

Article

GC-Olfactometric Analysis as a Tool for Comprehensive Characterization of Aromatic Profiles in Cocoa-Based Beverages with a Natural Chocolate Substitute

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Abstract: Cocoa is the third most important global agricultural export commodity. However, because it is a crop sensitive to climatic change, there has been an active search for cocoa substitutes worldwide. Roasted jackfruit seeds were previously described as having a chocolate aroma and are affordable and accessible. In this study, we characterized and identified by SPME-GC-O and SPME-GC-MS the aroma profile of cocoa-based beverages formulated with jackfruit seed flour as a natural cocoa substitute. Our analysis tentatively identified 71 odor-active aroma descriptors with some similarities between formulations. Overall, 15 odor-active aromas were present in all beverages. The formulation containing only cocoa/chocolate showed the following aroma descriptors: cocoa, hazelnut, peanut butter, earthy, and roast, which are mostly related to the presence of 2,3-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine. The fermented beverage had a content of complex pyrazines such as 2,3,5-trimethyl-6-isopentylpyrazine and methylpropylpyrazine. Our data indicated that both the control and fermented beverages showed a similar aromatic profile, mainly earthy, pyrazine, and chocolate. Qualitative similarities in the pyrazine content were observed between the fermented jackfruit seed flour and cocoa beverages. In conclusion, fermented jackfruit seed flour can be incorporated into cocoa-based beverages as a natural chocolate substitute, offering the potential to elevate the chocolate aroma.

Keywords: jackfruit seed; chocolate aroma; by-product; pyrazines; odor-active



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1. Introduction

Cocoa-based beverages, popular across generations, particularly with children, owe their widespread acceptance to sensory appeal, ease of preparation, and varied market availability. Offered in diverse forms, such as hot or cold drinks, with milk or water, and as ready-to-drink or instant options, these beverages are commonly derived from cocoa powder with a chocolate flavor [1,2].

The food industry has constantly improved the aroma of novel cocoa-based products, such as the aroma sensation of chocolate in cocoa substitutes [2–4]. The increasing demand for cocoa products has encouraged the search for novel chocolate substitutes. To date, cocoa is the third most important global agricultural export commodity after coffee and sugar [5]. Recent evidence has indicated that cocoa is sensitive to climatic change and expert projections estimate a warmer and drier climate in the Amazon basin, which could substantially reduce cocoa crop production [6,7]. One of the strategies to address this issue is to manage and utilize affordable and accessible ingredients. Previous studies on sensory characteristics and headspace volatiles were carried out to compare cocoa samples and cocoa substitutes [8]. An ideal cocoa substitute should exhibit aroma descriptors as similar as possible to those of raw cocoa.

Jackfruit is a tropical plant that is easy to grow and resistant to diseases, high temperatures, and drought. It is available despite climate change and can produce a huge amount of fruit in each tree [9]. A large number of jackfruit seeds are usually underutilized, yet they could be used as a plant-based ingredient [10]. Previous studies with jackfruit seed flours reported favorable conclusions about their preparation, composition, sensory acceptance, volatile profile, functional properties, and food applications.

This investigation centers on the scrutinization of beverage formulations derived from jackfruit seeds, wherein distinct drying (DJS) and fermentation before roasting (FJS) methodologies are applied [4,11]. The formulated beverages encompass a meticulously structured composition following the guidance proposed by Spada et al. (2018) [12]. Volatile compounds within the powder beverages were extracted utilizing solid-phase microextraction (SPME) and subsequently subjected to comprehensive analysis via gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O). The identification and characterization of diverse aroma components, encompassing attributes such as chocolate, honey, malty cocoa, peanuts, earthy green, flowery, cheese, fruity, and vinegar, constitute the pivotal outcomes of this inquiry. Statistical methodologies, including principal components analysis (PCA), hierarchical clustering, and a self-organizing map, were judiciously employed for data analysis and visualization.

In our previous study, waste jackfruit seeds were roasted to prepare a flour with a chocolate aroma. Different aroma profiles were obtained by fermenting jackfruit seeds roasted under different time/temperature combinations. Jackfruit seed flour was shown to have moisture, pH, and color characteristics like those of cocoa [11]. Spada et al. (2018) [12] pointed out that it could be used as a cocoa substitute since the fermentation, drying, and roasting temperature did not affect its solubility parameters. The intensity of the chocolate aroma was similar to or better than that of cocoa powder [12] while consumer preference was for fermented jackfruit seed flour. Samples that were fermented before drying had more esters and acids—compounds that provide the chocolate aroma—than those that were only dried. In addition, dry jackfruit seed flour was found to have more pyrazines compared to cocoa samples [4].

The incorporation of jackfruit seed flours into beverage formulations (50% and 75% substitution of cocoa powder) did not change their sensory acceptability or visual characteristics [12]. Thus, our study aimed to characterize and identify by SPME-GC-O and SPME-GC-MS, for the first time, the aroma profile of cocoa-based beverages formulated with jackfruit seed flour as a natural cocoa substitute. This endeavor seeks to provide novel and refined insights into the sensory nuances associated with these beverages.

2. Material and Methods

2.1. Samples

Jackfruit (*Artocarpus heterophyllus* Lam) samples were manually collected from a single tree in the southeast region of Brazil (22°46'24.3" S 47°38'38.4" W). Ripe fruits were rinsed with water, and seeds were removed and submitted to drying (DJS) or fermentation before roasting to obtain dry and fermented jackfruit seeds (FJS). To obtain DJS samples, jackfruit seeds were dried for 24 h (60 °C), then the spermoderms were manually removed and the seeds were dried again for an additional 24 h at the same temperature (the total process lasted 48 h at 60 °C). To obtain FJS samples, the cocoa processing was simulated as previously described [11]. Seeds were roasted in a rotary electric oven (laboratory sample roaster, Emmerich am Rhein, Germany) and tree batches were obtained from DJS and FJS with time and temperature parameters previously optimized [11] using a digital temperature control. DJS samples were roasted at 171 (±1) °C for 47 min and FJS samples were roasted at 154 (±1) °C for 35 min. Flour was packed under vacuum and stored protected from light at 8 (±1) °C.

