a | 2.0 ± 0.2a | 1.4 ± 0.2b  |
| Glycin                 | 7.1 ± 0.2c | 10.3 ± 0.2b | 12.4 ± 0.4a* |
| Isoleucine             | 4.8 ± 0.2b | 6.1 ± 0.3a | 6.6 ± 0.2a*  |
| Leucine                | 6.2 ± 0.0c | 12.3 ± 0.2a | 11.5 ± 0.5b  |
| Lysine                 | 5.6 ± 0.3a | 7.0 ± 1.0a | 6.2 ± 0.8a*  |
| Ornithine              | nd      | nd        | 0.8 ± 0.2   |
| Phenylalanine          | 5.3 ± 0.4c | 9.3 ± 0.5a | 7.9 ± 0.5b  |
| Proline                | 7.8 ± 0.3b | 10.6 ± 0.8a | 10.0 ± 0.5a* |
| Serine                 | 8.1 ± 0.4b | 9.2 ± 0.6a | 9.1 ± 0.3ab  |
| Threonine              | 5.4 ± 0.2c | 8.1 ± 0.4a | 7.4 ± 0.1b  |
| Tryptophan             | 0.6 ± 0.1c | 1.2 ± 0.1b | 1.7 ± 0.1a*  |
| Tyrosine               | 6.0 ± 0.4b | 8.4 ± 1.0a | 7.1 ± 0.6ab |
| Valine                 | 8.7 ± 0.2c | 12.5 ± 0.3b | 14.2 ± 0.4a* |

Means with different letters within a row are significantly different at p < 0.05 Tukey test.

nd = not detected.
Table 2. Odour description and intensity of the volatiles compounds detected by GC-O in headspace of jackfruit seeds and chocolate.

| LRI experimental | LRI GCMS | LRI autentical | Aroma perception | Compound | Dry | Acid | Fer | Choc | JFS mean |
|------------------|----------|----------------|------------------|----------|-----|------|-----|------|----------|
| 1                |          |                |                  | Toasted  | 1598| 0    | 0   | 7    | 0        |
| 2                |          |                |                  | Green earthy | 1520| 5    | 3   | 0    | 3        |
| 3                |          |                |                  | Oven chips | 1794| 8    | 0   | 3    | 0        |
| 4                |          |                |                  | Minty white chocolate | 1619| 11   | 0   | 0    | 4        |
| 5                |          |                |                  | Fruity | 1770| 7    | 4   | 0    | 4        |
| 6                |          |                |                  | Jam scum | 1827| 0    | 11  | 0    | 4        |
| 7                |          |                |                  | Fruity | 1128| 3    | 2   | 7    | 0        |
| 8                |          |                |                  | Fruity | 1347| 7    | 4   | 0    | 4        |
| 9                |          |                |                  | Broth  | 1799| 6    | 6   | 0    | 4        |
| 10               |          |                |                  | Earthy green | 1380| 8    | 5   | 0    | 4        |
| 11               |          |                |                  | Coffee peanuts | 1436| 8    | 3   | 0    | 8        |
| 12               |          |                |                  | Tropical fruit / Honey | 1715| 10   | 0   | 4    | 0        |
| 13               |          |                |                  | Jack fruit seeds/ Soap | 1654| 9    | 5   | 0    | 5        |
| 14               |          |                |                  | Mint terpene | 1406| 10   | 0   | 4    | 13       |
| 15               |          |                |                  | Hot dry | 1545| 15   | 0   | 0    | 5        |
| 16               |          |                |                  | Earthy green | 1935| 4    | 11  | 0    | 5        |
| 17               |          |                |                  | Rotting veg / savoury | 1078| 6    | 6   | 0    | 6        |
| 18               |          |                |                  | Sweet fruity juicy | 1417| 10   | 9   | 0    | 6        |
| 19               |          |                |                  | Faecal  | 1833| 10   | 9   | 0    | 6        |
| 20               |          |                |                  | Fruity melon | 976| 3    | 9   | 10   | 8        |
| 21               |          |                |                  | Fatty fruit | 1395| 8    | 6   | 8    | 7        |
| 22               |          |                |                  | Flowers  | 1643| 0    | 12  | 10   | 0        |
| 23               |          |                |                  | Fruity | 1237| 6    | 6   | 12   | 0        |
| 24               |          |                |                  | Fruity | 1178| 8    | 9   | 9    | 0        |
| 25               |          |                |                  | Green savoury | 1258| 16   | 10  | 0    | 9        |
| 26               |          |                |                  | Citrus orange, fruity | 1271| 12   | 14  | 0    | 0        |
| 27               |          |                |                  | Fried but also one green earth pyr | 1415| 9    | 11  | 7    | 0        |
| 28               |          |                |                  | Odd viney fruity poder | 1368| 10   | 10  | 9    | 11       |
| 29               |          |                |                  | Acid fruity | 1060| 8    | 12  | 10   | 0        |
| 30               |          |                |                  | Earthy green | 1423| 11   | 9   | 11   | 5        |
| 31  | 1815 | 1794 | 1814 | Oven chips | 2,4-decadienal | 12 | 0 | 19 | 0 | 10 |
| 32  | 1244 |   1 |   1 | Bread     | 1-(2-methyl/propyl)pyrrole | 10 | 10 | 12 | 14 | 11 |
| 33  | 1414 | 1412 |       | Fruity   | nutty/Earthly/green | soup/peanuts | 2-ethyl-3,4,5-trimethylpyrrole | 18 | 14 | 0 | 12 | 11 |
| 34  | 1410 |       |       | Peanuts  | 2,6-diethylpyrazine | 12 | 12 | 8 | 0 | 11 |
| 35  | 1527 | 1529 |       | Earthy green | 2,3-diethyl-5,6-dimethylpyrazine | 11 | 10 | 11 | 11 | 11 |
| 36  | 1691 | 1674 |       | Fried, oven chips | EE-2,4-nonadienal | 9 | 10 | 13 | 0 | 11 |
| 37  | 998  |       |       | Fruity   | ester (nl) | 3 | 15 | 15 | 7 | 11 |
| 38  | 1317 |       |       | Soup     | Furan sulfide | 10 | 17 | 6 | 0 | 11 |
| 39  | 1487 | 1489 |       | Jack fruit seeds/Carbolic soap cardboard | 2-methylpropylpyrazine | 6 | 18 | 9 | 0 | 11 |
| 40  | 1553 | 1553 |       | Jack fruit seeds, soap, cardboard | 2-ethylpropylpyrazine | 12 | 13 | 8 | 0 | 11 |
| 41  | 1574 | 1578 |       | Fly spray | floral violet | 6-methylhepta-3,5-dien-2-one | 13 | 20 | 0 | 2 | 11 |
| 42  | 1808 | 1800 | 1818 | Jammy, brown fruit | 2-phenylethyl acetate (b-damascenone) | 12 | 10 | 11 | 4 | 11 |
| 43  | 1309 | 1307 |       | Soup, savoury | 2-methyl-3-furanthiol | 18 | 6 | 10 | 7 | 11 |
| 44  | 1106 |       |       | Green    | nl          | 12 | 10 | 12 | 10 | 11 |
| 45  | 1240 | 1248 |       | Fatty, lamb fat | 4-heptenal | 11 | 11 | 12 | 0 | 11 |
| 46  | 1413 |       |       | Oil      | nl          | 10 | 12 | 12 | 0 | 11 |
| 47  | 1515 | 1517 |       | Fried oven chips | 3-methyl-(2-methylpropyl)pyrazine | 13 | 12 | 9 | 0 | 11 |
| 48  | off  | 907 | 822  | Malty cocoa | 2-methylpropanal | 17 | 11 | 8 | 16 | 12 |
| 49  | 1483 | 1484 |       | Green earthy | 3,5-dimethyl-2-methylpyrazine | 19 | 11 | 6 | 0 | 12 |
| 50  | 1487 |       |       | Butter/oil | nl          | 12 | 12 | 12 | 5 | 12 |
| 51  | 1523 | 1512 |       | Waxy, fatty | fresh laundry, makeup | 2-nonanenol | 13 | 13 | 11 | 0 | 12 |
| 52  | 1609 | 1614 |       | Hot dry   | 2-(3-methylbutyl)-methylpyrazine | 21 | 7 | 9 | 17 | 12 |
| 53  | 1597 |       |       | Cheese    | 2-methylpropanoic acid | 12 | 14 | 12 | 0 | 13 |
| 54  | 1079 | 1075 | 1063 | Green    | hexanal  | 9 | 11 | 20 | 4 | 13 |
| 55  | 1504 |       |       | Makeup    | nl          | 14 | 14 | 14 | 0 | 14 |
| 56  | 1064 | 1063 | 1050 | Fruity, Pineapple | Ethyl-3-methylbutanoate | 16 | 17 | 12 | 3 | 15 |
| 57  | 1187 | 1184 | 1182 | Fruity   | 2-methylpropyl 3-methylbutanoate | 18 | 14 | 14 | 11 | 15 |
| 58  | 1315 | 1314 | 1309 | Greenhouse earthy, coffee | 2,5-dimethylpyrazine | 24 | 22 | 0 | 0 | 15 |
| 59  | off  | 1063 |       | Fruity vegetal, poo | Methaenthiol/carbon disulfide | 18 | 17 | 12 | 15 | 16 |
| 60  | 1494 | 1495 |       | Jack fruit seeds, carbolic soap Cardboard sheets | 2-methylpropylpyrazine | 22 | 19 | 9 | 0 | 17 |
| 61  | 1390 | 1394 | 1396 | Mushroom, earthy, odd cooked Remains, peanuts | Trimethylpyrazine | 13 | 18 | 24 | 6 | 18 |
|   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|
| 62 | 1511 | 1512 | Green earthy | 3-methylbutylpyrazine | 20 | 23 | 13 | 10 |
| 63 | 1440 | 1454 | Potato | Methional | 20 | 19 | 20 | 20 |
| 64 | 1619 | 1619 | Fried oven chips, bread | 2-(2/3-methylbutyl)-3-methylpyrazine | 17 | 22 | 20 | 10 |
| 65 | 1636 | 1634 | Green herbs flowers | Ethyl-3-(2-methylbutyl)pyrazine | 20 | 16 | 23 | 12 |
| 66 | 909 | 911.0 | 925/928 | Malty cocoa | 2/3-methylbutanal | 22 | 19 | 20 | 17 |
| 67 | 1653 | 1653 | Broth/Meaty/Soup | 2-methyl-3-furanmethyl disulfide | 20 | 21 | 20 | 19 |
| 68 | 979 | 972 | 956 | Butter | 2,3-butanedione | 22 | 20 | 21 | 20 |
| 69 | 1048 | 1046 | 1032 | Fruity/Strawberry | Ethyl-2-methylbutanoate | 20 | 23 | 24 | 14 |
| 70 | 1435 | 1427 | 1427 | Vinegar | Acetic acid | 21 | 23 | 27 | 0 |
| 71 | 1100 | no | Stench beer headspace | 3-methyl-2-butene-1-thiol | 23 | 23 | 26 | 10 |
| 72 | 1628 | 1624 | 1624 | Honey/Violets/Fly spray/Rose | Phenylacetaldehyde | 25 | 24 | 25 | 24 |
| 73 | 1300 | 1302 | Raw mushroom/Metallic | 1-octen-3-one | 23 | 26 | 28 | 10 |
| 74 | 1658 | 1666 | 1645 | Parmesan cheese | 3-methylbutanoic | 26 | 25 | 31 | 22 |
| 75 | 1363 | 1360 | 1354 | Pickled onion | Dimethyl trisulfide | 33 | 29 | 29 | 32 |

ni = not identified. Off – out range. Acid – acidified; Fer – fermented; JFS – Jackfruit seeds.
Table 3. Amounts of Volatile Compounds Identified in Headspace of Jackfruit seeds flours and cocoa powdermg100g

