Discovering fine flavour in Asian single-origin cocoa: Fast GC Electronic-nose aroma fingerprinting of cocoa liquors and consumer acceptability of *Tablea* in the Philippines

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Summary

The recent global cocoa supply decline calls for investigating disease-resistant and single-origin cocoa varieties in Asia. While genetic analysis has identified promising clones, information on bean quality and flavour remains limited. This research aims to analyse quality attributes of single-origin cocoa beans and key volatile organic compounds in cocoa liquors, and to determine consumer acceptability of indigenous cocoa beverage (*Tablea*) from single-origin cocoa in the Philippines. Aroma fingerprinting of cocoa clones (UF18, BR25, W10) using fast-GC electronic nose revealed distinctive profiles. Cocoa clone UF18 exhibited high fat content (52.1%), surpassing African cocoa clones. Even more important is the discovery of elevated levels of desirable volatile compounds in cocoa clone W10 such as methyl decanoate (fruity) and phenylacetaldehyde (honey, floral), which marks the first study that identified fine aroma components in single-origin cocoa beans in Asia. Harnessing potential of these cocoa clones ensures consumer acceptability of the cocoa beverage *Tablea.*

Keywords: cocoa, electronic nose, fine-flavour, sensory analysis, volatile organic compounds, *Tablea*, The Philippines

Introduction

Cocoa (*Theobroma cacao* L.), a commodity of great economic significance, has increasingly gained remarkable attention on the global market as it remains to be one of the most profitable and heavily traded food commodities in the world (Afoakwa, 2014; Peña-Correa et al., 2022). It is an invaluable cash crop for many smallholder farmers in the world and its processing is an important global industry (Essegbey & Ofori-Gyamfi, 2012). However, since the last decade, several cocoa-producing nations have witnessed a notable decline in production due to factors such as aging trees, plant diseases, and inadequate rainfall (Schroth et al., 2016). Consequently, numerous countries have embarked on robust genetic improvement initiatives to address these challenges (Monteiro et al., 2009). A case in point is the Philippines, where a revival of the cacao industry is ongoing to be a significant global cocoa contributor. Since the Philippines is still an emerging cocoa bean producer, there is more incentive to focus on cocoa quality than quantity. Preliminary genetic analysis and productivity experiments on new cocoa varieties determined highly recommended clones that are high-yielding and more resistant to diseases (Cena, 2012). Despite these advancements, a critical gap exists in our understanding of the volatile organic compounds responsible for the aroma profile of local cocoa beans (Marseglia et al., 2020).

Recently, the chocolate industry has observed a growing trend towards labeling chocolates by their origin, as evidenced by the increasing popularity of single-origin chocolates (Hanifah et al., 2022; Kitani et al., 2022; Muhammad et al., 2022; Saltini et al., 2013). These chocolates are

produced using cocoa beans sourced exclusively from a specific location or country, highlighting the unique flavor profiles that different geographical regions can impart to cacao beans. This movement towards origin-specific labeling has been driven by a demand for chocolates offering more nuanced and distinctive taste experiences, such as chocolates with a more pronounced fruity flavor (Counet et al., 2004). This trend reflects consumers' growing interest in understanding the provenance of their chocolate and the distinct tastes associated with different cocoa-producing regions (Kumar et al., 2014). Although the geographical origin of cocoa beans significantly influences their volatile organic chemical content (Acierno et al., 2016), the chocolate industry and consumers historically associate quality with the chocolate's manufacturing location (e.g., Belgium, Switzerland) rather than the source of the cacao beans (i.e. Africa, South America & Asia) (Cidell & Alberts, 2006).

The quality of cocoa is multifaceted, with flavor emerging as a pivotal factor influencing consumer preferences and, consequently, cocoa bean prices (Araujo et al., 2014; De Pelsmaeker et al., 2015; Sukha et al., 2008). The global cocoa market distinguishes between "fine or flavor" cocoa beans, primarily from Criollo or Trinitario varieties, and "bulk" or "ordinary" cocoa beans, typically derived from Forastero trees (ICCO, 2019). Fine or flavour cocoa beans fetch a premium over terminal prices paid for bulk cocoa due to their distinctive flavour (Sukha et al., 2008).

While physico-chemical and sensory attributes play crucial roles in chocolate evaluation, they alone are insufficient for predicting consumer acceptability, as individual preferences vary

widely (Costell et al., 2010). Research on the acceptability of indigenous cocoa liquor beverages is limited and given that cocoa bean flavour determines acceptability of cocoa beans and cocoa products (Kongor et al., 2016), this study therefore was conducted to determine the quality attributes of new cocoa bean varieties, key volatile organic compounds in the cocoa liquor samples and to assess the acceptability of a traditional Philippine cocoa liquor beverage (*Tablea*) made from these new cocoa clones. This comprehensive approach addresses the industry's quest for cocoa sources that not only offer quantity but, more importantly, deliver the desirable flavors from Asian cocoa beans to consumers.

Materials and Methods

Cocoa beans selection and sample procurement

Cocoa bean clones (UF18, BR25 and W10) were selected based on preliminary investigation on productivity and disease resistance by the National Seed Inspection Council of the Philippines. Although there are ten (10) registered cocoa bean clones, only BR25, UF18 and W10 are widely planted based on key informant interviews conducted. The cocoa bean samples were sourced from a single farm in Calinan Dictrict, Davao City, Philippines. Cocoa beans were box-fermented for 5 days followed by sun drying for another 5 days. For each type of cocoa clone, three wooden rectangular boxes ($60cm^3$) on a platform lined inside with banana leaves were prepared. Approximately 40-45kg of wet cocoa beans were placed inside the boxes and

were thoroughly mixed. The bottom of the box had perforations to allow sweatings to drain. Turning was done on the 3rd day to allow aeration after temperature reached at least 47°C.