2.2. Beverage Formulations

One batch of beverage formulations was prepared (using a blend to DJS and FJS roasted batches) following the recommendations of Spada et al. (2018) [12]. The authors suggest 50% and 75% cocoa replacement in DJS- and FJS-added beverages, respectively. Accordingly, we developed three powder beverages, as shown in Table 1. Natural non-alkaline cocoa powder (IBB 1100 HQ 32318P001) was obtained from the Brazilian Industry of Cocoa (Rio das Pedras, São Paulo). The other ingredients (powdered sugar, powdered milk, sodium bicarbonate, cinnamon powder, soy lecithin, and xanthan gum) were purchased from a local market (Piracicaba, Brazil).

Table 1. Control and experimental beverage formulations.

Ingredients (%)	CTRLBev	FJSBev	DJSBev
Jackfruit seed flour	0	12.5	18.75
Cocoa powder	25.0	12.5	6.25
Powdered sugar **	41.5	41.5	41.5
Powdered milk	30.35	30.35	30.35
Sodium bicarbonate	1.75	1.75	1.75
Powdered cinnamon	0.4	0.4	0.4
Soy lecithin	0.5	0.5	0.5
Xanthan gum	0.5	0.5	0.5
Total	100	100	100

** supplemented with 2% corn starch. CTRLBev = control formulation containing non-alkaline cocoa powder; FJSBev = beverage formulation containing fermented jackfruit seed flour; DJSBev = beverage formulation containing dry jackfruit seed flour.

2.3. SPME-GC-MS and GC-O Analysis

The extraction of volatiles was carried out by solid-phase-microextraction (SPME) using the same method described by Spada et al. (2017) [11]. Briefly, powder beverages (1.0 ± 0.1 g) were added to 2 mL of DI in a 20 mL SPME vial. After equilibrium at 45 °C for 15 min, the SPME fiber (50/30 μ m divinylbenzene/carboxen/polydimethylsiloxane, 2 cm, Supelco, Bellefonte, PA, USA) was exposed to the headspace sample for 55 min, under magnetic agitation. The volatile compounds extracted in triplicate by SPME were separated and identified on a Shimadzu QP2010 GC-MS under the following experimental conditions: RTX5MS column (30 m \times 0.25 mm i.d. \times 0.25 μ m film thickness, Restek, Bellefonte, PA, USA); injector: splitless mode for 1.0 min at 250 °C; helium at 1.0 mL min⁻¹, oven temperature at 40 °C (1 min), increasing to 250 °C at 5 °C min⁻¹; interface temperature at 250 °C, electron ionization source at +70 eV; quadrupole mass analyzer SCAN mode at 35 to 350 *m/z*. A solution of n-alkanes C₈–C₂₀ was injected under the same GC-MS conditions to obtain linear retention indices (LRIs). The tentative identification of the volatiles was carried out by comparing the LRIs and mass spectra of the sample with those found in the literature (NIST 2011 and Willey 8), with a similarity of at least 85% for the mass spectra and maximum variation of ± 10 for the LRIs. The GC-O analysis was performed in the same gas chromatography system but using an ODO II (SGE) GC-O system. Two experienced examiners (each with >150 h of GCO experience) performed the detection and verbal description of the odor-active components of the extracts. The sniffing time was approximately 20 min. Examiners sniffed SPME extracts twice. Each odor was scored on a 7-point linear scale (1–7), where 3 = weak, 5 = medium, and 7 = strong. The scores of each sample were summed up based on the analysis of specific aroma descriptions (chocolate, honey, malty cocoa, peanuts, earthy green, flowery, cheese, fruity, and vinegar).

2.4. Statistical Analysis

The data were analyzed in Metaboanalyst v. 4.0. Principal components analysis (PCA) was carried out using a prcomp package. The calculation was based on singular value decomposition. Two-dimensional score plots and a biplot were computed from selected PCs. Hierarchical clustering was carried out using an hclust package. Clustering results were expressed as dendrograms and a heatmap was plotted indicating the Euclidean distance measures and the Ward clustering algorithm. A self-organizing map (SOM) was used to produce a two-dimensional “map” on which observations in proximal clusters have more similar values than those in distal clusters. Intergroup differences between the odor-active aromas in the beverages were determined by one-way analysis of variance (ANOVA) followed by a Tukey’s post hoc test ($p < 0.05$). The data were analyzed in a XLStat program, considering a 5% significance level.

3. Results

The prospection of flavor compounds is an ever-growing field that is continuously influenced by consumer demands. Hence, the identification of odor-active compounds is of extreme importance for the characterization of foods and new food ingredients.

In the first phase of our study, all 71 odor-active aromas detected in the beverages were shown to both examiners (control, dry, and fermented beverage). The PCA with these 71 descriptors is shown in Figure 1A,B. The PC1 × PC2 (50.9 × 32.4) showed three different groups, each one related to different sample formulations, i.e., control beverage, dry beverage, and fermented beverage. However, as seen in Figure 2, PC1 × PC3, PC1 × PC4, and PC1 × PC5 indicated similarities between the control and fermented beverages. This is also consistent with the Euclidian (Ward) dendrogram (Figure 1B). The dendrogram showed two groups (<60), namely: group I—control and fermented beverages; and group II—dry beverages. Some similarities in the aroma descriptions were observed between the cocoa and fermented jackfruit seed flour formulations. The Supplementary Materials shows a heatmap with all 71 odor-active aromas obtained by GC-O.