| Compound                  | Code | Wax | ID | Dry | Acidified | Fermented | Chocolate | LSD | Significance |
|---------------------------|------|-----|----|-----|-----------|-----------|-----------|-----|--------------|
| **Alcohols**              |      |     |    |     |           |           |           |     |              |
| Etanol                    | a1   | 936 | 928|     | nd        | 30.9 ± 4.7a | 26.3 ± 3.2a | nd  | 7.49         | ***           |
| 2,3-butanediol            | a2   | 1573 | 1570|     | A         | 4.5 ± 0.6b  | 3.9 ± 0.4b  | 37 ± 13.8a | 50.3 ± 8.1a | 20.88         |
| Phenylethanol             | a3   | 1898 | 1909|     | A         | 38 ± 4.8c  | 29.7 ± 1.1c | 47.9a | 24.6b        | 70.74         |
| Benzeneopropanol          | a4   | 2028 | 2029|     | A         | 2.6 ± 2.7b | 1.6 ± 2.7b | 27.8 ± 12.7a | 44.7 ± 1.7a | 17.44         |
| **Aldol condensates**     |      |     |    |     |           |           |           |     |              |
| 2-isopropyl-2-butenal? (3MB + Ac) | ad1 | 1167 | 1192|     | A         | 28 ± 2.7b  | 1.5bc      | 17.4 ± 1.7c | 57 ± 6.9a   | 10.16         |
| 5-methyl-2-hexenal (3MB + Ac) | ad2 | 1248 | 1271|     | A         | 23.8 ± 3.7b| 16.3 ± 2.9b| 9.3 ± 1.2b | 74.4 ± 11.2a | 15.86         |
| 6-Methyl-2,3-heptanediol (3mB + HP) | ad3 | 1268 |     |     | T         | 9.3 ± 1.7b | 8.1 ± 0.6b | 8.4 ± 0.5b | 22.9 ± 2.6a | 4.189         |
| 5-methyl-2-isopropyl-2-hexen-1-al (3MB + 3MB) | ad4 | 1347 | 1365| AS  | A         | 32.7 ± 1.7b| 40.6 ± 5.8b| 27.4 ± 5b  | 10.9a        | 17.61         |
| 2-phenyl-2-butenal (PA + Ac) | ad5 | 1911 |     |     | A         | 63.8 ± 4b  | 44.3 ± 4.6c| 50.4 ± 12bc | 96.2 ± 4.4a | 18.49         |
| 5-methyl-2-phenyl-2-hexenal (PA + 3MB) | ad8 | 2053 | 2087| AS  | A         | 54.6 ± 4.1a| 54.8 ± 8.7a| 84 ± 20.4a | 78.1 ± 4.4a | 30.117        |
| **Lipid-derived aldehydes** |      |     |    |     |           |           |           |     |              |
| Hexanal                   | d1   | 1072 | 1091|     | A         | 0.9ab      | 16.8 ± 0.7b| 7.6 ± 1.5c | 20.4 ± 0.5a | 2.58          |
| 2,4-decadienal            | d3   | 1749 |     |     | BT        | 2.5 ± 0.3a | nd         | 0.9 ± 0.2b | nd           | 0.44          |
| E,E,2,4-decadienal        | d4   | 1791 | 1814|     | A         | 8.6 ± 0.6a | 2.2 ± 0.9b | 3.3 ± 0.5b | nd           | 1.58          |
| **Esters**                |      |     |    |     |           |           |           |     |              |
| ethyl acetate             | e1   | <900 | 900 |     | A         | 23.4 ± 2.6b| 21.9 ± 1.3b| 89.5 ± 12.4a | 78.3 ± 6.3a | 18.50         |
| ethyl butanoate           | e2   | 1032 | 1045|     | A         | 1 ± 0.2b   | 1.6 ± 0.2b | 10.6 ± 1.5a | 11.8 ± 3.6a | 5.10          |
| ethyl-2-methylbutanoate   | e3   | 1046 | 1050|     | A         | 3.6 ± 0.6b | 4.4 ± 0.3b | 13.4 ± 1a  | nd           | 1.592         |
| ethyl-3-methylbutanoate   | e4   | 1063 | 1082|     | A         | 0 ± 0c     | 1 ± 0.1a  | 2.2 ± 0a   | nd           | 0.106         |
| ethyl hexanoate           | e5   | 1227 | 1237|     | A         | 13.1 ± 0.6c| 27.1 ± 1.6b| 52.1 ± 4.8a | 2.2 ± 0d    | 6.66          |
| 3-methylbutyl 3-methylbutanoate | e6 | 1285 | 1293|     | A         | 3.2 ± 0.6b | 4.7 ± 0.8b | 44.9 ± 9.8a | 6.9 ± 4.8b  | 14.38         |
| ethyl octanoate           | e7   | 1427 | 1438|     | A         | 16.5 ± 1.6b| 41 ± 8.7b  | 58.9a      | 5.1 ± 0.4b  | 77.79         |
| 2-furanmethyl acetate     | e8   | 1526 | 1523|     | A         | 24.4 ± 0.8c| 29 ± 0.9c  | 82.5 ± 2.9a | 41 ± 5.1b  | 7.84          |
| ethyl phenylacetate       | e9   | 1771 | 1768|     | A         | 1.9 ± 0.1b | 3.4 ± 0.5b | 43.2 ± 12.1a | 6.5 ± 2.9b | 16.30         |
| 2-phenylethyl acetate?    | e10  | 1800 | 1835|     | A         | 12 ± 0.5b  | 16.2 ± 2.1b| 34.8a      | 42.3 ± 2.1b | 45.62         |
| ester 71 79               | e11  | 1857 |     | U    |          | 11.3b      | 26.9 ± 13b | 23.5 ± 16.3b | 114.1a      | 52.43         |
| ethyl benzeneopropanoate  | e12  | 1869 |     | BT   |          | 2.5 ± 0.4b | 3.5 ± 0.6b | 35.2 ± 15a | 1.5 ± 0.8b  | 19.65         |
| **Maillard-derived furans** |      |     |    |     |           |           |           |     |              |
| Furfural                  | f1   | 1449 | 1481|     | A         | 12.8a      | 25.7 ± 3.1b| 18 ± 3.3b  | 57.3 ± 5.6a | 19.16         |
| 2-acetylpyruvan         | f2   | 1490 | 1503|     | A         | 45.6 ± 9.6a| 42.2 ± 5a  | 52 ± 1.7a  | 46 ± 4.3a  | 15.31         |
| 5-methylfurfural         | f3   | 1557 | 1574|     | A         | 31.5a      | 16.9a      | 66.3 ± 5.9b | 13.9a       | 50.71         |
| 2-furanmethanol           | f4   | 1653 | 1665|     | A         | 25.5a      | 25.9 ± 3.3a| 42.9 ± 3.2a | 46.6 ± 5.8a | 34.68         |
| Compound                      | p1 | p2 | p3 | p4 |
|-------------------------------|----|----|----|----|
| 1-(2-furanylmethyl)-1H-pyrrole| f1 | f2 | f3 | f4 |
| 3-phenylfuran                 | f5 | f6 | f7 | f8 |
| 2,3-butanedione               | bd | bd | bd | bd |
| 2-heptanone                   | k1 | k2 | k3 | k4 |
| 6-methyl-3,5-heptadiene-2-one | l1 | l2 | l3 | l4 |
| Butyrolactone                 | m1 | m2 | m3 | m4 |
| 2-pentylfuran                 | n1 | n2 | n3 | n4 |
| 1-methyl-1H pyrrole           | n5 | n6 | n7 | n8 |
| 1-(2-methylpropyl)pyrrole     | n9 | n10| n11| n12|
| 1-(2-methylbutyl)pyrrole      | n13| n14| n15| n16|
| 1-(3-methylbutyl)pyrrole      | n17| n18| n19| n20|
| 2-acetylpyrrole?              | n21| n22| n23| n24|
| 5-methyl-1H-pyrrole-2-carboxaldehyde | n25| n26| n27| n28|
| 2-acetyl-2,3,4,5-tetrahydripyrazine | n29| n30| n31| n32|
| Methylpyrazine                | o1 | o2 | o3 | o4 |
| 2,5-dimethylpyrazine          | o5 | o6 | o7 | o8 |
| 2,6-dimethylpyrazine          | o9 | o10| o11| o12|
| 2-ethylpyrazine               | o13| o14| o15| o16|
| 2,3-dimethylpyrazine          | o17| o18| o19| o20|
| 2-ethyl-5-methylpyrazine      | o21| o22| o23| o24|
| 2-ethyl-6-methylpyrazine      | o25| o26| o27| o28|
| 2-ethyl-3-methylpyrazine      | o29| o30| o31| o32|
| 2,3,5-trimethylpyrazine       | o33| o34| o35| o36|
| 2-propylpyrazine              | o37| o38| o39| o40|
| 2,6-diethylpyrazine           | o41| o42| o43| o44|
| 2,5 dimethyl-3-ethylpyrazine (and poss 2,3-dimethyl-5-ethylpyrazine) | o45| o46| o47| o48|
| 2,3-diethylpyrazine           | o49| o50| o51| o52|
| 2,5-diethyl pyrazine          | o53| o54| o55| o56|
| 3,5 dimethyl-2-ethylpyrazine (and poss 2,3-dimethyl-5-ethylpyrazine) | o57| o58| o59| o60|

**Note:** The table includes various pyrazines, pyrroles, ketones, lactones, and lipid-derived furans with their respective concentrations and statistical significance. The table format is as follows:

- **Ketones**
- **Pyroles**
- **Pyrazines**

Each compound is listed with its concentration in parts per million (ppm), along with their statistical significance levels (***, **, *, ns).
| Compound                                      | p   | Mass   | Mol. %  | Molar % | Mass % | Molar % |
|-----------------------------------------------|-----|--------|---------|---------|--------|---------|
| 2-methyl-6-propylpyrazine                     | p16 | 1454   | 1459    | 11 ± 1a | 12.2 ± 0.7a | 0 ± 0b  |
| 2.3.5.6-tetramethylpyrazine                   | p17 | 1465   | 1487    | A       | 7.7 ± 0.8b | 9.5 ± 0.1b |
| 2.3.5.6-tetramethylpyrazine                   | p18 | 1475   |         | BT      | 8.9 ± 1.1a | 5 ± 0.1b  |
| 2.3.5.6-tetramethylpyrazine                   | p19 | 1478   | 1477    | TM1     | 51.2 ± 3.9a | 54.1 ± 2.6a |
| 2.3-diethyl-5-methylpyrazine                  | p20 | 1481   | 1488    | A       | 7.2a      | 11.3a    |
| 2.3-diethyl-5-methylpyrazine                  | p21 | 1484   |         | BT      | 8.9 ± 1a  | 6 ± 0.4b  |
| 2.3-diethyl-5-methylpyrazine                  | p22 | 1489   |         |         | 126.2 ±   | 106.3 ±  |
| 2.5-dimethyl-3-propylpyrazine                 | p23 | 1500   |         |         |          |          |
| 3,5-diethyl-2-methylpyrazine or TMEP?         | p24 | 1504   | 1508    | A       | 7.8 ± 1.7b | 8.7 ± 0.7a |
| a diethyl-dimethylpyrazine                    | p25 | 1506   |         | U       | 0.7 ± 0.1a | 0.6 ± 0.1a |
| not 3-methylbutylpyrazine?/isobutyl           | p26 |         |         |         |          |          |
| a diethyl-dimethylpyrazine                    | p27 | 1512   |         | U       | 0.6 ± 0.1b | 0.8 ± 0a  |
| an ethyl-3-(2-methylpropyl)pyrazine?          | p28 | 1517   |         |         |          |          |
| 2-ethyl-5/6-vinylpyrazine                     | p29 | 1522   |         |         |          |          |
| 2,3-dimethylisoamyl                          |     |        |         |         |          |          |
| 2,3-dimethylisoamyl                          |     |        |         |         |          |          |
| 2,3-dimethylisoamyl                          |     |        |         |         |          |          |
| 2,3-dimethylisoamyl                          |     |        |         |         |          |          | **
| 2,3-dimethylisoamyl                          |     |        |         |         |          |          | ***
| 2,3-dimethylisoamyl                          |     |        |         |         |          |          | ***
| Component                                      | Retention Time | Species | Identification                      | Peak Height | Area | RSD % |
|------------------------------------------------|---------------|---------|-------------------------------------|-------------|------|-------|
| Phenols                                        |               |         |                                     |             |      |       |
| Guaiacol                                      |               | A       |                                     | 2.1 ± 0.3bc | 23.5 ± 6.2a | 4.5 ± 0.1c |
| 4-ethyl-2-methoxyphenol                       |               | A       |                                     | 1.1 ± 0.1bc | 21.1ab | 2.1 | 8.70 |
| 4-vinylguaiacol                               |               | A       |                                     | 7.0 ± 0.3a  | 64.2 ± 14.6c | 0.5 ± 0.5d |
| Sulfur compounds                              |               |         |                                     |             |      |       |
| dimethyl disulfide                            |               | A       |                                     | 31.5 ± 2.1c | 41.4 ± 1.1b | 13.9 ± 2.1d |
| dimethyl trisulfide                           |               | A       |                                     | 21.7 ± 0.7b | 26.6 ± 1.6b | 20.3 ± 3.6b |
| Strecker aldehydes                            |               |         |                                     |             |      |       |
| 2-methylbutanal                               |               | A       |                                     | 456.5 ±     | 289.6 ± 157.2 ± | 295.8 ± |
| 3-methylbutanal                               |               | A       |                                     | 163.6 ±     | 96.4 ± 135.6 ± | 492.3 ± |
| Benzenecetaldehyde                            |               | A       |                                     | 421.3 ±     | 335.9 ±     |       |
| Sugar derivatives*                            |               |         |                                     |             |      |       |
| maltol2                                       |               | A       |                                     | 5.6 ± 0.8a  | 5.5 ± 0.8a  | 5 ± 1.2a |
| cyclotene3                                     |               | A       |                                     | 3.3 ± 0.2a  | 2.5 ± 0.5b | 4.7 ± 1.1a |
| furaneol1                                     |               | A       |                                     | 24.5 ± 1.9a | 10.3 ± 2.1b | 11.8 ± 2.8b |
| Acids                                         |               |         |                                     |             |      |       |
| acetic acid                                   |               | A       |                                     | 102.8 ±     | 195.9 ± 786.9 ± | 385.8 ± |
| 2/3-methylbutanoic acid                       |               | A       |                                     | 63.5 ±      | 218.2 ± | 124.9 ± |
| hexanoic acid                                 |               | A       |                                     | 25.3 ± 8.2 ± | 3.9 ± 20.6a | 22.3b |
| Misc                                          |               |         |                                     |             |      |       |
| Phenylacetaledehyde                           |               | A       |                                     | 196.4 ±     | 152.7 ± | 168.4 ± |

