A STUDY ON FLAVOUR PROFILE OF SELECTED CACAO GERMPLASM COLLECTION IN COCOA RESEARCH AND DEVELOPMENT CENTRE BAGAN DATUK, PERAK

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Malaysian Cocoa J. 15 (1): 11-22 (2023)

ABSTRACT – *Previously, the development and production of superior genetic materials were concentrated on high yield and resistance to pests and diseases. But lately, there are a growing interest of cocoa flavours as the trait has been a popular demand from the cocoa industries worldwide. Cocoa beans are a crucial source for cocoa based products especially in the production of chocolate. World demand for cocoa and chocolate products has been increasing and related to their unique flavour, thus flavour profile of cocoa beans plays the main factor measuring cocoa quality, as flavour is central to acceptability of cocoa beans and cocoa products that lead to premium and higher price. Basically, cocoa beans from particular cocoa varieties could determine the potential of chocolate flavour produced as beans from different origin or genotype have different flavour or aroma profiles. Thus, there is a necessary to select cocoa beans with desired genetic background for the crop cultivation to maintain the cocoa bean quality especially with favourable flavour. In this paper eight selected cocoa genotypes were used to determine their potential flavours. ANOVA result revealed that floral attribute evaluated on eight selected cocoa genotypes was highly affected significantly (at $p \leq 0.01$) by the genotypes. Variation also was detected among the genotypes (at $p \leq 0.05$) for cocoa liquor attributes such as nutty and roasted. For the tree within genotype, most of the attributes indicated significant (at $p \leq 0.05$) and highly significant (at $p \leq 0.01$) for cocoa, bitterness, astringency, fresh fruits, woody and roasted, and for global quality.*

Key words: Cocoa, genotype, flavour, chocolate, quality

INTRODUCTION

Cocoa tree (*Theobroma cacao* L.), also known as chocolate tree is a perennial tropical tree species. It lives under shade to grow in an optimum condition. Cocoa is grown for its fruit, which is also known as the cocoa pod. It contains cocoa seeds (Kongor *et al.*, 2016) commonly known as cocoa beans in a sugary pulp. As the commercially important industrial tree crops, cocoa is cultivated throughout the world because of its economic importance i.e., the cocoa beans (Malhotra and Elain Apshara, 2017; Aprotosoaie *et al.*, 2016). These cocoa beans which is produced from fermented and dried cotyledons of the seeds are mainly used as raw materials (Krähmer *et al.*, 2015; Ho *et al.*, 2015; Kongor *et al.*, 2016; Espino *et al.*, 2017; Deus *et al.*, 2018; Dzelagha *et al.*, 2020) in cocoa based products industries especially in food (chocolate, beverages, cocoa butter) and non food production such as cosmetics, toiletry products, pharmaceuticals as well as antioxidant sources.

Raw beans of cocoa have an unpleasant astringent taste, high bitter and; typical aroma (Aprotosoaie *et al.*, 2016; Pedan *et al.*, 2017; Toker *et al.*, 2020) with high phenolics especially anthocyanins. Therefore, they need to undergo post-harvest process (pod storage, fermentation, drying) and roasting before developing fine or any unique cocoa and chocolate flavours (Aprotosoaie *et al.*, 2016; Kumari *et al.*, 2018). This will upgrade the quality of the cocoa beans and could raise higher prices at the cocoa market. Differences in the cocoa flavour among clones have been reported by Clapperton *et al.* (1994) which is to compare the flavours between cocoa beans from various genetic origins that was fermented under local standard condition.

Formerly, cocoa beans produced in Malaysia were described and characterized by excessive acidity with weak chocolate flavour and the presence of undesirable flavours. Chocolate products produced by this bean will lead to high percentage of bitter taste with burnt flavour and also weak in chocolate flavor. Most of the Malaysia cocoa beans are from Trinitario varieties which is considered to have fine flavour cocoa. As Malaysia holding more than 1000 genetic materials throughout Malaysian regions, an effort has been performed to evaluate and investigate the flavour of Malaysia cocoa beans. Through the right and perfect post harvest treatments such as fermentation and drying, cocoa beans quality especially in flavour could be improved.

