Aroma Properties of Cocoa Fruit Pulp from Different Origins

Thomas Bickel Haase 1,2,*, Ute Schweiggert-Weisz 1,3, Eva Ortner 1, Holger Zorn 2,4 and Susanne Naumann 1,5

Abstract: Cocoa pulp occurs as a by-product of cocoa bean production and can be repurposed to different food applications, such as jams, fruit preparations and beverages, improving the sustainability of cocoa production, as well as the livelihoods of cocoa farmers. In this work, aroma-active compounds of fresh cocoa fruit pulps from different origins were investigated by applying aroma extract dilution analyses in combination with gas chromatography-mass spectrometry/olfactometry for identification. In total, 65 aroma-active compounds were determined in four different pulps originating from Indonesia, Vietnam, Cameroon, and Nicaragua. Vietnamese pulp showed the highest number of aroma-active regions, while Cameroonian pulp accounted for the lowest. Moreover, Cameroonian cocoa pulp showed the lowest FD factors. Overall, the odorants with the highest FD factors were trans-4,5-epoxy-(E)-decenal, 2- and 3-methylbutanoic acid, 3-(methylthio)propenal, 2-isobutyl-3-methoxypyrazine, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal, 4-vinyl-2-methoxyphenol, δ-decalactone, 3-hydroxy-4,5-dimethylfuran-2(5H)-one, dodecanoic acid, and linalool. This study provides insights into the aroma composition of fresh cocoa pulp from different origins for future food applications.

Keywords: Theobroma cacao L.; by-product; aroma; aroma extract dilution analyses; gas chromatography-olfactometry; mass spectrometry

1. Introduction

The International Cocoa Organization [1] forecasted for 2020/2021 a production of over 4.8 million tons of cocoa beans mainly destined for the chocolate industry. Taking into account the by-products of the cocoa bean processing chain, which represent together about 70–80% of the dry weight of the fruit and comprise the cocoa pod husk, the bean shells, and the pulp [2]; approximately 19 million tons of residual biomass will be produced. As the disposal of these by-products causes several social and environmental concerns, some studies have focused on the use of cocoa by-products in the formulation of new and versatile ingredients for the food, pharmaceutical, and cosmetic industries. By adding value to these side fractions, cocoa farmers may profit from new sources of income and better livelihoods, allowing also a more sustainable cocoa production [3,4]. Due to its pleasant flavor, cocoa fruit pulp has recently gained increasing attention of the food sector.

Cocoa pulp is a moist white fibrous layer that covers the fresh cocoa beans. With a low pH, usually ranging between 3.3 and 3.9, fresh cocoa pulp contains 83–86% water, 11–13% sugars (D-glucose, D-fructose, and sucrose), 0.5–1.2% pectin, 0.2–3% hemicelluloses, 0.7–0.9% cellulose, 0.1–0.3% lignin, and 0.3–1.3% citric acid [5–7]. The cocoa pulp is hydrolyzed during the fermentation of cocoa beans and flows out of the fermentation boxes as the so-called cocoa sweatings and is, thereby, lost [8]. Previous reports suggest that a fraction of the pulp can be separated from the fresh cocoa beans without having a negative effect on the later fermentation of the beans [9,10].
Accordingly, alternative applications for the use of the aromatic pulp are gaining popularity. Possible applications for the cocoa pulp, such as the production of cocoa jelly, alcohol, vinegar, nata, and processed juices, were described by Figueira et al. [11]. Moreover, Puerari et al. [12] took advantage of the high sugar content of cocoa pulp and used kefir grains for the development of an alcohol-containing drink, while dos Santos Filho et al. [13] inoculated cocoa juice with Lactobacillus casei for the production of a probiotic beverage. Further approaches focused on the use of cocoa pulp for wine production [14] and as an adjunct for the production of beer [15]. Additionally, the chocolate industry has used cocoa pulp as a replacement for added sugar and released chocolate-like products to the European and Asian market [16–18]. This broad range of pulp-based products highlights the high application potential of cocoa pulp in foods, which can be mainly attributed to its pleasant tropical flavor.

To date, aroma research has primarily focused on the role of cocoa pulp for the later aroma quality of fermented cocoa beans. Pino et al. [19] studied the volatile composition of cocoa pulp from Colombia. The authors identified sixty-six different volatile organic compounds (VOCs) in the pulp, wherein the substances 2-heptyl acetate, 2-pentyl acetate, and linalool were present in the highest concentrations. Kadow et al. [20] quantified the VOCs of pulp and seeds of two fine flavor cocoas (SCA6, EET62) and a bulk cocoa (CCN51). Monoterpenes, present in SCA6, as well as methylketones, secondary alcohols, and their respective esters, present in EET62, were shown to be potential fine aroma components of cocoa beans originating in the pulp. Both studies investigated the cocoa pulp by means of headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME CG-MS), yet did not investigate the aroma activity of the determined VOCs using olfactometric methods. Furthermore, to gain insights on how the cocoa pulp odorants affect the aroma quality of the beans during fermentation, Chetschik et al. [21] studied odor-active constituents in three cocoa pulp varieties by means of aroma extraction dilution analysis (AEDA) and gas chromatography-mass spectrometry/olfactometry (GC-MS/O). The experiments were performed with two cocoa varieties from Costa Rica (UF654 and CCN51) and one from Colombia (FSV41). In terms of flavor dilution (FD) factors, the CCN51 cocoa pulp presented lower aroma intensities than the varieties FSV41 and UF654, which showed more intense floral and fruity notes. By means of headspace solid-phase microextraction and GC-MS, Hegmann et al. [22] studied the influence of season and ripening stage on the cocoa pulp aroma of five cocoa varieties from Costa Rica. The total aroma diversity and intensity were shown to increase during ripening and aroma profiles were found to be more diverse when fruits ripened during the dry season, whereas the aroma intensity of the pulp was higher in the wet season.

Several authors showed the influence of background (e.g., cocoa genotype, growing and harvesting conditions, and origin) as well as processing on the flavor of cocoa beans (e.g., fermentation, drying, and roasting); and how these factors determine the later quality of the cocoa beans [23–28]. In particular, the origin plays a decisive role on their final quality, as different countries cultivate botanically different subspecies of the cocoa tree. The cocoa cultivars can be divided into Criollo, Forastero, Trinitario, and Nacional [29,30]. Cocoa pulp is still an emerging market and little is known on how the origin of cocoa fruits determine the pulp’s flavor. In order to obtain cocoa pulps with constant and high qualities for the food sector, it is vital to study how the sourcing of cocoa pulp may affect its aroma composition and, thus, its suitability as a food ingredient. Investigations on the aroma-active compounds present in cocoa pulps from various origins and genetic backgrounds are a pre-requisite to identify possible application fields for the different cocoa pulps. Understanding the importance of certain aroma-active volatile organic compounds on the flavor of the cocoa pulps and elucidating the differences between the raw materials might help in the development of tailored food products for diverse markets and consumer groups. Therefore, the aim of this study was to investigate the differences in the main aroma-active compounds of four fresh cocoa pulps from different origins including comparative aroma extract dilution analysis (cAEDA) and GC-MS/O. In addition, the cocoa pulps
were analyzed by HS-SPME CG-MS and stir-bar sorptive extraction gas chromatographyolfactometry/mass spectrometry (SBSE-GC-MS/O). Fresh cocoa pulps from South East Asia (Indonesia and Vietnam), Central America (Nicaragua), and Africa (Cameroon) were investigated in this study.

