



## Effect of extraction methods on yield, oxidative value, phytosterols and antioxidant content of cocoa butter

<sup>1</sup>Roiaini, M., <sup>1</sup>Seyed H. M., <sup>2</sup>Jinap, S. and <sup>1\*</sup>Norhayati, H.

<sup>1</sup>Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

<sup>2</sup>Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

### Article history

Received: 19 May 2015

Received in revised form:

23 June 2015

Accepted: 7 July 2015

### Abstract

Cocoa beans are rich in numbers of beneficial bioactive compounds such as phenolics and phytosterols, which benefits to human being. The suitable extraction method is needed to produce high quality and quantity of cocoa butter and other bioactive compounds. There are many extraction method to extract these compounds such as Soxhlet extraction, supercritical fluid extraction, ultrasound extraction method and others. The objective of this study is to determine the effectiveness of the different extraction methods producing high yields of cocoa butter, lower oxidative value, stable phytosterols and antioxidant content. The cocoa beans were subjected to different extraction methods such as Soxhlet extraction (SE), Ultrasonic extraction method (USE), Supercritical carbon dioxide (SCO<sub>2</sub>) and Supercritical carbon dioxide with co-solvent (SCO<sub>2</sub>-Ethanol). Cocoa butter extracted using SCO<sub>2</sub>-Ethanol has significantly ( $p < 0.05$ ) obtained highest cocoa butter yield (37.05%) and phytosterols content (6441 µg/g of extract) compared to SE (28.87% and 4960 µg/g of extract), SCO<sub>2</sub> (31.32% and 5492 µg/g of extract) and USE (34.81% and 5106 µg/g of extract). Meanwhile, the oxidative value of SCO<sub>2</sub>-Ethanol was significantly ( $p < 0.05$ ) obtained lowest value compared to other extraction methods. Extraction method are crucial in cocoa industry to minimise the cost during processing, obtain maximum extraction yield and preserve the bioactive compounds thus will improve the value of cocoa butter. improve the value of cocoa butter.

© All Rights Reserved

### Keywords

Soxhlet extraction

Ultrasonic extraction

Supercritical fluid

extraction

Phytosterols

Antioxidant

Cocoa butter

### Introduction

Cocoa butter is responsible for the melting properties of chocolate and was obtained from cocoa beans around 50–57% yield dry weight (Steinberg *et al.*, 2003). It contain omega-6 and omega-9 fatty acids, and also natural antioxidants such as alpha-tocopherol and phytosterols, as well as nutrients which support mood and the immune system (Norton *et al.*, 2009). The major fatty acids in cocoa butter are palmitic acid (C16) 25–33.7%, stearic acid (C18:0) 33.7–40.2%, oleic acid (C18:1) 26.3–35% and linoleic acid (C18:2) 1.7–3% which contribute to about 98% of the total fatty acid (Asep *et al.*, 2008; Bracco, 1994). The uniqueness of cocoa butter includes brittle at room temperature and fast liquefies at body temperature. Due to their remarkable functional properties, cocoa butter was essentially being used in the food, cosmetic and pharmaceutical industries. In the food industry, the mild texture, mouth feel, flavour release and shimmer of chocolate products were produced from cocoa butter (Liendo *et al.*, 1997; Schilchler-Aronhime and Garti, 1988).

Li and Hartland (1996) stated that hexane has been broadly used to extract cocoa butter in solvent extraction similar to other fats and oils from oil-contained sources. The application of organic solvents has raised the concern for the health and safety hazards. The cleaner extraction method was chosen due to great responsibility over disposal of such toxic organic solvents and their effect on the environment (Jahurul *et al.*, 2014). In contrast, hydraulic method which has been widely used by the cocoa industries always initiates contaminants into the cocoa butter that must be removed later. This places a new alternative in acquiring cocoa butter to emerge clean and efficient technologies.

Latest extraction techniques suitable for cocoa butter extraction are ultrasound-assisted extraction (USE), pressurized liquid extraction, microwave extraction and supercritical fluid extraction (Zuoaga *et al.*, 1998; Schroeter *et al.*, 1999; Hawthorne *et al.*, 2000; Giergielewicz-Mozajska *et al.*, 2001). The differentiation of traditional methods with supercritical extraction, it is practical on producing a good product and has the ability to obtain higher

\*Corresponding author.

