NUTRITIONAL AND PHENOLIC CONTENTS OF DEVELOPED MARVELLES MILK, DARK, SUGARFREE AND WHITE CHOCOLATES

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Malaysian Cocoa J. 14: 62-65 (2022)

ABSTRACT – *Chocolate contains a wide range of nutritional values that includes fat, fiber, proteins, carbohydrates and energy. It also carries beneficial active compounds, antioxidants such as soluble phenolic compounds (phenolic acids, catechin, epicatechin and procyanidins), insoluble polymeric phenolics and methylxanthines. The objectives of this study was to determine nutritional values and phenolic contents in milk (MC), dark (DC), sugarfree (SC) and white (WC) chocolates developed by the Food Biotechnology Division, CITC, Nilai Negeri Sembilan. Nutritional values of the chocolates were determined using AOAC methods and total flavonoids were quantified using HPLC method. Total energy was highest in sugarfree chocolates followed by dark, white and milk chocolates. Dark chocolates exhibited the highest flavonoids contents followed by sugarfree and milk chocolates but not detected in white chocolates. Catechin and epicatechin were major flavonoids detected in dark chocolates. When nutrition and health promotion are of concern, sugarfree dark chocolates would be recommended over dark, milk and white chocolates owing to their higher contents of antioxidant phenolic compounds without exhibited insulin level.*

Key words: Chocolates, sugarfree, nutritional, catechin, epicatechin

INTRODUCTION

The delicious form of chocolates, as we like today, comes from the perfect mixture of quality ingredients such as cocoa liquor, cocoa butter, cocoa powder, milk and sugar constitutes. As well as ideal processing method and time to refine the mixture with correct tempering and proper storage. Selection of the origin and type of bean which produce cocoa liquor and butter for chocolate production are driving factors of purchase for chocolate manufacturers around the world. Today, there are three cacao cultivars known as criollo, the northern South American strain, forastero, the Amazon Basin strain, and trinitario, the hybrid strain born in Trinidad. The criollo and forastero are pure species; while trinitario arose from conscious cross pollination of the two, engineered to provide more abundant crop yield (Scharffenberger et al., 2006). The forastero bean is known to have a stronger and flat flavor, but not as complex and mellow as the light color criollo bean. The rise of trinitario created a heartier, distinct and different flavor of cacao than its parent ancestors. The hearty and bountiful forastero cacao, known as 'bulk beans', account for more than 95% of cacao used by the World's chocolate manufacturers today (Guittard et al., 2009).

Good chocolate comes from good cocoa beans, starting from the primary process which are harvesting, fermentation, drying, roasting, winnowing, refining, grinding and pressing, followed by secondary processing as well as conching, tempering and storage process. Harvesting was carried out for ripe cocoa fruits and undergo fermentation for 5-6 days. Prior to fermentation, cacao beans are bitter and astringent. Fermentation is the first step in the development of chocolate flavor. Once fermentation is complete, beans are spread on a concrete floor and dried in the sun for 5-6 days. During drying the beans develop their characteristic brown color. High temperatures (110-220°C) during roasting are important for flavor development due to Maillard reaction, non-enzymatic browning, where the cacao bean gains sweetness and floral/caramel notes. Its reduces acidity in bean flavor as indicated by a significant decrease in concentration of volatile acids (e.g., acetic acid) and non-volatile acids (e.g., oxalic, citric, tartaric, succinic, and lactic acids) (Jinap et al., 1995).

Refining and grinding of the cacao nib is necessary to produce chocolate liquor and reduce particle size. Nibs contain 53% fat, 14% protein, and have 1.5% maximum 9 moisture content; they are typically ground using roll or ball refiners to form a thick cocoa paste (Minifie et al., 1989). The paste is further refined to optimize particle size. The melangeur, first used in the 18th century, is one of many versatile machines used to refine cocoa paste, as it allows the mass to pass between two rotary stone slabs (Presilla et al., 2001). Beckett et al. (1999) describes the design of conching to optimize viscosity and flow properties by mechanically "smearing" the fat over the ground sugar and cacao surfaces to increase particle flow and further reduce particle size to <18 microns (Lucisano et al., 2006). Particle size is important for even melting, smooth