2. Results and Discussion

The aroma-active volatile compounds responsible for the aroma impression of cocoa pulps originating from Indonesia, Vietnam, Cameroon, and Nicaragua were isolated by extraction with dichloromethane (DCM) and separated from non-volatiles lipids using the solvent-assisted flavor evaporation (SAFE)-distillation technique (c.f. 3.2). The distillates obtained exhibited the typical characteristic overall aroma of each kind of pulp, proving the successful extraction of all key aroma compounds. The distillates were subjected to cAEDA by means of GC-O analyses. The cAEDA revealed a total of 65 aroma-active regions with an FD factor range of 2 to 1024 (Table 1). Of the detected aroma-active regions, five could not be conclusively identified. Substances identified by SBSE- and HS-SPME-GC-MS/O in the fresh pulps were also found in cocoa pulp distillates by GC-MS/O or did not exhibit aroma-active regions with FD > 2 in the cAEDA. Therefore, the results obtained with SBSE- and HS-SPME-GC-MS/O measurements are provided as Supplementary Materials. The identified aroma-active volatiles belonged to various chemical groups. Aldehydes were the most predominant group, followed by carboxylic acids, lactones, phenols, and ketones. Additionally, terpenes, alcohols, esters, pyrazines, furans, sulfides, and thiazolines were found in the sample distillates. Overall, 36 odorants could be perceived in all cocoa pulps (compounds 1–4, 6, 9–21, 23, 24, 26, 27, 29, 30, 32, 33, 35, 37, 42, 44, 47, 49, 51, 58, 64, and 65). Out of the 65 identified aroma-active regions, 24 odorants (No. 1, 2, 5, 10, 11, 17, 18, 19, 21, 23, 28, 29, 30, 31, 32, 35, 37, 42, 44, 45, 47, 51, 54, and 65) were previously reported in cocoa pulp by means of AEDA [21]. In total, the authors identified 37 aroma-active regions with FD factors below FD 128. Overall, higher FD factors (up to FD 1024) were determined in this work. This may result from the fact that distillates were concentrated to a final volume of 100 µL, compared to 300 µL in the previous study. Moreover, compared to Chetschik et al. [21], more odorants were identified in this work, which may also be explained by differences in the innate aroma compositions of the investigated cocoa pulp varieties.

Table 1. Aroma-active compounds identified in extracts of cocoa pulps grown in Indonesia, Vietnam, Cameroon, and Nicaragua.

| No. | Odorant                        | Odor Quality | DB-FFAP | DB-5 | Indonesia | Vietnam | Cameroon | Nicaragua |
|-----|--------------------------------|--------------|---------|------|-----------|---------|----------|-----------|
| 1   | 2,3-butanedione                | butter-like  | 1008    | 731  | 64        | 16      | 256      | 16        |
| 2   | methyl 2-methylbutanoate       | fruity, banana-like | 1017 | 776  | 4        | 32      | 4        | 4         |
| 3   | 2,3-pentanedione                | butter       | 1056    | 706  | 64        | 16      | 256      | 16        |
| 4   | hexanal                        | green        | 1089    | 802  | 4        | 128     | 32       | 32        |
| 5   | 3-methylbutyl acetate          | fruity       | 1118    | 880  | 16       | 16      | <2       | 128       |
| 6   | δ-carene                       | green        | 1140    | 1014 | 2        | 32      | 8        | 64        |
| 7   | 3-methylbutanol                | malty, roasty| 1200    | 760  | <2       | 32      | <2       | 8         |
| 8   | 2-heptanone                    | fruity, flowery| 1207 | 891  | 16       | <2      | 32       | <2        |
| 9   | (Z)-4-heptenal                 | fishy        | 1245    | 894  | 32       | 128     | 8        | 32        |
| 10  | octanal                        | citrus-like, green | 1280 | 1002 | 256      | 128     | 64       | 64        |
| 11  | 1-octen-3-one                  | mushroom-like| 1285    | 978  | 32       | 128     | 16       | 128       |
| 12  | (E)-2-heptenal                 | green, flowery| 1311   | 951  | 16       | 32      | 16       | 128       |
| 13  | 1-hexanol                      | green, grassy| 1338    | n.d. | 2        | 128     | 128      | 32        |
| 14  | 2-acetyl-1-pyrroline           | popcorn-like | 1342    | 930  | 64       | 128     | 128      | 32        |
| 15  | nonanal                        | citrus-like, soapy | 1376 | 1106 | 8        | 32      | 32       | 256       |
| 16  | (E)-2-octenal                  | fatty, grassy, green | 1417 | 1055 | 128      | 128     | 64       | 128       |
| 17  | acetic acid                    | vinegar-like | 1430    | n.d. | 128      | 128     | 256      | 16        |
| 18  | 3-(methylthio)propanal         | cooked potato-like | 1455 | 903  | 64       | 128     | 64       | 512       |
| 19  | (E),(E)-2,4-heptadienal        | fatty, roasty| 1486    | 1020 | 4        | 32      | 128      | 32        |
| 20  | (Z)-2-nonenal                  | green, fatty  | 1494    | 1140 | 256      | 256     | 128      | 128       |
Table 1. Cont.