Email: [aryatihussain@upm.edu.my](mailto:aryatihussain@upm.edu.my)

Tel: +603-89468536

yields and a superior quality cocoa butter replacers blends (Zaidul *et al.*, 2006; Zaidul *et al.*, 2007a, c). On the other hand, USE has been recognized to extract wide range of herbal and other plant extracts. The propagation of ultrasound pressure waves, develop from cavitation phenomena by ultrasound, resulting in enhancement of extraction (Vilkhu *et al.*, 2008). Ultrasounds cause mechanical effects due to the agitation of the solvent, the increase of contact surface area between solid and solvent and thus, greater infiltration of the solvent into the matrix (Zhang Zh-Sh *et al.*, 2008; Kowalski and Wawrzykowski 2009; Shalmash 2009). Recently, it was shown that the yield of oil by USE was similar to Soxhlet extraction (Da Porto *et al.*, 2013).

The extraction of oil using different extraction methods may produce oils with improved characteristics such as able to produce high yield and preserve the bioactive compounds. Therefore, the goal of this research was to investigate the impact of the different extraction methods of cocoa bean in obtaining maximum yield of cocoa butter, phytosterols and antioxidant content and lower oxidation value.

## Materials and Methods

### Materials

The cocoa beans were purchased from Lembaga Koko Jengka, Pahang, Malaysia. Cocoa shells were removed manually from cocoa beans and the nibs were smashed approximately below 1 mm with a mortar and pestle before being analysed to determine the yield, oxidative value, phytosterols and antioxidant content. The nibs were stored in a dark closed container to prevent from humidity and light at room temperature (24°C).

### Extraction procedures

The cocoa nibs were hydrolyzed with dilute hydrochloric acid (25%) and filtered (Whatman filter paper, 8.0 µm) before extraction processes. The cocoa nibs were extracted using applied methods such as (Soxhlet extraction, Ultrasonic extraction, Supercritical extraction CO<sub>2</sub> and ethanol. The cocoa butter obtained from the extraction procedure mentioned below was analysed for comparison of oxidative value, phytosterols and antioxidant content. The yield of cocoa butter was calculated using the formula below:

$$\text{Yield(\%)} = \frac{\text{Weight of flask (after)} - \text{Weight of flask (before)}}{\text{Weight of sample}} \times 100$$

### Soxhlet extraction

Approximately 5 g of cocoa nibs was weighted into the thimble. Then, 150 ml of ethanol (99.5%) added in 120 ml round bottom flask and refluxed for 6 h using Soxhlet apparatus. The extracts were filtered and concentrated under reduced pressure until dryness (AOCS 1998).

### Supercritical extraction: CO<sub>2</sub> and CO<sub>2</sub>-Ethanol

Supercritical extraction consist of Intelligent HPLC Pump Model PU-1580 (Jasco Corporation, Tokyo, Japan). A Back Pressure Regulator (BPR) Model BP- 1580-81 (Jasco Corporation, Tokyo, Japan) was used to control the extraction pressure. Separation was controlled using Back Pressure Regulator (BPR) (Model BP-1580-81, Jasco Corporation, Tokyo, Japan). Approximately 20 g of cocoa nibs were loaded into 50 ml extraction vessel. CO<sub>2</sub> was performed using carbon dioxide alone at 60°C and 35 MPa, while CO<sub>2</sub>-Ethanol using both CO<sub>2</sub> and ethanol (25%) at 60°C and 35 MPa and flow rate of ethanol was 2 ml/ min (Asep *et al.*, 2008).

### Ultrasonic extraction

Ultrasonic extractions were performed with an ultrasound cleaning bath—Delta/ DC150H. Approximately 20 g of cocoa nibs were extracted with 200 ml of ethanol (99.5%) in an ultrasonic apparatus. The temperature of the bath was controlled at 30°C. The cocoa nibs were immersed in ethanol and homogenized a few minutes before ultrasonicated for 1 h. After the process were repeated twice, the solvent was collected by filtering the extract using Whatman filter No. 1. The solvent containing cocoa butter then were applied with rotary evaporator to remove ethanol (Mariod *et al.*, 2011).

### Oxidative analysis

#### Iodine value (IV)

The IV measures the number of reactive double bonds present in cocoa butter. A higher IV number indicates more double bonds in the cocoa butter. IV was determined according to AOCS method Cd 1d-92 (1993b).

#### Peroxide value (PV)

The PV measures the oxidation processes in oil. PV of cocoa butter was determined according to AOCS method Cd 8-53 (1993c).

#### Acid value (AV)

The acid value (which is twice the free fatty acid (FFA) value) measures the amount of fatty acids

separated from glycerol molecules. The amount of free fatty acid present in cocoa butter was estimated by determining the quantity of alkali that must be added to the fat to become neutral. Acid value of cocoa butter was determined according to AOCS method Ca 5a-40 (1993).