Insufficient information of flavour in cocoa genetic materials, including Malaysia cocoa genotypes, would limiting the value of cocoa and their potential, in the world cocoa market. Due to unidentified flavour and less information of Malaysia cocoa beans, people perception of Malaysia cocoa genotypes is unfavourable, or could be off flavour, where the beans will only be used in blends. Consequently the beans sold at discounted price at the world market. Therefore, bean quality information especially on flavour profile need to be determined and evaluated. Thus, by getting flavour information of cocoa genetic materials, and by using high quality cocoa clones, could attract cocoa growers' interest to plant for the crop, and increase their household income, as well as providing job opportunity for the community.

A proper strategies and activities should be well-planned to address these challenges for more sustainable development of the cocoa planting and processing industry in Malaysia. The strength of this study is based on the usage of selected cocoa clones, from the germplasm collection of Cocoa Research and Development Centre (CRDC), of Malaysian Cocoa Board (MCB), Bagan Datuk, Perak and the method used in the study. This study was undertaken to evaluate the selected cocoa clones for variation in flavor profile. The evaluation of the selected cocoa clones is expected to show diverse flavour characteristics such as high and low cocoa flavour, bitterness, astringency, acidity or sourness and other unique flavour such as floral or fruity note.

MATERIALS AND METHODS

The source of the cocoa genotypes was from Field 18B (3.54 N and 100.51E), the collection of Cocoa Research and Development Centre (CRDC) of Malaysian Cocoa Board (MCB), Bagan Datuk, Perak, Malaysia. Eight cocoa genotypes were selected and utilized such as AMAZ 12, IMC 16, IMC 20, IMC 103, MCBC 5, PNG 296, SCA 16 and UF 12 (Table 1).

Table 1. List of cocoa genotypes evaluated in the study and the related information.

Genotype	Source Population	Accession Number	Donor Genebank
AMAZ 12	Reading University, UK	RUQ 334	ICG, T §
IMC 16	Reading University, UK	RUQ 829	ICG, T §
IMC 20	Reading University, UK	RUQ 985	ICG, T §
IMC 103	Reading University, UK	RUQ 862	ICG, T §
MCBC 5	CRDC, MCB, Malaysia	MCBC 5	MCB †
PNG 296	Reading University, UK	RUQ 1299	CIRAD-CP ≠
SLA 16	Reading University, UK	RUQ 1092	ICG, T §
UF 12	Reading University, UK	RUQ 962	ICG, T §

† Malaysian Cocoa Board
§ International Cocoa Genebank (Trinidad)
≠ Centre de cooperation international en recherché agronomique pour le développement ("-CP": Montpellier, "_GUF": French Guiana)

The trees are about fifteen years old, planted at 3 m x 3 m on the triangle, under twenty-five percent (25%) shade trees (*Gliricidia macculata*). The trees are maintained with normal agricultural practices for cacao. Fertilizers are applied at the rate of 250 g per tree using compound fertilizer, Nitrophoska Blue (N P K Mg 12:12:17:2+TE); three times per year. Weeds are controlled using several herbicides meanwhile pests and diseases by pesticides and fungicides. Pod sleeving also had been practiced for cocoa pod borer (CPB) control. Maintenance pruning of the trees are carried out every three months to improve air circulation around and within the trees, to reduce the risk of pest and diseases infestation and infection while forming appropriate shape for easy pods harvesting activity.

The experiment was carried out in nested design. Three factors were studied i.e., clones, individual trees and the pods. Nested design was employed with 4 replications (tree) within clone and three pods within tree.

Three healthy pods were sampled randomly for each genotype from four individual trees. The ripe pods were harvested and put in each labelled sack.

