ENRICHMENT OF COCOA POLYPHENOL EXTRACT IN CHEWABLE TABLET AS A NEW SOURCE OF HIGH ANTIOXIDANT PRODUCTS

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ABSTRACT - *Chewable tablet is a common sweets and breath refreshment among children and adults in the markets nowadays. Although its only plays a simple role in our lives such as breath refreshment and mouth exercise, enrichments of these chewable tablet with cocoa polyphenol extract may enhance its potential as healthy confectionary. Cocoa is well known for its high flavonoid content, mainly catechin and epicatechin which has been proven as a potent antioxidant that react as a highly effective chemo preventive agent against chronic diseases including cancer, heart disease, diabetes, neurodegenerative disease and ageing. Objectives of this study are to develop chewable tablet enrich with cocoa polyphenol extract and to determine the physicochemical properties of chewable tablet enrich with cocoa polyphenol extract. Total polyphenol content was determined using folin ciocalteu method and gallic acids as a standard, whereas the nutritional composition was carried out using AOAC method. Results shows chewable tablet enrich with cocoa polyphenol extract contains 16.26±1.63 mg/g which is twenty fold lower compared to cocoa polyphenol extract from unfermented cocoa beans which had a total polyphenol content of 335.70±27.51 mg GAE/g. Meanwhile, analysis nutrition shown on moisture (1.38%), ash (1.37%), fat (2.32%), crude protein (3.76%), crude fibre (0.53%), total carbohydrate (91.17%) and energy value (410.16%). The increasing global market confectionary potential about 6.2% from 2016 to 2021 by $76.88b shown chewable tablet enriches with cocoa polyphenol extract have a big potential in the market.*

Keywords: Cocoa, polyphenol, flavonoid, antioxidant, tablet

INTRODUCTION

Nowadays, chewable tablets are one of the favourite sweets among kids, teenagers and adults. Although its main function is mouth freshness and to remove unpleasant taste, it also can be enriched with functional ingredients such as vitamins, minerals and antioxidants to enhance its beneficial health. Chewable tablets may have different percentages of soluble base like fillers, sweeteners, antioxidants, flavouring agents, colouring agents and coating agents depending upon the base used and its properties. A flavouring agent is included to make it more palatable. These tablets are intended to disintegrate smoothly in the mouth at a moderate rate either with or without actual chewing, characteristically chewable tablets have a smooth texture upon disintegration, are pleasant tasting and leave no bitter or unpleasant taste. Various factors involved in the formulation of chewable tablets. The major formulation factors are flow, lubrication, disintegration, organoleptic properties, compressibility, compatibility and stability, which are common to regular (swallowed) and chewable tablets; however, organoleptic properties of the active drug substances are primary concern here (Kerimi & Williamson, 2015).

Cocoa is well known for its high polyphenol content, mainly catechin and epicatechin which has been proven as a potent antioxidant that react as a highly effective chemo preventive agent against chronic diseases including cancer, heart disease, diabetes, neurodegenerative disease and ageing (Lambert & Elias, 2010; Martin *et al.,* 2016; Martin *et al.,* 2013). In this sense, flavonoid compounds may be involved either directly or indirectly to

scavenge or detoxify free radicals to prevent chronic diseases and increase human health. Indeed, direct antioxidant effects of flavonoids seemed to be partly based on their structural characteristics via hydrogen donating (radical scavenging) properties and their metal chelating antioxidant properties (Nakagawa *et al*., 2004; Ramos, 2008; Renu *et al.*, 2015). More importantly, flavonoids can avert free radical-induced damage indirectly by modulating several enzymes related to oxidative stress, modifying the metabolism such as conjugating enzymes (glucuronidation, sulfation, acetylation, methylation and conjugation) as well as through regulation of certain transcription factors (Rosmawati *et al*., 2020). Recently, a previous study showed cocoa polyphenol extract may increase the first body defence mechanism (glutathione) in ethanol induce toxicity (Shahidi & Ambigaipalan, 2015). This finding also reported the protective properties of the cocoa extract, which was able to protect the hepatic function by preventing damages due to ethanol consumption. The purpose of this study was to develop high antioxidant chewable tablets as a new source of high antioxidant products from cocoa which can improve human health.

MATERIALS AND METHODS

Development of high antioxidant chewable tablets

Three high antioxidant chewable tablets were developed using different percentages of isomalt, cocoa powder, magnesium stearate and cocoa polyphenol extract. Cocoa polyphenol extract was supplied by Malaysia Cocoa Board (MCB) which contain 335.70 ± 27.51 mg GAE /g of total polyphenol content. High antioxidant chewable tablets developed consist of three doses which are 0%, 1.5% and 2.0%. The production will be carried out based on an existing MCB method using a medium scale tableting machine with 10 punchers. All ingredients were mixed, grind and dry before pouring into the tablet machine. Tablet thickness and weight was set during tablet forming.

Determination of Particle Size

Particle size of these high antioxidant dark 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.

Determination of Hardness Testing

Hardness of the sample was determined using texture analyser (TA.XT plus). 1 piece of sample was put on a texture analyser. Run the test and reading was recorded. Control parameter: Probe (HDP/3PB); pre-test speed (2.0 mm/sec); post-test speed (2.0 mm/sec); post-test (10.0 mm/sec); distance (15mm thick).