Quality assessments

Moisture content

Moisture content of the cocoa beans samples was determined using the gravimetric procedure described in AOAC (2005) method 931.04.

pH and titratable acidity

The pH and titratable acidity of the cocoa beans were done as described by Nazaruddin et al. (2006) with slight modifications (Nazaruddin et al., 2006). The beans were pulverized into 5 g pieces and mixed thoroughly for 30 seconds in 100 mL of heated distilled water. The mixture was then filtered under vacuum using Whatman filter paper No. 4. A 25 ml portion was transferred into a beaker and the pH was determined using a pH meter (model MP230 Mettler Toledo MP 230, Mettler Company Limited, Geneva, Switzerland). Another 25 ml portion was titrated until the pH reached 8.1 using 0.01N NaOH. The results were then expressed as moles of sodium hydroxide per 100 g of dry nibs. The analysis was performed in triplicate.

Fermentation index

Fermentative quality of cocoa beans from the selected farms was assessed using fermentation index. This was determined using the spectrophotometric method described by (Gourieva &

Tserevitinov, 1979) with slight modifications (Kongor et al., 2018). A solution containing 0.1 g of ground cocoa nibs was obtained by extracting it with a 50 ml mixture of Methanol and HCl in a ratio of 97:3. The homogenate was refrigerated at 8°C for 20 hours and subsequently filtered. The filtrate's absorbance was measured using a UV-visible spectrophotometer (Cary Bio 50) at wavelengths of 460 nm (for yellow oxidized polyphenols) and 530 nm (for anthocyanins) (Evina et al., 2016). The fermentation index of the sample was determined by computing the ratio of absorbance at 460 nm to the absorbance at 530 nm. Three duplicate measurements were acquired for each sample and the average values were reported.

Preparation of cocoa liquor

Sorted cocoa beans were roasted at 135°C for 30 minutes (min) using a coffee bean roaster (Gene Café, CBR-101, Gyeonggi- Do, Korea). Afterwards, the beans were manually broken and deshelled. About 500 g nibs of each sample were gradually grinded for 30 min in a preheated (45°C) mortar mill (Retch RM200, Haan, Germany) and subsequent refining using a conching machine (CocoaTown Melanger ECGC-12SLTA, Alpharetta, Georgia, USA) which resulted in the liquefaction of the cocoa mass (i.e. cocoa liquor). After cooling, the liquors were stored in sterile plastic containers (120 mL) wrapped in aluminum foil.

Total fat content

Total fat content of the liquor was determined using the Soxhlet extraction method (AOAC 2005 method 963.15).

140 **Volatile organic compound (VOC) determination**

141 To determine the volatile organic compounds contained in the cocoa liquors from different
142 varieties, an analytical fingerprint approach based on fast gas chromatography (GC) was used
143 (Śliwińska et al., 2016; Yang et al., 2020). Fingerprinting of aroma volatiles in the liquor was
144 done using Heracles II (Alpha M.O.S., Toulouse, France) a cooled Tenax trap, two capillary
145 columns, and two ultra-sensitive flame ionization detectors (μFIDs). The two columns have
146 different polarities, namely a non-polar MXT-5 (5% diphenyl) and a medium polar MXT-1701
147 (14% cyanopropylphenyl) column with dimensions of 10 m × 180 μm × 0.4 μm. (Rottiers,
148 Tzompa Sosa, Van de Vyver, et al., 2019).

149 About 2 g of solid cocoa liquor was weighed in 20 mL vials which were tightly capped with
150 PTFE/silicone seals. Each sample was analyzed five times, and blanks (empty vials) were added
151 in between. The vial was placed in the incubation oven for 20 min at 50°C. About 5000 μL of
152 volatiles in the headspace above the sample was taken and injected into the e-nose at a speed
153 of 125 μL s-1 with injector temperature set at 200°C. In the e-nose, the injected volatiles were
154 adsorbed on a Tenax trap kept at 20°C for 50 s while hydrogen gas, used as carrier gas, passed
155 through the volatiles in order to concentrate the analytes and to remove associated air and
156 moisture. Desorption was subsequently done by increasing the temperature of the trap up to
157 240°C for 30 s. From the trap, the concentrated volatiles were directed onto the column where
158 the individual volatile fractions were separated. The first column was a non-polar column (5%
159 diphenyl), MTX-5 (10 m × 0.18 mm × 0.4 μm film thickness) and the second column was a

medium polar stationary phase (14% cyanopropylphenyl), MXT-1701 (10 m × 0.18 mm × 0.4 μm film thickness). The separation of the volatiles was performed using the following chromatographic temperature program: initial column temperature and isotherm was 50°C and 2 s respectively. The temperature was increased to 80°C at 1.0°C s-1, held for 0 s and then increased to 250°C at 3.0°C s-1 and held for 21 s. Temperature of the two detectors was 260°C each and the acquisition time was 110 s. Samples were analysed five times.

The identification of volatile compounds was conducted using AroChemBase (V6, Alpha M.O.S, Toulouse, France) according to three criteria: (1) comparing the experimental Kovats Retention Index (RIexp) with the RI values found in the AroChemBase library data (RIlit), (2) comparing the relevance indices assigned to each volatile compound using the same software, and (3) verifying the results with literature sources. A standard solution of alkanes ranging from C6 to C16 (Restek, Bellefonte, USA) was subjected to analysis using the identical chromatographic conditions as the samples. This was done in order to determine the Kováts retention indices (RI). The Kováts retention index is a dimensionless measure that converts retention time to system-independent constants (Qu et al., 2021). This value is unaffected by numerous experimental variables and, thus, is regarded as a nearly universal characteristic that describes the time it takes for a substance to be retained on a chromatography column.

179 **Consumer Acceptability testing of cocoa liquor beverage**

180 Cocoa liquor (locally known in the Philippines as *Tablea*) is the primary ingredient of a popular
181 cocoa liquor beverage which is a hot chocolate drink. About 240 g solid cocoa liquor from each
182 of the cocoa clones was dissolved in 1.5 L purified boiling water (CLSU Aqua). Next, 120 g
183 table sugar (Victoria, Metro Manila) and 30 g non-dairy creamer (Coffee-Mate, Nestle
184 Philippines) were added. Afterwards, the mixture was removed from heat and 200 mL of
185 sterilized milk (Bear Brand, Philippines) was added and mixed. The same preparation was used
186 for a commercial *Tablea* sample (Rosario's Delicacies, Davao City, Philippines) using mixed
187 cocoa clones from the Philippines used as control.