1,2,3 and 4 were determined by SPE. nd = not detected. A = MS and LRI agree with authentic standard on BPXWax; AS = MS and LRI agree with compound synthesised via aldol condensation; BT = Tentative identity base on lit MS and LRI; RM1T = Tentative, found in reaction mixture 1; RM2T = Tentative, found in reaction mixture 2; RM3T = Tentative, found in reaction mixture 3; T = Tentative based on match library match; ns = no significant difference between means (p>0.05); * significant at the 5% level; ** significant at the 1% level; *** significant at the 0.1% level at Tukey test.
Table Supplementary 1. Cocktails used in SIDA Method

| Cocktail | Pyrazine group | Internal standard                      | Rpi  | Ion | Quantity (uL) |
|---------|----------------|----------------------------------------|------|-----|---------------|
|         |                |                                        |      |     | Lab           | Unlab |
| A       | C2             | 2,5-dimethylpyrazine                   | 6.747| 114 | 100           |
| C       | C2             | 2,6-dimethylpyrazine                   | 6.747| 108 | 100           |
| C       | C2             | ethylpyrazine                          | 9.877| 108 | 100           |
| B       | C3             | trimethylpyrazine                      | 0.654| 122 | 100           |
| A       | C3             | 3,5-dimethyl-2-ethylpyrazine           | 4.376| 125 | 100           |
| B       | C4             | tetramethyl-pyrazine                   | 0.540| 136 | 100           |
| C       | C4             | 2-methyl-3-propylpyrazine              | 0.675| 136 | 100           |
| A       | C5             | 3,5-diethyl-2-methylpyrazine           | 6.473| 153 | 10            |

Figure supplementary 1. SIDA method
4. CHARACTERIZATION OF FUNCTIONAL PROPERTIES AND SENSORY AROMA OF ROASTED JACKFRUIT SEED FLOURS COMPARED TO COCOA POWDER AND COMMERCIAL CHOCOLATE POWDER

Abstract

Jackfruit seeds are an under-utilized waste product in many tropical countries. In this study, we demonstrated the functional properties of roasted jackfruit seeds for the development of foodstuffs rich in starch and chocolate aroma compared to the properties of cocoa and chocolate powder. Three types of jackfruit seed flour, namely dry (DJS), acidified (AJS), and fermented (FJS) flour, were evaluable for solubility, swelling power, wettability, apparent density, viscosity, sensory preference, and intensity of chocolate aroma. The central composite design was used to optimize solubility and swelling power. Water temperature and flour exposure were the response variables. Solubility was optimized ($r^2>0.7$) by adjusting the temperature and time of FJS flour and comparing its properties to those of commercial chocolate (CC). The FJS flour had higher solubility and wettability than other flours. The viscosity of jackfruit seed flours was low with high solubility, properties that are desirable in cocoa powder (CP). The swelling power was the same (p≤0.05) for CP, AJS, and FJS. Chocolate aroma was more intense for FJS flour and sensory preference was not different (p≤0.05) among all flours. Therefore, jackfruit seed flours were proven to have similar or better technological properties and chocolate aroma compared to CP and CC. Thus, jackfruit seed flours could be used as an innovative cocoa replacer.

Keywords: *Artocarpus heterophyllus* Lam; Chocolate replacer; Waste utilization; Starch; Cocoa aroma

4.1. INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* Lam.) seeds represent 18–25% (db) of the fruit weight (Mahanta & Kalita, 2015). They have high carbohydrate content, 77%(db), (Ocloo et al., 2010; Singh et al., 1991), but generally are rejected as agroindustrial waste (John & Narasimham, 1993; Kee & Saw, 2010). A portion is consumed in local culinary preparation by boiling, steaming, and roasting (Kee & Saw, 2010); typically, in the Asian industry, jackfruit seeds are boiled and canned in brine or in tomato sauce (Mahanta & Kalita, 2015). Recently,
Spada et al., (2017) Chapter 1, found chocolate aroma and volatiles compounds similar to those in cocoa powder (pyrazines, esters, alcohols, aldehydes, and others) in roasted jackfruit seeds after drying, acidification, and fermentation processes. Thus, roasted jackfruit seeds have the potential to replace chocolate aroma in food and other products.

Jackfruit seeds are a great alternative source of starch, and thus have the potential for use in foods such as cakes, gum candies and fillings, and beauty products with a low cost compared to other starchy ingredients (Madruga et al., 2014; Mahanta & Kalita, 2015). Functional properties of jackfruit seeds are also compatible with properties of other starch sources (Do et al., 2011; Madruga et al., 2014), and are better than corn in some applications (Rengsutthi & Charoenrein, 2011). Therefore, several studies have used jackfruit seed starch in food preparations such as cookies, breads, and sweet products (Madrigal-Aldana et al., 2011; Mukprasirt & Sajjaanantakul, 2004; Santos et al., 2009). In addition, restrictions on the use of chemical texture modifiers provide an increased demand for starches with ideal characteristics such as transparency, stability, solubility, and the absence of synergism. Therefore, research is being conducted to identify natural sources of starch with different properties.

The biological origin of starch affects its functional properties and consequently its applications. In jackfruit seeds, the variety, environment, and ripeness changes the amylose content; in jackfruit the amylose content is approximately 27%, similar to that of potato starch (Dutta, Paul, Kalita, & Mahanta, 2011; Mahanta & Kalita, 2015; Rengsutthi & Charoenrein, 2011). Reduced viscosity and high solubility are associated with breakdown of amylose by cooking temperature and acidification (Dutta et al., 2011; Mahanta & Kalita, 2015). Temperature and time exposure are responsible for increased intermolecular breakdown and water incorporation, thus increasing carbohydrate solubility (Mason, 2009; Thomas & Atwell, 1999).

In chocolate products, the important functional properties are viscosity, solubility, and swelling power. Especially in chocolate milk beverages, instantaneity is a desirable characteristic, and is correlated with wettability (Dogan et al., 2016). However, it is expensive to improve instantaneity and solubility in cocoa powder because cocoa is hydrophobic. Therefore, it would be advantageous to produce a natural chocolate replacer with similar or better functional properties, such as low viscosity, high solubility, and wettability, associated with high levels of starch. Thus, this study aimed to characterize the
functional properties, mainly solubility at different temperatures and times, and compare sensory aromas of roasted jackfruit seed flours with those of cocoa powder and commercial chocolate powder.

4.2. MATERIALS AND METHODS

4.2.1. Jackfruit seed flours

Several ripe jackfruits of a hard pulp variety (Artocarpus heterophyllus Lam.) were manually collected between October 2013 and January 2014 in the countryside of São Paulo, Brazil, cleaned in running water, and the fruits were opened in a clean environment. The seeds were separated from the fruit and the attached pulp was completely removed. Three different treatments were executed as follows.

For dry jackfruit seed flours, seeds were dried in an oven (Probat laboratory sample roaster, Germany) at 60°C with air circulation for 24 h. Afterward, the spermoderms were manually removed and the seeds were dried for additional 24 h at 60°C. Finally, the seeds were stored at 4°C prior to blending with all dried seeds of different jackfruits. Portions of 200 g of dried seeds were roasted in a rotary electric oven for 47 min at 171°C.

Seeds destined for acidification were allocated in polypropylene trays with an aqueous solution of 1% acetic acid for 5 days at 25 ± 3°C. The solution volume was twice the weight of the seeds. After five days, the solution was removed and the acidified seeds were subjected to the same drying process described above, except that they were roasted for 40 min at 180°C.

For fermented jackfruit seeds, pulp and banana leaves were placed in a closed bucket between three and five days to encourage anaerobic fermentation and alcohol production. Over the next days, the bucket was opened daily, and the fermenting mass was upturned manually to promote alcohol oxidation and acetic acid production. After eight days, the pulp and banana leaves were removed, and the fermented seeds were subjected to the same drying process described above, except that they were roasted at 154°C for 35 min. In the final stages, 200 g seed portions were ground using unheated mill hammers to preserve the volatile compounds of the flour. Flours were vacuum-packed and stored in the
dark at 8±1°C. Thus, three kinds of jackfruit seed flours were produced: dried (DJS), acidified (AJS), and fermented (FJS) flours.

The others flours used were: cocoa powder (CP) that was fermented, not alkalized, roasted, and of Brazilian origin. This powder was commercially available through Cargill. Bournville cocoa (CC) is a commercial chocolate powder produced with 100% cocoa beans, made under license from Cadbury UK Ltd.

### 4.2.2. Solubility and swelling power

Solubility and swelling power were determined based on a previously described method (Madruga et al., 2014). Briefly, 0.1 g of jackfruit seed flours, CP, or CC were placed in weighed centrifuge tubes and 10 mL of distilled water was added. The suspension was stirred and placed in a water bath, using various combinations of time and temperature (Table 1). Next, the tubes were centrifuged (NT 825/Piracicaba/Brazil) at 25°C for 15 min at 4420 rpm. The supernatants (5 mL) were transferred into Petri dishes and placed on the stove at 105°C for 24 h to determine the weight of the solid. The solubility was determined by equation 1 (Eqn 1). Finally, the tubes were dried (outer walls) and carefully weighed, to determine swelling power (Eqn 2).

\[
\text{Solubility} \% = \frac{\text{[weight of plate with sample after evaporation} - \text{weight of plate]} \times 100}{\text{Eqn 1.}}
\]

\[
\text{Swelling power} = \frac{\text{[(weight of tube + residue after centrifugation)-(weight of tube+ sample on dry basis)]/(weight of sample)}}{\text{Eqn 2.}}
\]

### 4.2.3. Wettability and apparent density

Wettability was measured using the immersion method described by Hogekamp & Schubert (2003). The time between placing a powder sample (2.5 g) of a given height (5 cm) on a liquid surface at 80°C (optimal temperature for best solubility) and achieving complete wetting was determined. The measurement enabled free sinking of particles, so that
unsteady and steady state wetting occurred (Hogekamp & Schubert, 2003). To calculate wettability, Eqn 3 was applied and results were expressed in seconds$^{-1}$ to reflect a direct association with time and wettability. The apparent density was measured based on the method described by Micha (1983) by adding the required weight of flour to generate 30 mL in a 100 mL graduated cylinder.

\[ \text{Wettability} = \frac{1}{\text{time (s)}} \]

Eqn 3

### 4.2.4. Viscosity

Viscosity was evaluated using a Rapid Visco Analyser (RVA-S4A, Newport Scientific, Warriewood, NSW, Australia), and viscoamylograms were generated using 3 g of sample (14% moisture) in 25 g of water with the Thermocline for Windows software version 2.3, Newport Scientific Pty. Ltd, and according to the N°162 methodology proposed by the ICC using the Standard one analysis program (Lawal & Adebowale, 2005). Unroasted dry jackfruit seed flour was added as a marker to determine changes in viscosity that occur after the roasting process.

### 4.2.5. Ranking tests

Sensory evaluations were approved by the Ethics Committee of Human Research of the ESALQ/USP (COET/077/131). First, to detect the intensity of chocolate aroma in roasted jackfruit seed flours (dried, acidified, and fermented), their aromas were compared with the aroma of cocoa powder. A ranking test was used to evaluate each sample (Meilgaard, Civille, & Carr, 2007), using ascending scores: 1 from least and 4 to most intense chocolate aroma. A randomized complete block design was employed on the four samples (DJS, AJS, FJS, and CP) for 72 panelists (nonsmokers and 60% female) who received four random samples with different three-digit numbers. Thus, the complete block was applied three times with twenty four combinations each (Cochran & Cox, 1950).

Finally, the same 72 panelists were asked about aroma preference, and they were given the same four samples in a randomized complete block (Cochran & Cox, 1950;
Meilgaard et al., 2007) design using ascending scores: 1 from least and 4 to most preferred aroma.

