Antioxidants and Characterization of Stability and Organoleptic Properties of Cocoa Facial Mask

Siti Salwa Abd Gani1,2, Alyaa Nurathirah Abd Halim2, Uswatun Hasanalh Zaidan3, Mohd Izuan Effendi Halmi4, Norliza Abdul Wahab5

1 Department of Agriculture Technology, Faculty of Agriculture,
2 Halal Products Research Institute, Putra Infoport,
3 Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences,
4 Department of Land Management, Faculty of Agriculture,
5 Malaysia Cocoa Board, Cocoa Innovative and Technology Centre, Lot 12621 Nilai Industrial Area, 71800 Nilai, Negeri Sembilan, Malaysia
1, 2, 3, 4 Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ssalwaag@upm.edu.my

Abstract. Beauty products plant based are getting increasing interest from users and the cosmetic industry. Cocoa liquor is a natural source of antioxidants from (Theobroma cacao L.) cocoa beans, with potential health benefits. The present study was conducted to determine the stability of cocoa facial mask in different temperature, the durability of CFM in extreme high and low temperature and the prospect of integrating CFM offers additional skin defence capability by eliminating free radicals from the environment. CFM shown stabled with different storage conditions. The amount of ferric ion reducing power (FRAP) in CFM was evaluated by the reduction of ferric-TPTZ to blue ferrous-TPTZ color, while β-carotene linoleate bleaching (β-CB) assay were determined by using β-carotene emulsion. FRAP and β-carotene recorded values of 252.31 ± 0.001 mmol Fe2+/g and 83.42 ± 0.03%, respectively. The EC50 of β-carotene linoleate bleaching (β-CB) reported at 2.92 ± 0.03 mg/mL. The results suggest that CFM is a potential source of phytochemicals. The study presents scientific validation on its phytochemical contents showing presence of bioactive compounds with nutritional and therapeutic values which may have positive impact on skin health and suggesting its prospective use in value-added products such as skin care cosmetics.

1. Introduction
Skin aging is an unavoidable process that occurs daily resulting in slower functionality of the skin. Changes in face appearance are caused by the apparent of skin wrinkles, sagging skin, sunspot or age spot, less firmness and elastic, dryness of skin, slower skin turnover and other signs. The science of skin aging involves soft-tissues and bone structure [1]. There are two types of aging process which are intrinsic aging that naturally occurs in the body which is uncontrollable and extrinsic aging is caused by external factors and the aging process is controllable. Extrinsic aging linked with sun exposure, pollution and chemicals. However, the process of skin aging can be delayed with topical anti-aging products.
Empirical studies have documented various beneficial effects of cocoa liquor, also called chocolate liquor, unsweetened chocolate, cocoa mass or simply liquor on skin [2]. The effects of cocoa on skin either by consumption or topical applications are giving positive feedbacks on products for skin tone, hydration, elasticity, wrinkles reduction, improvement on skin complexion, improvements in skin thickness and density [2], [3], [4]. Polyphenols compounds found in cocoa are particularly rich in flavonoids, specifically flavanols or flavan-3-ols such as catechin and epicatechin. However, other existing compounds also contributes in antioxidant activity against free radical for example tannin[5]. Thus, β-carotene linoleate bleaching (β-CB) and ferric ion reducing power (FRAP) used to confirm the ability of compounds in cocoa facial mask by neutralizing free radicals.

Upon this background, the present study was aim to prepare the cocoa facial mask and study the stability of facial mask in different temperature. The antioxidant contents in cocoa facial mask with a view of determining its potentiality in skin care cosmetics giving the product an added ability in protecting the skin in fighting free radicals from the environment.

2. Materials and Methods

2.1. Cocoa Liquor

2.1.1. General. Cocoa liquor was directly purchased from Pusat Inovatif dan Teknologi Koko (Cocoa Innovative and Technology Centre) Nilai, Negeri Sembilan, Malaysia. Ingredients that made up CFM such as emulsifier, viscosity inducing agent, emollient, white clay powder and wetting agent were purchased from Gattefossé.