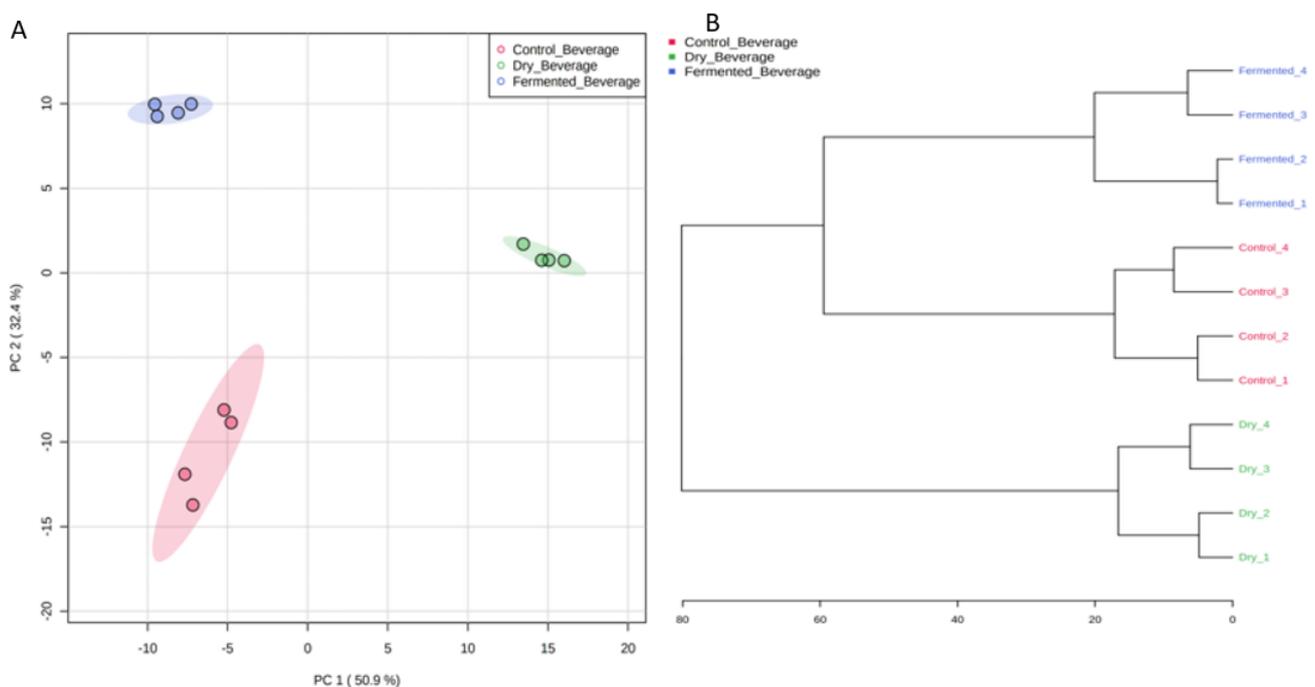


Figure 1. (A) Principal components analysis (PCA) of all (71) odor-active aromas (GC-O) detected in the beverage formulations; (B) GC-O dendrogram data (Euclidean—Ward) of the beverage formulations. group I—control and fermented beverages; and group II—dry beverages.

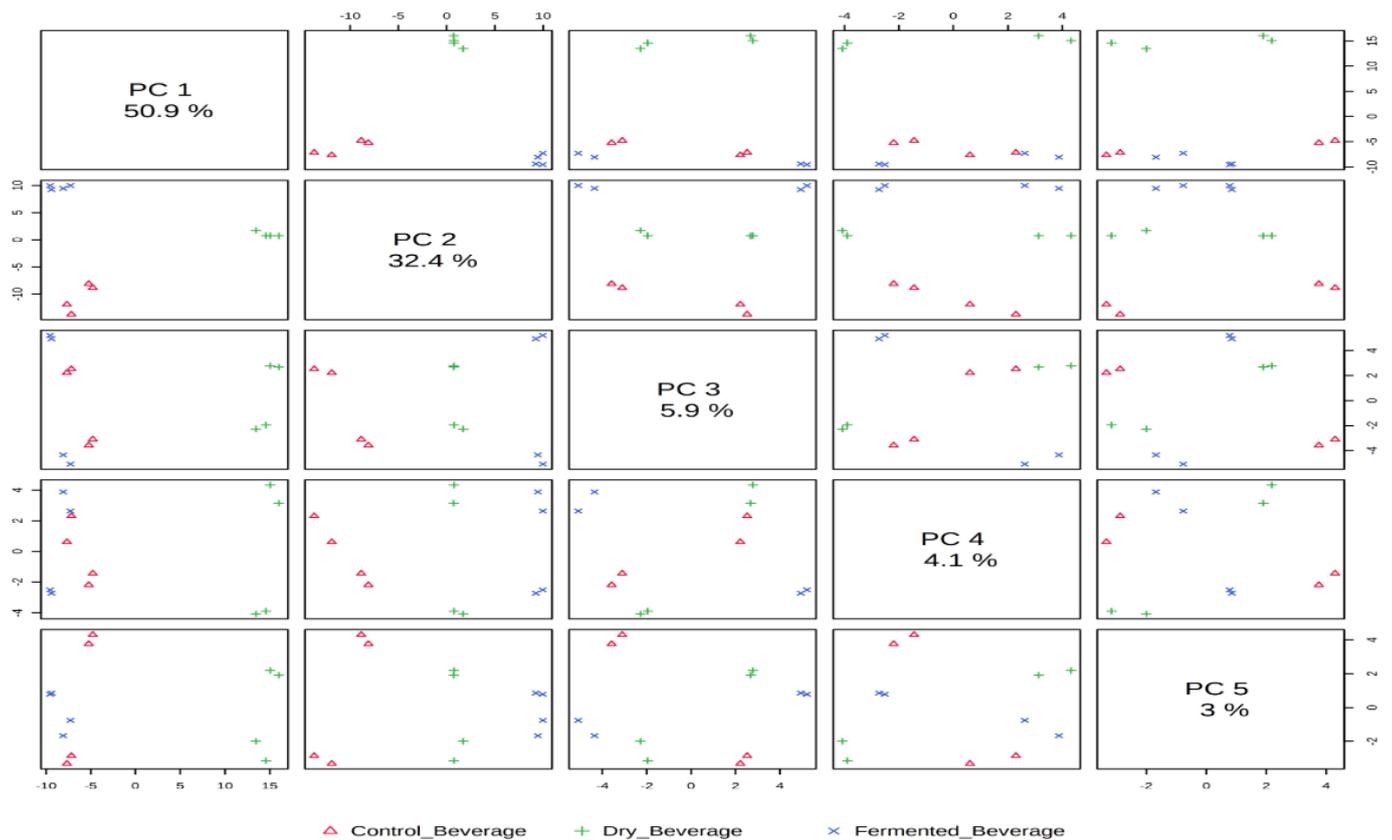


Figure 2. Pairwise score plots between the selected PCs. The explained variance of each PC is shown in the corresponding diagonal cell.