mouthfeel, and volatile release in chocolate. The human tongue can detect particles a minimum size of 20-30 microns (Liang et al., 2004); thus, it is important during refining to reduce particles below the human detection threshold to uphold a favorable chocolate texture. Conching is also essential for chocolate flavor development by removing the distasteful bitter, astringent, and sour flavors. Once conching is complete, the flavorful chocolate product undergoes its final step of processing. Tempering involves controlled cooling of melted chocolate that will promote a stable crystalline structure for a finished product. Stable cocoa butter crystals will provide desirable properties such as snap, gloss, proper texture, contraction for de-molding, and less permeability (Hartel et al., 2001). However, storage of chocolate results in structural changes consequently affecting the favorable texture and flavor attributes. With improper storage, these changes are magnified, causing an increase in particle size, and the development of either fat bloom or sugar bloom, which compromises mouthfeel, visual, and textural quality (Morgan et al., 1994).

Standard of Identity (SOI)

According to the Food and Drug Administration (FDA), the standards of identity for dark chocolate are as follows: must contain at least 35% chocolate liquor and a maximum of 12% milk solids (Code of Federal Regulations Title 21, 2003). Milk chocolate (based on dry matter basis) must contain no less than 25% cocoa solids and a minimum of specified milk solids between 12-14%, including a minimum of milk fat between 2.5-3.5%. Whereas, white chocolate must contain no less than 20% cocoa butter and no less than 14% milk solids, including a minimum milk fat between 2.5-3.5%.

Chemical Properties of Chocolate

Chocolate is a suspension of cocoa, sugar and/or milk solid particles in a continuous fat (butter) phase. Cocoa butter accounts for 50% to 70% of the dry weight of cocoa beans and is responsible for the melting properties of chocolate. The predominant fatty acids in cocoa butter are saturated (stearic, 35% and palmitic 25%) and monounsaturated (oleic, 35%), with remaining fat being primarily polyunsaturated (linoleic 3%). In spite of its high saturated fat content, chocolate does not appear to raise cholesterol levels in normal humans. Surprising that stearic acid has a neutral or cholesterol-lowering effect as well as oleic acid which has beneficial effects on health including reduction of coronary heart disease (Thijssen et al., 2005). Fiber in cocoa beans comes from seed coat, also called bran, which contains of good source of insoluble fiber (44%) and also soluble fiber (11%) that contribute to lower serum lipids (Fernandez et al., 2001 & Jenkins et al., 2000). One of the fascinating things about chocolate is that it has a long shelf life with virtually no microbial concerns. The objectives of this study was to determine nutritional values and phenolic contents in milk (MC), dark (DC), sugarfree (SC) and white (WC) chocolates developed by the Food Biotechnology Division, CITC, Nilai Negeri Sembilan.

MATERIALS AND METHODS

Chocolate formulation and development

Different types of chocolates such as milk, dark, sugarfree and white may have different percentages of cocoa liquor, cocoa powder, cocoa butter, sugar/substitute and milk powder. Melted fat components and dry powders were mixed homogeneously in a kneader and the chocolate mass (75% fats) was pre-refined on a lab scale three roller refiner. Otherwise for white chocolate, it does not contain cocoa powder and cocoa liquor so too sugarfree chocolate which using isomalt as sugar replacer. Then, the mass was transferred to a lab scale conch and remaining cocoa butter (refined separately) was added for a total of 10 hours. Then, tempering of finished chocolate mass manually will take place on a marble slab and moulded to 10g shape. Finally, the chocolate was stored at 16^{o}C after demoulded them into praline shape. Nevertheless, all samples produced met the legal standard of 'quality chocolate'.

Determination of Particle Size

Particle size of these chocolate was measured using Malvern mastersizer. Firstly, 20ml oil was added into the 0.2g sample. After, 2 min sonication at room temperature sample was inserted into the chamber until the obscuration reading reached to 20%. Reading was recorded.

Proximate analysis of chocolates.

Chocolate samples were sent to accredited MS ISO/ISE 17025 Analytical Services Laboratory, MCB, Cocoa Innovation and Technology Centre (CITC) for proximate analysis. Proximate analysis was include ash (AOAC 13.003), moisture (ISO8534:1996E), crude fat (IOCCC:Pg8a-E 1978), crude protein (AOAC 13.009), crude fibre (AOAC 962.06), total carbohydrate (calculation) and total calories (calculation).