2.1.2. Formulation of Cocoa Facial Mask (CFM). Firstly, CL melted in an oven at 40°C. The ingredients in each phases were weighted according to Table 1 Each phases was mixed in a beaker using a homogenizer Heindolph, Germany) at 1000 rpm for about 40.0 ± 5.0 minutes. Fresh CFM was left to rest for 24 hours before any evaluation. After 24 hours, the pysico-chemical properties of CFM was evaluated.

| Table 1. Formulation List of CFM |
|----------------------------------|
| Ingredients                  | Amount (%) |
| Oil Phase                    |            |
| Cocoa Liquor                 | 50         |
| Emulsifier                   | q.s        |
| Emollient                    | q.s        |
| Wetting agent                | 8          |
| Dry Phase                    |            |
| White Clay Powder            | 20         |
| Preservatives                | q.s        |
| q.s = quantum satis (Latin term, meaning the amount which is enough) |

2.2. Stability Test
CFM was weighed about 100 g and poured in opaque polyethylene bottle and placed in the following storage conditions in order to evaluate its preliminary physico-chemical stability [6], [7]: low temperatures (5.0 ± 1.0°C), oven (40.0 ± 2.0°C), room temperature with exposure to sunlight (22.0 ± 5.0°C) and room temperature protected from sunlight (22.0 ± 2.0°C). The procedure was counted as Day 0 after 24 hours in each condition. CFM sample was analyzed weekly for 28 days according to the following parameters: organoleptic characteristics (visual aspect, application and touch, color, odor, pH value and rheology).

2.3. Freeze-thaw cycles
CFM formulation was freshly prepared and weighed about 100 g and placed in a chiller for freezing at -10°C temperature for 24 hours. Subsequently, the CFM was thawed at room temperature for 24 hours and later placed in an oven at higher temperature 40°C for 24 hours and again placed at room temperature for 24 hours. The CFM was analyzed for significant changes. This completes one cycle. The process was repeated for 4 cycles.

2.4. Organoleptic Properties (Aspect, Odor, Application, pH, viscosity, color)
The pH value of CFM was detected by Delta 320 pH meter (Mettler-Toledo, Schwerzenbach). Standard buffer solutions of both pH 4.01 and pH 7.00 were used for calibration purposes of the pH meter. The CFM sample was weighted (1 g) and dissolved in (9 ml) of distilled water. Determination of viscosity using AR-G2 controlled stress rheometer (TA Instruments®, New Castle, USA), linked to a computer system (TA Instruments Universal Analysis 2000 Software). For color was determined using spectrophotometer (Konica Minolta CM-5, Tokyo, Japan) and reported using (ΔE) calculated from “L”, “a”, “b” systems. The CFM was comparable with pure cocoa liquor and selected commercial brand.

2.5. CFM extraction and drying
The melted CFM containing bioactive antioxidant compounds were prepared following the procedure described by [8] with some minor modifications. The CFM defatted with hexane and after air-drying to remove excess hexane. The defatted CFM soaked with solvent overnight and filtered. The CFM extracts were concentrated in a rotary evaporator (IKA, Germany) until the solvent was evaporated. The extracts CFM placed in freeze dryer turns in powder form. The extract was kept at 2°C prior to further analyses.

2.6. Determination of Ferric Ion Reducing Power (FRAP)
For FRAP assay was determined based on the reduction of ferric-TPTZ to blue ferrous-TPTZ color measured at 593 nm using the methods from [9]. For FRAP solution involved two solutions of ferric chloride solution (20 mM of FeCl3.6H2O) and TPTZ solutions (2,4,6-tri((2-pyridyl)-s-triazine; 10 mM in 40 mM hydrochloric acid) were prepared. Twenty μL of CFM was added to 300 μL to FRAP solution and immediately measured the absorbance using spectrophotometer. The FRAP value calculated from the standard of ferrous sulphate (FeSO4.7H2O) and express the result as mM Fe2+/g DW.

2.7. Determination of β-carotene Linoleate Bleaching (β-CB) Assay
Antioxidant activity was determined according to method as described by [10] with minor modifications. Two milligram of β-carotene powder (MP Biomedicals) was dissolved in 0.2 mL chloroform (Merck), followed by addition 0.2 mL of linoleic acid (Sigma-Aldrich), and 2 mL of Tween 20 (Merck) in round bottom flask. Chloroform was removed with a rotary evaporator at 50 °C. Following the procedure, 100 mL of distilled water was added and shaken vigorously to form β-carotene emulsion. 200 μL of the β-carotene emulsion was added to 20 μL of the tested solution CFM extracts. A blank was prepared as described above without the addition of β-carotene solution. Measurement of the mixture at 450 nm was carried out after incubation for 20 minutes at 50 °C using
UV spectrophotometer (Infinite M200, Tecan). Measurement was monitored for 2 hours at 30 minutes intervals. Calculation of antioxidant activity was expressed as percent of degradation rate of CFM to degradation rate of control. The EC50 (effective concentration at 50%) was determined and BHT as positive control.

2.8. Statistical analysis
All data were expressed as mean ± standard deviation and independent analyses were performed in triplicates. Data were evaluated using one-way (unstacked) analysis of variance (ANOVA) using Tukey test by Minitab Software version 14.

3. Results and Discussion

3.1. Formulation of CFM
The formulation of CFM was a moisturizing cream clay mask and after homogenizer with high shear and pressure, was cocoa cream clay mask for therapeutic value.