Proximate analysis of high antioxidant chewable tablets.

Tablet 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, 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 curves 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 DISCUSSION

Tables below show results for particle sizes, hardness testing, proximate analysis and total polyphenol content of three different doses of high antioxidant chewable tablets. Table 1 show particle size of these tablets was between 68-79 μm and tablet hardness was between 716-3041 G. Hardness of tablet F1 was low compared to tablet F2 and F3. It was shown that the texture of tablet F1 was smoother compared to F2 and F3.

Table 1: particle size and hardness testing of high antioxidant chewable tablet

Type of analysis	Tablet formulations		
	F1	F2	F3
Particle size (μm)	68.86	83.45	79.48
Hardness testing (force; G)	716.06±34.39	3001.67±54.06	3041.37±62.28

Data were expressed as mean±SE. N=3.

Table 2 below shows proximate analysis of three different doses of high antioxidant chewable tablets. Moisture content in all tablets was in a range of 1.38-2.85%. It shows that this high antioxidant chewable tablet was highly hygroscopic. Percent of ash was also low which was between 1.24-1.42% 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 2.13-2.32%, 3.76-4.14% and 0.52-0.57% respectively. These tablets have high total energy which was between 407-410 kCal/100g and this energy may heve come from total carbohydrate contents which was 89-91%.

Table 2: Proximate analysis of high antioxidant chewable tablet

Type of analysis	Nutritional value (dry basis)		
	F1	F2	F3
Moisture (%)	2.34	1.38 ± 0.2	2.85
Ash (%)	1.24	1.37 ± 0.0	1.42
Crude fat (%)	2.13	2.32 ± 0.3	2.24
Crude protein (%)	3.79	3.76 ± 0.8	4.14
Crude fibre (%)	0.52	0.53 ± 0.0	0.57
Total carbohydrate (%)	90.50	91.17 ± 0.0	89.35
Total energy (kCal/100g)	408.15	410.16 ± 0.0	407.00

Data were expressed as mean±SE. N=3

Total polyphenol content of high antioxidant chewable tablets was between 6.61-17.67 mg GAE/g and higher in a scale of F3>F2>F1 during 6 month shelf-life study as shown in Table 3 below. It also showed the content of polyphenol also reduced during shelf-life study for all samples. It might be due to the improper packaging and storage of tablets.

Table 3: Total polyphenol content of high antioxidant chewable tablet after 6 months

Tablet formulations	Shelf life (month)/Total polyphenol content (mg GAE/g)			
	0	1	3	6
F1	7.25±0.80	7.30±0.56	6.91±0.07	6.61±0.33
F2	13.33±1.36	12.33±1.07	13.28±0.50	11.64±0.14
F3	15.56±1.28	17.67±0.81	17.38±0.60	14.71±0.30

- Gallic acid as a standard
- Data were expressed as mean±SE. N=3

CONCLUSION

In conclusion, these high antioxidant chewable tablets have to undergo further analysis to identify its friability, disintegration and dissolution properties.

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REFERENCES

Kerimi, A. and Williamson, G. (2015) The cardiovascular benefits of dark chocolate. *Vascular Pharmacology* **71**:11-5.

Lambert, J.D. and Elias, R. (2010) The antioxidant and pro-oxidant activities of green tea polyphenols: A role in cancer prevention. *Archieves of Biochemistry and Biophysics* **501**:65-72.

Martin M.A., Goya L. & Ramos S. (2016) Antidiabetic actions of cocoa flavonols. *Molecular Nutrition and Food Research* doi:10.1002/mnfr.201500961.

Martin, M.A., Goya, L. and Ramos, S. (2013) Potential for preventive effect of cocoa and cocoa polyphenols in cancer. Food and chemical toxicology: *An international Journal Published for the British Industrial Biological Research Association* **56**:336-51.

Nakagawa, H., Hasumi, K., Woo, J., Nagai, K. and Wachi, M. (2004) Generation of hydrogen peroxide primarily contributes to the induction of Fe(II)-dependent apoptosis in Jurkat cells by (-)-epigallocatechin gallate. *Carcinogenesis* **25**:1567-74.

Ramos, S. (2008) Cancer chemoprevention and chemotherapy: Dietary polyphenols and signaling pathways. *Molecular Nutrition and Food Research* **52**:507-26.

Renu, J. D., Pawan, J., Balvinder S. (2015) Chewable Tablets: A Comprehensive Review. *The Pharma Innovation Journal* **4(5)**:100-105.

Rosmawati, M.S., Maskat, M.Y., Ayob, M.K. and Rosmin, K. (2020). Protective Effect of Cocoa Extract on Ethanol Induced Liver Injury in Sprague-Dawley Rats. *Science Malaysiana* **49 (11)**:93-100 Doi.Org/10.17576/Jsm-2020-4901-08.

Shahidi, F. and Ambigaipalan, P. (2015) Phenolics and polypenolics in foods, beverages and spices: Antioxidant activity and health effects-A review. *Journal of Functional Foods* **18**:820-97.