SCALING UP COCOA POLYPHENOL EXTRACTS: A COMPARATIVE ANALYSIS OF TOTAL PHENOLIC CONTENT

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ABSTRACT - *Polyphenols, renowned for their robust antioxidant properties, play a pivotal role in promoting human health by mitigating the risk of various ailments, including diabetes, cardiovascular disease, specific cancers, and indigestion. Widely distributed in numerous food plants, these beneficial compounds are particularly abundant in cocoa beans, sourced from Theobroma cacao, which serves as a primary reservoir for chocolate and cocoa powder. Our study aimed to identify the total polyphenol content in upscaling extraction of unfermented cocoa beans sourced from the Cocoa Research and Development Centre in Jengka (Pahang). The investigation encompassed diverse production methods, namely laboratory scale (60g/8h), medium scale (3kg/6d), and large scale (100kg/3d). The process involved deshelling and defatting unfermented dried cocoa beans, with polyphenols subsequently extracted using 70% undenatured ethanol. The extracts underwent meticulous analysis using the Folin-Ciocalteu method (UV spectrophotometry) at 765nm. The findings unveiled a compelling disparity in the total phenolic content of cocoa extract, with the large-scale production (IBS UTM) demonstrating a twofold increase compared to medium scale, followed by laboratory scale—measuring 502.18 mg GAE/g, 335.70 mg GAE/g, and 141.255±14.716 mg GAE/g, respectively. In conclusion, our comprehensive analysis of polyphenol content in upscaled production of cocoa polyphenol extract reveals an intriguing finding. Contrary to expectations, increasing the production scale did not result in a reduction of the total polyphenol content under control conditions. This suggests that the benefits of cocoa polyphenols, particularly in terms of antioxidant properties, remain consistent even with the upscale in production. These findings hold significance for the potential applications and benefits of cocoa polyphenol extract at different production scales. Further research is warranted to delve into the nuances of this relationship and explore the implications for both production efficiency and the health-related aspects of cocoa polyphenols.*

***Keywords**:* Cocoa, polyphenol, antioxidant, total polyphenol content, cytotoxicity

INTRODUCTION

Cocoa beans, the primary ingredient in chocolate and cocoa products, are rich in polyphenols, particularly flavan-3-ols such as epicatechin, catechin, and procyanidins (Knight, 2000; Scalbert et al., 2000; Hammerstone et al., 2000; Adamson et al., 1999; Lamuela-Raventos et al., 2001; Lazarus 1999). The antioxidant properties of cocoa polyphenols have sparked significant interest due to their potential health benefits. Epidemiological studies have suggested that polyphenols may help reduce the risk of cardiovascular diseases (Hertog *et al.,* 1995; Knekt *et al.,* 1996). Previous in vivo studies have also indicated that cocoa products may have the potential to lower the risk of degenerative diseases (Amin *et al.,* 2004; Ruzaidi *et al.,* 2005; Ruzaidi *et al.,* 2008; Abbe Maleyki *et al.,* 2008).

Initially, cocoa fruit was primarily used for chocolate and beverages, but its utilization expanded after it was recognized for its health-enhancing properties due to its high polyphenol content compared to tea and red wine (Lee *et al.,* 2003). The polyphenol content in cocoa beans can reach up to 10% of their dry weight (Richelle *et al.,* 1999). This composition varies depending on factors such as location, bean preparation methods (Natsume *et al.,* 2000; Hay *et al.,* 2006), and analytical techniques employed (Kim & Keeney, 1983; Hammerstone *et al.,* 1999). Othman *et al.* (2007) found that ethanol extraction of Malaysian cocoa beans yielded the highest polyphenol content, followed by beans from Sulawesi, Ghana, and the Ivory Coast. This elevated polyphenol content may contribute to the bitter taste characteristic of Malaysian cocoa beans.

Our objective was to identify the total polyphenol content at different scales of extraction process which are laboratories, medium and large scale unfermented cocoa beans sourced from the Cocoa Research and Development Centre in Jengka (Pahang).

MATERIALS AND METHODS

Preparation Of Cocoa Polyphenol Extracts

Laboratory Scale Method

Cocoa fruits were provided by the Cocoa Research and Development Centre, Jengka. Cocoa beans were separated from the cocoa pod husk and soaked in boiling water for 20 minutes. The beans were then dried in an oven (Memmert, Germany) with a blower at 60°C for 4 to 5 days, or until the moisture content reached 4-6%. The dried cocoa beans were manually deshelled using a knife and coarsely ground (Waring, USA) to optimize the extraction process.