GC–O is a widely recognized analytical and sensorial tool extensively documented in the literature [4,13–16]. The introduction of the GC–O technique marked a pivotal moment in analytical aroma research, enabling the discrimination of a multitude of odor-active and non-odor-active volatiles based on their concentrations in the food matrix. This breakthrough led to the emergence of new flavor creations [17].

The similar aroma descriptors identified in the samples are presented in Figure 3 and described as follows: cheese; chocolate; earth, pyrazine, and chocolate; floral; fresh; fruity; green; mint; mushroom soup; musty; and sweet (Figure 3). Both the control and fermented beverages exhibited similar descriptions for cheese; chocolate; earth, pyrazine, and chocolate; and green. Fruity and sweet notes were more pronounced in beverages formulated with jackfruit seed flour. The musty aroma was absent in the control beverage, while mushroom soup was more intense in the dry and fermented beverages.

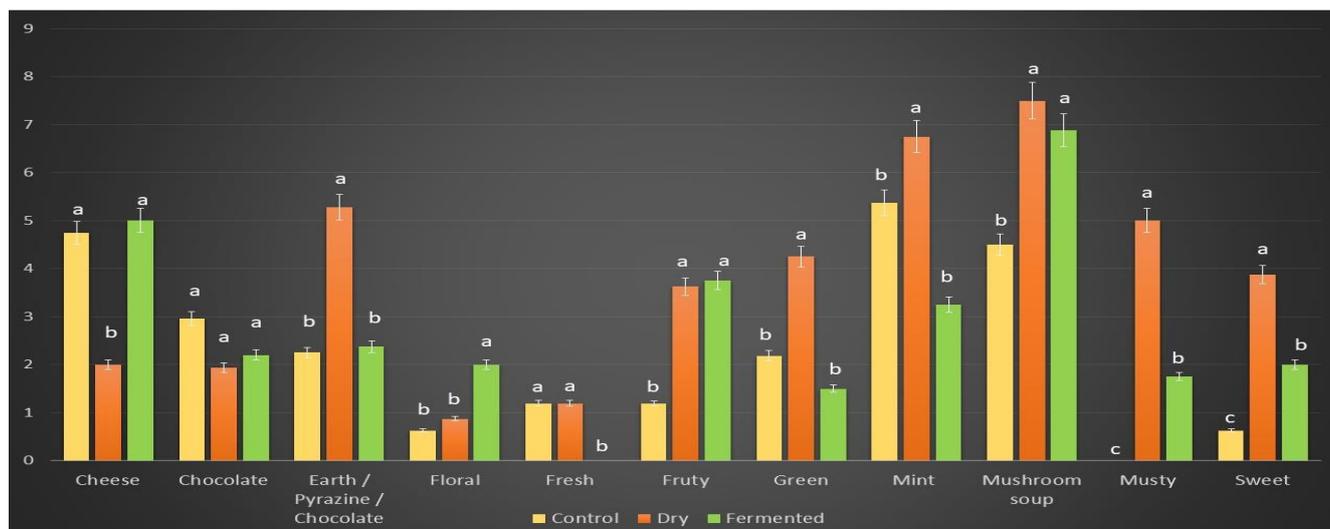


Figure 3. Aroma attributes of the beverages by GC-O analysis. Means with different letters for each attribute indicate statistically significant differences ($p < 0.05$).

The active aromas uncovered in this study may be linked to the presence of specific complex pyrazines, such as ethyl (3-methylbutyl) pyrazine (Z36) and ethyl 2-methylpropylpyrazine (Z32). These compounds were detected by GC-MS in beverages formulated with dry jackfruit seeds and garnered high scores in GC-O evaluations. Z36 and Z32 are associated with aroma descriptors reminiscent of soup and pyrazines, respectively. Consequently, elevated concentrations of Z36 and Z32 may signify the presence of off-aromas in beverages containing dry jackfruit seed flours.

In the second phase of our study, 15 odor-active aromas present in all samples were analyzed by both examiners. The PCA with these 15 descriptors is shown in Figure 4A,B. The PC1 \times PC2 (40.3 \times 25.0) presented three different groups. The Euclidian (Ward) dendrogram showed two groups (<20), as follows: Group I—control and fermented beverages; and Group II—dry beverage (Figure 5A); these findings are consistent with the PCA analysis with all 71 odor-active aromas. The 15 odor-active aromas represent the variability between the samples. Thus, we could find some specific differences between them (Figure 4B). Pyrazine is the volatile group characteristic of chocolate flavor. In the control beverage, chocolate, cocoa, hazelnut, peanut butter, earthy and roast descriptors were mainly related to the presence of 2,3-dimethylpyrazine (A8) and 2,3-diethyl-5-methylpyrazine (B15). The fermented beverage showed a content of complex pyrazines, which were also found in the cocoa category—2,3,5-trimethyl-6-isopentylpyrazine (A20) and methylpropylpyrazine (A15) (Figure 5B). The fact that the dry beverages were clustered in a separate group could be explained by the presence of green (A6) citrus (A6), and earth (A16) odor-active descriptors (Figure 5B). Heptanal (A6) and 2-phenylethyl ester (B8) were also found in the dry beverage (Figure 5B), mischaracterizing a typical cocoa beverage.