Determination of total polyphenol content using Folin-ciocalteau assay

Total polyphenol content in unfermented cocoa seeds extracted were determined according to the Folin-ciocalteau method (Waterhouse et al., 2001). Briefly, the defatted cocoa seeds in both methods were dissolved in 70% (v/v) acetone and were sonicated for 10 minutes. Samples were centrifuged at 5 000 rpm for 15 minutes. 100 µl of the

supernatant was added with 7.9 ml distilled water followed by 0.5 ml folin-ciocalteau reagent (Merck) (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5-8 min. Then, 1.5 ml of 20% sodium carbonate (Sigma) solution was added to the mixture. Mixtures were leaved at 20˚C for 2 hour and absorbance of each mixture was determined at 765 nm using UV-vis spectrophotometer (Shimadzu, Japan). A standard calibration curve was obtained from 0, 50, 100, 150, 250, 500, 750 and 1000 mg/l gallic acid (Sigma Co., USA). Results were expressed as gallic acid equivalents (GAE) in milligrams per gram extract.

Data analysis

Data were expressed as means ± S.E.M. One-way ANOVA was applied to find the difference among means. Results are considered significantly different at the level of $p<0.05$.

RESULTS AND DISCUSSIONS

Particle size is important for chocolate melting, smooth mouthfeel, and volatile release in chocolate. Tables below show results for particle sizes of milk, dark, sugarfree and white chocolates. Tables 1 shows the particle size of these chocolates was between 32.9-103 μm. It was shown that the particle size in dark chocolate was smoother followed by sugarfree, milk and white chocolates. According to Liang and Hartel (2004), the human tongue can detect particles a minimum size of 20-30 microns thus, it is important during refining to reduce particles below the human detection threshold to uphold favorable chocolate texture. Results show that the lesser powder ingredients used in chocolate formulation, particle size will be smaller such as only 5 ingredients were used in dark chocolate compared to 7 ingredients in white chocolate.

Table 1: Particle size of several chocolates

Type of analysis	Nutritional value (dry basis)			
	Milk	Dark	Sugarfree	White
Particle size (μm)	48.4	32.9	37.5	103

Data were expressed as mean±SE. N=3.

Table 2 below shows proximate analysis of three different doses of milk, dark, sugarfree and white chocolates. Moisture content in all tablets was in a range of 1.96-3.66%. It shows that all types of chocolates were highly hygroscopic. Percent of ash was also low which was between 1.70-3.17% and it's shown that all ingredients were soluble and absorbed in the body. It also contains a low percentage of crude fat, crude protein and crude fibre which was between 36.7-39.02%, 0.85-12.04% and 1.95-5.64% respectively. These chocolates have high total energy which was between 557.4-568.42 kCal/100g and this energy may have come from total carbohydrate contents which was 40.83-52.19%.

Table 2: Proximate analysis of several chocolates

Type of analysis	Nutritional value (dry basis)			
	Milk	Dark	Sugarfree	White
Moisture (%)	2.59	1.96	2.46	3.66
Ash (%)	2.48	1.70	1.75	3.17
Crude fat (%)	39.02	36.70	37.11	38.35
Crude protein (%)	7.22	4.55	0.85	12.04
Crude fibre (%)	3.20	4.69	5.64	1.95
Total carbohydrate (%)	45.49	50.37	52.19	40.83
Total energy (kCal/100g)	568.42	559.63	557.44	560.55

Type of analysis	Nutritional value (dry basis)			
	Milk	Dark	Sugarfree	White
Catechin	0.0089	0.0648	0.0229	0.0000
Epicatechin	0.0989	0.3928	0.2164	0.0000

Quantification of flavonoid content using HPLC analysis shows that the catechin and epicatechin content was between 0-0.0648 mg/g and 0-0.3928 mg/g, respectively. Similarly, as reported by Kim et al. (2014) has shown that the content of catechin and epicatechin were 0.006 mg/g and 0.023

mg/g, respectively. It shows that white chocolate does not contain flavonoid because in chocolate formulation there was no additional of cocoa liquor and powder. However, flavonoid content in dark chocolate was highest because it contains 60% cocoa mass compared to only 20-30% in milk and sugarfree milk chocolate.

CONCLUSIONS

In conclusion, cocoa content in chocolate will determined the flavonoid content in chocolate. The highest cocoa content used in chocolate formulation, the highest flavonoid content was found.

ACKNOWLEDGEMENTS

This study was supported by the Development Fund provided by MASTECC Project Code PTJ7710910 (Pembangunan Harta Intelek dan Pengkomersialan). The authors would like to acknowledge MCB, Food Biotechnology Division, CITC and all teams either directly or indirectly for their excellent technical assistance throughout the entire study.

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