4.2.6. Experimental design

The central composite design, Statistics (2014), was used for two key responses: solubility and swelling power; using nine points with three repetitions in central point, randomized and ordained by the central rotation for the two factors (time and temperature). The two independent variables of the design, time of sample exposure (min) and water temperature (°C), were coded as \( x \) and \( y \), respectively (Table 1). Equation 4 shows the quadratic polynomial model that was fitted to each response, where \( b_0, b_1, b_2, b_{11}, b_{12}, \) and \( b_{22} \) are the regression coefficients (Cochran & Cox, 1950).

\[
z = b_0 + b_1x + b_2y + b_{11}x^2 + b_{22}y^2 + b_{12}xy
\]  
Eqn 4

The analysis of variance (ANOVA) tables were generated and regression coefficients of individual linear, quadratic, and interaction terms were determined using design expert software (Statistics). The significance (p≤0.05) of all terms in the polynomial model was judged statistically by computing the F value. Non-significant effects were eliminated and removed, thus improving the coefficient of determination.

Wettability and apparent density analyses were performed in triplicate. Analysis of variance (ANOVA) was carried out to analyze the results and comparisons between treatments were performed by a Tukey's test (p≤0.05). The ranking tests were available using Compusense Five software and the Tukey's test (p≤0.05).

4.3. RESULTS AND DISCUSSION

4.3.1. Solubility

Solubility affects the sensory attributes and development characteristics in instant products containing cocoa powder. In this study, CP and DJS flours exhibited a linear (L) effect of temperature on solubility, \( p=0.02 \) and \( 0.003 \), respectively (Table supp). Thus,
increased temperature improved the solubility (Table 1, Fig. 1A and 1B). However, the correlation coefficients ($r^2$) were not predictive, lower than 0.7 (Table supp), making it impossible to use the polynomial model. Solubility of AJS flour was not significantly affected by time and temperature (p≤0.05) (Table 1 and Table supp), indicating no influence of water temperature and time exposure on its solubility (Table supp). It is possible that other factors influence AJS solubility, such as pH, which is known to change starch solubility (Mason, 2009; Thomas & Atwell, 1999).

The solubility for FJS was optimal, with a significant linear effect of temperature (p=0.05) and also a predictive (Eqn 5) correlation coefficient ($r^2=0.92$). The best solubility was found near 80°C after approximately 20 minutes (Table supp; Table 1; Fig. 2A and 2B).

$$z = -0.241 -0.016x -0.000722x^2 +0.03538y -0.000264y^2 +0.000516xy$$

Eqn 5

The CC showed a significant quadratic (Q) effect of time (p=0.01), and also L temperature (p=0.02) and Q (p=0.003) (Table supp). The quadratic polynomial model shown in Eqn 6 was extremely predictive ($r^2=0.98$) (Table 1; Fig. 2C and 2D). Thus, increasing water temperature and time improved the solubility. It is worth mentioning that this product has been subjected to technologies that increase its solubility.

$$z= 7.388 -0.1199x +0.00104x^2 -0.1563y +0.000927y^2 +0.00106xy$$

Eqn 6

The equations for FJS and CC were validated and the optimal conditions for solubility of FJS were 80°C and 15 min. Since for DJS and CP, increased temperature also linearly improved solubility for wettability, these conditions (80°C for 15 min) were utilized.

These results are in accordance with previous studies (Madrigal-Aldana et al., 2011; Madruga et al., 2014; Tongdang, 2008), which also found that increasing temperature improved the solubility of jackfruit seed starch, particularly above 75°C. Madrizgal-Aldana et al., (2011) found that treating jackfruit seed starches at temperatures of approximately 80°C makes them suitable for consumption; this conclusion is similar to the results obtained in our study using roasted jackfruit seed flours. Thus, fermentation, drying, and a range of roasting temperatures did not influence the solubility of jackfruit seed flours.

Native starches exhibit increased solubility at high temperatures because of leaching of starch chains (amylose), and external long chains (amylopectin), toward the
continuous phase. Amylose acts as a diluent and amylopectin is responsible for granule swelling. Solubility could be affected by the amylose to amylopectin ratio, starch granule size, and amylopectin structure and architecture.

According to Omobuwajo, Busari, & Osemwegie, (2000), the main reasons for low solubility of cocoa powder are agglomeration, due to its high fat content (10–25%), air bubbles in capillary structures, and also its large number of hydrophobic polysaccharide and carbonyl groups. For acidified flour, seed exposure to acetic acid is likely to degrade starches with lower molecular weights. Thus, pH could be another factor correlated with solubility (Thomas & Atwell, 1999).

The FJS flour had the highest solubility (Table 2), while the solubilities of DJS, AJS, and CP were not different from each other (p≤0.05). In cocoa powders, fermentation results in biochemical modifications (oxidation and polymerization) that reduce their solubility. In the first three fermentation days, coinciding with bean death, protein solubility reduces due to polyphenol-protein interactions (Zak & Keeney, 1976). Polymerization with polyphenol oxidases, mainly epicatechin and free anthocyanidins, produces quinones. Polyphenols and quinones form complexes with peptides, proteins, and others polyphenols (Gu et al., 2013). These processes were also observed upon roasting carob powder, a cocoa replacer, but roasting conditions increased the phenolic content and antioxidant activity due to Maillard product formation and enhanced polyphenol solubility (Srour, Daroub, Toufeili, & Olabi, 2016). In carob powder, the native polysaccharides cellulose and hemi-cellulose increased the protein-polyphenol solubility when combined with milk proteins (Srour et al., 2016), thus making this powder applicable in the food industry.

Unprocessed roasted jackfruit seed flours have solubility characteristics superior or similar to those of cocoa. Thus, these flours could be used as cocoa replacers. Their high solubility could be also exploited for food coatings and they can replace more costly gums currently used in many applications. The ideal solubility conditions found in this study (80°C at 15 min) are suitable for soups, sauces, hot beverages, and quick-cooking products. Notably, to improve the solubility of cocoa powder, sophisticated processes are required, including expensive additives, and technical apparatuses (Wieland, 1972).

Drying changes the moisture available to starches, and the roasting process is shown to be more active in decreasing the moisture. In contact with water, jackfruit seed flours showed improved solubility and reduced viscosity. Mason, (2009) found it is easy to
dissolve starch upon drying (60–75°C) with continuous rotation in under pressure, with moisture content changing from around 18% to 11% in the end, then heating to 90–128°C for 45–90 minutes. These conditions are similar to those used to produce jackfruit seed flours in this study: 60°C for drying, 154–180°C and 35–47 min for roasting, which resulted in 6, 1, and 5% moisture for DJS, AJS, and FJS, respectively.

High solubility and low viscosity are typical in yellow dextrin and pyrodextrins. Generally, these dextrins are produced by heating dry, acidified, starch with agitation. Low pH facilitates glycosidic hydrolysis by a complex pyrolysis reactions; depending upon the dextrinization conditions, both hydrolysis and repolymerization can occur. Pyroconversion generally yields α-1,4 and α-1,6 linkages and glycosidic bonds (Thomas & Atwell, 1999).

4.3.2. Swelling power

For all jackfruit seed flours, the values of swelling power were close to values for cocoa powder and commercial cocoa, even at different conditions of water temperature and exposure time. There were no significant effects (p≤0.05) for any evaluated variable (time or temperature). The coefficients of determination (r²) were small and residues were not statistically significant (p≤0.05) (Table 1 and Table supll). Thus, the surface responses that did not represent these results were not shown in this study.

Swelling power and solubility were directly correlated in CC and DJS (Table 2). Carbohydrates become solubilized when temperature increases, and when the temperature is above the gelatinization point, swelling power increases (Fortuna, Januszewska, Juszczak, Kielski, & Palasinski, 2000). Jackfruit seed flours had intermediate swelling power, and swelling power of CP did not differ (p<0.05) between swelling powers of CC and jackfruit seed flours. Knowing that commercial cocoa products are commercialized after improving solubility (Afoakwa, 2010; Beckett, 1999; Wieland, 1972), jackfruit seed flours have a considerable potential, since their swelling power was similar (Table 2) to that of CP.
4.3.3. Wettability and apparent density

Wettability is the susceptibility of particles to water penetration. Table 3 shows the FJS and other jackfruit seed flours with the best wettability. Generally, food powder products with high wettability time tend to form lumps when added or mixed with water. For cocoa powders, a capillary structure is formed, which traps pockets of air, thus reducing water influx (Omobuwajo et al., 2000). Wettability is associated with hydrophobicity and solubility.

Medeiros & Lannes, (2009) evaluated the wettability of cocoa powder replacers such as a carob and cupuassu and observed 10% less wettability in comparison to the jackfruit seed flours, as determined by this study (Table 3). Gutcho, (1997) produced beverages with cocoa powder and observed cocoa particle sedimentation, leading to low wettability, similar to our results (Table 3).

Accordingly, wettability is an important factor for food powder development, and powders should not have lumps such as those occurring with cocoa. Thus, jackfruit seed flours have an advantage when used as a substitute for cocoa powder and commercial chocolate, as well as other flour replacers such as cupuassu and roasted carob.

The apparent densities of cocoa powder and commercial chocolate were significantly lower ($p \leq 0.05$) than those of jackfruit seed flours. According to a previous study (Azevedo, Mileib, Vissotto, & de Carvalho-Silva (2011), high values of apparent density improve wettability, because samples with high apparent density will have high granule porosity and surface area to expose particles. This was confirmed by our wettability results. Jackfruit seed flours were shown to have wettability characteristics superior to CC and CP.

However, the apparent density modifies sample volume. Thus, high density values increase package charge. Therefore, further studies related to this property are necessary; perhaps different methods of grinding jackfruit seed flours in smaller particle sizes could yield better standardization and reduce apparent density to levels similar to those of cocoa flours.
4.3.4. Viscosity

DJS and AJS began with the same viscosity levels upon exposure to the same temperatures and times (Fig. 3). FJS showed a notably improved viscosity, but still was similar to the viscosity of roasted DJS and AJS (Fig. 3). The low viscosity values of jackfruit flours were compatible with those of cocoa powder (Do et al., 2011). Viscosity profiles can vary significantly as a function of pH. Acid treatment degrades amylose in different ways, directly attacking the amylose or amyllopectin (Do et al., 2011). Generally, pH extremes also tend to have a negative impact on viscosity by hydrolyzing bonds and disrupting the molecular integrity of starch granules (Thomas & Atwell, 1999).

Food products prepared by modifying starch through dry roasting or acidification are subject to dextrinization; depending on reaction conditions (moisture, pH, temperature, duration of toast and roast), the range of yield products changes and the viscosity is affected. Low viscosity is desirable to form a good film, which occurs in commercial dextrins like yellow dextrin (Thomas & Atwell, 1999). Using dry heating in corn starch increased the enthalpy of gelatinization while decreasing peak viscosity, which is similar to the results of this study (Mason, 2009).

Unroasted dry jackfruit seed flour was the only sample that showed real starch behavior with a viscosity peak at high temperatures, comparable to the results of a previous study (Madrigua et al., 2014). Thus, roasting temperature depolymerized the starchy matrix and limited the expansion capacity of the starch granules during the gelatinization process, consequently reducing the viscosity, a characteristic related to amylose content. Dutta et al., (2011) reported high amylose content in jackfruit seed starch. In conclusion, roasted jackfruit seed flours could replace cocoa powder owing to their viscosity properties and maybe applied in food products independent of drying, acidification, or fermentation processes.

4.3.5. Intensity of chocolate aroma and preference

The ranking test for intensity of chocolate aroma showed the highest (p≤ 0.05) intensity for FJS (Fig.4). No tably, CP had a low value of chocolate aroma (Fig. 4), and DJS and
AJS showed no difference in intensity (p≤0.05) when compared with the other flours. Owing to the volatile composition, FJS has a higher concentration of pyrazines and esters than DJS.

The average chocolate aroma intensity scores for jackfruit seed flours (DJS and FJS) were 12% and 48% higher, respectively, than that of cocoa powder. This suggests that roasted jackfruit seed flours may be used as potential replacers for chocolate aroma, since other cocoa replacers such as roasted cupuassu showed 44% less intense chocolate aroma compared to cocoa (Queiroz & Garcia, 2000). Medeiros & Lannes, (2009) reported cupuassu and carob powder to have 24% and 15% lesser chocolate aroma intensities, respectively, than that of cocoa powder and natural chocolate aroma.

The preference ranking test did not show any difference between samples (p≤0.05) (Fig. 4). A previous study by Medeiros & Lannes, (2009) using cupuassu and carob powder showed low preference scores compared to chemical chocolate aroma (RBP 10886), cocoa powder, and an aroma identical to that of natural cocoa.

Accordingly, roasted jackfruit seed flours, mainly FJS, had a chocolate aroma intensity and consumer preference similar to or better than cocoa powder; thus, they are an innovative alternative to chocolate aroma in product development.