#### *Determination of phytosterols*

The phytosterols composition of the cocoa butter was determined by Careri *et al.* (2001). About 200 mg of cocoa butter was added into 50 ml round bottomed flask followed by the addition of 250  $\mu$ l of 5- $\alpha$  cholestane (1000  $\mu$ g/ml) in methanol as internal standard and 2 ml of 2 M KOH in methanol. The mixture was heated under reflux at 90°C for 1 h. After that, 4 ml of water and ethyl acetate were added to the mixture. The aqueous phase was washed three times with diethyl ether. Finally, the diethyl ether solution was dried with sodium sulfate anhydrous, filtered and then dried with a nitrogen stream. Solid phase extraction (SPE) was applied to perform the clean-up procedure. Silica gel (packing 500 mg/6 ml, Silicycle) SPE tubes were used and the mixture was dissolved in 5 ml hexane-ethyl acetate (95:5, v/v) and 6 ml of hexane-ethyl acetate (60:40) v/v) to perform washing step. The eluate was dried under a nitrogen stream and re-dissolved in 1 ml of hexane. The extracts obtained were analysed by HPLC with UV detection (HPLC-UV). HPLC Waters system consisted of an auto sampler and a spectrophotometric UV-Vis variable-wavelength detector. Separation was carried out using C18 column (150x2.1 mm, 5  $\mu$ m) (Silicycle) under isocratic condition with a mixture of acetonitrile-water (86:14, v/v). The operative wavelength was set at 200.4 nm. The injection volume was 1  $\mu$ l. The samples were injected triplicate.

#### *Determination of antioxidant activity*

Approximately 1 $\pm$ 0.01 g of cocoa butter was added with 10 ml of methanol in water (90:10). Then the solution was placed in an ultrasonic bath for 5 min. Subsequently, the sample was centrifuged for 10 min at 4,000 rpm at 24°C. The extraction procedure was carried out three times. All methanol extracts were combined, filtered and treated by rotary evaporator to dryness. The extracts were kept at -20°C, and just before spectrophotometric determination, they were mixed with 2.5 ml of the concentration of methanol (10:90) and centrifuged as previously. The analysis was done in triplicate (Malićanin *et al.*, 2014).

#### *DPPH assay*

About 3.2 mg of DPPH powder was diluted in 80% methanol. Then, 3.9 ml of DPPH solution was

added to 0.1 ml of extracts. The mixture was kept in dark for 2 h. The absorbance was measured at 517 nm.

#### *Total phenolic content (TPC)*

About 500  $\mu$ l Folin-Ciocalteu and 1.5 ml sodium carbonate (20%) were added in 100  $\mu$ l of the extracts. The mixture was incubated in the dark at room temperature for 90 min. The absorbance was measured at 730 nm. A calibration curve was obtained against standard Gallic acid. The results were expressed as mg of Gallic acid per 100 g of oil.

#### *Statistical analysis*

All analysis were done in triplicate (n=3) and the data were statistically analyzed by one-way analysis of variance (ANOVA) procedure, using Minitab software. Significant differences (p<0.05) between means were determined.

## **Results and Discussion**

From the preliminary study, ethanol was used as solvent since the yield of phytosterols and antioxidant value was higher (4974 mg/100 g of extract, DPPH; 64.87% and TPC; 22.38 mg GAE/100 g of extract) compared to hexane, petroleum ether and 2-propanol.

#### *Yield determination*

The SCO<sub>2</sub>-Ethanol (37.05%) and USE (34.81%) produced significantly (p<0.05) higher yield of cocoa butter compared to SE and SCO<sub>2</sub> (28.87% and 31.32%) (Table 1). The physical and chemical of cocoa butter is very complex and changes depending on the processing it receives. The greatest loss of cocoa butter from cocoa bean was observed in SE due to high temperature applied and no agitation provided during extraction. Each extraction was applied with different temperature. The higher temperature was set on SE at 78°C, while both SCO<sub>2</sub> and SCO<sub>2</sub>-Ethanol was set at 60°C and USE was set at 30°C. Different temperature applied will effect different yield of cocoa butter. The major fatty acid present in cocoa butter are oleic acid (34.5%), stearic acid (34.5%) and palmitic acid (26.0%) (Jahurul *et al.*, 2014). Stearic acid have a nonpolar chain that confers solubility in organic solvent. Thus, cocoa butter can be extract using solvent but the amount of yield obtained was influenced by the processing of the extraction.