Sensory evaluation of cocoa liquor was conducted following the procedure and method established by Malaysian Cocoa Board with slight modification. The remain of dry cocoa beans were broken into nibs and the shell removed. The nibs were roasted in oven (Memmert, U.K) at 135°C for 35 minutes. The nibs were grinded into smooth cocoa paste/liquor in Mortar and pestle mill (Pascal, U.K) for 3 hours and kept warm in oven at 45°C.

Sensory evaluation of cocoa liquor was carried out using descriptive analysis with scale of "0" to "10" where "0" indicates the absence or minimum intensity and "10" indicates the maximum intensity. Ghana cocoa liquor was used as a reference sample where the standard score for its flavour attributes has been agreed during earlier discussion. The score for reference sample were marked on the sensory form and used as a basis for scoring of flavour attributes for other cocoa samples.

Each sample was labeled with randomly selected 3-digits numerical code. Seven to ten trained panelists from Malaysian Cocoa Board were participating in the evaluation and the flavour characteristics evaluated are cocoa, bitter, astringent, acid, fruity and thickness/viscous.

Three cocoa liquor samples and the reference were served at the same temperature in one evaluation session. During the evaluation the panelists work individually in a sensory booth and they are free to describe any other taste that may be present in the samples and note down any comment in the sensory form.

Statistical analyses

Data collection were analysed using the Statistical Analysis Software (SAS), version 8.2. The analysis of variance (ANOVA) was used to determine the significance of variation among genotypes, trees and pods. Means performance among the genotypes were separated using the Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSIONS

Sensory evaluation of cocoa liquor

Flavour quality of cocoa beans basically depends on the genotype and also origin of the cocoa tree which produced the beans (Kongor *et al*., 2016). They have distinct flavour characteristics. Results of the variance analysis (ANOVA) carried out revealed that, floral attribute evaluated on eight selected cocoa genotypes was highly affected significantly (at $p \leq 0.01$) by the genotypes (Table 2). Variation also was detected among the genotypes (at $p \leq 0.05$) for cocoa liquor attributes such as nutty and roasted. Meanwhile, for other attributes such as cocoa, bitterness, astringency, acidity, fresh fruits, browned fruits, spicy, and woody, there were no significant difference detected among the genotypes. Results also revealed that, there were no variation among the genotypes tested for off-flavours such as dirty/dusty, meaty/animal, overfermented/putrid, smoky and mouldy (Table 3). No significant difference detected for global quality from all of the genotypes (Table 4) but viscous affected them with high significant.

For the tree within genotype, most of the attributes indicated significant (at $p \leq 0.05$) and highly significant (at $p \leq 0.01$) for cocoa, bitterness, astringency, fresh fruits, woody and roasted (Table 2), and for global quality (Table 4). Meanwhile, there were, no significant difference for acidity, sweet, browned fruits, floral and nutty attributes and for all off-flavours characters (Table 3), and viscous (Table 4).

Performance of cocoa genotypes for cocoa liquor flavour profiles

Table 5 shows results of comparison of mean values among the eight selected cocoa genotypes for cocoa liquor attributes, using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$. The performances of the genotypes score ranged as: cocoa (6.13 – 6.67), bitterness (2.30 – 2.83), astringency (2.11 – 2.58), acidity (1.31 – 1.64), sweet (0.03 – 0.17), fresh fruits (0.96 – 1.26), browned fruits (1.13 – 1.31), floral (0.69 – 1.14), spicy (0.02 – 0.08), woody (0.07 – 0.32), nutty (1.56 – 2.20) and roasted (4.70 – 5.08). The mean value of the genotypes for the cocoa liquor attributes were 6.39 (cocoa), 2.56 (bitterness), 2.33 (astringency), 1.42 (acidity), 0.09 (sweet), 1.07 (fresh fruits), 1.20 (browned fruits), 0.91 (floral), 0.05 (spicy), 0.21 (woody), 1.87 (nutty) and 4.90 (roasted).