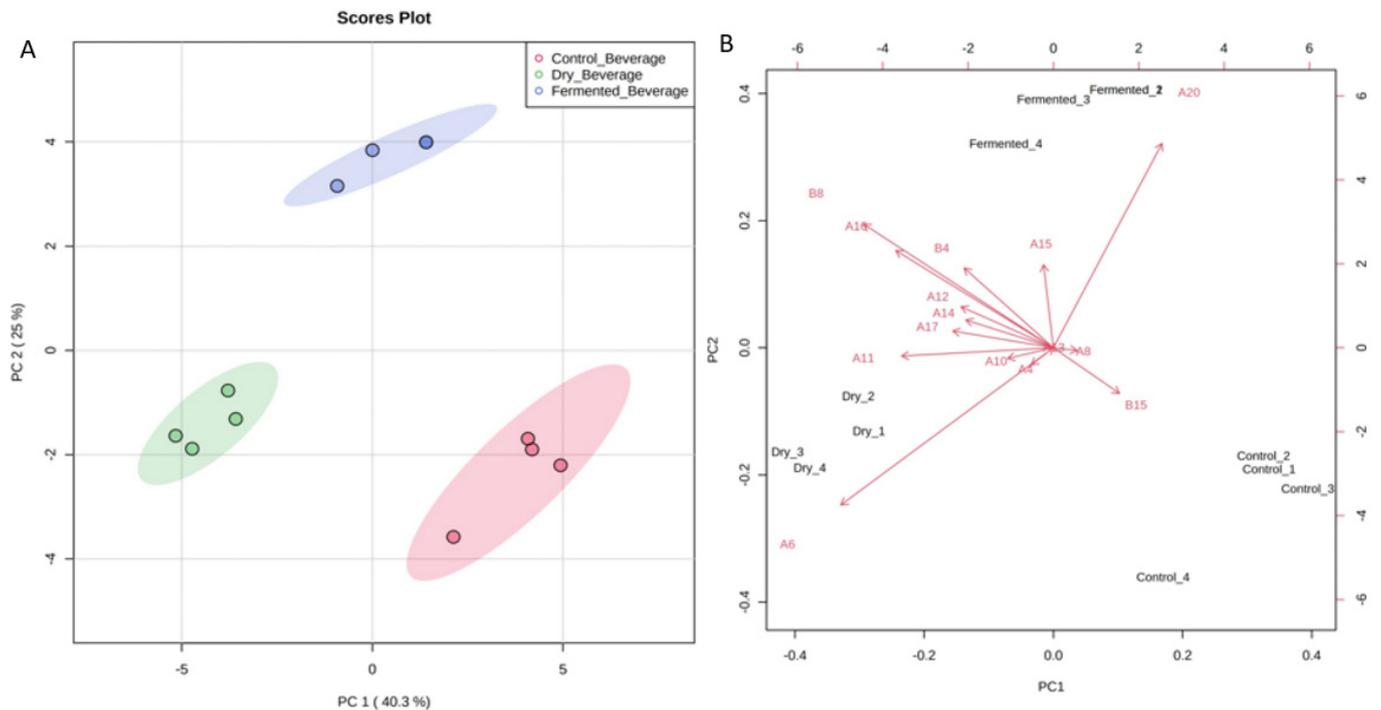


Figure 4. (A) Principal components analysis (PCA) of 15 odor-active aromas (GC-O) that were detected in all beverage formulations; (B) Principal components analysis (PCA) of the projection of 15 volatile constituents (GC-O) contained in the beverage formulations.

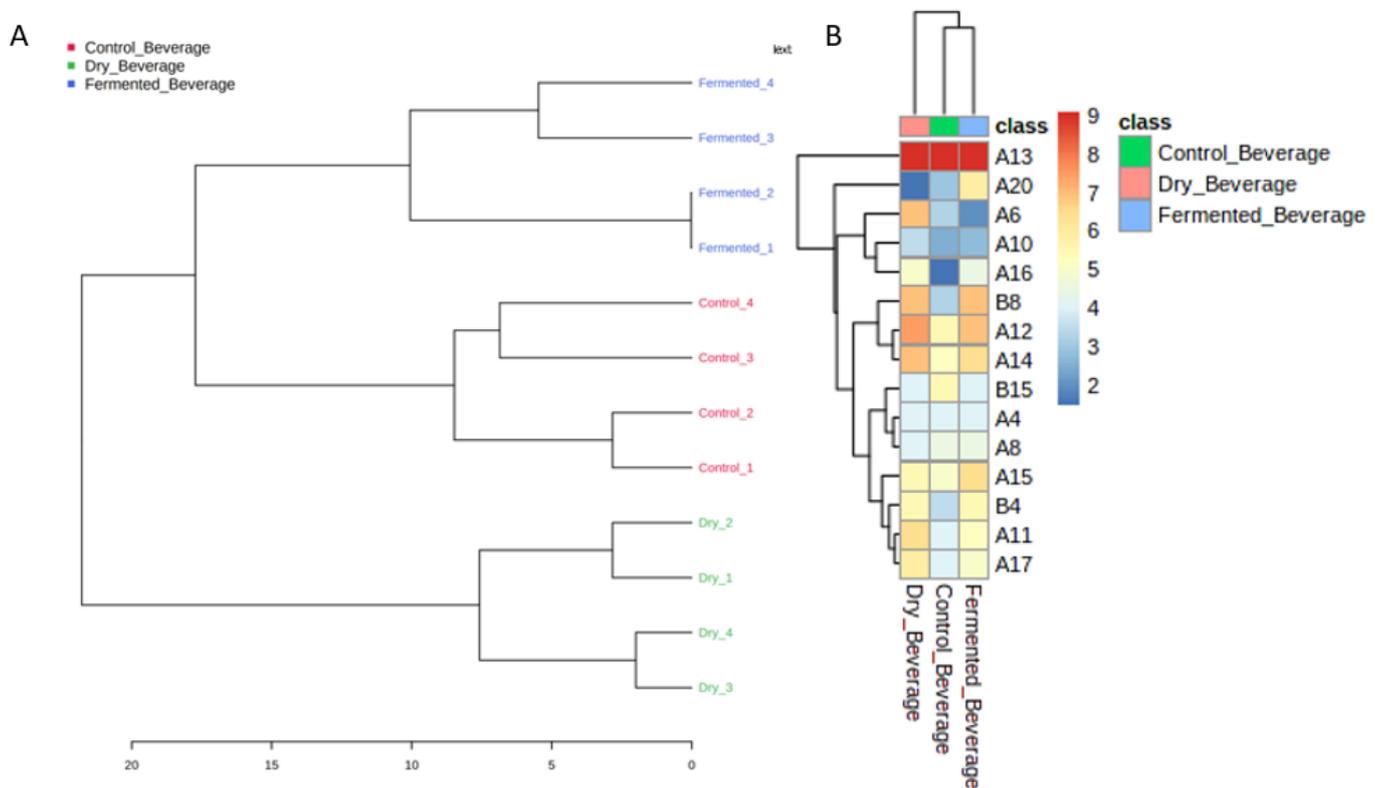


Figure 5. (A) GC-O dendrogram data (Euclidean—Ward) of the beverage formulations using 15 odor-active aromas that occurred in all samples; (B) Heatmap data showing the concentration of volatile compounds in the samples. The color scale represents the variation in the relative concentration of compounds in the beverage formulations, from low (blue) to high (red) content.