188 Consumers who served as respondents in this study were recruited at Central Luzon State
189 University, Philippines following standard criteria for eligibility (Meilgaard et al., 2006).
190 Informed consent was obtained from the respondents in writing after they were oriented about
191 the study. Ethical approval was provided by Central Luzon State University, Science City of
192 Munoz, Nueva Ecija, Philippines. Respondents were seated in individual sensory booths and
193 were given a brief instruction about the procedure for the test. Approximately 30 mL of sample
194 was placed in extruded polystyrene foam cups and kept warm (about 50°C). The samples were
195 marked with random three-digit codes before being served to the respondents in randomized,
196 monadic order. The preparation was executed 5 min before the evaluation to keep the warmth
197 of the samples. The respondents received table napkins and were encouraged to drink still

198 mineral water (CLSU Aqua) in between samples to cleanse their palate. The average time for a
199 respondent to complete the evaluation was about 10 min.

200 Of the 80 individuals who qualified after screening, 68 participated in consumer testing at the
201 Sensory Laboratory, with 50 completing the questionnaire satisfactorily. Despite this, the
202 respondent count still exceeds the minimum threshold of 40 required for statistical significance
203 in affective testing, ensuring robust results (Bower, 2013; Gacula Jr & Rutenbeck, 2006). A
204 total of 50 consumers (26 females, 24 males, mean age 19.2 (± 0.7) evaluated the overall liking
205 and individual attribute liking using a 9-point hedonic scale (1 = extremely dislike, 9 =
206 extremely like), sensory attributes (colour, mouthfeel, aroma, taste, aftertaste using just-about-
207 right scale and purchase intention (1 = definitely will not buy, 5 = definitely will buy) of the
208 samples (Brewer et al., 2001; Kemp et al., 2011; Narayanan et al., 2014).

209 **Data analysis**

210 Data obtained from quality attribute assessment (moisture, pH, fat, titratable acidity) was
211 subjected to one-way ANOVA as well as the peak area of the volatile compounds (Rottiers et
212 al., 2019). Principal component analysis (PCA) was used for linear dimensionality reduction
213 and to create a visual map of the VOC's associated with the different samples (Xu et al., 2021).
214 For a better visualization of the obtained data, PCA plots were created among all cocoa liquors
215 to check natural clustering in the data (Owusu et al., 2013). This statistical unsupervised model
216 helps to reduce the variables and visualize data trends after fingerprinting (Kumar et al., 2014).
217 For the consumer test, data obtained from each respondent were recorded as a sample-attribute

matrix. One-way ANOVA was performed on overall liking and individual attribute liking of chocolate beverage (*Tablea*) samples. When significant differences were found, Tukey HSD post hoc test was used to determine where the differences occurred. The significance of the differences was defined at $p < 0.05$. Equality of variance was checked using Levene's test. Multiple Factor Analysis (MFA) was conducted to examine the relationship between the volatile organic compounds, overall liking and the cocoa clones (Mandha et al., 2022). To determine the whether the sensory attributes are in their optimal level and examine its effect on acceptability, Penalty Analysis combining Just-About-Right (JAR) and overall liking data was conducted (Narayanan et al., 2014). All statistical analyses were conducted by means of XLSTAT version 2023.1.1 software package (Addinsoft, New York, NY, USA).

Results

Physico-chemical analysis of fermented cocoa beans

The important quality parameters for cocoa beans are shown in Table 1. In terms of moisture content, UF18 had the significantly higher ($p < 0.05$) moisture (5.34%) compared to the other two clones. The moisture content of the samples (4.7-5.34%) however are lower than those reported in literature for dried and fermented cocoa beans (Beckett, 2008). In terms of acidity, cocoa clone W10 had the significantly lower ($p < 0.05$) pH, followed by BR25 and finally UF18. The titratable acidity of the cocoa beans followed the same trend as the pH, where W10 significantly higher TA (6.27 meq NaOH/g) compared to BR25 and UF18.

238 **Table 1.** Physico-chemical properties of selected Philippine cocoa bean varieties.

	BR25	UF18	W10
Moisture content (%)	4.77 ± 0.01^{b}	5.34 ± 0.99^{a}	4.68 ± 0.01^{b}
pH (20 °C)	6.01 ± 0.01^{b}	6.56 ± 0.01^{a}	4.79 ± 0.02^{c}
Titratable acidity (meq NaOH/g)	3.10 ± 0.05^{b}	1.73 ± 0.03^{c}	6.27 ± 0.06^{a}
Fermentation index	1.12 ± 0.03^{a}	1.03 ± 0.08^{b}	1.17 ± 0.05^{a}
Crude fat (%)	41.6 ± 0.6^{b}	52.1 ± 0.3^{a}	36.4 ± 0.9^{c}

239 Values represent means ± standard deviations. For each row different letter superscript represent
240 significant differences (α=0.05) among samples according to Duncan's multiple range test

241

242 Moreover, UF18 also had the highest fat content (52.1%) among the cocoa clones which is also
243 higher than West African cocoa beans (Dand, 2011). The fermentation index (FI) range of 1.03–
244 1.17 confirmed that all three samples were fully fermented. Compared to bean cut test, the FI
245 presents a more objective tool for the estimation of the quality and extent of fermentation of
246 cocoa beans. Well-fermented beans should have an FI ≥ 1 after the required fermentation time
247 (Gourieva & Tserevitinov, 1979).

248

249 **Identification of volatile organic compounds**

250 The Principal Component Analysis scores plot in Fig. 1 illustrates three main clusters: BR25,
251 UF18 and W10 which are clearly separated from each other due to the differences in levels of
252 VOCs. In PC1, W10 was distinctly separated from UF18 and BR25. The first principal

component accounted for 90.13% of total variance. Hence, PCA was able to fully discriminate all cocoa liquors according to cocoa variety (Owusu et al., 2013).