| No. | Odorant b | Odor Quality c | Retention Index on | FD Factor d |
|-----|-----------|----------------|-------------------|------------|
|     |           |                | DB-FFAP           |            |
| 21  | 2-isobutyl-3-methoxypyrazine | bell pepper-like, earthy | 1510 1090 | 2 512 32 4 |
| 22  | (E)-2-nonenal | fatty, cardboard-like | 1524 1164 | <2 128 128 <2 |
| 23  | linalool | flowery | 1539 1103 | 32 512 512 512 |
| 24  | 2-methylpropanoic acid | cheesy | 1562 782 | 256 128 256 64 |
| 25  | (E,Z)-2,6-nonadienal | cucumber-like, fatty | 1574 1158 | <2 64 <2 <2 |
| 26  | (E)-2-decenal | green, fatty | 1616 1252 | 16 128 64 128 |
| 27  | butanoic acid | cheesy | 1626 804 | 128 256 32 4 |
| 28  | phenylacetaldehyde | flowery, honey-like | 1634 1040 | <2 <2 <2 32 |
| 29 a/b | 2- and 3-methylbutanoic acid | rancid, cheesy | 1662 860 | 1024 128 128 128 |
| 30  | (E,E)-2,4-nonadienal | fatty, deep fried | 1696 1216 | 512 64 128 |
| 31  | (E)-2-nonenal | fatty, cardboard-like | 1524 1164 | <2 128 128 <2 |
| 32  | linalool | flowery, earthy | 1841 1428 | 64 <2 16 |
| 33  | 2-methylfuran | smoky, ham-like | 1848 1096 | 16 128 128 128 |
| 34  | ethyl (E,E)-2,4-decadienoate | metallic, pear-like | 1890 n.d. | <2 128 <2 <2 |
| 35  | 2-acetyl-2-thiazoline | popcorn-like, roasty | 1747 n.d. | 16 256 32 4 |
| 36  | (E,E)-2,4-decadienal | fatty, deep fried | 1800 1280 | <2 128 128 128 |
| 37  | ß-damascenone f | fruity | 1808 1374 | 2 256 64 |
| 38  | ß-nonalactone | fruity, coconut-like | 1908 1154 | <2 4 <2 <2 |
| 39  | unknown | metallic | 1920 1545 | <2 8 <2 <2 |
| 40  | unknown | metallic | 1947 1325 | <2 16 <2 <2 |
| 41  | 2-methoxy-4-methylphenol | clove-like, vanilla-like | 1994 1379 | 1024 512 1024 |
| 42  | γ-decalactone | fruity, coconut-like | 2014 1364 | 16 64 32 8 |
| 43  | γ-octalactone | fruity, coconut-like | 2011 n.d. | <2 8 <2 <2 |
| 44  | γ-nonalactone | fruity, coconut-like | 2014 1364 | 16 64 32 8 |
| 45  | 4-hydroxy-2,5-dimethyl-3(2H)-furanone | caramel-like | 2026 1080 | <2 64 <2 <2 |
| 46  | octanoic acid | green, soapy | 2043 1180 | <2 32 4 <2 |
| 47  | 4-methylphenol | feral | 2073 1085 | 64 32 256 8 |
| 48  | ß-nonalactone | fruity, coconut-like | 2084 1380 | 32 64 <2 128 |
| 49  | unknown | metallic | 2103 1200 | <2 4 64 |
| 50  | 2,3-dimethylphenol | phenolic | 2105 1200 | 256 <2 <2 <2 |
| 51  | 2-hydroxy-4-methoxymethanol | clove-like, vanilla-like | 2128 1326 | 128 512 256 |
| 52  | γ-decalactone | fruity, peach-like | 2133 1474 | <2 16 <2 <2 |
| 53  | γ-decalactone | clove-like, vanilla-like | 2128 1326 | 128 512 256 |
| 54  | 3-hydroxy-4,5-dimethylfuran-2(5H)-one | maggi-like, celery-like | 2194 1106 | 16 512 <2 128 |
| 55  | 3-propylphenol | medical | 2247 1285 | 64 128 <2 256 |
| 56  | undecanoic acid | soapy, cardboard-like | 2323 1475 | <2 64 <2 <2 |
| 57  | unknown | smoky, phenolic | 2341 1345 | <2 1024 <2 <2 |
| 58  | γ-dodecalactone | caramel-like, flowery | 2371 1667 | 256 128 64 64 |
| 59  | ß-dodecalactone | phenolic | 2388 1071 | <2 <2 <2 <2 |
| 60  | δ-dodecalactone | peach-like | 2393 1700 | <2 16 <2 <2 |
| 61  | coumarin | cinnamon-like | 2435 1440 | 256 <2 <2 <2 |
| 62  | indole | feral | 2485 1320 | <2 16 <2 <2 |
| 63  | dodecanoic acid | fatty, wax-like | 2496 1574 | 1024 <2 128 256 |
| 64  | unknown | smoky, flowery | 2516 n.d. | 64 256 128 16 |
| 65  | phenylacetic acid | honey-like | 2545 1256 | 16 128 64 256 |

a Consecutive numbering of odorants according to their retention indices on capillary column DB-FFAP. b Odorant was identified by comparison of its odor quality and intensity and retention indices on capillaries DB-FFAP and DB-5 as well as mass spectra (EI mode) with data of reference compounds. c Odor quality perceived at the odor detection port by four trained panelists. d Flavor dilution factor determined on DB-FFAP. e n.d. not detected. f No unequivocal mass spectrum was obtained; identification is based on the remaining criteria given in footnote b.

In the investigated cocoa pulps, Vietnamese pulp displayed the highest number of aroma-active regions with a total of 57 odorants. It was followed by Nicaraguan cocoa pulp with 47, and Indonesian pulp with 46 aroma-active regions. Cocoa pulp originating from Cameroon accounted for a total of 43 aroma-active volatiles. Among others, all investigated cocoa pulps exhibited fatty (e.g., No. 19, 20, 26, 30, 32); fatty, cheesy (e.g., No. 24, 27, 29); green, grassy (e.g., No. 4, 6, 10, 13, 16); flowery (e.g., No. 12, 23, 37, 49, 58); fruity (e.g., 2, 33, 44); and smoky (e.g., No. 35, 51, 64) smelling compounds. The highest FD factors of 1024 and 512 were determined for 3-(methylthio)propanal (No. 18, cooked potato-like, RI 1455), 2-isobutyl-3-methoxypyrazine (No. 21, bell pepper-like, earthy RI 1510), linalool (No. 23, flowery, RI 1539), 2- & 3-methylbutanoic acid (No. 29 a/b, rancid, cheesy, RI 1662),...
With regard to aroma diversity, the intensity of the flowery smelling linalool showed a lower FD in Indonesian pulp (FD 32) compared to the other samples (FD 512). This odorant is thought to be a fine flavor aroma compound in cocoa beans, where it is present in higher concentrations compared to bulk cocoa [40]. It has been also proposed that certain aroma compounds present in the cocoa pulp, including the odorant linalool, may originate in the
pulp and migrate to the beans during cocoa bean fermentation [41]. In addition, odorants of the group of phenols were identified in this work. The compound 4-vinyl-2-methoxyphenol (No. 51, smoky, clove-like, RI 2128) was found in all cocoa pulp samples with similar FD factors. The odorant 2,3-dimethylphenol (No. 50, phenolic, RI 2109) was exclusively found in Indonesian pulp with factor FD 256. On the other hand, the unknown substance No. 57 (RI 2341), which exhibited a smoky and phenolic odor quality, was only found in pulp from Vietnam. Phenols can be released by the degradation of polyphenols and lignin by enzymes and microbes [42]. Phenols and their derivatives can be potent odor-active compounds. Methylphenols usually impart phenolic and smoky odor qualities, while methoxyphenols are often described with a broad range of aroma qualities such as smoky, vanilla, leather, meat-, and ham-like. Terpene-derived phenols can be herbal and spice-like odorants [34]. Further odorants present only in Indonesian cocoa pulp were the lactone coumarin (No. 61, cinnamon-like, RI 2435, FD 256) and the heterocyclic compound indole (No. 62, fecal, RI 2485, FD 16). Coumarin can be found naturally in many food products such as fruits, oils, nuts, and spices [42], and was identified by Balladares et al. [43] in cocoa pulp sweatings from Ecuador. Additionally, Mashuni et al. [44] found coumarin derivatives in the polyphenol fraction obtained from cocoa pod husks by means of a microwave-assisted extraction method. The compound indole is a nitrogen-containing odorant that imparts a floral taint with a fecal note to food products [24] and is often produced by bacteria [45]. Indonesia is mainly a bulk cocoa producer and only 1% of its total cocoa exports is classified as fine and flavor cocoa [46]. Therefore, it is likely that the analyzed SUL2 variety, categorized as a Trinitario cocoa [47,48], would be also considered to have a bulk cocoa quality. Altogether, the low FD factor of linalool in Indonesian cocoa pulp and the fatty, cheesy, and phenolic notes suggest that the pulp from this origin, specifically of the variety SUL2, is less suitable for products in which predominant attributes such as fresh, fruity, and floral are desired (e.g., juices, jams, fruit snacks).