4.4. CONCLUSION

Solubility was the main functional properties evaluated and the higher solubility was found in fermented treatment. Apparent density was higher in the jackfruit seed flours than cocoa powder. Others functional properties like swelling power, wettability and viscosity were similar to cocoa powder.

Aroma of jackfruit seed flours acidified and dried compared with cocoa powder and commercial chocolate showed similar and fermented showed better according sensorial panel.

Jackfruit seed flours may be used as a cocoa replacer in food development because they have similar or better technological properties and chocolate aroma compared to cocoa powder and commercial chocolate.
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Figure 1. Central composite design using two factors each at five levels; A: values; B: Scheme.
Table 1. Experimental results for solubility and swelling power in central composite design

| Codified | Real values | DJS | AJS | FJS | CC | CP |
|----------|-------------|-----|-----|-----|----|----|
| x        | y           | Time | Temperature | Solubility | Swelling power |
| 0        | -1.41       | 20  | 54  | 0.53 | 47.47 | 45.48 | 38.43 | 48.09 |
| 1        | -1          | 30  | 60  | 0.22 | 45.97 | 47.97 | 47.47 | 49.98 |
| -1       | -1          | 10  | 60  | 0.90 | 49.61 | 49.60 | 49.42 | 49.83 |
| -1.41    | 0           | 6   | 75  | 1.03 | 49.33 | 49.00 | 49.00 | 48.09 |
| 0        | 0           | 20  | 75  | 0.39 | 48.04 | 50.54 | 50.75 | 49.83 |
| 0        | 0           | 20  | 75  | 0.42 | 48.04 | 50.54 | 50.75 | 49.83 |
| 0        | 0           | 20  | 75  | 0.51 | 51.04 | 63.78 | 63.78 | 53.78 |
| 1.41     | 0           | 34  | 75  | 0.56 | 50.29 | 50.29 | 50.29 | 49.34 |
| -1       | 1           | 10  | 90  | 0.91 | 48.22 | 48.22 | 48.22 | 49.34 |
| 1        | 1           | 30  | 90  | 0.63 | 48.65 | 48.65 | 48.65 | 49.34 |
| 0        | 1.41        | 20  | 96  | 0.91 | 50.75 | 50.75 | 50.75 | 49.34 |

x = exposition time (min); y = water temperature (°C); DJS = dry jackfruit seed flour; AJS = acidified jackfruit seed flour; FJS = fermented jackfruit seed flour; CC = Commercial cocoa; CP = cocoa powder.
Figure 1. (A) Temperature effect in solubility of dry jackfruit seed flour. (B) Temperature effect in solubility of cocoa powder.
Figure 2. Response surface for FJS (A and B) and CC (C and D). A and C: three-dimensional; C and D: planned. FJS = Fermented Jackfruit seed flour; CC = Commercial chocolate. 

Solubility FJS = \(-0.241 - 0.016x - 0.000722x^2 + 0.03538y - 0.000264y^2 + 0.000516xy\)

Solubility CC = \(7.388 - 0.1199x + 0.00104x^2 - 0.1563y + 0.000927y^2 + 0.00106xy\)

x = time; y = temperature.
Table 2. Solubility and swelling power in the central point (75°C to 20 minutes) to flours.

|         | Solubility | Swelling power |
|---------|------------|----------------|
| DJS     | 0.44\(^c\) ± 0.06 | 42.05\(^b\) ± 5.72 |
| AJS     | 0.43\(^c\) ± 0.06 | 49.69\(^ab\) ± 1.57 |
| FJS     | 1.09\(^a\) ± 0.06 | 49.43\(^ab\) ± 0.70 |
| CC      | 0.90\(^b\) ± 0.10 | 54.62\(^a\) ± 7.96 |
| CP      | 0.49\(^c\) ± 0.02 | 51.50\(^ab\) ± 2.22 |

DJS = dry jackfruit seed flour; AJS = acidified jackfruit seed flour; FJS = fermented jackfruit seed flour; CC = Commercial cocoa; CP = cocoa powder.
Table 3. Wettability (80°C) and apparently density in cocoa powder, commercial chocolate and jackfruit seed.

|     | Wettability (s⁻¹) | Apparently density (g/mL) |
|-----|-------------------|---------------------------|
| DJS | 0.07 ± 0.01<sup>cb</sup> | 0.67 ± 0.030<sup>a</sup> |
| AJS | 0.10 ± 0.03<sup>ab</sup> | 0.61 ± 0.020<sup>b</sup> |
| FJS | 0.11 ± 0.01<sup>a</sup> | 0.58 ± 0.010<sup>c</sup> |
| CC  | 0.03 ± 0.01<sup>d</sup> | 0.33 ± 0.010<sup>e</sup> |
| CP  | 0.03 ± 0.001<sup>cd</sup> | 0.40 ± 0.001<sup>d</sup> |

DJS = dry jackfruit seed flour; AJS = acidified jackfruit seed flour; FJS: fermented jackfruit seed flour; CC = Commercial cocoa; CP = cocoa powder.
Figure 3. Viscoamylographic obtained by RVA.
Nature – unroasted dry jackfruit seed flour; DJS = dry jackfruit seed flour; AJJS = acidified jackfruit seed flour; FJS: fermented jackfruit seed flour.
Figure 4. Ranking test for chocolate aroma and preference.
DJS = dry jackfruit seed flour; AJS = addified jackfruit seed flour; FJS: fermented jackfruit seed flour;
CP = cocoa powder; Capital letters compare preference and small intensity of chocolate aroma. Different letters in
the same characteristic differ significantly ($p \leq 0.05$) by the Tukey's test.
Supplementary content

| Effect (p-value) | Time | Temperature | Equations |
|------------------|------|-------------|-----------|
|                  | L    | Q           | L         | Q         | 1L by 2L   | r²        |
| DJS              |      |             |           |           |            |           |
| Solubility       | 0.16 | 0.57        | 0.003     | 0.05      | 0.17       | 0.67      |
| Swelling power   | 0.70 | 0.31        | 0.41      | 0.73      | 0.70       | 0.50      |
| AJS              |      |             |           |           |            |           |
| Solubility       | 0.66 | 0.31        | 0.31      | 0.24      | 0.10       | 0.61      |
| Swelling power   | 0.58 | 0.86        | 0.48      | 0.20      | 0.80       | 0.58      |
| FJS              |      |             |           |           |            |           |
| Solubility       | 0.11 | 0.12        | 0.05      | 0.15      | 0.13       | 0.92      |
| Swelling power   | 0.10 | 0.65        | 0.65      | 0.85      | 0.24       | 0.61      |
| CC               |      |             |           |           |            |           |
| Solubility       | 0.20 | 0.01        | 0.02      | 0.003     | 0.006      | 0.98      |
| Swelling power   | 0.16 | 0.16        | 0.23      | 0.13      | 0.17       | 0.57      |
| CP               |      |             |           |           |            |           |
| Solubility       | 0.25 | 0.71        | 0.02      | 0.09      | 0.12       | 0.51      |
| Swelling power   | 0.64 | 0.36        | 0.89      | 0.32      | 0.86       | 0.40      |

L – linear effect; Q – quadratic effect. r² - coefficient determination. DJS = dry jackfruit seed flour; AJS = acidified jackfruit seed flour; FJS = fermented jackfruit seed flour; CC = Commercial chocolate; CP = cocoa powder.
Abstract

Jackfruit seeds are an under-utilized waste product in many tropical countries. In this work, we demonstrate the potential of roasted jackfruit seeds as a substitute for cocoa powder. Two different flours were produced from a hard variety of jackfruit by the seeds of the fruit. Next, seven formulations were prepared with 50%, 75%, and 100% substitution of cocoa powder with jackfruit seed flours. The consumer acceptance (n=126) of cappuccinos and quantitative descriptive analysis (QDA®) were used to describe the preparations. Physicochemical properties were also evaluated. When 50% and 75% cocoa powder was replaced to dry jackfruit seed flour, there was no change in sensory acceptability or technological properties; however, it is possible to identify advantages to using dry jackfruit seed flour, including moisture reduction and high wettability, solubility and sensory acceptance of the chocolate aroma. The principal component analysis of QDA® explained 90% variances; cluster analysis enabled the definition of four groups for six cappuccino preparations. The primary characteristics of cappuccinos with dry jackfruit seeds were cappuccino, chocolate, cinnamon and coffee aromas, and cappuccino and chocolate tastes. Dry jackfruit seed flour can be incorporated as an ingredient in cappuccino formulations; 50% and 75% substitution of cocoa powder by dry jackfruit seed flour did not change sensory acceptability or characteristics.

Keywords: Jackfruit seeds; Chocolate aroma; Waste utilization; QDA; Cocoa substitute; Artocarpus heterophyllus Lam

5.1. INTRODUCTION

Jackfruit (Artocarpus heterophyllus Lam.) is a syncarp native to India, that is present in tropical regions and composed of stuffs pulp and seeds (Gohain Barua & Boruah, 2004). Ripe jackfruits are large, with weights ranging from 2 to 36 kg; seeds represent 18%–25% the fruit weight (Madrigal-Aldana et al., 2011). Each seed is covered with a golden-yellow fleshy aril and has a strong, sweet taste (Saxena, Bawa,
& Raju, 2011). Generally, jackfruit is eaten raw and processed (canned juice and leather), and seeds are eaten after boiling, steaming and roasting (Madrigal-Aldana et al., 2011; Saxena; Bawa; Raju, 2011). However, jackfruit is still underused due to seasonality, difficulty in logistics and conservation, and low consummation due to a high sensory intensity of taste and aroma in addition to an association of jackfruit with poor communities. Thus, jackfruit is seldom added to other products.

Jackfruit seeds are a source of fiber: approximately 2% and 26% (dry base), soluble and insoluble dietary fiber, respectively (Mahanta & Kalita, 2015). These seeds provide positive physiological effects when consumed. Jackfruit seed flour also has high levels of potassium, calcium and sodium (Ayala-Zavala et al., 2011). In recent years, jackfruit seeds gained the attention of researchers as an alternative source of starch and protein that can be industrially exploited (Madrigal-Aldana et al., 2011; Madruga et al., 2014; Saxena et al., 2011).

In addition, roasting jackfruit seeds (after drying and/or fermentation processes) produced changes in the aroma sensory profile that resulted in an agreeable chocolate aroma (Spada et al., 2017) Chapter 1 and 2. Roasting jackfruit seeds, using the proper combination of temperature and time provides the Maillard reaction with characteristic cocoa flavor, but it also decrease undesirable odors, such as aliphatic acids. The main final volatile composition included pyrazines, Strecker aldehydes, alcohols, esters and furanes.

Jackfruit seed flour is a chocolate substitute because of the chocolate aroma and similar color (Spada et al., 2017) Chapter 1 and 2; it has the added advantage of having less calories than cocoa due to the lower lipid content of jackfruit seeds (0.7%–2.2%) compared to Forastero cocoa beans (53%–39%) (Afoakwa, 2010; Gu et al., 2013). Recently, the price of cocoa has climbed, so there is an incentive in the food industry to find a cocoa substitute (Fadel et al., 2006). In addition, the estimates of cocoa beans demand by 2020 is large; however, production is not expected to grow significantly in the next 10 years (FAO, 2010). In this context, jackfruit seed is an agreeable chocolate substitute and could be provide an alternative to revenue streams for communities in tropical countries.

Generally, studies with cocoa substitutes formulate chocolate with milk (Dogan et al., 2016; Medeiros & Lannes, 2009; Srour et al., 2016). Cappuccino is a
potential product that uses milk as an ingredient because consumers expect a chocolate aroma without strong chocolate taste, as is characteristic of cappuccinos. In this study, jackfruit seed flour was substituted while producing cappuccino with similar sensory acceptability to preparations using just cocoa powder. This study characterized and developed a food application for jackfruit seed flour and consequentially, utilizes this tropical fruit waste by incorporating it as an ingredient in a common product of the human diet.

5.2. MATERIALS AND METHODS

5.2.1. Jackfruit

Jackfruits, hard pulp variety, was manually collected between October 2013 and January 2014 in the countryside of São Paulo, Brazil, and fruits of similar size (5 ± 1 kg) and maturity, as indicated by the yellow color of the shell, were collected. Jackfruits were cleaned manually in running water, and the seeds were removed. Cocoa powder was provided by Cargil®. The other ingredients were bought at a local market; the same lot for all ingredients was used to standardize the cappuccino formulations.