The compounds predominantly discriminating (i.e., PC loadings > 0.90) (Rottiers, Tzompa Sosa, Van de Vyver, et al., 2019) the cocoa liquors are summarized in Table 3. As shown in Fig. 3, BR25, UF18 and W10 were clearly separated from each other. The cocoa clone W10 showed a higher concentration of volatile compounds based on the average peak areas, followed successively by BR25 and UF18. The cocoa clone W10 was richest in acetic acid, 3-methylbutanal, pyrrole, phenylacetaldehyde and 3-hexanone which are associated with among others sour, chocolate, fruity, nutty and popcorn-like notes. Similarly, BR25 showed a relatively high peak area for 2/3-methylbutanal, 3-hexanone, and acetic acid.

The highest peak area in all liquors was identified as acetic acid, which was significantly highest in W10. Remarkably, the acetic acid content in liquors BR25 and UF18 was very low, and even lower when compared with the content in the West African cocoa liquors in literature (Afoakwa et al., 2008). This is reflected in the pH of W10 (4.79) is significantly lower than the two other samples.

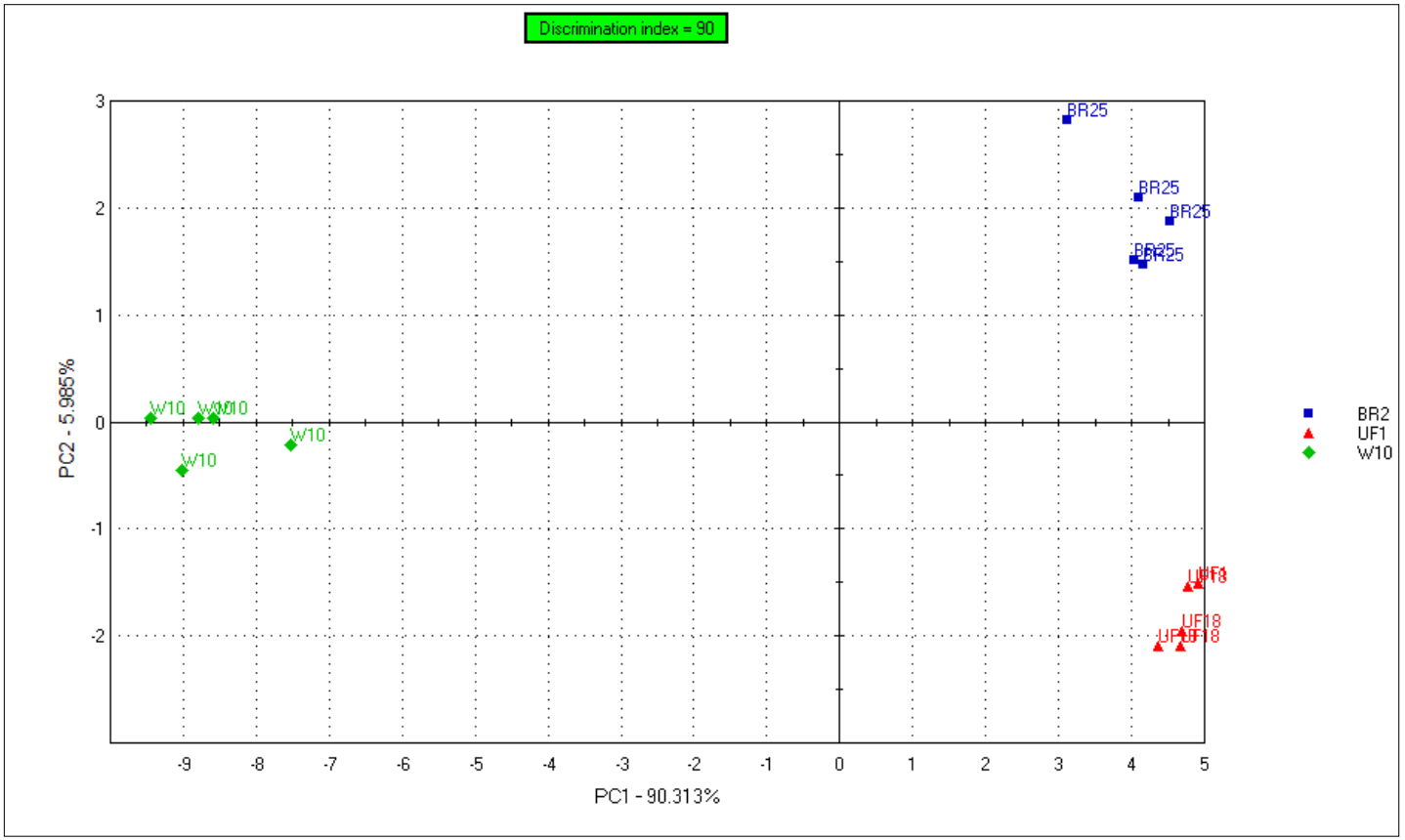


Figure 1

Principal Component Analysis showing discrimination of cocoa liquors based on cocoa clones

264

265 **Acceptability testing of cocoa liquor beverage from Philippine cocoa beans**

266

267 Table 2 shows the mean overall liking of the samples. Significant differences were found on
268 the consumer's overall liking for the samples ($p < 0.05$). Post-hoc analysis using Tukey
269 adjustment on pairwise comparisons revealed that that W10 was significantly the most liked
270 sample (6.6) followed by UF18 (6.1) and BR25 (5.8). BR25 had significantly lower ($p < 0.05$)
271 overall liking which may be due to its intense brown color, bitterness and aftertaste based on
272 just about right (JAR) scores (Figure 2). Although *Tablea* made from W10 cocoa liquor had
273 significantly lower pH and higher titratable acidity, it obtained significantly higher overall
274 acceptability.

275

276 **Table 2.** Overall liking of *Tablea* (cocoa liquor beverage) from selected Philippine cocoa
277 bean varieties

Variety	Overall liking	
	Mean*	SD
BR25	5.8^{c}	± 1.6
UF18	6.1^{ab}	± 1.6
W10	6.6^{a}	± 1.5

Control (commercial *Tablea*)	5.4[c]	± 1.8

Note: Means with different letter superscript within a column represent significant differences ($p < 0.05$) among samples based on Tukey's HSD post hoc test. Overall liking measured on 9-point hedonic scale ranging from 1 (extremely dislike) to 9 (extremely like)

Cursory inspection of the individual attributes of the different cocoa liquor samples showed that the samples were statistically distinguishable ($p < 0.05$) in terms of chocolate flavour and aftertaste (Annex). All samples were equally liked in terms of their brown color. Samples did not have significant liking differences in other sensory attributes.