Cocoa pulp from Vietnam also showed unique aroma compounds. The substances (E,Z)-2,6-nonadienal (No. 25, cucumber-like, fatty, RI 1574, FD 64), ethyl (E,E)-2,4-decadienoate (No. 36, metallic, pear-like, RI 1890, FD 128), γ-octalactone (No. 38, fruity, coconut-like; RI 1908, FD 4), 4-methylhexanoic acid (No. 43, sweaty, fishy, RI 2011, FD 8), 4-hydroxy-2,5-dimethyl-3(2H)-furanone (No. 45, caramel-like, RI 2026, FD 64), γ-decalacton (No. 52, fruity, peach-like, RI 2133, FD 16), undecanoic acid (No. 56, soapy, coriander-like, RI 2323, FD 64), substance No. 57 (unknown, smoky, phenolic, RI 2341, FD 1024), and δ-dodecalactone (No. 60, peach-like, RI 2393, FD 16) could not be determined in Indonesian, Cameroonian, and Nicaraguan cocoa pulp. The odorant 4-hydroxy-2,5-dimethyl-3(2H)-furanone was first identified as a Maillard reaction product [49]. In addition, it has been determined in strawberries [50], pineapples [51], tomatoes [52], grapes [53], and raspberries [54]. Chetschik et al. [21] identified 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol) in cocoa pulp from Colombia and Costa Rica. Furthermore, Vietnamese pulp displayed the most perceivable odorants from the group of lactones (n = 7) compared to the three counterparts (n < 5). However, lactones were determined with low FD factors, accentuating the predominance of odorants with green, fatty, and smoky notes. Moreover, compared to other varieties, the odorant 2-isobutyl-3-methoxypyrazine (No. 21, bell pepper-like, earthy, RI 1510) was more intense (FD 512) in the South East Asian pulp. This pyrazine is characteristic for bell peppers [55] and certain types of grapes [56]. Vietnamese pulp also shared odorants with other single samples. The odorant 3-methylbutanol (No. 7, RI 1200), which exhibits malty and roasty odor qualities, was only perceived in Vietnamese and Nicaraguan pulp.

Odorants (E)-2-nonenal (No. 22, fatty, cardboard-like, RI 1524), compound No. 39 (metallic, RI 1920), and octanoic acid (No. 46, green, soapy, RI 2043) were found only in pulp from Vietnam and Cameroon. Although cocoa pulps from Vietnam and Indonesia came both from South East Asia and were expected to show strong resemblances in their aroma compositions, they did not share odorants exclusively. ICCO recognized that 40% of the total cocoa exports of Vietnam can be classified as fine and flavor cocoa [46]. Along with
this, the predominance of green, fatty, and smoky notes suggests that Vietnamese cocoa pulp investigated in this work originated from a bulk cocoa variety.

The cocoa variety TSH 565, short for Trinidad Selected Hybrid 565, was obtained from the crossing of varieties ICS1 (Imperial College Selection 1) and SCA6 (Scavina 6). While the variety ICS1 has been reported to be original to Trinidad as well as to produce superior beans [57], Scavina cocoa varieties originated from the Ucayali River basin [58]. Scavina cocoa is known for its floral aroma notes in the fruit pulp and raw cocoa [20]. The resulting hybrid TSH 565 can be associated to fine flavor cocoa due to its high concentrations of terpenes such as β-myrcene and β-cis-ocimene [59]. Compared to other origins investigated in this work, cocoa pulp from the TSH 565 variety grown in Nicaragua exhibited higher FD factors in aroma compounds with the odor qualities fruity and flowery. For instance, the odorant 3-methylbutyl acetate (No. 5, fruity, RI 1118) was determined with FD 128 in Nicaraguan, FD 16 in both South East Asian samples, and FD < 2 in Cameroonian pulp. In addition, the odorant (E)-2-heptenal (No. 12, green, flowery, RI 1311) displayed an FD factor of 128 in Nicaraguan, 16 in Indonesian and Cameroonian, and 32 in Vietnamese cocoa pulp. The aldehyde nonanal (No. 15, citrus-like, soapy, RI 1376) showed FD factors of 256, 8, 32, and 32, respectively. The odorants (E)-2-heptenal and nonanal have been reported previously in cocoa pulp from Colombia [19], but could not be found in five cocoa pulp varieties from Costa Rica [22]. Furthermore, cocoa pulp from Nicaragua stood out for having the lower intensity of acetic acid (FD 16) compared to the Indonesian (FD 128), Vietnamese (FD 128), and Cameroonian (FD 256) counterparts. Odorant No. 53, δ-decalactone (coconut-like, RI 2188), was only perceived in Cameroonian and Nicaraguan cocoa pulp (FD 128 and FD 512, respectively). The odorants phenylacetaldehyde (No. 28, flowery, honey-like, RI 1634, FD 32) as well as 2-methoxy-4-methylphenol (No. 41, clove-like, vanilla-like, RI 1962; FD 128) were also exclusive for pulp from Nicaragua (FD 32). Together with the honey-like phenylacetic acid (No. 65, RI 2545, FD 256), the odorants may have contributed to the overall honey-like impression of the Nicaraguan extracts. The combination of fruity, flowery, honey-like, and vanilla-like odor qualities of Nicaraguan cocoa pulp make it interesting for the development of food products. The results may possibly relate to the fact that Nicaragua is solely a fine flavor cocoa exporter [46]. An industrial implementation as well as the existing infrastructure in the country would have to be revised, as Nicaragua is a small cocoa producer compared to countries such as Indonesia and Cameroon [60,61].

3. Materials and Methods

3.1. Separation and Storage of the Fresh Cocoa Pulp

Cocoa pulps from Indonesia (SUL2), Cameroon (unknown variety), Vietnam (unknown variety), and Nicaragua (TSH 565) were investigated. Cameroonian, Vietnamese, and Nicaraguan fresh cocoa fruits, harvested in 2019, were imported to Germany in a cool shipment directly after harvest. At Fraunhofer IVV, the fruits were washed, cut open and the cocoa pulp was separated mechanically immediately upon arrival. After de-pulping, the pulp was vacuum-sealed in odorless plastic bags (PA/PE 90/130 × 280 mm, Dagema eG, Willich, Germany) and immediately frozen at −50 °C. Indonesian cocoa pulp was supplied by the Indonesian Cocoa and Coffee Research Centre (ICCRI) in Jember, East Java. The cocoa pulp was separated mechanically on-site (Indonesia) directly after cocoa pod harvest (wet season 2019) and frozen immediately. The material was shipped in a frozen state to Germany, where it was stored at −50 °C in the same manner as its counterparts.