A two-dimensional “map” (SOM) can make high-dimensional data easier to visualize and analyze. Figure 6 shows a SOM map with dimensional PC1 X PC2. In that map, the control and fermented beverages were allocated within the same cluster, while the dry beverage was categorized into a different group similar to Figure 5A,B.

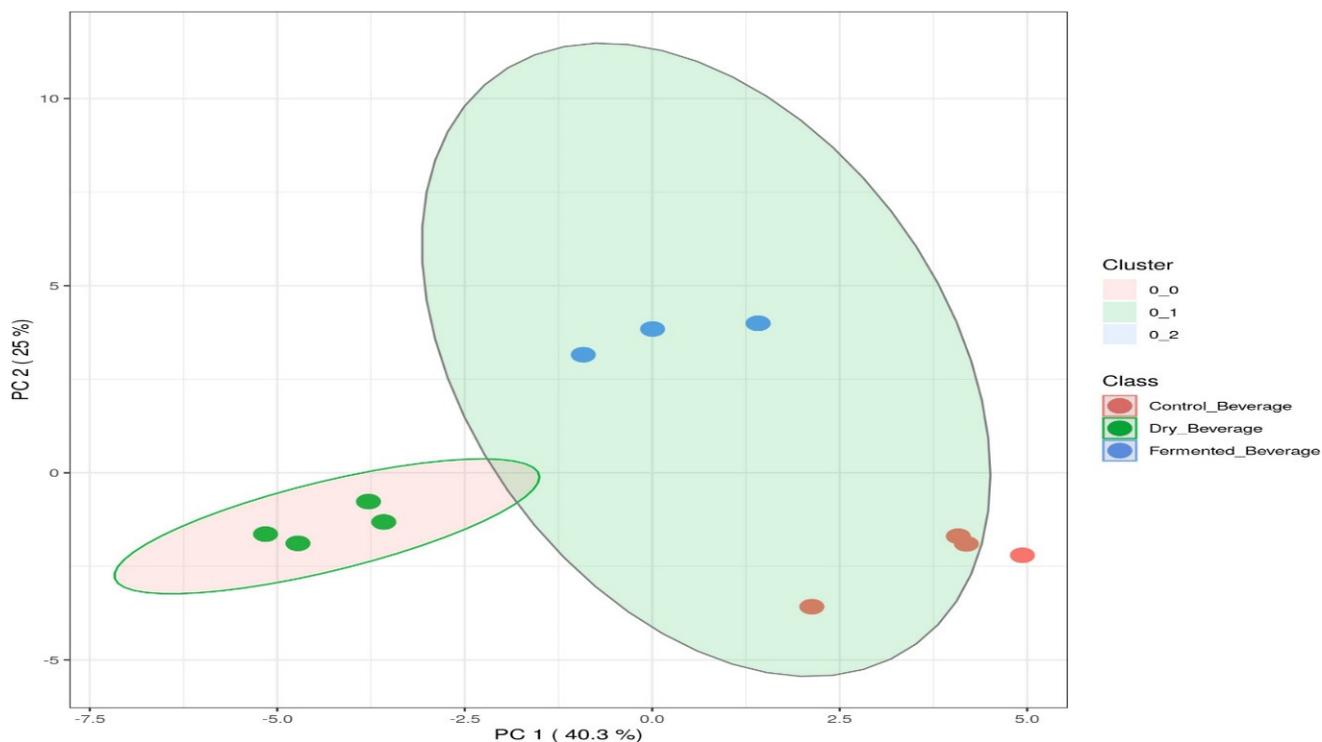


Figure 6. The GC-O score plot shows SOM clusters in the PCA. In the overview plot, darker lines represent the median intensity of each cluster.

In a previous study, Spada et al. (2021) [4] detected five important aroma descriptors typically found in chocolate and cocoa aroma in samples of jackfruit seed flour and cocoa powder. In our study, we found the same compounds in the samples—2-methyl butanal and 3-methyl butanal (A4); phenylacetaldehyde (A10); trimethylpyrazine (A11); 2,3-dimethyl-5-ethylpyrazine (A12) and 2-phenylethyl ester (B8) (Table 2). These constituents most likely provided the chocolate aroma in the fermented, dry, and cocoa beverages, even when available in low concentrations (12.5% in fermented jackfruit seed flour; 18.75% in dry jackfruit seed flour; and 25% in cocoa powder). Consistent with the findings by Spada et al. (2021) [4], methyl-2-methylpropylpyrazine compounds (Z28, A13, and A15) were found in beverages formulated with cocoa and jackfruit seed flour. They were likely responsible for the carbolic soup and jackfruit seed descriptors. The formulations also contained other complex pyrazines (methylbutyl and methylpropylpyrazines) with aroma descriptors such as cocoa, pyrazine, green, herbs, flowery, and jackfruit seed, namely: 3-methylbutyl-methylpyrazine (A17), ethyl-2-methylpropylpyrazine (Z32), ethyl-3-(2-methylbutyl) pyrazine (Z37), and ethyl (3-methylbutyl)pyrazine (Z36) (Table 2). In addition, the formulations also contained 2,3-dimethylpyrazine (A8), 2,3-diethyl-5methylpyrazine (B15), and 2,3,5-Trimethyl-6-isopentylpyrazine (A20), which are associated with descriptors such as pyrazines and typical chocolate and cocoa.