Table 3. Volatile organic compounds (VOC) identified in selected cocoa liquor samples from Philippine cocoa clones based on fast GC electronic nose

VOC	Average peak area ± standard deviation			Aroma description	RI_{exp}	RI_{lit}
	BR25	UF18	W10			
MXT-5 (FID1)						
1-Propanol	6535 ± 323b	7136 ± 799ab	7262 ± 124a	Sweet, candy	557	543
3-Methylbutanal	19,569 ± 1639b	15,724 ± 2332c	27,681 ± 1645a	Chocolate	655	652
3-Hexanone	12,120 ± 3686b	5113 ± 857c	21,378 ± 789a	Fruity, sweet	779	786
2,3-Butanediol	6861 ± 1223b	2925 ± 753c	13,146 ± 1905a	Buttery	790	788
Hexanal	1307 ± 722b	0 ± 0c	4464 ± 565a	Green	804	801
Butanoic acid	1307 ± 722b	0 ± 0c	4464 ± 565a	Rancid, cheese	814	812
Ethyl isovalerate	1167 ± 899b	513 ± 486b	6444 ± 1182a	Fruity, apple	853	854
Ethyl pyrazine	699 ± 126b	0 ± 0 c	2409 ± 639a	Peanut butter, musty, nutty	922	921
2-Acetyl-1-pyrroline	1826 ± 283	625 ± 357	17,681 ± 1464	Popcorn	932	932
Phenylacetaldehyde	0 ± 0b	0 ± 0b	966 ± 305a	Honey, floral	1042	1045

MXT-1701 (FID2)						
1-Propanol	7275 ± 304	7775 ± 775	7684 ± 88	Sweet, candy	641	666
2/3-Methylbutanal	24,892 ± 2534a	18,435 ± 4658b	19,150 ± 1776b	Chocolate	743	729
Acetic acid	112,847 ± 24183c	104,992 ± 20436b	949,603 ± 44478a	Sour, vinegar	774	776
2-Methylthiophene	3620 ± 2615b	2713 ± 1735b	20,450 ± 3718a	Sulfurous	826	827
3-Methyl-1-butanol	0 ± 0b	0 ± 0b	8697 ± 2396a	Malty, fruity, banana	847	846
Pyrrole	7463 ± 2238b	7193 ± 1711b	22,386 ± 518a	Nutty	922	915
Isoamyl acetate	3083 ± 996b	1778 ± 684b	11,344 ± 1989a	Fruity, banana	950	945
Heptan-2-ol	7966 ± 858b	8673 ± 1087b	13,968± 3347a	Citrus	1007	1000
2-Ethyl-6-methylpyrazine	2325 ± 263b	922 ± 108c	16,779 ± 369a	Nutty, raw potato	1096	1096
3-Octanol	1504 ± 120c	2064 ± 262b	3494 ± 425a	Earthy, mushroom, herbal	1110	1105
Tetramethylpyrazine	1259 ± 100c	1037 ± 90b	2678 ± 164a	Chocolate, cocoa, coffee	1180	1172
Acetophenone	319 ± 291b	104 ± 233b	2682 ± 118a	Floral	1208	1208
Methyl decanoate	0 ± 0b	0 ± 0b	1791 ± 156a	Fruity, oily, winey	1409	1403

For each row, different lowercase letters indicate significant differences ($p < 0.05$) among cocoa liquors

Kovat Ri_{exp}, experimental retention index obtained using alkanes; Ri_{lit}, retention index obtained from AroChemBase (V6, AlphaM.O.S, Toulouse, France)

Penalty analysis using JAR data

The penalty analysis and mean drop plot of chocolate beverages from the different cocoa clones are shown in Figure 2. W10 obtained >70% JAR score for the attributes color and aroma and >50% for mouthfeel, creaminess and thickness. UF18 obtained 70% for the attribute thickness. BR25 is evidently negatively perceived because of its intense bitterness, gritty mouthfeel, little sweetness and intense aftertaste. Too much bitterness (70%) and too much aftertaste (40%) caused a significant mean drop (1.06 and 1.21) for BR25. For W10, too little sweetness (50%), too much bitterness (55%), and too much aftertaste (35%) significantly lowered mean liking. Significant mean drop for UF18 were caused by too much bitterness (55%) , too much aftertaste (50%). The significant dichotomous presence of too little chocolate taste (20%), too much chocolate taste (30%) in the plot may indicate the presence of segmentation in the consumer respondents' preference for level of chocolate taste (Lawless & Heymann, 2010). For the commercial control, significant mean drop was due to too much bitterness (65%) and too little sweetness (70%).