Asep *et al.* (2008) stated that SCO<sub>2</sub>-Ethanol has a lot of benefit due to its potential to obtain products clear from sample residues, fast and alternative methods for environmental reasons by the minimum

Table 1. Yield percentage, Iodine value, Peroxide value and Acid value in cocoa butter using different extraction methods

Method of Extraction	Yield (%)	Iodine value (g I <sub>2</sub> /100 g)	Peroxide value (meq O <sub>2</sub> /kg)	Acid value (mg KOH/g oil)
SE	28.87 ± 0.53 <sup>c</sup>	33.97±0.59 <sup>a</sup>	2.20±0.12 <sup>b</sup>	1.58±0.01 <sup>b</sup>
USE	34.81 ± 1.03 <sup>ab</sup>	34.04±0.27 <sup>a</sup>	1.96±0.21 <sup>b</sup>	1.61±0.01 <sup>a</sup>
SCO <sub>2</sub>	31.32 ± 0.78 <sup>bc</sup>	34.31±0.99 <sup>a</sup>	2.49±0.08 <sup>a</sup>	1.60±0.01 <sup>a</sup>
SCO <sub>2</sub> -Ethanol	37.05±1.63 <sup>a</sup>	34.56±0.36 <sup>a</sup>	2.02±0.06 <sup>b</sup>	1.62±0.02 <sup>a</sup>

Results were expressed as means ± SD for triplicates measurement. Means in the same column with different letters were significantly different ( $p < 0.05$ ).

usage of large solvent volumes. The higher yield of cocoa butter obtained from SCO<sub>2</sub>-Ethanol may be due to the attraction of ethanol in extracting fat content in the cocoa bean. Based on study from Saldana *et al.* (2002), they found supercritical carbon dioxide without the use of co-solvent does not able to extract theobromine from cocoa seed. Li and Hartland (1996) also showed that it is difficult to extract either xanthenes or cocoa butter from cocoa nibs using carbon dioxide. There was significant value from 0.10% to 11.91% of xanthenes and 0.06% and 1.45% of cocoa butter was obtained from 0 to 23% of ethanol added in supercritical CO<sub>2</sub> system. Similar to this study, SCO<sub>2</sub> was significantly have a lower cocoa butter obtained compared to SCO<sub>2</sub>-Ethanol. The solubility of ethanol can be markedly enhanced compared with SCO<sub>2</sub> under similar condition.

Kopcak and Mohamad (2005) showed that using ethanol or iso-propanol as co-solvent with supercritical fluid increased the yield of caffeine extraction. Salajegheh *et al.* (2013) reported that the quantity of oil obtained from whole cocoa beans by SCO<sub>2</sub> was too low to form a basis for a commercial extraction process. Therefore, the addition of polar co-solvent ethanol greatly had enhanced cocoa butter solubility, thus produce high yield of cocoa butter from the extraction. Salajegheh *et al.* (2013) stated that supercritical extraction using ethanol as co-solvent has a higher yield due to the solubility of ethanol on carbon dioxide. The extract yield from USE also had a higher value compared to SE and SCO<sub>2</sub>. A study from Péres *et al.* (2006) found that the extraction of USE was higher in mass yield of 4.36% at 60 min compared to SE; 0.42%. The contact of ethanol with cocoa beans during the process of USE may enhanced and accelerated the cocoa butter extraction from cocoa beans compared to SE and SCO<sub>2</sub>.

#### Oxidative analysis

Iodine value (IV) of cocoa butter was insignificantly ( $p > 0.05$ ) affected by the different extraction methods (33.97-34.56 g I<sub>2</sub>/100 g) (Table 1) due to the similarity in the fatty acid composition of the extracted oils. As reported by Bhatnagar and Krishna (2013), the IV of Niger seed was insignificant when applied with the different type of solvents (petroleum ether, hexane, chloroform, acetone, methanol and ethanol).

Similar pattern was shown in the Peroxide values (PV) of the cocoa butter (Table 1). The PV for SE, USE and SCO<sub>2</sub>-Ethanol (2.20, 1.96 and 2.02 meqO<sub>2</sub>/kg) was not significantly different ( $p > 0.05$ ) from others except for SCO<sub>2</sub> (2.49 meqO<sub>2</sub>/kg). The high values of PV from SCO<sub>2</sub> occur maybe due to the oxidation by carbon dioxide. Oxidation of CO<sub>2</sub> may cause unwanted effects during extraction. Calvo *et al.* (1994) stated that oxidative stability of supercritical extracted sunflower oil could be enhanced using ultra-pure CO<sub>2</sub> (without oxidation). The lower the PV will contributed to the better quality of oil.

The level of acid value (AV) in the cocoa butter was not significantly different ( $p > 0.05$ ) except for SE (1.58 mg KOH/g oil) (Table 1). High temperature during the extraction process and period of ethanol exposed to the cocoa nibs during USE may have either separated lots of fatty acid from glycerol molecules thus accelerate the hydrolytic rancidity. The level of AV value was higher in SCO<sub>2</sub> and SCO<sub>2</sub>-Ethanol also might be caused by oxidation of CO<sub>2</sub> thereby produced water vapour that increases the hydrolytic rancidity (Crowe *et al.*, 2002).