5.2.2. Jackfruit seed flour

Two different treatments were executed. For dry jackfruit seed flour: seeds were dried in an oven (Probat® laboratory sample roaster, Germany) at 60°C with air circulation, for 24 h. After that time, the spermoderms were manually removed and the seeds dried for 24 h more at 60°C. Portions of 200 g of dry seeds were roasted in a rotary electric oven for 47 min at 171°C. For fermented jackfruit seed flour, seeds were fermented with pulp and banana leaves by placing them in a closed bucket for six days to encourage anaerobic fermentation and alcohol production. Daily over the next six days, the bucket was opened, and the fermenting mass was upturned manually to encourage alcohol oxidation and acetic acid production. After twelve days, the pulp and banana leaves were removed, and the fermented seeds were treated with the
same method as the drying process, they were roasted at 154°C for 35 min. In the final stages, 200 g seeds portions were ground, using unheated mill hammers, to preserve the volatile compounds of the flour (Spada et al., 2017) chapter 1 and 2. Flours were packed under vacuum and stored without light at 8±1°C.

5.2.3. Cappuccino formulations

The formulations were elaborated based on American patent US5721003A (Zeller, 1998), ingredients of Melitta® cappuccino, Nescafé® cappuccino capsule, the Brazilian food legislation and ingredients recommendation (BRASIL, 2000). Seven kinds of cappuccinos were produced: one control with cocoa powder, three products that replaced cocoa powder for dry jackfruit seed flour roasted at 171°C for 47 min, and others three using fermented jackfruit seed flour roasted at 154°C for 40 min. Cappuccinos with jackfruit seeds were formulated with 50%, 75% and 100% control cocoa composition (Table 1 and Fig. 1A).

It is noteworthy that the commercial cappuccino Melitta® was added to determine the similarity of color with the developed product.

5.2.4. Physicochemical analysis

Water activity was measured from the temperature of the dew point (Aqualab®), and moisture was determined by a standard gravimetric method using infrared light (Bel Engineering Modelo B-TOP-Ray). The wettability was measured using the immersion method based on the work of Hogekamp & Schubert (2003). The time between placing a powder sample (2.5 g) of given height (5 cm) on a liquid surface (80°C - optimized temperature for best solubility for jackfruit seeds) and achieving complete wetting was determined. The measurement enabled free sinking of particles, so that unsteady and steady state wetting occurred (Hogekamp & Schubert, 2003). For calculations, the equation one (Eqn 1) was applied. The apparent density was measured, based on the work of Micha (1983), in a 100 mL graduated cylinder, by addition of the required weight of the cappuccino formulation to generate 30 mL.

\[
\text{Wettability} = \frac{1}{\text{time} \ (s)}
\]
Solubility was determined based on the method used by (Madruga et al., 2014) by weighing 0.1 g cappuccino formulations into weighed centrifuge tubes and adding 10 mL of distilled water. The suspension was stirred and placed in a water bath for 20 min at 80°C, and then the tubes were centrifuged (NT 825) for at 25°C for 15 min at 4420 rpm. An aliquot (5 mL) was transferred from the supernatant to a Petri dish and placed on the stove at 105°C for 24 h to determine the weight of the solid. The solubility was determined by Eqn 2:

\[
\text{Solubility (\%) = \frac{\left(\text{weight of plate with sample after evaporation} - \text{weight of plate}\right) \times 100}{\text{weight of plate}}}
\]

Eqn 2.

5.2.5. Instrumental color analysis

Color was measured using a Minolta® colorimeter, with illuminant C, previously calibrated with a white surface (Y = 93.7, x = 0.3135 and y = 0.3195) based on the CIE-lab L*, a* and b* scale. The Chroma (C), which sets the actual color of the cappuccino mixes from each treatment, was expressed as in (Eqn 3) and the indicator angle of color saturation, Hue Angle (H°), was expressed in (Eqn 4). The pH was determined in triplicate using two grams of the cappuccino formulation added to 20 mL distilled water (Bible & Singha, 1993).

\[
C = \sqrt{a^*^2 + b^*^2}
\]

Eqn 3.

\[
H^\circ = \arctan \frac{b^*}{a^*}
\]

Eqn 4.
5.2.6. Sensory analysis

Sample preparation. One day before sensory tests, ingredients (Table 1 and Table suppl) were weighed according to each formulation and homogenized. The cappuccino formulations were portioned into 10 g samples. During the sensory tests, 50 mL water at 60°C was added to cappuccino formulations in a polystyrene thermal cup. Every sample was prepared immediately after the panelist arrived.

5.2.7. Consumer study

The acceptance test was carried out on a laboratory scale (Meilgaard et al., 2007; Stone & Sidel, 1998) with one session using 126 consuming assessors (55% female; 18–42 years old; nonsmoker) recruited from the students and staff of the University of São Paulo - ESALQ, selected because they liked and consumed coffee. The available control, dry and fermented cappuccino preparations had 50%, 75% and 100% jackfruit seed flour. The consuming assessors evaluated the appearance, aroma, taste and overall impression using a nine points hedonic scale (1=disliked extremely; 9=liked extremely) to determine how much they liked the cappuccino preparations. Samples were randomly evaluated; the consumers were considered repetitions in an incomplete block reply 18 times (T = 7, k = 4, r = 4, B = 7, L = 2, E = 0.88) where T = number of samples; k = number of samples in each ranking test; r = number of times each sample was shown in each block; B = number of panelists in each block; L = number of times the samples were shown together; and E = dependability of the analysis (Cochran & Cox, 1950).

5.2.8. QDA®

Sensory evaluations were approved by the Ethics Committee of Human Research of the ESALQ/USP (COET/077/131). Conventional profiling using QDA® was applied according to Meilgaard et al., (2007); Moskowitz, (1983) and Stone & Sidel, (1998). In the first stage, 20 nonsmoker volunteers were recruited among students and employees of the University of São Paulo – ESALQ, and a pre-selection was performed
to evaluate their ability to discriminate tastes and odors through basic taste tests, odor recognition tests and sequential analysis using triangle tests. For the second stage, 12 panelists were selected, all females, 18–35 years old, to define the descriptive terminology for the sensory attributes of cappuccino with dry or fermented jackfruit seeds during six training sessions. The panelists were trained in sensory analysis of cappuccino to present them with the sensorial attributes identified to be important in cappuccino preparations.

Each attribute was provided, together with definitions and physical references using formulated cappuccinos, similar to commercial (Table suppl and Fig. 1B) and cappuccinos added to jackfruit seed flours (Table 2). The generation of a unique list of attributes was achieved by consensus (two sessions), and the discrepant terms were eliminated. The final attributes were chocolate (choaro), cappuccino (caparo), coffee (cofaro), cinnamon (cinaro), and fermented (feraro) as attributes for aroma; chocolate (chotas), cappuccino (captas), and fermented (fertas) as attributes for taste; brown (broapp) as the attribute for appearance; gritty (gritex) as the attribute for texture; and overall impression (oveimp). Thus, the reference material was established and the intensity scores were determined for each attribute (Table 2), which were used in the sensory analysis stage.

The sensorial evaluation of the samples was performed in three sessions for cappuccinos with dry jackfruit seeds and another three sessions for cappuccinos with fermented seeds (50%, 75% and 100% substituted). Samples were prepared with 10 g powder in 50 mL water (60°C), kept at room temperature (25°C) in random order, and numbered with three digits in a random manner to eliminate errors due to residual taste in individual booths under white light. The tasters were instructed to describe the sensations perceived regarding choaro, caparo, cofaro, cinaro, feraro, chotas, captas, fertas, broapp, gritex and oveimp of the samples using a nine-point intensity scale ranging from less intense to more intense for attributes.
5.2.9. Statistical analysis.

Physicochemical analyses were available in triplicate, and analysis of variance (ANOVA) was carried out to analyze the results. The comparisons of treatments were performed with Tukey's test (p≤0.05). The acceptance test was determined using Compusense Five® by the Tukey's test (p≤0.05). The QDA® results were submitted to multivariate analysis using the correlation analysis (CORR), principal component analysis with biplot graph (PCA) and cluster analysis (CA). The CORR was performed to evaluate the interdependence among variables, calculating the correlation matrix. PCA was used to determine the sensorial characterization of the cappuccinos with 50%, 75% and 100% replacing cocoa powder with dry and fermented jackfruit seed flour. In the CA, the cutoff was the average method with the Euclidean distance as the similarity coefficient with a cutoff at 0.70. The statistical software used for all tests was SAS 9.3 (Statistical Analysis System Institute – SAS, 2005).

5.3. RESULTS AND DISCUSSION

5.3.1. Physicochemical analysis

The density was similar (p≤0.05) for all available treatments (Table 3). The addition of more than 50% fermented jackfruit seed flour reduced the pH compared to the cappuccino formulated with cocoa powder. Moisture and water activity (aW) were lowest in cappuccino made with jackfruit seeds. Wettability and solubility were higher in cappuccino with jackfruit seed flour compared to the control (Table 3).

The pH would affect the intensity of the sour taste, which is linearly related to the summation of the molar concentrations of organic acid species that contain at least one protonated carboxyl group plus the concentration of free hydrogen ions (Ohanningsmeier & Oger, 2005). In addition, salt of the organic acid in the food further lowers the ionization by common effect (Meyer, 1960). In cocoa nibs, the alkalization process raises the pH. This process provides cocoa nibs at pH 6.0 with chocolate flavor or nibs at pH 7.2 to 8.1 that is typical of dark chocolate with the sour, bitter, fruity and
moldy characteristics. This may influence the acceptability reductions in cappuccinos with fermented jack fruit seed flour.

Formulated cappuccinos have aW similar to that found by Srour et al., (2016) when they developed milk beverages with some varieties of carob powder. The values of aW were between 0.29 and 0.41. However, Yousif & Alghzawi, (2000) identified 20% higher aW using carob powder, and moisture was three times larger in comparison to cappuccinos: 9.6% and 9.0% for carob and cupuassu, respectively. These are technological advantages because they reduce potential microorganism proliferation and change compaction and mechanical proprieties (Ostrowska-Ligeza & Lenart, 2015).

The low wettability in the cappuccino control was influenced by high cocoa concentration (Fitzpatrick et al., 2016), and this value was compatible to Dogan et al., (2016). Cappuccino formulations had higher wettability, which predisposed for higher levels of solubility, probably because cocoa beans have ten times more lipids than jack fruit seeds. In effect, cappuccinos with jack fruit seeds are similar or better than other natural substitutes currently in use (carob and cupuassu).

5.3.2. Consumer study

These results demonstrated the innovate potential of dry jackfruit seeds as a cocoa powder replacer. Carob and cupuassu are established substitutes for cocoa powder; however, when they were used in milk beverages, the sensory acceptation was reduced (Dogan et al., 2016; Medeiros & Lannes, 2009). Before this study, the low acceptability of cocoa substitutes was justified due to low lipid concentrations, but jackfruit seeds also have low lipid content did not reduce the consumer acceptance when 50% or 75% cocoa powder was submited. Most likely, the pH reduction in cappuccino using fermented flours (75% and 100% substitution) improved sour and moldy tastes; arrange the acceptance (ps0.05) for taste, and this may have aroma and overall impression.

The dry jackfruit seed flour utilization with 50% and 75% substitution improved the aroma and cappuccino acceptability. However, fermented flour used for 75% and 100% cocoa powder substitution reduced the aroma acceptability (Table 5).
The taste was still similar to the control using dry jackfruit seed flour (p≤0.05), but fermented flour reduced the acceptance of taste (Table 5). The overall impression was still equal to the control when dry jackfruit seed flour was used (p≤0.05).

Bull et al. (2015) determined that the flavor profile was maintained to be the most important attribute for the consumer, and an even better prediction of all of the sensory attributes would be expected if a larger number of instrumental and/or chemical measurements were considered. Thus, the next analysis (QDA) will define which sensory attributes were responsible for improving acceptance or rejection of cappuccinos with dry seeds flour or fermented flour, mainly to explain aroma, taste and overall impression results.

**5.3.3. Instrumental color and consumer studies**

Color results demonstrated the 50% cocoa substitution had similar chroma values compared to the cappuccino control (p≤0.05) (Table 4). Independent of the kind of jackfruit seed flour, 75% produced formulations with the same yellowness (b*) as the control, but the clearest were observed with Hue results (Table 4). The commercial standard was the clearest (low L*, chrome and Hue) (Table 4 and Fig. 1A).

Other evidence of similar color was related by consumers, because they not only found appearance modifications between preparations (p≤0.05), but the values indicated greater acceptability, with scores higher than seven (Table 5). In fact, cappuccino color did not change the sensory acceptation.