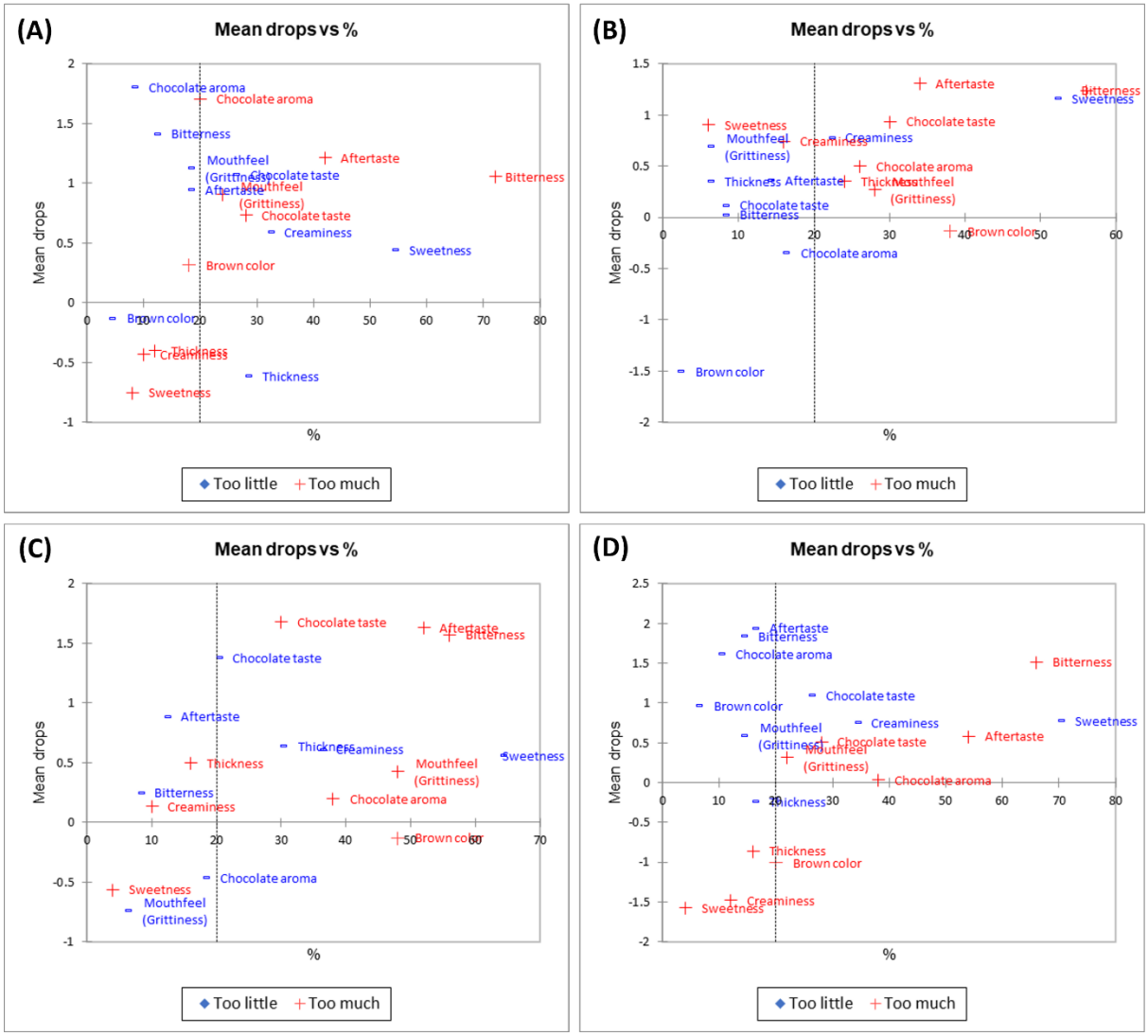
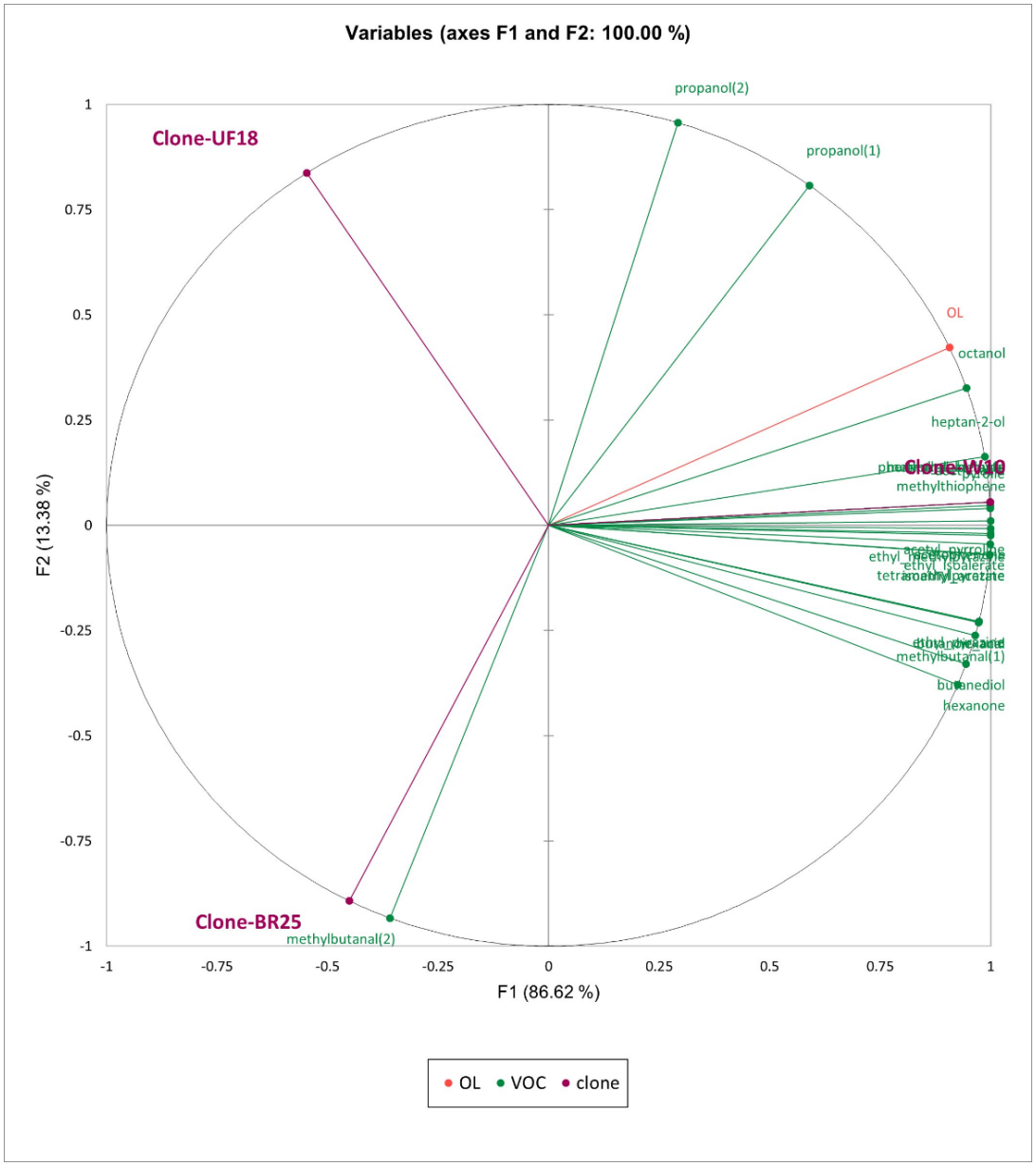


Figure 2

Penalty analysis and mean drop plot of chocolate beverage (*Tablea*) from different cocoa clones. (A) – BR25, (B) – W10, (C) – UF18, (D) – Commercial. Dashed line represents threshold of 20% of consumer respondents. Too low attributes are shown in blue, too high attributes are shown in red. The term "mean drop" describes the variation between the mean overall liking score for the just-about-right group and the mean overall liking score of the "too much" or "too little" groups.