3.2. Isolation of Volatile Organic Compounds (VOC)

To isolate the volatile organic compounds, 50 g (±0.1) fresh cocoa pulp was extracted by stirring vigorously with 200 mL dichloromethane (DCM) for one hour at room temperature in a closed vessel. Dichloromethane (DCM) was purchased from Merck KGaA (Darmstadt, Germany) and distilled for purification prior to use. After decanting of 150 mL DCM, the volatiles were separated from the non-volatiles by means of the Solvent Assisted
Flavor Evaporation (SAFE) technique [62]. The distillation was carried out under high vacuum, maintaining the round flask in a water bath at 50 °C and thermostating the SAFE apparatus to 55 °C. The obtained distillates were dried over anhydrous sodium sulfate (Merck KGaA, Darmstadt, Germany), filtered, and concentrated at 50 °C to ~3 mL by a Vigreux column (50 cm × 1 cm i.d.) and finally to a volume of ~100 µL by microdistillation [63]. To enable a comparison between the pulps, the same amounts were extracted, subjected to SAFE distillation, concentrated to the same final volume, and, finally, the same volume was used for GC-O.

3.3. Comparative Aroma Extract Dilution Analysis (cAEDA)

To enable a comparison between all pulps, the same volumes of the extracts were used for gas chromatography-olfactometry (GC-O). To avoid a potential overlooking of aroma-active areas, the original distillates were evaluated by four trained panelists of the Fraunhofer IVV sensory panel using GC-O. The panelists undergo trainings once a week on an in-house established odor-language with selected reference compounds, corresponding to about 150 different odorants. All panelists exhibited normal olfactory function and had no known illnesses at the time of examination. A blank was performed for each sample by applying the same work-up procedure (c.f 3.2). The flavor dilution (FD) factors of the odorants were determined by diluting the distillate stepwise (1 + 1, v + v) with dichloromethane up to factor 1024 and analyzing the dilutions with GC-O [64]. For each aroma-active area, the respective FD factor, correlating to the highest dilution in which the compound was perceived by the panelists at the odor detection port for the last time, was assigned.

3.4. Gas Chromatography-Olfactometry (GC-O)

GC-O was carried out by means of a Trace GC Ultra (Thermo Fisher Scientific GmbH, Dreieich, Germany) equipped with either a DB-FFAP or DB-5 capillary column (both 30 m × 0.32 mm, 0.25 µm film thickness) (J&W Scientific, Agilent Technologies GmbH, Waldbronn, Germany). Aliquots (2 µL) of the sample distillates were injected manually by the cold on-column technique at 40 °C, using helium as carrier gas at a constant flow mode (2.2 mL/min). The initial temperature of 40 °C was held for 2 min, raised at 6.0 °C/min to 235 °C (DB-FFAP) or 250 °C (DB-5), respectively, and held for 5 min. At the end of the column, the effluent was split 1:1 by volume through two deactivated fused silica capillaries of the same length (70 cm × 0.2 mm) leading to a flame ionization detector (FID) held at 250 °C and to an odor detection port (ODP) held at 235 °C. Linear retention indices (RI) were calculated using a homologous series of n-alkanes ranging from C₆ to C₂₆ for the DB-FFAP and C₆ to C₁₈ for the DB-5 column [65].

3.5. Gas Chromatography-Mass Spectrometry/Olfactometry (GC-MS/O)

For identification of the VOCs present in the distillates, GC-MS/O was performed using a Trace GC Ultra and a Trace dual stage quadrupole (DSQ) mass spectrometer (both Thermo Fisher Scientific GmbH) equipped with a DB-FFAP column (30 m × 0.32 mm, 0.25 µm film thickness, J&W Scientific, Waldbronn, Germany). The distillates (2 µL) were injected automatically by a multipurpose autosampler MPS 2 (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) using the cold on-column technique. The initial temperature of 40 °C was held for 2 min, raised at 6.0 °C/min to 235 °C, and held for 5 min. The flow rate of the helium carrier gas was 2.2 mL/min. At the end of the capillary column, the effluent was split between an ODP and the MS using deactivated fused silica capillaries (50 cm × 0.2 mm). Mass spectra were recorded in positive electron ionization (EI) mode at 70 eV (m/z range 35–250).

3.6. Stir-Bar Sorptive Extraction Gas Chromatography-Olfactometry/Mass Spectrometry (SBSE-GC-MS/O)

The SBSE method was applied to confirm the presence of VOCs and to compensate for possible losses and changes in the aroma composition of the pulp during the isolation
step (c.f. 3.2). Fresh cocoa pulp samples (2.0 g ± 0.01) were diluted individually 1:1 (w/w) in distilled water in headspace vials (volume 20 mL), sealed airtight, and the suspension was stirred at room temperature for 15 min with a preconditioned SBSE Twister® (polydimethylsiloxane sorbent (PDMS), 20 mm length, 0.5 mm coating thickness; Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany). Preconditioning was performed at 280° C for five hours. After extraction, the Twister® was automatically transferred to the Thermal Desorption Unit at 40 °C (TDU, Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany). Desorption started at a temperature of 40 °C (initial time: 0.1 min), then increased to 250 °C at a rate of 12.0 °C/s, before being held at 250 °C for 5 min. The desorbed volatiles were transferred to the cold-injection-system (CIS, Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany), cooled at −70 °C using liquid nitrogen, and then transferred onto the GC-MS/O system (cf. Section 3.5) by thermal desorption. After an initial time of 2 min at 40 °C, the oven temperature was raised at 6 °C/min to 235 °C and held for 5 min. The column flow of the helium carrier gas was adjusted to 50 mL/min. Mass spectra were generated in full scan mode (m/z range 35–400, EI 70 eV) on the same GC-MS system as described in Section 3.5.

3.7. Headspace Solid-Phase Microextraction Gas Chromatography-Olfactometry/Mass Spectrometry (HS-SPME GC-O/MS)

Highly volatile aroma-active compounds were additionally analyzed using HS-SPME-GC-O/MS to rule out any losses during the concentration steps by the liquid extraction technique. Fresh cocoa pulp samples (1.0 g ± 0.01) were diluted individually 1:1 (w/w) in distilled water and air tightly sealed in a 20 mL headspace glass vial. By means of an orbital shaker (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany), each vial was agitated for 10 min at 50 °C and 250 rpm, changing the direction every 90 s. Prior to use, the SPME fiber (PDMS, 100 µm, Supelco, Bellefonte, PA, USA) was conditioned at 250 °C for 5 min. The SPME fiber was introduced automatically into the vial for volatile adsorption, exposed to the fresh cocoa pulp for 10 min at 50 °C during HS equilibration, and analyzed using the MPS autosampler and GC-O/MS system equipped with a DB-FFAP capillary column as described in Section 3.5. Thermal desorption was performed using a PTV (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) for 180 s at 250 °C. After an initial time of 2 min at 40 °C, the GC-oven temperature was raised at 6 °C/min to 150 °C, then raised at 20 °C/min to 235 °C and held for 5 min. The column flow of the helium carrier gas was adjusted to 50 mL/min. Mass spectra were generated in full scan mode (m/z range 35–250, EI 70 eV).