Among the genotypes, the highest bitterness was indicated by UF 12 (Figure 8) with scoring 2.83 and the lowest by SLA 16 (Figure 7) with 2.30 scored, the highest astringency attribute was from UF 12 (2.58) and the lowest by IMC 16 (2.11), the highest acidity was revealed by IMC 20 (Figure 3) with the scoring of 1.64, followed by SLA 16 (1.55) and the lowest by IMC 103 (Figure 4) with 1.31 scored, the sweetest was showed by IMC 16 (0.17) and the least sweet attribute was by UF 12 (0.03), the highest fresh fruit taste was indicated by IMC 16 (1.26) and the lowest by MCBC 5 (0.96), the highest browned fruit attributes was detected by IMC 20 (1.31) meanwhile the least by MCBC 5 (0.69), the highest spicy flavour was from IMC 20 (0.08), followed by PNG 296 (Figure 6) with 0.07 scored and the lowest from UF 12 (0.02), the highest woody taste was showed by IMC 103 (0.32) and the lowest by IMC 16 (0.07), the highest nutty flavour was scored by AMAZ 12 (Figure 1) with scoring 2.20, followed by IMC 16 (2.16) and the lowest by SLA 16 (1.56) meanwhile, the highest roasted beans was from MCBC 5 (5.08), followed by UF 12 (5.01) and the lowest by SLA 16 (4.70).

The coefficients of variation for the cocoa liquor attributes were 6.62 (cocoa), 12.61 (bitterness), 11.64 (astringency), 17.32 (acidity), 112.56 (sweet), 19.47 (fresh fruits), 17.37 (browned fruits), 22.66 (floral), 129.21 (spicy), 62.60 (woody), 20.39 (nutty) and 4.15 (roasted).

Table 6 shows results of comparison of mean values among the eight selected cocoa genotypes for cocoa liquor off-flavours, using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$. The performances of the genotypes score ranged as: dirty/dusty (0.00 – 0.04), meaty/animal (0.00 – 0.03), overfermented/putrid (0.01 – 0.06), smoky (0.03 – 0.06) and mouldy (0.01 – 0.06). The mean value of the genotypes for the cocoa liquor off-flavours were 0.01 (dirty/dusty), 0.01 (meaty/animal), 0.04 (overfermented/putrid), 0.04 (smoky) and 0.03 (mouldy).

Among the genotypes, highest dirty/dusty off-flavour was showed by UF 12 (0.04) and the lowest by MCBC 5 and IMC 16 (0.00), highest meaty/animal off flavour was indicated by IMC 20 (0.03) and the lowest by IMC 16, IMC 103 and MCBC 5 (0.00), the highest overfermented/putrid off-flavour was from PNG 296 (0.06) and the lowest by IMC 20 (0.01), the highest smoky off-flavour was revealed by IMC 103 (0.06) and the lowest by IMC 16, UF 12, AMAZ 12 and PNG 296 (0.03) meanwhile, the highest mouldy off-flavour was showed by IMC 103 (0.06) and the lowest was by AMAZ 12 and MCBC 5 (0.01).

The coefficients of variation for the cocoa liquor off-flavours were 339.12 dirty/dusty), 430.26 (meaty/animal), 206.83 (overfermented/putrid), 151.19 (smoky) and 216.29 (mouldy).

Table 7 shows results of comparison of mean values among the eight selected cocoa genotypes for cocoa liquor global quality and viscous, using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$. The performances of the genotypes score ranged as: global quality (5.96 – 6.70) and viscous (1.83 – 4.07). The mean value of the genotypes for the cocoa liquor global quality and viscous were 6.34 (global quality) and 2.90 (viscous).

Among the genotypes, the highest global quality score was indicated by AMAZ 12 (6.70) followed by IMC 16 (6.67), PNG 296 (6.55) and IMC 20 (6.45) while, the least score was by MCBC 5 (5.96). The most viscous was revealed by PNG 296 (4.07) meanwhile the least viscous was from SLA 16 (1.83).