Combination of Volatile Organic Chemical data, Cocoa clones and Overall liking

Multiple Factor Analysis (MFA) was conducted to examine the relationship between the volatile organic compounds, overall liking and the cocoa clones (Mandha et al, 2022). The prevalence of volatile organic compounds (VOCs) within the examined cocoa clones exhibited significant differences, with clone W10 showing a markedly elevated average peak area (Table 4) when compared with BR25 and UF18. The cocoa clone W10 contained high amounts of methyl decanoate (fruity) and phenylacetaldehyde (honey, floral) which are not present in the other samples. Additionally, clone W10 exhibits significantly higher concentrations of 2,3 methylbutanal and 2-ethyl-6-methylpyrazine, which are primary contributors to the olfactory profile associated with chocolate. Furthermore, it also contains high level of pyrrole which is source of nutty attributes. The VOC responsible for popcorn-like aroma (2-acetyl-1-pyrroline) is present in W10 ten times more than the other two samples. On the other hand, BR25 is characterized by its association with the VOC 2/3-Methylbutanal, a key contributor to the chocolate aroma spectrum. Conversely, UF18 conspicuously lacks six (6) VOCs, primarily responsible for imparting floral and fruity aromatic nuances, and generally exhibits the lowest average peak area relative to the other samples. Overall liking is most closely associated with the cocoa clone W10 with the VOCs methylbutanal (chocolate), heptanol (citrus), which are present in high levels in the cocoa clone W10.



334

335 **Figure 3**

336 Multiple Factor Analysis (MFA) plot showing the relationship between the cocoa clones (BR25, UF18,

337 W10), volatile organic compounds (VOC) and overall liking (OL).

338

339 **Discussion**

340 Analyzing cocoa liquors from three varieties using fast GC electronic nose analysis revealed

341 over 70 volatile compounds (peak area > 500), comparable to findings for a Brazilian cocoa

342 variety (Bastos et al., 2019). This discrimination may be related to the difference in
343 concentration of volatile compounds among the liquors since chemical composition varies
344 according to cocoa varieties (Barbin et al., 2018). Previously, Southeast Asian cocoa beans are
345 known for their high acidity (Afoakwa et al., 2008). However, this study showed that some
346 Philippine cocoa clones (W10 and BR25) contained high amounts of methyl decanoate (fruity)
347 and phenylacetaldehyde (honey, floral). These volatile compounds are perceived as desirable
348 flavours in cocoa liquors (Barišić et al., 2023) and are normally only found in fine cocoas
349 (Herrera-Rocha et al., 2023; Hinneh et al., 2018; Rottiers, Tzompa Sosa, De Winne, et al.,
350 2019). To our knowledge, this is the first study that has identified fine flavour components in
351 single-origin cocoa beans in Asia which has always been classified as mostly acidic or sour
352 (Holm et al., 1993; Jinap & Dimick, 1990).
353 The variability in titratable acidity observed in cocoa beans can be attributed to an interplay of
354 genetic factors, environmental conditions, and processing techniques, particularly fermentation
355 (Kongor et al., 2016). This diversity in acidity levels is often linked to the differential utilization
356 of pulp carbohydrates, such as fructose, glucose, and sucrose, by microorganisms during
357 fermentation. Such metabolic activity leads to a pronounced increase in the concentration of
358 lactic and acetic acids (Gálvez et al., 2007; Guehi et al., 2010). On a molecular level, the distinct
359 acid profiles observed among cocoa beans may stem from the intrinsic variances in precursor
360 compounds present in different cocoa clones. These genetic variations result in clones having
361 differing capacities for acid production, thereby contributing to the observed differences in

362 titratable acidity levels. The pH of W10 (4.79) is significantly lower compared to the other
363 clones which may be due to the inherent acidity of this cocoa clone considering that
364 fermentation conditions are the same for all samples. Nonetheless, this pH is still optimal for
365 the rate of enzyme activity for the production of flavour precursors (Afoakwa et al., 2013;
366 Sakharov & Ardila, 1999). Titratable acidity and pH are inversely related to a degree. Typically,
367 a high titratable acidity means that a substance contains a larger quantity of acidic components
368 (acetic, citric, lactic in cocoa) which usually corresponds to a lower (more acidic) pH value.
369 A study on Criollo cocoa by Alvarez et al., (2012) reported cocoa beans with fat content ranging
370 from 33.2% to 54.9%. They classified cocoa beans into high fat content (50-54%), intermediate
371 fat content (40-50%), and low fat content (33-39%). Our experimental sample W10 then could
372 be classified as a low fat content cocoa clone (36%). Fat content tended to be lower for the
373 Criollo cocoa type compared to Forastero and Trinitario types (Álvarez et al., 2012). This
374 further supports the results of the fine flavour VOC content of W10 which may indicate that
375 W10 could be from Criollo type.
376 The indigenous Philippine chocolate beverage *Tablea* made from W10 cocoa liquor obtained
377 significantly higher overall acceptability which may be due to the presence of higher amounts
378 of volatile organic compounds associated with chocolate flavour and fruity aroma such as 3-
379 methylbutanal, pyrazine (chocolate), and 3-hexanone (fruity) (Owusu et al., 2013).
380 Moreover, the relatively higher JAR scores of W10 also contributed to its superior overall liking
381 score. To be considered that an attribute is at JAR level, there should be a minimum of 70%

382 respondents' vote (Meullenet et al., 2008). UF18 obtained greater than 70% for the attribute
383 thickness. The JAR level score for thickness of UF18 may be attributed to its highest crude fat
384 content. Fat content or yield is an important quality index for cocoa processors during
385 purchasing of fermented cocoa beans (Afoakwa et al., 2013). Only UF18 had comparable fat
386 content with that found in other studies while BR25 and W10 are lower (Dand, 2011; Grassia
387 et al., 2019; Samaniego et al., 2021). The results of the high JAR score for aroma of W10 may
388 be due to its high concentration of volatile compounds which are associated with chocolate,
389 fruity, nutty and popcorn-like notes as revealed by the electronic nose analysis.