4. Conclusions

Our study shows that cacao fruit pulp may present great qualitative differences in the composition of volatiles depending on their origin, which then may determine their suitability for diverse types of food products. By means of cAEDA, 65 aroma-active areas were detected in cocoa pulps from various origins, 60 of which were unequivocally identified by GC analysis on two columns of different polarity and comparison to authentic reference standards. Of the identified compounds, 36 were found in all four cocoa pulps. The highest odorant diversity was found in Vietnamese pulp. Cocoa pulp from Cameroon exhibited the lowest flavor intensities in terms of FD factors. In the higher dilutions, the Indonesian cocoa pulp extract was predominantly fatty, cheesy, and phenolic. The Vietnamese cocoa pulp extract displayed high FD factors with fatty, green, and smoky notes. Cameroonian cocoa pulp presented butter-like, popcorn-like, flowery, and fruity odor qualities in the higher extract dilutions, whereas the Nicaraguan cocoa pulp extract was primarily fruity, flowery, but also possessed honey, clove, and vanilla-like traits. Given that odor impressions result from complex interactions between many aroma-active substances, correlations between a single volatile and an olfactory perception are often not conclusive [66]. Synergistic, additively or suppressive effects between substances present in the sample may affect its overall aroma impression unpredictably [66,67]. In order to understand the final aroma as well as the aroma compound diversity in cocoa pulp better, a quantification of the VOCs
would be needed. Furthermore, the influence of genetic background on the aroma quality of the cocoa pulp should be investigated, as the genetic backgrounds of samples analyzed in this work were unclear. Studies with bigger cocoa pulp cohorts could provide important insights on how cocoa genetics determine the aroma composition of cocoa pulps. Finally, investigations using one commercial cocoa genotype, e.g., CCN51, grown in different regions of the world could help us understand better how other factors, such as the harvesting season, age of the cocoa tree, and growing conditions, may affect the aroma quality of cocoa pulp and its later application as a food ingredient.

Supplementary Materials: The following are available online: results obtained by HS-SPME-GC-MS/O and SBSE-GC-MS/O.

Author Contributions: T.B.H., conceptualization, methodology, investigation, formal analysis, writing—original draft, review and editing; S.N., conceptualization, review and editing, supervision, project administration; U.S.-W., conceptualization, funding acquisition, review and editing, supervision; E.O., methodology, data curation, review and editing, supervision; H.Z., review and editing, supervision. All authors have read and agreed to the published version of the manuscript.

Funding: The project on which this paper is founded was funded by the German Federal Ministry of Education and Research (BMBF) under the grant number 031B0819. The responsibility for the content of this publication lies with the authors.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors thank the panelists contributing in the aroma analyses as well as the scientific staff at Fraunhofer Institute for Process Engineering and Packaging, Freising, Germany, for their constructive criticism. We greatly thank Hauke Will (Alfred Ritter GmbH & Co., KG, Waldenburg, Germany) for supplying the Nicaragua fresh cocoa pods. A special thanks to the Indonesian Coffee and Cocoa Research Center (ICCRI) for supplying fresh cocoa pulp for the experiments.

Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of the compounds are not available from the authors.

References
1. The International Cocoa Organization. *ICCO Quarterly Bulletin of Cocoa Statistics*; The International Cocoa Organization: Abidjan, Côte d’Ivoire, 2021; Volume XLVIII, No. 1, Cocoa Year 2020/2021.
2. Prabhakaran Nair, K.P. (Ed.) *The Agronomy and Economy of Important Tree Crops of the Developing World*, 1st ed.; Elsevier: Amsterdam, The Netherlands, 2010; ISBN 9780123846778.
3. Vásquez, Z.S.; de Carvalho Neto, D.P.; Pereira, G.V.M.; Vandenberghme, L.P.S.; de Oliveira, P.Z.; Tiburcio, P.B.; Rogez, H.L.G.; Góes Neto, A.; Soccol, C.R. Biotechnological approaches for cocoa waste management: A review. *Waste Manag.* 2019, 90, 72–83. [CrossRef] [PubMed]
4. Beyond Beans. Increasing Farmers’ Incomes through Cocoa Juice. Notes from the Field 15/12/2020. Available online: https://mcusercontent.com/5346a19420bc321403f2088/files/039cfeaa-f64d-4d0b-98ca-4e1b68ade138/Cocoa_Juice_NftF_General.pdf (accessed on 7 October 2021).
5. Schwan, R.F.; Wheals, A.E. The microbiology of cocoa fermentation and its role in chocolate quality. *Crit. Rev. Food. Sci. Nutr.* 2004, 44, 205–221. [CrossRef] [PubMed]
6. Pettipher, G.L. Analysis of Cocoa Pulp and the Formulation of a Standardized Artificial Cocoa Pulp Medium. *J. Sci. Food Agric.* 1986, 37, 297–309. [CrossRef]
7. Roelofsen, P.A. Fermentation, Drying, and Storage of Cacao Beans. *Adv. Food Res.* 1958, 8, 225–296.
8. Afoakwa, E.O. Industrial chocolate manufacture—Processes and factors influencing quality. In *Chocolate Science and Technology*; Wiley-Blackwell: Oxford, UK, 2010; pp. 35–57.
9. Schwan, R.F.; Lopez, A.S. Mudanca no perfil da fermentacao de cacau ocasionada pela retirada parcial da polpa da semente. *Rev. Theobroma* 1988, 18, 247–257.
10. Bangerter, U.; Beh, B.H.; Callis, A.B.; Pilkington, I.J. Treatment of Cocoa Beans for Improving Fermentation. European Patent 91101882.8, 11 February 1991.
11. Figueira, A.; Janick, J.; BeMiller, J.N. New Products from Theobroma cacao: Seed Pulp and Pod Gum; Academic Press: New York, NY, USA, 1993.