3.2. Stability test, freeze-thaw cycles with organoleptic characteristics
The results of stability test and freeze-thaw cycle were obtained from organoleptic characteristics in formulation of CFM with pH, color and viscosity values determination was shown in Table 3 and Table 4.

The Aspect evaluation for chiller (low temperature), oven (high temperature) and room temperature with or without sun exposure depicted same consistency along the fourth weeks (visual observation) in stability test whilst the same resultants showed in freeze-thaw in 4 cycles. For Odor assessment of CFM was preserved due to no added fragrance and natural scents via cocoa liquor. The evaluation of Application and Touch showed the pleasant touch with acceptable adherence and spreadability. The CFM easy to spread on the skin. The color values was measured using “L”, “a”, “b” systems and the results expressed in ∆E indicates both stability test and freeze-thaw cycles there were no changed in color since the colorant through cocoa liquor. To compare with pure cocoa liquor showed the color value of 22.2 ± 0.06. The color values of CFM increased with addition of other ingredients and the values was sustained until fourth weeks and cycles. From the Table 2, pH values varied over the time. However, still in the normal skin pH ranging about 4-6 to maintain the skin barrier [11], [12]. The viscosity values for both stability test and freeze-thaw cycles were maintained and near to each other’s. The viscosity of commercial brand was measured and recorded at 25.17 ± 2.16 Pa.s which closed to CFM viscosity.

3.3. Determination of Ferric Ion Reducing Power (FRAP)
The FRAP absorbance data were calculated against a series of dilutions of ferrous sulphate FeSO₄.7H₂O as standard calibration curve. The result was expressed as mmol Fe²⁺/g. In the present study on determining the potentiality of CFM extracts act as reducing power, data were comparable with 3,5-tert-4-butylhydroxytoluene (BHT) as a standard. The results of the serial dilutions in both BHT and CFM extracts were performed from 1000 to 31.25 mg/mL.

Table 2 shows that at 1000 mg/mL concentration, both CFM extracts and BHT exhibited the highest antioxidant potential based on the FRAP assay. As concentration of CFM extracts increased, the antioxidant potential (of CFM extracts) increased. According to Benzie and Strain (1996), FRAP measured the ability of a compound in an extract particularly in CFM to reduce Fe³⁺ to Fe²⁺ by breaking free radical chain through donating hydrogen atom and these reaction could be seen by the changing of the color to deep blue with low pH requirement.

A study by [13] reported that the determination of FRAP on CL in methanol extracts was highest antioxidant potential compared with water extracts which exhibited lower. In addition, CFM extracts in ethanol showed higher FRAP value of 252.31 ± 0.001 mmol Fe²⁺/g. Other reports claimed that CL
had the highest antioxidant capacity due to contribution of phenolic compounds such as catechin and epicatechin [14]. The present study confirmed that the antioxidant compounds of cocoa liquor in CFM acted as reducing agents in combating ferric ions to ferrous ion, indicating a good reducing power in protecting the skin from free radicals.

**Table 2. FRAP values for CFM and BHT**

| Concentration (mg/mL) | FRAP value (x10⁻⁴ mmol Fe²⁺/g) |
|-----------------------|---------------------------------|
|                       | BHT                             | CFM                             |
| 1000                  | 1129.02 ± 0.001                 | 252.31 ± 0.001                  |
| 500                   | 514.87 ± 0.013                  | 100.34 ± 0.006                  |
| 250                   | 151.60 ± 0.009                  | 46.40 ± 0.001                   |
| 125                   | 89.56 ± 0.003                   | 22.12 ± 0.001                   |
| 62.5                  | 33.81 ± 0.001                   | 10.43 ± 0.003                   |
| 31.25                 | 12.23 ± 0.00                   | 3.057 ± 0.001                   |

Data are expressed as mean ± standard deviation, triplicates of three independent samples

Significant differences between the concentrations (p<0.005) using Tukey’s test

3.4. Determination β-carotene Linoleate Bleaching Assay

β-carotene is a secondary metabolite that belongs to a group of carotenoids. Chemical structure of β-carotene represents a long chain of conjugated carbon-hydrogen double bond which also presents in red and yellow color oranges [15]. In β-carotene bleaching assay, hydroperoxides act as free radicals from linoleic acid that bleaches the β-carotene at 50°C. Protecting β-carotene from being bleached, antioxidants in the CFM extracts are used to estimate the potentiality as protecting agents.