390 *Tablea* made from cocoa clone W10 had significantly highest chocolate flavour and aftertaste
391 which may be explained by the results of the fast GC electronic nose where it obtained the
392 highest peak area for desirable sensory attributes for cocoa beverage such as chocolate and
393 fruity aroma (Barišić et al., 2023). Taste, which is a component of flavour (Lawless &
394 Heymann, 2010), is a very important attribute and it drives the likelihood of consumers to pay
395 more for *Tablea* with good taste (Ballesteros et al., 2023). As such, cocoa farmers and
396 processors may be able to improve their products by means of harnessing the fine-flavour
397 potential of these identified cocoa clones.

398 Previous research have determined that flavour quality is influenced by many factors such as
399 cacao bean variety and genotype (Reineccius, 2005), polyphenol/proanthocyanadin content of
400 the bean (Jinap et al., 2004), fermentation practices (Zhao & Fleet, 2014), roasting conditions
401 (Kothe et al., 2013), drying methods (Guehi et al., 2010), as well as manufacturing methods and

ingredient formulation (Torres-Moreno et al., 2012). However, recent research has shown that that certain flavour qualities are not limited to only one type of cocoa tree or variety (Ullrich et al., 2023). While specific compounds may exhibit high VOCs in particular types, the overall flavour profile of dark chocolates is determined by the complete makeup of flavour compounds. The chocolates derived from Criollo and Trinitario beans exhibited significant diversity in their taste compound profiles which was shown on a molecular level, indicating that the variety itself does not solely dictate a certain flavour profile (Ullrich et al., 2022). As such, future research could examine the characteristics of chocolate products as affected by flavour precursors present in cocoa beans and those that are generated during post-harvest treatments and transformed into desirable odour notes in the manufacturing processes. Further studies may be conducted to analyze the non-volatile components of cocoa beans and its synergistic relationship with VOCs on flavour perception. This study was limited only to the disease-resistant and productive cocoa clones in the Philippines. Experimental samples in other cocoa-growing locations and consumer segments in Asia could be further explored. Nonetheless, the comprehensive approach undertaken in this study addresses the industry's quest for cocoa sources that not only offer quantity but, more importantly, deliver the desirable flavours that are of prime important for consumers.

Conclusions

This study evaluated the quality attributes and consumer acceptability of disease resistant and highly productive cocoa clones in the Philippines. The cocoa bean and cocoa liquor samples showed significant differences in key quality attributes. W10 is a promising cocoa clone in terms of key volatile aroma contents but had relatively low fat content. Although the cocoa clone W10 had significantly lower pH, higher titratable acidity, W10 was the most liked sample when prepared as cocoa liquor beverage which is an indigenous drink in the Philippines due to the presence of higher amount of volatile organic compounds associated with chocolate flavour and fine-flavour. This is the first study that explored the quality characteristics and consumer acceptability of highly recommended cocoa beans from the Philippines and showed the presence of volatile organic compounds that are responsible for fine aroma. This can guide processors in harnessing the flavour potential of local cocoa varieties as well as direct efforts in pushing for a more robust cacao industry, not only in the Philippines but also in the South-East Asian region, and contribute to a more vigorous world-wide supply of quality cocoa.

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Data Availability Statement

The sensory acceptability data are not publicly available since respondents did not provide their consent to share their data.

Ethical Guidelines

Informed consent was obtained from all respondents involved in the study. Ethics approval was by the Research Ethics Committee of Central Luzon State University, Philippines. The respondents of the study comprised adult volunteers who were duly apprised of the research objectives and methodology. In exchange for their voluntary participation, these individuals granted informed consent and retained the right to revoke their consent at any phase without incurring any adverse repercussions. The respondents were provided with the contact information for the principal investigator and the institution's data protection officer, who could be reached with any inquiries. Personal identifying information was not gathered, and all data records underwent anonymization prior to storage and subsequent analysis.

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This research investigated the effect of cocoa beans' botanical and geographical origins, as well as brand-related processing, on the volatile organic compound (VOC) profiles of 90 dark

chocolates using Proton-Transfer-Reaction-Mass Spectrometry (PTR-MS). Their results revealed that while chocolate VOC profiles are significantly influenced by brand processing, the original botanical and geographical signatures of the cocoa beans are also discernible. However, cocoa beans from the Philippines were not included.

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683 **Table S1.** Mean liking scores of the sensory attributes of *Tablea* (cocoa liquor beverage) from

684 selected Philippine cocoa beans

	Color	Chocolate flavour	Sweetness	Bitterness	Smoothness	Viscosity	Creaminess	Aftertaste
BR25	7.0 [a]	5.7[ab]	5.0 [a]	4.5 [a]	5.9 [a]	5.8 [a]	5.5 [a]	5.0[ab]
W10	6.9 [a]	6.6[a]	5.7 [a]	4.8 [a]	5.9 [a]	6.4 [a]	6.1 [a]	5.7[a]
UF18	6.4 [a]	6.0[ab]	5.3 [a]	5.1 [a]	5.3 [a]	5.8 [a]	5.5 [a]	5.4[ab]
Control	6.9 [a]	5.4[b]	5.0 [a]	5.1 [a]	5.4 [a]	6.0 [a]	5.6 [a]	4.6[b]

685 Note: Means with different letter superscript within a column represent significant differences ($p < 0.05$) among

686 samples based on Tukey's HSD post hoc test. Overall liking measured on 9-point hedonic scale ranging from 1

687 (extremely dislike) to 9 (extremely like)

688