12. Puerari, C.; Magalhaes, K.T.; Schwan, R.F. New cocoa pulp-based kefir beverages: Microbiological, chemical composition and sensory analysis. Food Res. Int. 2012, 48, 634–640. [CrossRef]

13. dos Santos Filho, A.L.; Veloso Freitas, H.; Rodrigues, S.; Gonçalves Abreua, V.K.; de Oliveira Lemos, T.; Faria Gomes, W.; Narain, N.; Fernandes Pereira, A.L. Production and stability of probiotic cocoa juice with sucralose as sugar substitute during refrigerated storage. LWT-Food Sci. Technol. 2019, 99, 371–378. [CrossRef]

14. Dias, D.R.; Schwan, R.F.; Freire, E.S.; Serodio, R.D. Elaboration of a fruit wine from cocoa (Theobroma cacao L.) pulp. Int. J. Food Sci. Technol. 2007, 42, 319–329. [CrossRef]

15. Nunes, C.S.O.; da Silva, M.L.C.; Camilloto, G.P.; Machado, B.A.S.; Hodel, K.V.S.; Koblitz, M.G.B.; Carvalho, G.B.M.; Uetanabaro, A.P.T. Potential Applicability of Cocoa Pulp (Theobroma cacao L.) as an Adjunct for Beer Production. Sci. World J. 2020, 2020, 3192585. [CrossRef]

16. Nestec, S.A. Foodstuff Products, Ingredients, Processes and Uses. Patent WO2019115735, 13 December 2018. Available online: https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2019115735 (accessed on 27 October 2021).

17. Alfred Ritter GmbH & Co. KH. Cacao Y Nada. Available online: https://www.ritter-sport.com/de/cacao-y-nada (accessed on 27 October 2021).

18. Lindt und Sprüngli GmbH. EXCELLENCE Cacao Pur | Lindt Deutschland. Available online: https://www.lindt.de/onlineshop/marken/excellence/cacopur (accessed on 27 October 2021).

19. Pino, J.A.; Ceballos, L.; Quijano, C.E. Headspace Volatiles of Theobroma cacao L. Pulp From Colombia. J. Essent. Oil Res. 2010, 22, 113–115. [CrossRef]

20. Kadow, D.; Bohlmann, J.; Phillips, W.; Lieberei, R. Identification of main fine or flavour components in two genotypes of the cocoa tree (Theobroma cacao L.). J. Appl. Bot. Food Qual. 2013, 86, 90–98. [CrossRef]

21. Chetschik, I.; Kneubühl, M.; Chatelain, K.; Schlüter, A.; Bernath, K.; Hühn, T. Investigations on the Aroma of Cocoa Pulp (Theobroma cacao L.) and Its Influence on the Odor of Fermented Cocoa Beans. J. Agric. Food Chem. 2018, 66, 2467–2472. [CrossRef] [PubMed]

22. Hegemann, E.; Niether, W.; Rohsius, C.; Phillips, W.; Lieberei, R. Besides variety, also season and ripening stage have a major influence on fruit pulp aroma of cacao (Theobroma cacao L.). J. Appl. Bot. Food Qual. 2020, 93, 266–275. [CrossRef]

23. Biehl, B.; Brunner, E.; Passern, D.; Quesnel, V.C.; Adomako, D. Acidification, proteolysis and flavour potential in fermenting cocoa beans. J. Sci. Food Agric. 1985, 36, 583–598. [CrossRef]

24. Ziegleder, G. Verfahrenstechnische Einflüsse auf Kakaoaroma. II. Zucker-Und Süsswaren-Wirtsch. 1993, 66, 199, 2467–2472. [CrossRef] [PubMed]

25. Ziegleder, G. Verfahrenstechnische Einflüsse auf Kakaoaroma. J. Zucker-Und Süsswaren-Wirtsch. 1993, 46, 131–133. [CrossRef]

26. Msinawi; Jinap, S.; Jamilah, B.; Nazamid, S. Sensory properties of cocoa liquor as affected by polyphenol concentration and duration of roasting. Food Qual. Prefer. 2004, 15, 403–409. [CrossRef]

27. Ziegleder, G. Composition of flavor extracts of raw and roasted cocoas. Eur. Food Res. Technol. 1991, 192, 521–525. [CrossRef]

28. Kongor, J.E.; Hinneh, M.; de van Walle, D.; Afoakwa, E.O.; Boeckx, P.; Dewettinck, K. Factors influencing quality variation in cocoa (Theobroma cacao) bean flavour profile—A review. Food Res. Int. 2016, 82, 44–52. [CrossRef]

29. Rusconi, M.; Conti, A. Theobroma cacao L., the Food of the Gods: A scientific approach beyond myths and claims. Pharm. Res. 2010, 61, 5–13. [CrossRef] [PubMed]

30. Lima, L.J.R.; Almeida, M.H.; Nout, M.J.R.; Zwietering, M.H. Marken/excellence/cacaopur (accessed on 27 October 2021).

31. dos Santos Filho, A.L.; Veloso Freitas, H.; Rodrigues, S.; Gonçalves Abreua, V.K.; de Oliveira Lemos, T.; Faria Gomes, W.; Narain, N.; Fernandes Pereira, A.L. Production and stability of probiotic cocoa juice with sucralose as sugar substitute during refrigerated storage. LWT-Food Sci. Technol. 2019, 99, 371–378. [CrossRef]

32. Torres-Moreno, M.; Torrescasana, E.; Salas-Salvadó, J.; Blanch, C. Nutritional composition and fatty acids profile in cocoa beans and chocolates with different geographical origin and processing conditions. Food Chem. 2015, 166, 125–132. [CrossRef] [PubMed]

33. Sánchez-Sevilla, J.F.; Cruz-Rus, E.; Valpuesta, V.; Botella, M.A.; Amaya, I. Deciphering gamma-decalactone biosynthesis in strawberry fruit using a combination of genetic mapping, RNA-Seq and eQTL analyses. BMC Genom. 2014, 15, 218. [CrossRef]

34. Parker, J.K.; Elmore, S.; Methven, L. (Eds.) Flavour Development, Analysis and Perception in Food and Beverages; Woodhead Publishing: Cambridge, UK; Waltham, MA, USA; Kidlington, UK, 2015; ISBN 1782421114.

35. Wessel, M.; Quist-Wessel, P.F. Cocoa production in West Africa, a review and analysis of recent developments. NJAS—Wagening. J. Life Sci. 2015, 74–75, 1–7. [CrossRef]

36. Fowler, M.S. Cocoa Beans: From Tree to Factory. In Industrial Chocolate Manufacture and Use, 4th ed.; Beckett, S.T., Beckett, S.T., Eds.; Wiley-Blackwell: Chichester, UK; Ames, IA, USA, 2009; pp. 10–47, ISBN 9781444301588.