Cocoa butter was removed from the cocoa beans using a Soxhlet unit (Buchi, Switzerland) with n-hexane (Merck, Germany). Following this, cocoa polyphenols were extracted using 70% undenatured ethanol (Systerm, USA). The Soxhlet unit was equipped with 6 sets of extraction chambers, condensers, round bottom flasks, and cellulose thimbles, each capable of holding up to 10g of defatted ground cocoa nibs. The extraction process took approximately 6-8 hours to extract all polyphenols from the cocoa nibs. The cocoa polyphenol extract was then concentrated using a rotary evaporator (N-N-series, EYELA, USA) under partial vacuum at 70°C to remove undenatured ethanol. The aqueous extract was freeze-dried (LABCONCO, USA) to remove water and stored at -20°C until use.

Figure 2.1 Cocoa beans oven drying for freeze drying for laboratory methods of preparation of cocoa polyphenol extracts

Figure 2.2 Defatting & extracting for freeze drying for laboratory methods of preparation of cocoa polyphenol extracts

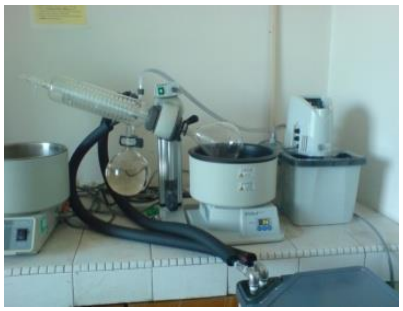

Figure 2.3 Concentrating for freeze drying for laboratory methods of preparation of cocoa polyphenol extracts

Figure 2.4 Aqueous extract for freeze drying for laboratory methods of preparation of cocoa polyphenol extracts

Figure 2.5 Concentrated extract Freeze drying for laboratory methods of preparation of cocoa polyphenol extracts

Figure 2.6 Freeze drying for laboratory methods of preparation of cocoa polyphenol extracts

Medium Scale Method

Cocoa fruits were provided by the Cocoa Research and Development Centre, Jengka. The cocoa beans were separated from the cocoa pod husk and soaked in boiling water for 20 minutes. They were then sun-dried for 3 to 4 days, or until the moisture content reached 4-6%. The dried cocoa beans were manually deshelled using a knife and coarsely ground to optimize the extraction process.

Approximately 40-45% of the cocoa butter was removed from the cocoa beans using a mechanical oil press at 100°C. Following this, cocoa polyphenols were extracted using 70% undenatured ethanol in a medium-scale extractor. The extractor unit included a condenser, extraction chamber, 12L round bottom flask, and thimbles capable of holding up to 3kg of defatted ground cocoa nibs. The extraction process took about 30 hours to extract all polyphenols from the cocoa nibs. The cocoa polyphenol extract was concentrated using a rotary evaporator under partial vacuum at 70°C to remove undenatured ethanol. The aqueous extract was then freeze-dried to remove water and stored at -20°C until use.

Figure 2.7 Cocoa beans sun drying for the medium scale method preparation of cocoa polyphenol extracts

Figure 2.8 Defatting cocoa beans for the medium scale method preparation of cocoa polyphenol extracts

Figure 2.9 Extracting cocoa nibs for the medium scale method preparation of cocoa polyphenol extracts

Figure 2.10 Concentrating for the medium scale method preparation of cocoa polyphenol extracts

Figure 2.11 Freeze drying for the medium scale method preparation of cocoa polyphenol extracts

Pilot Scale Method

Cocoa fruits were supplied by the Cocoa Research and Development Centre, Jengka. The cocoa beans were separated from the cocoa pod husk and soaked in boiling water for 20 minutes. Then, the cocoa beans were sun-dried for 3 to 4 days, or until their moisture content reached 4-6%. The cocoa beans were heated at 150-180°C using an infrared micronizer, then deshelled using a winnower and breaker machine.

Approximately 40-45% of the cocoa butter was removed from the cocoa beans using a mechanical oil press at 100°C, followed by the extraction of cocoa polyphenols with 70%

undenatured ethanol using a jacketed extractor. The extractor unit included an extraction chamber capable of holding up to 30kg of defatted ground cocoa nibs. The extraction process took about 4 hours at 40-50°C to extract all polyphenols from the cocoa nibs. The cocoa polyphenol extract was concentrated using a concentrator under partial vacuum at 70°C to remove undenatured ethanol. The aqueous extract was then spray-dried to remove water and stored at -20°C until use.

Figure 2.12 Cocoa beans drying for the pilot scale method preparation of cocoa polyphenol extracts

Figure 2.13 Cocoa beans deshelling for the pilot scale method preparation of cocoa polyphenol extracts

Figure 2.14 Defatting nibs for the pilot scale method preparation of cocoa polyphenol extracts

Figure 2.15 Extracting for the pilot scale method preparation of cocoa polyphenol extracts

Figure 2.16 Concentrating for the pilot scale method preparation of cocoa polyphenol extracts

Figure 2.17 Freeze drying for the pilot scale method preparation of cocoa polyphenol extracts

Determination Of Total Polyphenol Content Using Folin-Ciocalteau Assay

Total polyphenol content in cocoa extract was determined according to the *Folin-ciocalteau* method (Waterhouse, 2002). Chemicals used in this methos was purchase from Sigma-Aldrich, Switerland. Briefly, 0.1 g defatted cocoa seeds were dissolved in 10 ml 70% (v/v) acetone and were sonicated (SONICATOR, USA) for 30 minutes. Samples were centrifuged (Universal 32®, Hettich Zentrifugen, Germany) at 5 000 rpm for 20 minutes. An amount of 100 µl of the supernatant was added with 7.9 ml distilled water followed by 0.5 ml *folin-ciocalteau* reagent (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 (Na_2SO_3) solution was added to the mixture. Mixtures were left 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 and catechin. Results were expressed as gallic acid equivalents (GAE) in milligrams per gram extract.