Table 2. GC-MS and aroma descriptors by GC-O detected in the control, dry, and fermented beverages.

ID		LRI (Calculated)	LRI (Literature)	Similarity (%)	GC-MS			GC-O Scores (Mean)			Aroma Descriptor	Ref. Aroma
					DB5	Ctrl	Dry	Fermented	Ctrl	Dry		
Compounds typically found in chocolate or cocoa aroma												
A4	2 or 3-methyl butanal	<650	642	90	X	X	X	4	4	4	Malty cocoa	[18–23]
A11	trimethylpyrazine	995–998	997	96	X	X	X	4	6	5	Peanuts	[18–23]
A10	phenylacetaldehyde	1046–1047	1045	98	X	X	X	2	3	3	Rose	[18–23]
A12	2,3-dimethyl-5-ethylpyrazine	1079	1080	96	X	X	X	5	7	7	Pyrazines and cocoa	[21]
B8	2-phenylethyl ester	1262	1244	95	X	X	X	3	7	7	Jam scum	[24–26]
Major compounds found in the beverages												
B4	2,3-butanediol	800	795	95	X	X	X	3	5	5	Cream, butter, and fruit	[27,28]
A6	heptanal	905	903	92	X	X	X	3	7	2	Green	[29,30]
A8	2,3-dimethylpyrazine	929	930	94	X	X	X	4	4	4	Cocoa, hazelnut, peanut butter, and roasted	[27]
Z28	a methyl-2-methylpropylpyrazine	1137	1138	96		X		ni	3	ni	Carbolic soap or jackfruit seeds	[4]
A13	a methyl-2-methylpropylpyrazine	1137	1140	96	X	X	X	9	9	9	Carbolic soap or jackfruit seeds	[4]
A15	a methyl-2-methylpropylpyrazine	1137	1149	96	X	X	X	5	5	5	Carbolic soap or jackfruit seeds	[4]
A14	eucalyptol	1140	1132	96	X	X	X	5	7	6	Cool, mint, and sweet	[28]
B15	2,3-diethyl-5-methylpyrazine	1159	1156	94	X	X	X	5	4	4	Chocolate, earth, and roast	[26]
A16	4-terpineol	1194	1182	96	X	X	X	2	5	4	Camphoraceous and earth	
Z32	an ethyl-2-methylpropylpyrazine	1245	1240	95		X		ni	6	ni	Pyrazines and cocoa	[25]
A17	a (3-methylbutyl)-methylpyrazine	1254	1258	90	X	X	X	4	6	5	Pyrazines, hot dry and cocoa	[25]
Z37	an ethyl-3-(2-methylbutyl)pyrazine	1320	nf	91		X	X	ni	3	4	Green, roast, and sweet	[27]
Z36	an ethyl (3-methylbutyl) pyrazine	1329	nf	86		X		ni	ni	ni	Carbolic soap or jackfruit seeds	[4]
A20	2,3,5-trimethyl-6-isopentylpyrazine	1392	1387	73	X	X	X	3	2	6	Cocoa category	[27]

ni = unidentified; nf = not found.

In our study, the beverages also showed other important aroma descriptors, including cream, butter, and fruity, related to the occurrence of 2,3-butanediol (B4); green, citrus, and lamb fat provided by heptanal (A6); cool, mint, and sweet provided by eucalyptol (A14); and camphoraceous and earth, related to the presence of 4-terpineol (A16).

Cocoa powder demonstrated a lower concentration of 2,3-butanediol compared to beverages formulated with jackfruit seed flour (Figure 5B). Kowalska et al. (2020) [18] associated these findings with the concentration of 2,3-butanediol and its correlation with sucrose availability. Notably, sugar is commonly added to alleviate bitterness and enhance solubility in cocoa-based beverages [1].

Furthermore, sucrose was included in the beverage formulations (Table 1), resulting in distinct volatile compounds. Consequently, the sucrose content is expected to impact the 2,3-butanediol concentration during fermentation or the seed-roasting process [4].

4. Discussion

In our study, we described odor-active aromas in beverage formulations using the 15 most important volatiles in their composition. In a previous study, Spada et al. (2018) [12] found no differences between cappuccino formulations with cocoa, coffee, and jackfruit seed flour based on a sensorial evaluation. In this study, GC-MS and GC-O data showed that beverage samples were different, but the beverage with fermented jackfruit seed flour was similar to that formulated with cocoa powder only. It was mainly associated with 2,3-dimethylpyrazine (A8), Isopentylpyrazine (A20), and 2,3-diethyl-5-methylpyrazine. The volatile compounds found in the beverages were recently described by Spada et al. (2021) [4] in cocoa powder and jackfruit seed flour. Thus, partial cocoa substitution by jackfruit seed flour could maintain the aroma sensation of chocolate in the formulations. Another aspect to consider is the similarity between control and fermented beverages in terms of cocoa character. The most important odor-active aromas were described as earth, pyrazine, and chocolate. Interestingly, qualitative similarities between pyrazines were observed in the beverages containing fermented jackfruit seed flour and cocoa.

Generally, pyrazines are produced during thermal processing (>100 °C). Pyrazines are nitrogen heterocyclic intermediates of the Maillard reaction, predominantly formed from the reaction between amino ketones [19]. Frequently, a Strecker aldehyde is incorporated as a substitute for the pyrazine ring. In the Maillard reaction, these intermediates are produced from the breakdown of glucose, initiated by an amino acid. Pyrazine and methylpyrazine are the major products when glucose and glycine are present. Side chains can also be introduced into the pyrazine ring via the incorporation of an aldehyde, which can react with a dihydropyrazine in model systems [20] and foods [21]. Strecker aldehyde 2-ethylpropanal is generated by the incorporation of valine into a glucose/glycine system, and the incorporation of this into the pyrazine molecule produces pyrazines with a 2-methylpropyl substituent [31,32].