The coefficients of variation for the cocoa liquor global quality were 7.99 while the viscous was 24.12. Every cocoa genotype has a unique potential flavour character (Kongor *et al.*, 2016). Differences and variation in flavour and final flavour formation of the beans could be attributed by inherent genetic composition of the bean, botanical origin, climatic conditions of cocoa growing trees, soil conditions, ripening and time of harvesting, bean fermentation and drying, roasting and other bean processing.

Table 2. Mean squares in ANOVA table for cocoa liquor attributes evaluated on eight selected cacao genotypes.

Source of variation	d.f. †	Mean squares				
		Cocoa	Bitterness	Astringency	Acidity	Sweet
Genotypes	7	0.63 ns	0.39 ns	0.43 ns	0.16 ns	0.03 ns
Tree / Genotypes	24	0.38**	0.18*	0.20**	0.07 ns	0.01 ns
Error	64	0.18	0.10	0.07	0.06	0.01
C.V. (%)		6.62	12.61	11.64	17.32	112.56

Source of variation	d.f. †	Mean squares			
		Fresh Fruits	Browned Fruits	Floral	Spicy
Genotypes	7	0.12 ns	0.06 ns	0.29**	0.01 ns
Tree / Genotypes	24	0.08*	0.07 ns	0.06 ns	0.01**
Error	64	0.04	0.04	0.04	0.00
C.V. (%)		19.47	17.37	22.66	129.21

Source of variation	d.f. †	Mean squares		
		Woody	Nutty	Roasted
Genotypes	7	0.09 ns	0.61*	0.23*
Tree / Genotypes	24	0.04**	0.17 ns	0.09**
Error	64	0.02	0.15	0.04
C.V. (%)		62.60	20.39	4.15

† d.f. = Degrees of freedom; C.V. = Coefficient of variation; *, **, ns = significant at $p \leq 0.05$, significant at $p \leq 0.01$, and non-significant, respectively.

Table 3. Mean squares in ANOVA table for cocoa liquor off-flavours evaluated on eight selected cacao genotypes.

Source of variation	d.f. †	Mean squares				
		Dirty/Dusty	Meaty/Animal	Overfermented/	Smoky	Mouldy

			putrid			
Genotypes	7	0.003^{ns}	0.001^{ns}	0.003^{ns}	0.002^{ns}	0.003^{ns}
Tree / Genotypes	24	0.003^{ns}	0.002^{ns}	0.006^{ns}	0.003^{ns}	0.004^{ns}
Error	64	0.002	0.002	0.005	0.004	0.003
C.V. (%)		339.12	430.26	206.83	151.19	216.29

Table 4. Mean squares in ANOVA table for cocoa liquor global quality and viscous evaluated on eight selected cacao genotypes.

Source of variation	d.f. †	Mean squares	
		Global Quality	Viscous
Genotypes	7	1.02^{ns}	7.97**
Tree / Genotypes	24	0.52*	0.73^{ns}
Error	64	0.26	0.49
C.V. (%)		7.99	24.12

† d.f. = Degrees of freedom; C.V. = Coefficient of variation; *, **, ^{ns} = significant at *p≤ 0.05*, significant at *p≤ 0.01*, and non-significant, respectively.

Table 5. Mean performance of eight selected cacao genotypes for the liquor cocoa attributes.