#### Phytosterols content

Table 2 shows the phytosterols content in cocoa butter from three different extraction methods. The campesterol content in the extract using SCO<sub>2</sub> and USE was not significantly higher ( $p > 0.05$ ) (2001

Table 2. Phytosterols content in cocoa butter using different extraction method

Method of Extraction	Phytosterol composition ( $\mu\text{g/g}$ of extract)			
	Beta sitosterol	Campesterol	Stigmasterol	Total phytosterols
SE	1911 $\pm$ 0.02 <sup>a</sup>	1404 $\pm$ 0.03 <sup>b</sup>	1645 $\pm$ 0.02 <sup>c</sup>	4960 $\pm$ 0.01 <sup>b</sup>
USE	2030 $\pm$ 0.01 <sup>a</sup>	1404 $\pm$ 0.04 <sup>b</sup>	1672 $\pm$ 0.02 <sup>c</sup>	5106 $\pm$ 0.02 <sup>b</sup>
SFE-CO <sub>2</sub>	2001 $\pm$ 0.01 <sup>a</sup>	1525 $\pm$ 0.01 <sup>b</sup>	1967 $\pm$ 0.01 <sup>b</sup>	5492 $\pm$ 0.01 <sup>b</sup>
Co-solvent SFE	1925 $\pm$ 0.27 <sup>a</sup>	1721 $\pm$ 0.07 <sup>a</sup>	2795 $\pm$ 0.08 <sup>a</sup>	6441 $\pm$ 0.11 <sup>a</sup>

Results were expressed as means  $\pm$  SD for triplicates measurement. Means in the same column with different letters were significantly different ( $p < 0.05$ ).

and 2030  $\mu\text{g/g}$  of extract) compared to SE and SCO<sub>2</sub>-Ethanol (1911 and 1925  $\mu\text{g/g}$  of extract). Nevertheless, no significant difference ( $p > 0.05$ ) was found for stigmasterol content among SE, USE and SCO<sub>2</sub> in extracted cocoa butter except for SCO<sub>2</sub>-Ethanol. On the other hand, beta-sitosterol content was significantly higher ( $p < 0.05$ ) in SCO<sub>2</sub>-Ethanol, followed by SCO<sub>2</sub> and no significant difference was found between SE and USE. Total phytosterols in SCO<sub>2</sub>-Ethanol (6441  $\mu\text{g/g}$  of extract) was significantly higher compared to SCO<sub>2</sub> (5492  $\mu\text{g/g}$  of extract), USE (5106  $\mu\text{g/g}$  of extract) and SE (4960  $\mu\text{g/g}$  of extract). The solubility of phytosterols in CO<sub>2</sub> in the presence of ethanol increases greatly compared to other extraction methods. Without co-solvent, the separation of solute in supercritical CO<sub>2</sub> from the solvent will be not easy. Distillation or evaporation are needed to obtained the desired products and regenerate the solvent (Li and Hartland, 1996).

The total phytosterols content in cocoa butter using SCO<sub>2</sub> was not significant with SE and USE similar with the study by Bernardo-gil and Grenha (2002). They stated that the contents of free fatty acids, phytosterols, triacylglycerols and tocopherols in the hazelnut oil extracted by SCO<sub>2</sub> were equivalent with soxhlet extraction. From the four methods used in this study, SE method showed the lowest amount of total phytosterols (4960  $\mu\text{g/g}$  of extract) compared to the others. SCO<sub>2</sub>-Ethanol method was among the suitable method in extracting phytosterols due to moderate pressure and temperature, higher diffusion coefficient, lower viscosity and surface tension compared to solvent and able to generate excellent mass transfer (Wang and Weller 2006). The operating temperature in both SCO<sub>2</sub> and SCO<sub>2</sub>-Ethanol (60°C) was much lower than SE method (78°C). Thus, higher thermal applied to the cocoa beans might degrade more phytosterols compared to moderate

temperature. The homogenization procedure during USE with ethanol for a few minutes also may degrade the phytosterols composition. Chemat *et al.* (2004) stated that Scanning electron micrographs (SEM) had shown the wrecking of cell walls and liberation of cell contents due to great agitation from USE in the system, thus encourage the ruptured and damaged the compounds.

#### *Antioxidant assay (AA) and Total phenolic content (TPC)*

Figures 1 and 2 shows the results of DPPH assay and TPC of the cocoa butter from different extraction methods. From Figure 1, the DPPH assay of cocoa butter from SCO<sub>2</sub>-Ethanol and SCO<sub>2</sub> extraction show significant value ( $p < 0.05$ ) (84% and 82.92% DPPH) compared to SE and USE (44.72% and 37.91%).