The dark brown color in jackfruit seeds was produced by the Maillard reaction, also typical in cocoa nibs due to ideal roasting conditions, reducing sugars and amino groups (Afoakwa, 2010; Beckett, 1999; Gu et al., 2013). In this study, it was independent of the fermentation process. The color in cappuccinos with jackfruit seeds did not change the sensory acceptance; thus, cappuccino formulations with jackfruit seed flour had compatible or better color in comparison to control and commercial cappuccinos, which is also another indication of the high quality of this natural cocoa substitute sample.
5.3.4. Quantitative descriptive analysis (QDA)

In the CORR analysis, based on the method of Sokal & Rohlf (1980), values above |0.70| were considered accentuated, and 22 correlations were thus selected (Table 6). There are seven above 0.90, which were regarded as very strong, and there are 13 others above 0.70. All variables had at least one correlation; for caparo, strong correlations were observed with captas, broapp, cinaro and oveimp. The captas was correlated (>0.9) with broapp and cinaro; even as cofaro was also correlated with oveimp (>0.9). High intensity of feraro and fertas reduced chocolate taste and consequentially oveimp (Table 5), other factors correlated with cocoa substitution were gritex and choaro, which were both strongly correlated.

Principal component analysis (PCA) and cluster analysis (CA). PCA is represented in a biplot graph divided by the projection of variables (Fig. 2A) and observations (Fig. 2B). In this study, two PCs were taken from the total data set, explaining 90.22% of the variance. The variable oveimp was not correlated in PCs because they were not relevant to the scientific foundation of the results. The first principal component (PC1) explained 62.41% of the statistical variance and was positively correlated (right side) with the variables caparo, captas, chotas, cinaro and cofaro and negatively correlated (left side) with feraro and fertas. The second principal component (PC2) explained 27.81% of the statistical variance and was positively correlated (top) with the variables broapp and feraro and negatively (bottom) correlated with gritex.

In CA, observations were separated into four groups (D100; D50 and D75; F100; and F50 and F75) by the cutoff held at 0.70, shown by the dotted line (Fig. 3). The same cutoff was used to separate the groups (dotted circles) shown in the projection of observations (Fig. 2B). Observations about samples D100 and F100 were individually separated in CA (Fig 3. and Table 2) because they had different characteristics from the other preparations.

Generally, treatments D100 and F100, with a higher substitution of jackfruit seed flour, received the lowest scores compared to other groups (D50-D75 and F50-F75). However, the D100 formulation receives the maximum value for gritex, and F100 had highly fermented aroma and taste; different from D100, in F100 choaro is higher.
For captas, F100 and D100 were similar, which was characterized by close values to the overall average.

For other cappuccino formulations using fermented flours (F50 and F75), the main characteristics were low gritex, caparo, cofaro, cinaro and chotas, and high values of broapp, choaro, fertas, and fearo were observed, as well. In F75 group, low coffee, cappuccino and cinnamon aromas and chocolate and cappuccino tastes were noted. The appearance was light brown, and the fermented tastes and aromas were more evident.

The group formed by D50 and D75 had high caparo, captas, choaro, chotas, cinaro and cofaro. The addition of jackfruit seed flour (D75) reduced broapp and increased gritex.

The use of jackfruit seed with a replacing of cocoa powder in cappuccino formulations is possible, dry seeds have more potential because it not has taste and aroma fermented. The ideal level of cocoa substitution in cappuccino formulations are nearest 50 and 75%, it is possible find some vantages using jackfruit seed flour, as a moisture reduce, high wettability, solubility, sensory acceptation by chocolate aroma and other similarities, such as color, density, pH, aW and appearance. Fermented attributes under-characterized the cappuccino preparations; PCA explaining 90% variance; using CA was possible define four groups to seven cappuccino preparations.

In the new cappuccino preparation, the characteristic aroma and taste were interdependent, with the attributes brown color and cinnamon aroma having a positive impact on overall impressions. Briefly, the aromas of cappuccino, coffee, cinnamon, and the taste of cappuccino were highly correlated and expected for a good overall impression of cappuccinos. The fermented aroma and taste were very interdependent and not characteristic in these preparations. This explained the few overall impressions of cappuccinos made with fermented jackfruit seed flour.

The addition of jackfruit seed flour, independent of quantity, reduces Choaro in cappuccino preparation. Nevertheless, this study observed a sensory acceptable substitution (50 and 75%) using jackfruit seed flour.

Principal component analysis (PCA) and cluster analysis (CA). The choaro was higher in F100, which is possible because the fermentation process improves volatile compounds, such as pyrazines and esters (Spada et al., 2017) Chapter 1 and 2, and
because dark color is generally associated with high chocolate concentration, meaning that broapp was great in F100. However, the high values for feraro and fertas in F100 produced an over-taste not characteristic of cappuccinos; thus D100 received more caparo and chotas in comparison to F100, even when D100 was less soluble.

The other group for CA corresponding to F50 and F75 was characterized as little gritex, caparo, cofaro and chotas; medium cinaro and higher capta, browapp, choaro, fertas and feraro. Thus, even the high chocolate aroma of the characteristic cappuccino aroma was reduced because other fermented flavors were found; these flavors were not expected in cappuccino preparations. The recognized food was related to the construction of different sensory systems to name foods or first to cause our survival (Prescott & Monteleone, 2015). To minimize the fermented flavor, appropriate amounts of flavors could be produced depending on fermentation conditions. For example, the mode of fermentation, natural or inoculated, which is usual for cocoa beans (Afoakwa, 2010; Kongor et al., 2016). In fact, better standardization of the chocolate fermentation process could be generate a chocolate with different sensory characteristics (Menezes et al., 2016). Thus, future studies will know the flavor characteristics of jackfruit seeds using inoculated cultures of microorganisms.

The key to effective perception is that sensory information is interpreted as qualities that belong to the object itself (Prescott & Monteleone, 2015). Thus recognizing the familiar cappuccino, chocolate taste and coffee, cinnamon, chocolate, cappuccino and aromas explained the reason why the group with D50 and D75 was characterized as similar to commercial cappuccinos; in these samples, cappuccino identification improved sensory acceptability, as well. Januszewska & Viaene, (2001) reported the familiarity limit of chocolate preferences, suggesting that familiarity with the product imposed a major influence on preference. Regarding the reduced broapp and increased gritex, which was expected when jackfruit seed flour was added in high levels, actually not have a perfect natural replacer to cocoa powder. Thus, it could be inferred that the D50 and D75 preparations are able to provide a cappuccino with sensory characteristics similar to using cocoa powder.
In summary, 100% replacement of cocoa powder with jackfruit seed flour resulted in preparations that were not as high and not characterized as cappuccinos preparations, but the use of 75% and 50% of cocoa substitutes resulted in similar profiles. Fermented flour did not have the expected attributes of cappuccinos preparations (fermented taste and aroma); thus, preparations with dry seeds were more similar to cappuccinos (control) with 15% cocoa.

5.4. CONCLUSIONS

It is possible used jackfruit seed flours to do cappuccino formulations. Six cappuccino formulations were tested to replace cocoa powder by jackfruit seeds. Dried jackfruit seed flour can be incorporated as an ingredient in cappuccino formulations; 50% and 75% substitution of cocoa powder by dried jackfruit seed flour did not change sensory acceptability or characteristics. Fermented attributes were not characteristic of cappuccinos, but they improved the chocolate aroma. The primary characteristics responsible for the character of cappuccinos with dry jackfruit seeds were cappuccino, chocolate, cinnamon and coffee aromas, and cappuccino and chocolate tastes.

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Table 1. Cappuccino formulations with dry or fermented jackfruit seed flours, replaced with 50%, 75% and 100% cocoa powder. *Proportions with 50%, 75% and 100% substitution to cocoa powder. ** with 2% corn starch.

| Ingredients (g)                      | Controle | 50%* | 75% | 100% |
|-------------------------------------|----------|------|-----|------|
| Jackfruit seeds flour (dry or fermented) | 0.00     | 7.50 | 11.25 | 15.00 |
| Cocoa                               | 15.00    | 7.50 | 3.75 | 0.00 |
| Powdered sugar**                    | 41.50    | 41.50 | 41.50 | 41.50 |
| Powdered milk                       | 30.35    | 30.35 | 30.35 | 30.35 |
| Soluble coffee                      | 10.00    | 10.00 | 10.00 | 10.00 |
| Sodium bicarbonate                  | 1.75     | 1.75 | 1.75 | 1.75 |
| Cinnamon powder                     | 0.40     | 0.40 | 0.40 | 0.40 |
| Soy lecithin                        | 0.50     | 0.50 | 0.50 | 0.50 |
| Xanthan gum                         | 0.50     | 0.50 | 0.50 | 0.50 |
| TOTAL                               | 100      | 100  | 100  | 100  |

*Proportions with 50%, 75% and 100% substitution to cocoa powder. ** with 2% corn starch.
Table suppl. Cappuccino formulations for QDA® reference scale extremes.

| Ingredients (g)                  | I      | II     | III    | IV     | V      | VI     | VII    |
|----------------------------------|--------|--------|--------|--------|--------|--------|--------|
| Dry flour jackfruit seeds        | 0.00   | 0.00   | 0.00   | 16.50  | 0.00   | 0.00   |        |
| Cocoa                            | 15.00  | 15.00  | 0.00   | 15.00  | 0.00   | 18.75  | 14.93  |
| Powdered sugar **                 | 41.50  | 41.50  | 41.50  | 41.03  | 39.94  | 41.31  |        |
| Powdered milk                    | 30.35  | 30.75  | 45.35  | 40.35  | 28.87  | 30.61  |        |
| Soluble coffee                   | 10.00  | 10.00  | 10.00  | 0.00   | 9.88   | 9.60   | 9.95   |
| Sodium bicarbonate               | 1.75   | 1.75   | 1.75   | 1.73   | 1.66   | 1.74   |        |
| Cinnamon powder                  | 0.40   | 0.40   | 0.40   | 0.39   | 0.38   | 0.46   |        |
| Soy lecithin                     | 0.50   | 0.50   | 0.50   | 0.49   | 0.40   | 0.50   |        |
| Xanthan gum                      | 0.50   | 0.50   | 0.50   | 0.49   | 0.40   | 0.50   |        |
| Total                            | 100    | 100    | 100    | 100    | 100    | 100    | 100    |

I - Cappuccino with cocoa powder (Control); II - cappuccino base without cinnamon; III - cappuccino base without cocoa powder; IV - cappuccino base without coffee; V - cappuccino plus 10% dry jackfruit seed flour; VI - cappuccino plus 25% cocoa powder; VII - cappuccino with 15% cinnamon. ** with 2% corn starch.
Table 2. QDA® attributes, definitions and descriptions for standard, with results of Cappuccino samples.

| Reference description | Scale extremes | Preparations |
|-----------------------|----------------|--------------|
| **Modality** | **Attribute** | **Definition** | **Minimum** | **Maximum** | **D50** | **D75** | **D100** | **F50** | **F75** | **F100** |
| | | Intensity of colour, from pale to dark | | | |
| Appearance | Brown | (Fig.1B) | Cappuccino with 0; 3.75; 7.5; 11.25 and 15% of cocoa powder | 7.46 | 6.61 | 5.18 | 7.32 | 6.27 | 5.21 |
| Aroma | Chocolate | Intensity of chocolate odour | Cappuccino base without Cappuccino with plus 25% of cocoa powder | 6.89 | 5.97 | 4.28 | 6.03 | 7.18 | 4.66 |
| Aroma | Cappuccino | Odour associated with Cappuccino | Cappuccino base without Cappuccino with cocoa powder | 7.27 | 6.48 | 4.67 | 6.36 | 5.38 | 4.55 |
| Aroma | Coffee | Intensity of coffee odour | Cappuccino base without Cappuccino with plus 25% of coffee | 4.19 | 3.78 | 2.90 | 1.79 | 1.63 | 1.27 |
| Aroma | Cinnamon | Intensity of cinnamon odour | Cappuccino base without Cappuccino with plus 15% of cinnamon | 3.40 | 2.63 | 1.94 | 1.44 | 1.12 | 0.83 |
| Aroma | Fermented | Odour associated with cell room or beer | Cappuccino base without Cappuccino with cocoa flour to fermented jackfruit seed powder (Control) in water 1:2 | 1.66 | 3.08 | 4.39 |
| Taste | Chocolate | Intensity of chocolate flavour | Cappuccino base without Cappuccino with plus 25% of cocoa powder | 5.65 | 6.62 | 5.65 | 6.03 | 4.96 | 4.15 |
| Taste | Cappuccino | Intensity of cappuccino flavour | Cappuccino base without Cappuccino with cocoa powder | 7.29 | 6.59 | 4.77 | 6.40 | 5.47 | 4.56 |
| Taste - Aftereffect | Flavour sensation which occurs after the swallow of the product and the sensations perceived in the mouth |
|---------------------|--------------------------------------------------------------------------------------------------|
| Fermented           | Cappuccino with cocoa Powder (Control) \(^1\) in water 1:2                                    |
|                     | Flour to fermented jackfruit seed in water 1:2                                                 |
|                     | ![Table](suppl_1)                                                                                 |
| Texture - Mouthfeel | Gritty The presence of small, hard particles.                                                    |
|                     | Cappuccino with cocoa Powder (Control) \(^1\) to dry jack seeds \(^3\)                          |
|                     | Cappuccino base without cocoa Powder (Control) \(^1\)                                            |
| Overall impression  |                                                                                                 |
| Global perception   |                                                                                                 |