GENOTYPE	Cocoa	Bitterness	Astringency	Acidity	Sweet	Fresh Fruits
IMC 16	6.67a	2.40b	2.11b	1.35b	0.17a	1.26a
IMC 20	6.60a	2.59ab	2.43ab	1.64a	0.10ab	1.07ab
IMC 103	6.18a	2.60ab	2.32ab	1.31b	0.10ab	1.06ab
UF 12	6.21a	2.83a	2.58a	1.43ab	0.03b	1.00ab
SLA 16	6.19a	2.30b	2.13b	1.55ab	0.06b	0.99b
AMAZ 12	6.60a	2.40b	2.13b	1.34b	0.12ab	1.15ab

MCBC 5	6.13a	2.69ab	2.51ab	1.39ab	0.05b	0.96b
PNG 296	6.53a	2.68ab	2.45ab	1.40ab	0.10ab	1.11ab
MEAN	**6.39**	**2.56**	**2.33**	**1.42**	**0.09**	**1.07**
CV	**6.62**	**12.61**	**11.64**	**17.32**	**112.56**	**19.47**

Mean values followed by the same letter in the same column are not significantly different at p≤0.05, based on DMRT.

Table 5 (continued).

Table 5. Mean performance of eight selected cacao genotypes for the liquor cocoa attributes.

GENOTYPE	Browned Fruits	Floral	Spicy	Woody	Nutty	Roasted
IMC 16	1.27a	1.14a	0.03a	0.07b	2.16ab	4.91ab
IMC 20	1.31a	0.93ab	0.08a	0.27a	1.81bcd	4.92ab
IMC 103	1.16a	0.95ab	0.06a	0.32a	1.71cd	4.70b
UF 12	1.15a	0.76bc	0.02a	0.26a	1.78bcd	5.01a
SLA 16	1.14a	0.76bc	0.06a	0.19ab	1.56d	4.70b
AMAZ 12	1.29a	1.02a	0.03a	0.14ab	2.20a	4.92ab
MCBC 5	1.13a	0.69c	0.05a	0.30a	1.74cd	5.08a
PNG 296	1.20a	1.01a	0.07a	0.18ab	2.00abc	4.99a
MEAN	**1.20**	**0.91**	**0.05**	**0.21**	**1.87**	**4.90**
CV	**17.37**	**22.66**	**129.21**	**62.60**	**20.39**	**4.15**

Mean values followed by the same letter in the same column are not significantly different at p≤0.05, based on DMRT.

Table 6. Mean performance of eight selected cacao genotypes for the liquor cocoa off-flavours.

GENOTYPE	Dirty/Dusty	Meaty/Animal	Overfermented/ Putrid	Smoky	Mouldy
IMC 16	0.00a	0.00a	0.02a	0.03a	0.02a
IMC 20	0.01a	0.03a	0.01a	0.04a	0.03a

IMC 103	0.03a	0.00a	0.04a	0.06a	0.06a
UF 12	0.04a	0.01a	0.05a	0.03a	0.03a
SLA 16	0.01a	0.01a	0.04a	0.05a	0.03a
AMAZ 12	0.01a	0.02a	0.02a	0.03a	0.01a
MCBC 5	0.00a	0.00a	0.04a	0.05a	0.01a
PNG 296	0.01a	0.01a	0.06a	0.03a	0.03a
MEAN	**0.01**	**0.01**	**0.04**	**0.04**	**0.03**
CV	**339.12**	**430.26**	**206.83**	**151.19**	**216.29**

Mean values followed by the same letter in the same column are not significantly different at p≤0.05, based on DMRT.

Table 7. Mean performance of eight selected cacao genotypes for the liquor cocoa global quality and viscous.

GENOTYPE	**Global Quality**	**Viscous**
IMC 16	6.67a	2.17cd
IMC 20	6.45ab	3.70a
IMC 103	6.07ab	2.87bc
UF 12	6.18ab	2.89bc
SLA 16	6.11ab	1.83d
AMAZ 12	6.70a	3.51ab
MCBC 5	5.96b	2.13cd
PNG 296	6.55ab	4.07a
MEAN	**6.34**	**2.90**
CV	**7.99**	**24.12**

Mean values followed by the same letter in the same column are not significantly different at p≤0.05, based on DMRT.