The TPC from SCO<sub>2</sub> and SCO<sub>2</sub>-Ethanol was significantly higher ( $p < 0.05$ ) (29.5 mg GAE/100 g extract and 28.77 mg GAE/100 g extract) compared to SE and USE (21.77 mg GAE/100 g extract and 14.42 mg GAE/100 g extract). The TPC value was the highest in SFE-CO<sub>2</sub> extracts, but unlikely for DPPH value. Low DPPH and TPC from SE and USE method might be due to high thermal involved during extraction, direct contact of cocoa bean with ethanol during processing and homogenization of cocoa nibs in ethanol possibly affected the polyphenolic content. Arlorio *et al.* (2008) also stated that various heat-based treatment steps, pressure or collision of ultrasound waves changed the content and the composition of polyphenolic proportion of cocoa beans. Azizah *et al.* (1999) stated that various causes such as variation of cocoa, stage of beans mature, the use of solvent and the technique of extraction influenced the efficiency of polyphenols extraction. Thus, the application of different techniques such as different temperature, pressure and level of homogenization in each

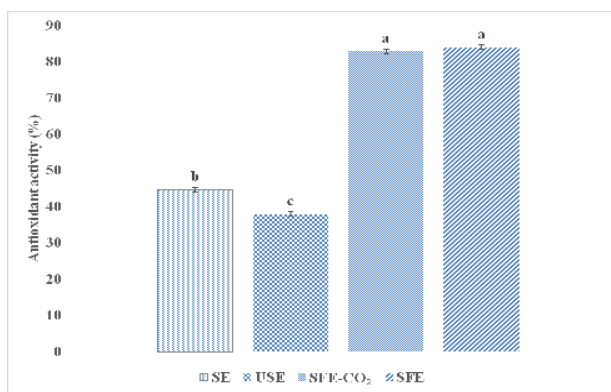


Figure 1. DPPH assay for different types of extraction methods

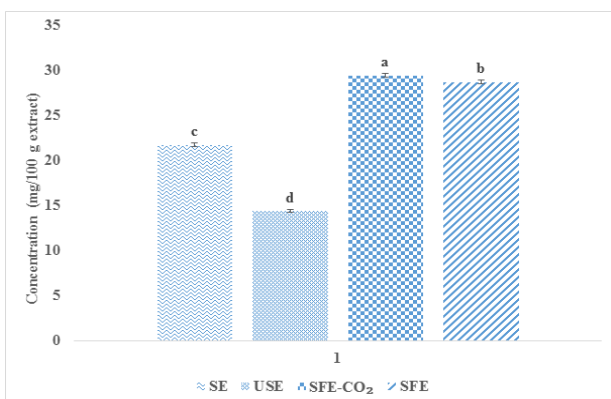


Figure 2. TPC values for different types of extraction methods

extraction method of cocoa nibs in this study may deteriorated the phenolic content in different amount.

From the results, high DPPH does not indicate the presence of phenols in cocoa extracts. Othman *et al.* (2007) stated that total antioxidant/antiradical capacity was influenced by the present of methyl xanthines (theobromine and caffeine), minor flavonoids and pigments in the cocoa extracts. The solubility of other compounds in water, ethanol and other solvents also contribute to the high antioxidant content other than phenolics.

## Conclusion

The different extraction methods significantly affected the yield, phytosterols and antioxidant content of cocoa butter. The SFE with co-solvent able to extract more cocoa butter, higher yield of phytosterols and antioxidant compared to other methods. However, their oxidative value does not affected by extraction methods due similar types of cocoa bean used in study. Carbon dioxide present in the SCO<sub>2</sub> promotes oxidation of the oil extract. The SCO<sub>2</sub>-Ethanol is much simpler and more effective than SE, USE and SCO<sub>2</sub> method in obtaining high phytosterols and antioxidant content. SE was not recommended for extraction because it requires

extensive extraction time and have minimum efficiency. Furthermore, as the temperature is elevated during the extraction, the natural constituent may be deteriorating because of their irresistible to thermal heat. Moreover, this method required a long extraction time and large amount of organic solvent. The requirement of large amount of solvent may cause additional costs and danger to environmental due to waste of poisonous solvents. This finding may be benefits the cocoa industry to produce cocoa butter with high yield and preserve the bioactive and antioxidant content.

## Acknowledgement

This research was supported by Universiti Putra Malaysia with grant project number GP-IPS/2013/9399841.