Statistical Analysis

All data are presented as mean ± S.E.M. (standard error mean). The data were analyzed using ANOVA test and Duncan New Multiple Range Test through Statistic Analysis System version 2.0 (SAS Institute, Cary, NC). Mean difference between groups of variables were tested with variance analysis (ANOVA). Two ended *p* values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSIONS

Our study evaluated the total polyphenol content in unfermented cocoa beans sourced from the Cocoa Research and Development Centre in Jengka (Pahang) across various production scales. The analysis revealed significant differences ($p<0.05$) in the total phenolic content of cocoa extracts, with large-scale production demonstrating a markedly higher polyphenol concentration compared to medium and laboratory scales.

In laboratory extraction using a Soxhlet unit, six extraction chambers, each containing 20g of defatted cocoa nibs, were extracted with 100ml of 70% ethanol for 8 hours. However, a total of 120g of defatted cocoa nibs produced less than 12g of a less water-soluble, brown-colored cocoa polyphenol extract. Results from the *folin-Ciocalteu* method using UV-spectrophotometer detection showed that this freeze-dried extract contained approximately 141.255±14.716 mg GAE/g of total polyphenol content.

Meanwhile, in medium-scale extraction, 3kg of defatted cocoa nibs was extracted with 10L of 70% ethanol in a glass chamber for up to 30 hours. About 250g of purple-colored, water-soluble cocoa polyphenol extract was produced. Results from the *folin-Ciocalteu* method using UV-spectrophotometer detection showed that this freeze-dried extract contained approximately 335.70 mg GAE/g of total polyphenol content.

In large-scale extraction, approximately 70kg of defatted cocoa nibs was extracted with 100L of 70% ethanol in a jacketed stainless-steel chamber with a condenser for 4 hours at 40-50°C. The extract was then concentrated and spray-dried together with a mixture of cocoa polyphenol extract and maltodextrin to produce about 10kg of purple-colored, water-soluble cocoa polyphenol extract powder, which contained approximately 502.18 mg GAE/g of total polyphenol content. Results shows the total phenolic content of cocoa extract, with the large-scale production (IBS UTM) demonstrating a twofold increase compared to medium scale, followed by laboratory scale measuring 502.18 mg GAE/g, 335.70 mg GAE/g, and 141.255±14.716 mg GAE/g, respectively.

These findings are particularly noteworthy as they contradict the common assumption that scaling up production may dilute the concentration of beneficial compounds due to increased handling and processing. Instead, our results indicate that large-scale production can maintain, if not enhance, the polyphenol content in cocoa beans under controlled conditions. This aligns with studies by Cooper *et al.* (2008) and Rusconi and Conti (2010), which emphasize the robustness of cocoa polyphenols during processing.

The significant increase in polyphenol content observed in large-scale production may be attributed to several factors, including optimized extraction techniques and controlled environmental conditions. The use of 70% undenatured ethanol for polyphenol extraction proved effective, aligning with findings from similar studies employing ethanol as a solvent for polyphenol extraction from plant materials (Waterhouse, 2002).

Furthermore, the *folin-Ciocalteu* method employed for polyphenol quantification has been validated in numerous studies for its accuracy and reliability (Singleton *et al.,* 1999). The UV spectrophotometry at 765nm provided consistent and precise measurements, corroborating the high polyphenol content in our cocoa extracts.

Our results also underscore the health implications of cocoa polyphenols. Given their potent antioxidant properties, high polyphenol content in large-scale production suggests that commercial cocoa products could retain significant health benefits, including reduced risks of chronic diseases such as diabetes, cardiovascular disease, and certain cancers (Katz *et al.*, 2011; Scholey and Owen, 2013).

CONCLUSIONS

Our study demonstrated that large-scale production of cocoa polyphenol extracts maintains, and even enhances, the polyphenol content significantly ($p<0.05$) compared to medium and laboratory scales. This contradicts the assumption that scaling up dilutes beneficial compounds, highlighting the robustness of cocoa polyphenols during processing. The high polyphenol content suggests significant health benefits, including reduced risks of chronic diseases. Further study should be carried out to investigate the specific factors contributing to higher polyphenol retention in large-scale production of cocoa polyphenol extract.

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