Dry seeds flour (D); Fermented seeds flour (F). Proportions with 50, 75 and 100% of substitution. I, II, III, IV, V, VI and VII are formulated in Table suppl 1.
Table 3. Physicochemical properties of cappuccino formulations (mean ± standard deviation).

|          | aW     | Moisture | pH      | Wettability | Apparently density | Solubility |
|----------|--------|----------|---------|-------------|-------------------|------------|
| Control  | 0.39 ± 0.004<sup>a</sup> 3.35 ± 0.16<sup>c</sup> 6.76 ± 0.005<sup>ab</sup> 0.07 ± 0.06<sup>c</sup> 17.93 ± 0.02<sup>a</sup> | | | | 3.08 ± 0.243<sup>de</sup> |
| D50      | 0.38 ± 0.001<sup>b</sup> 2.84 ± 0.04<sup>ab</sup> 6.75 ± 0.002<sup>b</sup> 0.28 ± 0.10<sup>b</sup> 19.01 ± 0.04<sup>a</sup> | | | | 3.77 ± 0.081<sup>b</sup> |
| D75      | 0.36 ± 0.003<sup>c</sup> 3.12 ± 0.08<sup>ab</sup> 6.71 ± 0.002<sup>b</sup> 0.20 ± 0.03<sup>bc</sup> 18.64 ± 0.04<sup>a</sup> | | | | 3.71 ± 0.015<sup>bc</sup> |
| D100     | 0.33 ± 0.007<sup>f</sup> 3.09 ± 0.02<sup>ab</sup> 6.82 ± 0.002<sup>a</sup> 0.29 ± 0.02<sup>b</sup> 18.92 ± 0.02<sup>a</sup> | | | | 2.97 ± 0.210<sup>f</sup> |
| F50      | 0.37 ± 0.001<sup>c</sup> 2.83 ± 0.04<sup>ab</sup> 6.73 ± 0.003<sup>b</sup> 0.32 ± 0.10<sup>b</sup> 18.18 ± 0.01<sup>a</sup> | | | | 3.53 ± 0.312<sup>bode</sup> |
| F75      | 0.38 ± 0.001<sup>b</sup> 2.75 ± 0.05<sup>ab</sup> 6.55 ± 0.002<sup>c</sup> 0.24 ± 0.04<sup>bc</sup> 18.74 ± 0.02<sup>a</sup> | | | | 3.58 ± 0.225<sup>bcd</sup> |
| F100     | 0.35 ± 0.001<sup>d</sup> 2.77 ± 0.04<sup>ab</sup> 6.56 ± 0.003<sup>c</sup> 0.39 ± 0.04<sup>ab</sup> 18.46 ± 0.00<sup>a</sup> | | | | 3.19 ± 0.031<sup>bde</sup> |

Control: Cappuccino with 15% cocoa powder; proportions with 50%, 75% and 100% substitution. D50: cappuccino with 7.5% dry jackfruit seed flour and 7.5% cocoa powder; D75: cappuccino with 11.25% dry jackfruit seed flour and 3.75% cocoa powder; D100: cappuccino with 15% dry jackfruit seed flour; F50: cappuccino with 7.5% fermented jackfruit seed flour and 7.5% cocoa powder; F75: cappuccino with 11.25% fermented jackfruit seed flour and 3.75% cocoa powder; and F100: cappuccino with 15% fermented jackfruit seed flour. Different letters in the same column differ significantly (p ≤ 0.05) by Tukey’s test.
Table 4. Color characterization of cappuccino formulations (mean ± standard deviation).

|                  | Lightness (L*) | Redness (a*) | Yellowness (b*) | Chroma (C) | Hue (H°)  |
|------------------|----------------|--------------|-----------------|------------|----------|
| Control          | 40.67 ± 0.19a | 11.02 ± 0.17a| 11.66 ± 0.17ab  | 16.05 ± 0.01a | 46.62±0.00' |
| D50              | 44.07 ± 0.37d | 10.35 ± 0.06b| 11.71 ± 0.1ab   | 15.63 ± 0.01abc | 48.51±0.01d |
| D75              | 47.40 ± 0.55c | 9.72 ± 0.17c | 11.73 ± 0.11ab  | 15.23 ± 0.01bc | 50.35±0.01c |
| D100             | 52.48 ± 0.62b | 8.17 ± 0.12d | 11.23 ± 0.07b   | 13.89 ± 0.01e | 53.96±0.01b |
| F50              | 44.37 ± 0.33d | 10.53 ± 0.16b| 11.89 ± 0.18a   | 15.88 ± 0.02ab | 48.49±0.00d |
| F75              | 44.99 ± 0.55cd| 9.41 ± 0.12c | 11.53 ± 0.08ab  | 14.89 ± 0.01cd | 50.78±0.01c |
| F100             | 51.30 ± 2.68b | 8.14 ± 0.42d | 11.63 ± 0.72ab  | 14.19 ± 0.06de | 55.01±0.01a |
| Commercial       | 61.09 ± 0.41a | 7.99 ± 0.12d | 7.33 ± 0.11c    | 10.84 ± 0.01f | 42.56±0.01f |

Control: produced with 15% cocoa powder; dry seeds flour (D); and fermented seeds flour (F). Proportions with 50%, 75% and 100% substitution. D50: cappuccino with 7.5% dry jackfruit seed flour and 7.5% cocoa powder; D75: cappuccino with 11.25% dry jackfruit seed flour and 3.75% cocoa powder; D100: cappuccino with 15% dry jackfruit seed flour; F50: cappuccino with 7.5% fermented jackfruit seed flour and 7.5% cocoa powder; F75: cappuccino with 11.25% fermented jackfruit seed flour and 3.75% cocoa powder; and F100: cappuccino with 15% fermented jackfruit seed flour. Commercial: Melitta® powder for traditional cappuccino preparation. Different letters in the same column differ significantly (p≤ 0.05) by Tukey's test.
Table 5. Average values of sensory scores of cappuccino preparations at the acceptance test.

|                | Appearance | Aroma     | Taste     | Overall impression |
|----------------|------------|-----------|-----------|--------------------|
| Control        | 7.29<sup>a</sup> | 6.74<sup>bc</sup> | 6.60<sup>a</sup> | 6.83<sup>a</sup>    |
| D50            | 7.05<sup>a</sup> | 7.55<sup>a</sup> | 6.54<sup>a</sup> | 6.88<sup>a</sup>    |
| D75            | 7.06<sup>a</sup> | 7.04<sup>ab</sup> | 6.31<sup>ab</sup> | 6.71<sup>a</sup>    |
| D100           | 6.99<sup>a</sup> | 6.46<sup>bc</sup> | 5.95<sup>abc</sup> | 6.30<sup>ab</sup>   |
| F50            | 7.10<sup>a</sup> | 6.06<sup>cd</sup> | 5.65<sup>bc</sup> | 6.02<sup>bc</sup>   |
| F75            | 7.19<sup>a</sup> | 5.56<sup>d</sup> | 4.51<sup>d</sup> | 5.30<sup>d</sup>    |
| F100           | 7.06<sup>a</sup> | 5.62<sup>d</sup> | 5.12<sup>ed</sup> | 5.54<sup>cd</sup>   |

Control: produced with 15% cocoa powder; dry seeds flour (D); and fermented seeds flour (F). Proportions with 50%, 75% and 100% substitution. D50: cappuccino with 7.5% dry jackfruit seed flour and 7.5% cocoa powder; D75: cappuccino with 11.25% dry jackfruit seed flour and 3.75% cocoa powder; D100: cappuccino with 15% dry jackfruit seed flour; F50: cappuccino with 7.5% fermented jackfruit seed flour and 7.5% cocoa powder; F75: cappuccino with 11.25% fermented jackfruit seed flour and 3.75% cocoa powder; and F100: cappuccino with 15% fermented jackfruit seed flour.
Table 6. Correlation coefficients observed for the sensory variables studied.

| Variables         | Correlation coefficients |
|-------------------|--------------------------|
| Caparo - Captas   | 0.99                     |
| Feraro - Fertas   | 0.99                     |
| Cinaro - Cofaro   | 0.98                     |
| Oveimp - Cofaro   | 0.96                     |
| Broapp - Caparo   | 0.95                     |
| Broapp - Captas   | 0.95                     |
| Oveimp - Cinaro   | 0.92                     |
| Cofaro-Feraro     | -0.89                    |
| Fertas-Cofaro     | -0.88                    |
| Arofer-Cinaro     | -0.85                    |
| Fertas-Chotas     | -0.85                    |
| Fertas-Cinaro     | -0.85                    |
| Chotas-Feraro     | -0.84                    |
| Feraro-Chotas     | -0.84                    |
| Gritex - Cinaro   | 0.78                     |
| Captas - Cinaro   | 0.77                     |
| Broapp - Choaro   | 0.77                     |
| Caparo - Cinaro   | 0.76                     |
| Feraro-Oveimp     | -0.76                    |
| Choaro-Gritex     | -0.76                    |
| Fertas-Oveimp     | -0.75                    |
| Broapp-Gritex     | -0.73                    |

Caparo – cappuccino aroma; captas – cappuccino taste; feraro – fermented aroma; fertas – fermented taste; cinaro – cinnamon aroma; cofaro – coffee aroma; oveimp – overall impression; broapp – brown appearance; gritex – gritty texture; and choaro – Chocolate aroma.
Figure 1A. Cappuccino formulations. Control: produced with 15% cocoa powder, dry jackfruit seed flours (D); and fermented jackfruit seed flours (F). Proportions with 50%, 75% and 100% substitution. Commercial: Melitta® powder for traditional cappuccino formulation.

Figure 1B. Cappuccino preparations for QDA® reference scale extremes. Cappuccino with 0.00%; 3.75%; 7.50%; 11.25% and 15.00% cocoa powder.
Fig 2. Principal component analysis using the sensory attributes of cappuccinos formulated with jackfruit seed flours.

Projection of variables (A) and group observations (B). PC1 and PC2: principal components 1 and 2. 1- gritex: gritty texture; 2- oveimp: overall impression; 3-cofaro: coffee aroma; 4- cinaro: cinnamon aroma; 5- chotas: chocolate taste; 6- captas: cappuccino taste; 7- caparo: cappuccino aroma; 8- broapp: brown appearance; 9- choaro: chocolate aroma; 10 -feraro: fermented aroma; and 11- fertas: fermented taste. Dry seed flours (D); fermented seed flours (F). Proportions with 50%, 75% and 100% substitution. D50: cappuccino with 7.5% dry jackfruit seed flour and 7.5% cocoa powder; D75: cappuccino with 11.25% dry jackfruit seed flour and 3.75% cocoa powder; D100: cappuccino with 15% dry jackfruit flour; F50: cappuccino with 7.5% fermented jackfruit seed flour and 7.5% cocoa powder; F75: cappuccino with 11.25% fermented jackfruit seed flour and 3.75% cocoa powder; and F100: cappuccino with 15% fermented jackfruit seed flour.
Fig 3. Dendrogram from the QDA® results of cappuccino formulations.

Dry seed flours (D); fermented seeds flours (F). Proportions with 50%, 75% and 100% substitution. D50: cappuccino with 7.5% dry jackfruit seed flour and 7.5% cocoa powder; D75: cappuccino with 11.25% dry jackfruit seed flour and 3.75% cocoa powder; D100: cappuccino with 15% dry jackfruit seed flour; F50: cappuccino with 7.5% fermented jackfruit seed flour and 7.5% cocoa powder; F75: cappuccino with 11.25% fermented jackfruit seed flour and 3.75% cocoa powder; and F100: cappuccino with 15% fermented jackfruit seed flour.
6. CONSIDERATIONS

The production of chocolate aroma from jackfruit seeds using roasting conditions as time and temperature was improved. The best dried flour was 47 min at 171°C, to fermented was 35 min at 154°C, and acidificated was 40 min at 180°C.

The volatile composition and aroma active in jackfruit seeds was similar to cocoa powder samples. According to the volatile composition, jackfruit seeds produced high amount of pyrazines, thus it has potential for others studies and some applications. Important compounds in cocoa were found in high concentration 2-phenylethyl acetate and 3-methylbutanal.

Technological properties of jackfruit seed flour are similar to commercial flowers with starch and could be applied in food development and solubility was higher.

Sensory attributes showed that jackfruit seed flours have a high potential for application in mixtures for cappuccino, since they showed an acceptance similar to the commercial cappuccinos formulations. However, fermentation processes could be improved, in order to eliminate some residuals flavours when flours are used in water solutions. Thus, more studies evaluating fermentative specific microorganisms should be developed.

Finally, the utilization of this local waste can provide a new revenue stream for local farms and boost local economies.