## References

- American Oil Chemists' Society (AOCS). 1993. Method Ca 5a-40. Official Methods and Recommended Practices of the American Oil Chemists' Society Champaign.
- American Oil Chemists' Society (AOCS). 1993b. Method Ca 5a-40. Official Methods and Recommended Practices of the American Oil Chemists' Society Champaign.
- American Oil Chemists' Society (AOCS). 1993c. Method Cd 1d-92. Official Methods and Recommended Practices of the American Oil Chemists' Society Champaign.
- American Oil Chemists' Society (AOCS). 1998. Method 945.16. Official Methods and Recommended Practices of the American Oil Chemists' Society Champaign.
- Asep, E.K., Jinap, S., Tan, T.J., Russly, A.R., Harcharan, S. and Nazimah, S.A.H. 2008. The effects of particle size, fermentation and roasting of cocoa nibs on supercritical fluid extraction of cocoa butter. *Journal of Food Engineering* 85: 450–458.
- Azizah, A., Nik Ruslawati, N. and Swee Tee, T. 1999. Extraction and characterization of antioxidant from cocoa by-products. *Food Chemistry Journal* 64: 199–202.
- Bernardo-gil, M.G., Grenha, J., Santos, J. And Cardoso, P. 2002. Supercritical fluid extraction and characterisation of oil from hazelnut. *European Journal of Lipid Science and Technology* 107: 402–409.
- Bhatnagar, A.S. and Krishna, A.G.G. 2013. Effect of Extraction Solvent on Oil and Bioactives Composition of Commercial Indian Niger (*Guizotia abyssinica* (L.f.) Cass.) Seed. *Journal of American Oil Chemists Society* 90: 1203–1212.
- Bracco, U. 1994. Effect of triglyceride structure on fat absorption. *The American Journal of Clinical Nutrition* 60: 1002–S1009.
- Calvo, L., Cocero, M.J. and Diez, J.M. 1994. Oxidative