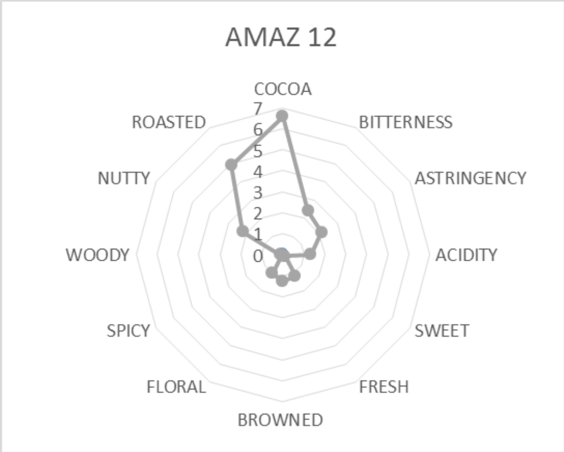

Figure 1: Cocoa liquor flavour profile for AMAZ 12

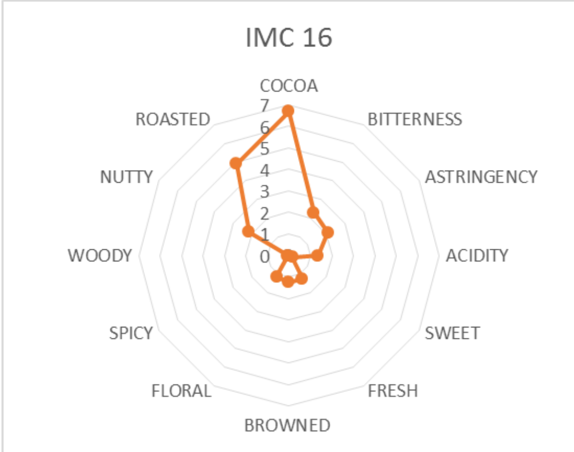

Figure 2: Cocoa liquor flavour profile for IMC 16

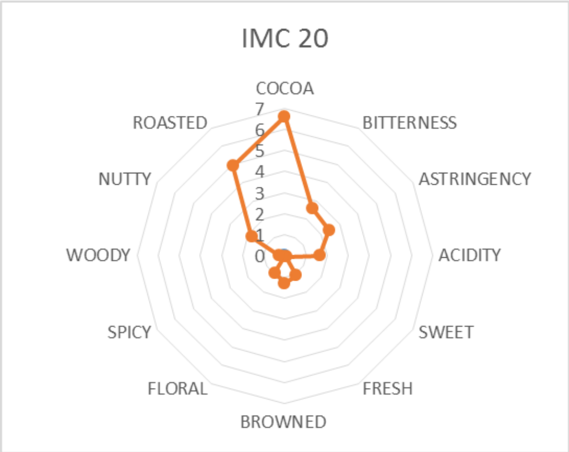

Figure 3: Cocoa liquor flavour profile for IMC 20

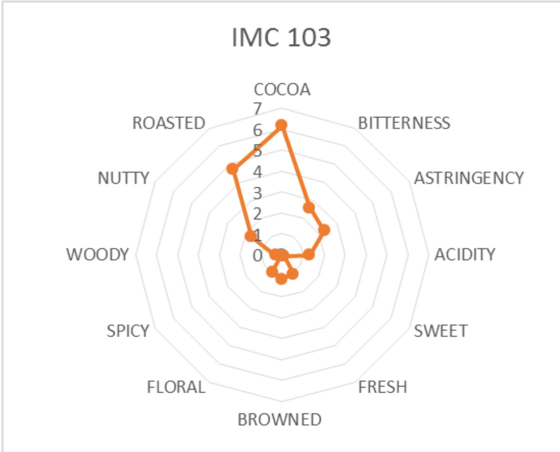

Figure 4: Cocoa liquor flavour profile for IMC 103

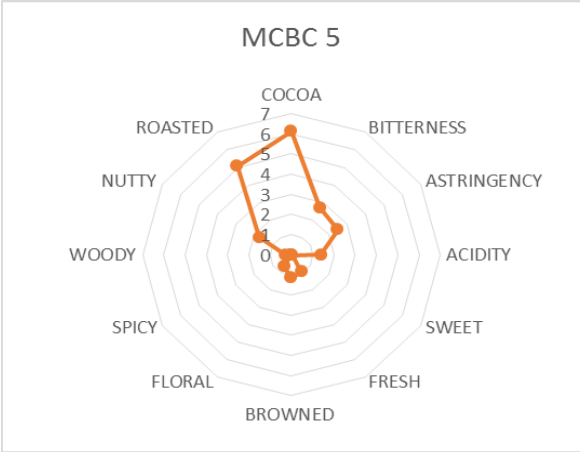

Figure 5: Cocoa liquor flavour profile for MCBC 5

Figure 6: Cocoa liquor flavour profile for PNG 296

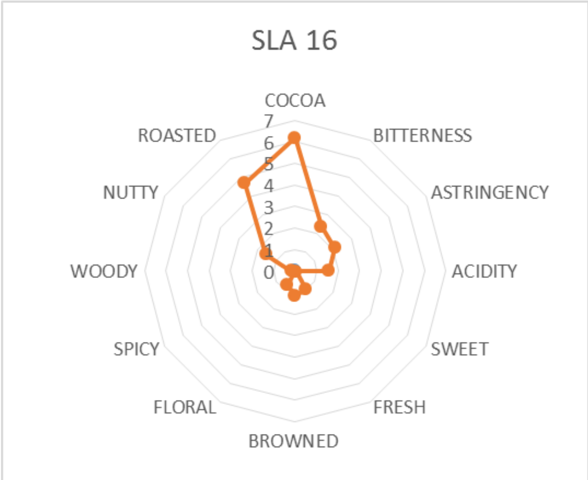

Figure 7: Cocoa liquor flavour profile for SLA 16

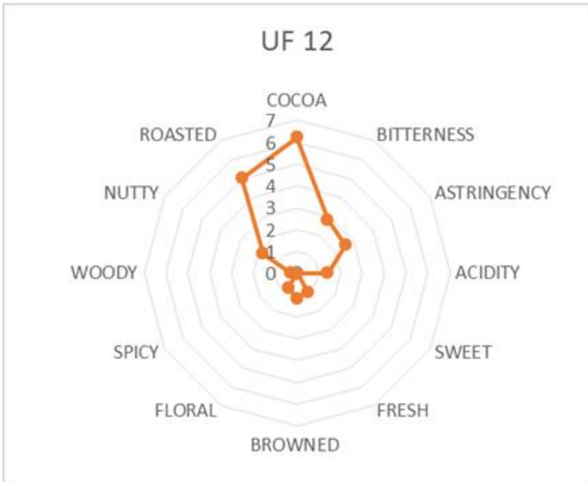

Figure 8: Cocoa liquor flavour profile for UF 12

CONCLUSIONS

Flavour and quality of chocolate products are important for the consumer acceptability. Thus, it is a vital to produce and use good quality cocoa beans. This paper showed that eight selected cocoa genotypes indicating variation in flavor profile especially for floral, nutty and roasted attributes, in cocoa liquor sensory evaluation. The second result indicated that most of the attributes affected significantly by the tree within genotype for cocoa, bitterness, astringency, fresh fruits, woody and roasted, and for global quality score. As MCB has many genetic materials in her collection to be explored, it is necessary to conduct further study on the flavour of the potential materials.

ACKNOWLEDGEMENTS

The authors express their appreciation to the Malaysian Cocoa Board's Director General, Deputy Director General R&D, the Director of Cocoa Upstream Technology for the permission to present this paper. The authors also would like to extend sincere gratitude to Hj. Zailaini Mohd. Jamil and staff of planting material development section, CRDC Bagan Datuk for their assistance in collecting samples. This paper is part of 11^{th} Malaysia Plan, Development Project 'Bahan Tanaman Koko Unggul' (P20001001031413).

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