The group of two precursor (C2)-substituted pyrazines with MW 108 [2,3-dimethylpyrazine (A8); methyl-2-methylpropylpyrazine (A15)] is derived from glyoxal and methylglyoxal, which are highly reactive intermediates formed from reducing sugars in the Maillard reaction [x + x mechanism formation [30]. C3-substituted pyrazines MW 122, such as trimethylpyrazine (A11); 2,3-diethyl-5-methylpyrazine (B15), suggest that trimethylpyrazine levels are not driven by time and temperature and that other events may be limiting their formation. Trimethylpyrazine is one of the main components of cocoa aroma and is responsible for the nutty, roasted, and chocolate flavor notes [18,31,32]. In previous studies, 2,3-diethyl-5-methylpyrazine was described as an important compound in chocolate aroma in cocoa samples [24,25,29]. Differently, C4-substituted pyrazines MW 136 (2,3,5-trimethyl-6-isopentylpyrazine (A20)) can be formed both by the "x + x" pathway and the "x + x + y" pathway, where "y" can be formaldehyde or acetaldehyde. This group of pyrazines, especially the trisubstituted ones, is often related to the chocolate aroma.

Similarly, in carob [22], ethyl-methyl-pyrazines, formed from the reaction of glucose with aspartic acid, emerged as significant contributors to the "cocoa, coffee, roasted nutty"

aroma. Interestingly, these compounds were found in higher concentrations in fermented jackfruit seed flour compared to cocoa powder.

Aldehydes are very common components of any food or flavoring and often have a low odor threshold [30]. They could display a very chemical aroma note, particularly when assessed by GC-O [33]. In our study, odor-active aromas described as green, lamb fat, and mussels were more intense in the dry beverage due to the occurrence of heptanal (A6). Heptanal is usually undesirable in beverages; thus, this aldehyde may be the main factor responsible for the allocation of the dry beverage formulation in a different group (Figure 4B). Consistent with the high levels of heptanal detected in the dry beverage, Spada et al. (2022) [34] found the highest heptanal concentration in dry jackfruit seed flour compared to cocoa and fermented jackfruit seed flour. Present in all samples with the same intensity, the compounds aldehyde 2-methylbutanal and 2-methylbutanal (A4) provide malty, bitter cocoa notes that are essential for malty and cocoa aromas [34].

The compound 2,3-butanediol (B4) is associated with a fruity and natural odor of cocoa butter, frequently described in cocoa. This compound was described to have a bitter and fruity taste. Yet, volatile 2,3-butanediol had a positive impact on the aromatic profile of the fermented beverage formulation (Figure 4B). Kowalska, et al. (2020) [18] studied the influence of sugar in beverages formulated with cocoa and further highlighted the occurrence of 2,3-butanediol. They suggested that its combination with non-alkaline cocoa powder, fermented jackfruit seed flour, and sugar could improve the aroma sensation of cocoa and chocolate.

In alternative cocoa replacements, such as carob [22], the concentration of active odor compounds, such as phenylacetaldehyde, was lower compared to cocoa. However, in the case of fermented jackfruit seed flour, both the concentration of phenylacetaldehyde measured by GC-MS and the sensory intensity assessed by GCO were higher than those found in cocoa powder.

In contrast with the findings reported by Fadel et al. (2006) [8], our data showed that beverages with cocoa substitutes had similar and/or higher scores for sweet/caramel and roast/earthy descriptors compared to beverages with cocoa powder. On the other hand, odor-active aromas of camphoraceous and earth were higher in beverages formulated with dry jackfruit seed flour. Thus, fermented jackfruit seed flour can be considered an effective substitute to provide a cocoa aroma.

Within the scope of our study, an analysis of beverages formulated with jackfruit seed flour revealed a volatile profile akin to that of cocoa powder, consistent with prior investigations demonstrating the propensity of jackfruit seeds to augment the concentration of volatile compounds associated with chocolate descriptors.

5. Conclusions

The three beverage formulations tested in our study were different in terms of odor-active aromas, but the control and fermented beverages showed a similar aromatic profile. The most important odor-active aromas that could act as cocoa substitutes were found in all beverages. Pyrazines were the main group of volatile compounds responsible for the major odor-active aromas in the beverages. Based on the GC-olfactometric analysis, this ingredient could be incorporated into cocoa-based beverages to improve the chocolate aroma. As a corollary, fermented jackfruit seed flours emerge as a prospective partial additive, offering the potential to elevate the chocolate aroma in diverse food formulations, especially considering cocoa's susceptibility to climate change.

6. Final Considerations about the GC-Olfactometric Analysis

In our study, the GC-O analysis was able to differentiate the beverage formulations with a high degree of sensibility. Our findings strongly suggest that GC-O associated with GCMS could be an effective technique for the identification of food modifications.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fermentation10050228/s1>. Table S1. Tentative identification of compounds with odor-active aroma descriptors in cocoa-beverages replaced with chocolate made from natural jackfruit seed flour; Figure S1. Heatmaps for odor-active aroma descriptors in cocoa-beverages replaced with chocolate made from natural jackfruit seed flour.

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Abbreviations

A10	phenylacetaldehyde
A11	trimethylpyrazine
A12	2,3-dimethyl-5-ethylpyrazine
A14	eucalyptol
A15	methylpropylpyrazine
A16	4-terpineol
A17	3-methylbutyl-methylpyrazine
A20	2,3,5-Trimethyl-6-isopentylpyrazine
A4	2-methyl butanal and 3-methyl butanal
A6	heptanal
A8	2,3-dimethylpyrazine
B15	2,3-diethyl-5methylpyrazine
B4	2,3-butanediol
B8	2-phenylethyl ester
CTRLBev	control formulation containing non-alkaline cocoa powder
DJS	dry jackfruit seed flour
DJSBev	beverage formulation containing dry jackfruit seed flour
FJS	fermented jackfruit seed flour
FJSBev	beverage formulation containing fermented jackfruit seed flour
GC-MS	gas chromatography-mass spectrometry
GC-O	gas chromatography-olfactometry
LRIs	linear retention indices
PCA	principal components analysis
SPME	solid-phase microextraction
Z28, A13, and A15	methyl-2-methylpropylpyrazine compounds
Z32	ethyl 2-methylpropylpyrazine
Z36	ethyl (3-methylbutyl)pyrazine
Z37	ethyl-3-(2-methylbutyl) pyrazine

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