- Stability of Sunflower Oil Extracted with Supercritical Carbon Dioxide. *Journal of American Oil Chemists Society* 71: 1251–1254.
- Careri, M., Elviri, L. and Mangia, A. 2001. Liquid chromatography-UV determination and liquid chromatography-atmospheric pressure chemical ionization mass spectrometric characterization of sitosterol and stigmaterol in soybean oil. *Journal of Chromatography A* 935: 249–257.
- Chemat, S., Lagha, A., AitAmar, H., Bartels, P.V. and Chemat, F. 2004. Comparison of conventional and ultrasound-assisted extraction of carvone and limonene from caraway seeds. *Flavour and Fragrance Journal* 19: 188–195.
- Crowe, T.D., Crowe, T.W., Johnson, L.A. and White, P.J. 2002. Impact of Extraction Method on Yield of Lipid Oxidation Products from Oxidized and Unoxidized Walnuts. *Journal of American Oil Chemists Society* 79: 453–456.
- Da Porto, C., Porretto, E. and Decorti, D. 2013. Comparison of ultrasound- assisted extraction with conventional extraction methods of oil and polyphenols from grape (*Vitis vinifera* L.) seeds. *Ultrasonics Sonochemistry Journal* 20: 1076–1080.
- Giergielewska-Mozajska, H., Da Browski, L. and Namiesnik, J. 2001. Accelerated Solvent Extraction (ASE) in the Analysis of Environmental Solid Samples - Some Aspects of Theory and Practice. *Critical Review in Analytical Chemistry* 31: 149-65.
- Hawthorne, S.B., Grabanski, C.B., Martin, E. and Miller, D.J. 2000. Comparisons of Soxhlet extraction, pressurized liquid extraction, supercritical fluid extraction and subcritical water extraction for environmental solids: recovery, selectivity and effects on sample matrix. *Journal of Chromatography A* 892: 421-433.
- Jahurul, M.H.A., Zaidul, I.S.M., Norulaini, N.A.N., Sahena, F., Abedin, M.Z., Ghafoor, K. and Mohd Omar, A.K. 2014. Characterization of crystallization and melting profiles of blends of mango seed fat and palm oil mid-fraction as cocoa butter replacers using differential scanning calorimetry and pulse nuclear magnetic resonance. *Food Research International Journal* 55: 103-109.
- Kopcak, U. and Mohamed, R.S. 2005. Caffeine solubility in supercritical carbon dioxide/co-solvent mixtures. *Journal of Supercritical fluids* 34: 209-214.
- Kowalski, R. and Wawrzykowski, J. 2009. Effect of ultrasound assisted maceration on the quality of oil from the leaves of thyme, *Thymus vulgaris* L. *Flavour and Fragrance Journal* 24: 69–74.
- Li, S. and Hartland, S. 1996. A new industrial process for extracting cocoa butter and xanthines with supercritical carbon dioxide. *Journal of American Oil Chemists Society* 73: 423–429.
- Liendo, R., Padilla, F.C. and Quintana, A. 1997. Characterization of cocoa butter extracted from Criollo cultivars of *Theobroma cacao*. L. *Food Research International Journal* 30: 727–731.
- Malićanin, M., Rac, V., Antić, V., Antić, M., Palade, L.M., Kefalas, P. and Rakić, V. 2014. Content of Antioxidants, Antioxidant Capacity and Oxidative Stability of Grape Seed Oil Obtained by Ultra Sound Assisted Extraction. *Journal of American Oil Chemists Society* 91: 989–999.
- Mariod, A.A., Matthäus, B. and Ismail, M. 2011. Comparison of Supercritical Fluid and Hexane Extraction Methods in Extracting Kenaf (*Hibiscus cannabinus*) Seed Oil Lipids. *Journal of American Oil Chemists Society* 88: 931–935.
- Steinberg, F.M., Bearden, M.M., Keen, C.L. 2003. Cocoa and chocolate flavonoids: implications for cardiovascular health—a review. *Journal of the American Dietetic Association* 103, 215–223
- Othman, A., Ismail, A., Abdul Ghani, N. and Adenan, I. 2007. Antioxidant capacity and phenolic content of cocoa beans. *Food Chemistry Journal* 100: 1523–30.
- Péres, V. F., Saffi, J., Melecchi M. I. S., Abad, F. C., Jacques, R. A., Martinez, M. M., Oliveira, E. C. and Caramão, E. B. 2006. Comparison of soxhlet, ultrasound-assisted and pressurized liquid extraction of terpenes, fatty acids and Vitamin E from *Piper gaudichaudianum* Kunth. *Journal of Chromatography A* 1105: 115-118.
- Salajegheh, D., Vaziri, A. and Bastani, D. 2013. Supercritical Extraction of Cocoa Butter from Cocoa Seed, Using Pure Carbon Dioxide, Carbon Dioxide with Ethanol as Co-Solvent and Ethane. *Middle East Journal of Scientific Research* 13: 1010-1015.
- Saldana, M.D.A., Mohamed, R.S. and Mazzafera, P. 2002. Extraction of cocoa butter from brazilian cocoa beans using supercritical CO<sub>2</sub> and ethane. *Fluid Phase Equilibria Journal* 194-197: 885-894.
- Schlichter-Aronhime, J. and Garti, N. 1988. Solidification and polymorphism in cocoa butter and the blooming problems. In: N. Garti and K. Sato, ed. *Crytallization and polymorphism of fats and fatty acids*. New York: Marcel Dekker, 363-393.
- Schroeter, F., Anastassiades, M. and Scherbaum, E. 1999. Comparison of ASE (accelerated solvent extraction) with Traditional Extraction Methods. *Chemistry Labor Biotechnology Journal* 50: 4-6.
- Vilkhu, K., Mawson, R., Simons, L. and Bates, D. 2008. Applications and opportunities for ultrasound assisted extraction in the food industry- A review. *Innovative Food Science and Emerging Journal* 9: 161–169.
- Wang, L. and Weller, C.L. 2006. Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science and Technology Journal* 17: 300–312.
- Zaidul, I.S.M., Norulaini, N.A.N., Mohd Omar, A.K. and Smith Jr, R.L. 2006. Supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction and fractionation of palm kernel oil from palm kernel as cocoa butter replacers blend. *Journal of Food Engineering* 73: 210–216.
- Zaidul, I.S.M., Norulaini, N.A.N., Mohd Omar, A.K. and Smith Jr, R.L. 2007a. Separation of palm kernel oil from palm kernel with supercritical carbon dioxide using pressure swing technique. *Journal of Food Engineering* 81: 419–428.
- Zaidul, I.S.M., Norulaini, N.A.N., Mohd Omar, A.K. and

- Smith Jr, R.L. 2007c. Blending of supercritical carbon dioxide (SC-CO<sub>2</sub>) extracted palm kernel oil fractions and palm oil to obtain cocoa butter replacers. *Journal of Food Engineering* 78: 1397–1409.
- Zhang, Zh-Sh, Wang, L.J., Li, D., Jiao, Sh-Sh, Chen, X.D. and Mao, Z.H. 2008. Ultrasound-assisted extraction of oil from flaxseed. *Separation and Purification Technology Journal* 62: 192–98.
- Zuloaga, O., Etxebarria, N., Fernandez, L.A., Madariaga, J.M. 1998. Comparison of accelerated solvent extraction with microwave-assisted extraction and Soxhlet for the extraction of chlorinated biphenyls in soil samples. *Trends in Analytical Chemistry* 17 (10): 642-647.