37. Jahurul, M.; Zaidul, I.; Norulaini, N.; Sahena, F.; Jinap, S.; Azmiz, J.; Sharif, K.M.; Omar, A.M. Cocoa butter fats and possibilities of substitution in food products concerning cocoa variety, alternative sources, extraction methods, composition, and characteristics. J. Food Eng. 2013, 117, 467–476. [CrossRef]

38. Fowler, M.S. Fine or flavour cocoas: Current position and prospects. Cocoa Grow. Bull. 1994, 48, 17–23.

39. International Cocoa Organization. Fine or Flavor Cocoa. Available online: https://www.icco.org/fine-or-flavor-cocoa/ (accessed on 3 November 2021).
40. Ziegleder, G. Linalool contents as characteristic of some flavor grade cocoas. Eur. Food Res. Technol. 1990, 191, 306–309. [CrossRef]
41. Eskes, A.; Ahnert, D.; Garcia Carrion, L.; Seguire, E.; Assemat, S.; Guardia, D.; Garcia, R. Evidence on the Effect of the Cocoa Pulp Flavour Environment during Fermentation on the Flavour Profile of Chocolates. Improving the profitability of small and medium-sized farms: The principal key to a global sustainbale cocoa economy. In Proceedings of the 17th International Cocoa Research Conference (COPAL), Yaoundé, Cameroon, 15–20 October 2012.
42. Belitz, H.-D.; Grosch, W.; Schieberle, P. Food Chemistry, 3rd Rev. ed.; Springer: Berlin, Germany, 2004; ISBN 3540408177.
43. Balladares, C.; Garca, J.; Chez Guaranda, I.; Prez, S.; Gonzalez, J.; Sosa, D.; Viteri, R.; Barragan, A.; Quijano Aviles, M.; Manzano, P. Physicochemical characterization of Theobroma cacao L. mucilage, in Ecuadorian coast. Emir. J. Food Agric. 2016, 28, 741–745. [CrossRef]
44. Mashuni; Hamid, F.H.; Muzuni; Kadidae, L.O.; Jahiding, M.; Ahmad, L.O.; Saputra, D. The determination of total phenolic content of cocoa pod husk based on microwave-assisted extraction method. In Proceedings of the 8th International Conference of the Indonesian Chemical Society (ICICS) 2019, Bogor, Indonesia, 6–7 August 2019; p. 30013.
45. Lee, J.-H.; Lee, J. Indole as an intercellular signal in microbial communities. FEMS Microbiol. Rev. 2010, 34, 426–444. [CrossRef]
46. Follana, C. ICCO Panel Recognizes 23 Countries as Fine and Flavour Cocoa Exporters. International Cocoa Organization [Online]. Available online: https://www.icco.org/icco-panel-recognizes-23-countries-as-fine-and-flavour-cocoa-exporters/ (accessed on 24 September 2021).
47. Febrianto, N.A.; Zhu, F. Diversity in Composition of Bioactive Compounds Among 26 Cocoa Genotypes. J. Agric. Food Chem. 2019, 67, 9501–9509. [CrossRef] [PubMed]
48. Anita-Sari, I.; Susilo, A.W.; Mawardi, S. Seleksi dan Penulisan Kakao; Gadjah Mada University Press: Yogyakarta, Indonesia, 2015.
49. Hodge, J.E.; Fisher, B.E.; Nelson, E.C. Dicarbonyls, reductones, and heterocyclics produced by reactions of reducing sugars with secondary amine salts. Proc. Ann. Meet. Am. Soc. Brew. Chem. 1963, 21, 84–92. [CrossRef]
50. Willbalm, B.; Stoll, M.; Thomas, A.F. 2,5-dimethyl-4-hydroxy-2,3-dihydrofuran-3-one. Chem. Ind. 1965, 18, 1629–1630.
51. Rodin, J.O.; Himel, C.M.; Silverstein, R.M.; Leeper, R.W.; Gortner, W.A. Volatile flavor and aroma components of pineapple. 1. Isolation and tentative identification of 2,5-dimethyl-4-hydroxy-3(2H)-furanone. J. Food Sci. 1965, 30, 280–285. [CrossRef]
52. Schwab, W. 4-hydroxy-3(2H)-furanes: Natural and Maillard products. Recent Res. Dev. Phytochem. 1997, 1, 643–673.
53. Ohloff, G. Chemie der Geruchs-und Geschmacksstoffe; Springer: Heidelberg, Germany, 1969.
54. Honkanen, E.; Pyysalo, T.; Hirvi, T. The aroma of Finnish wild raspberries, Rubus idaeus, L. Eur. Food Res. Technol. 1980, 171, 180–182. [CrossRef]
55. Roth, K. The Biochemistry of Peppers; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2014. [CrossRef]
56. Belitz, H.-D.; Grosch, W.; Schieberle, P. Food Chemistry, 2nd ed.; Springer: Heidelberg, Germany, 1999. [CrossRef]
57. Johnson, E.S.; Bekele, F.L.; Schnell, R.J. Field Guide to the ICS Clones of Trinidad: Morphological Characterisation of the Cocoa Accessions at the International Cocoa Genebank Trinidad (ICGT)—Safeguarding the Local and Regional Cocoa Industry. Available online: https://www.researchgate.net/publication/235456464_Field_Guide_to_the_ICS_Clones_of_Trinidad (accessed on 3 November 2021).
58. Hort, K. The Biochemistry of Peppers; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2014. [CrossRef]
59. van den Dool, H.; Kratz, P.D. A generalization of the retention index system including linear temperature programmed gas—Liquid partition chromatography. J. Chromatogr. A 1963, 11, 463–471. [CrossRef]
60. van den Dool, H.; Kratz, P.D. A generalization of the retention index system including linear temperature programmed gas—Liquid partition chromatography. J. Chromatogr. A 1963, 11, 463–471. [CrossRef]
61. van den Dool, H.; Kratz, P.D. A generalization of the retention index system including linear temperature programmed gas—Liquid partition chromatography. J. Chromatogr. A 1963, 11, 463–471. [CrossRef]
62. Follana, C. ICCO Panel Recognizes 23 Countries as Fine and Flavour Cocoa Exporters. International Cocoa Organization [Online]. Available online: https://www.icco.org/icco-panel-recognizes-23-countries-as-fine-and-flavour-cocoa-exporters/ (accessed on 24 September 2021).
63. Bemelmans, J. (Ed.) Cocoa Producing Countries—Forum Nachhaltiger Kakao. Available online: https://www.kakaoforum.de/en/news-service/ (accessed on 24 September 2021).
64. Follana, C. ICCO Panel Recognizes 23 Countries as Fine and Flavour Cocoa Exporters. International Cocoa Organization [Online]. Available online: https://www.icco.org/icco-panel-recognizes-23-countries-as-fine-and-flavour-cocoa-exporters/ (accessed on 24 September 2021).
65. Garcia Carrion, L.; Seguire, E.; Assemat, S.; Guardia, D.; Garcia, R. Evidence on the Effect of the Cocoa Pulp Flavour Environment during Fermentation on the Flavour Profile of Chocolates. Improving the profitability of small and medium-sized farms: The principal key to a global sustainbale cocoa economy. In Proceedings of the 17th International Cocoa Research Conference (COPAL), Yaoundé, Cameroon, 15–20 October 2012.
66. Belitz, H.-D.; Grosch, W.; Schieberle, P. Food Chemistry, 3rd Rev. ed.; Springer: Berlin, Germany, 2004; ISBN 3540408177.
67. Buettner, A.; Schieberle, P. Influence of mastication on the concentrations of aroma volatiles—Some aspects of flavour release and flavour perception. Food Chem. 2000, 71, 347–354. [CrossRef]