In β-carotene assay, CFM as an antioxidant preventing bleaching events from hydroperoxides of linoleic acid, BHT was used as a standard. In the present study, serial dilutions of CFM and BHT were performed from 1000 to 7.81 mg/mL as shown in Figure 1. In the present study, CFM exhibited the highest antioxidant value at concentration of 1000 mg/mL with a value of 83.42 ± 0.03%. At decreasing concentrations from 500 mg/mL to 7.81 mg/mL, antioxidant activities were recorded lower with decreasing percentage. BHT as a comparison showed slightly higher antioxidant values in protecting β-carotene. Higher antioxidant β-carotene activity in CFM was due to other compounds beside phenolic acids present in cocoa liquor that were soluble in ethanol. Results from previous study showed that antioxidant activities (β-carotene) in cocoa pod increased with increasing concentration [16] while cocoa beans in water extracts showed good protecting agent. According to [17], the presence of antioxidant specifically CFM prevented β-carotene radicals to form by slowing discoloration of β-carotene emulsion without rapid lost of double bond. This suggests that CFM was a good antioxidant agent in protecting the skin from lipid peroxyl radicals.

Effective concentration in 50% (EC₅₀) value was determined from the plotted graph of antioxidant activity in β-carotene against the concentration of CFM extract. EC₅₀ is the amount of antioxidant necessary to decrease the initial protecting β-carotene concentration by 50% [18]. The EC₅₀ of CFM extracts was recorded at 2.92 ± 0.03 mg/mL whilst BHT showed a value of 1.44 mg/mL. The slight difference in values of both CFM and BHT extracts indicated that a low concentration was required to protect β-carotene bleaching effect of linoleic acid.
Table 3. The Organoleptic Properties of Accelerated Stability test of CFM

| Parameters            | Storage Conditions |   |   |   |   |   |   |   |   |   |   |   |   |   |
|-----------------------|--------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|
|                       | Chiller            | Oven | RT with sun exposure | RT without sun exposure |
|                       | Weeks             | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Aspect                | N                 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Application/Touch     | A                 | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A |
| Odor                  | N                 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Color                 | 25.31 ± 0.35      | 26.29 ± 0.01 | 24.72 ± 0.02 | 25.78 ± 0.01 | 25.78 ± 0.01 | 24.74 ± 0.01 | 25.66 ± 0.01 | 24.89 ± 0.01 | 26.05 ± 0.01 | 26.12 ± 0.01 | 25.47 ± 0.01 | 24.97 ± 0.01 | 25.97 ± 0.01 | 25.55 ± 0.01 |
| pH                    | 5.76 ± 0.03       | 5.62 ± 0.18 | 5.40 ± 0.17  | 5.51 ± 0.17  | 5.54 ± 0.17  | 5.37 ± 0.17  | 5.43 ± 0.17  | 5.42 ± 0.17  | 5.44 ± 0.17  | 5.45 ± 0.17  | 5.55 ± 0.17  | 5.56 ± 0.17  | 5.46 ± 0.17  | 5.33 ± 0.17  |
| Viscosity             | 24.46 ± 1.01      | 25.80 ± 3.51 | 24.16 ± 3.81 | 24.84 ± 2.70 | 24.14 ± 3.70 | 22.04 ± 2.70 | 24.80 ± 2.70 | 24.98 ± 3.70 | 25.11 ± 3.70 | 25.57 ± 3.70 | 24.86 ± 3.70 | 25.10 ± 3.70 | 25.53 ± 3.70 | 25.55 ± 3.70 |

Legend: Chiller; oven; room temperature with sun exposure; room temperature without sun exposure; Aspect: N-Normal; M-Modified; IM-Intensely Modified; Application: A-Pleasant touch, easy skin application (spreadability); D-Unpleasant touch, sticky, difficult to apply on skin (spreadability); MD –Very unpleasant touch, very sticky, compromises skin application; Odor: N-Normal; M-Modified; IM-Intensely Modified; Color: ∆E values; pH: pH values; Viscosity: values measured in Pa.s using rheometer.

Table 4. Organoleptic Properties of Freeze-Thaw cycle of CFM[19]

|   | 1  | 2  | 3  | 4  |
|---|----|----|----|----|
|   | N  | N  | N  | N  |
|   | N  | N  | N  | N  |
| Application/Touch | A  | A  | A  | A  |
| pH          | 5.67 ± 0.04 | 5.48 ± 0.02 | 5.53 ± 0.01 | 5.58 ± 0.01 |
| Color       | 26.97 ± 0.06 | 27.41 ± 0.1  | 27.37 ± 0.2  | 27.42 ± 0.1  |
| Viscosity   | 24.91 ± 1.58 | 24.82 ± 2.37 | 24.82 ± 3.35 | 25.55 ± 2.79 |

Legend: freeze-thaw cycles 1-4; Aspect: N-Normal; M-Modified; IM-Intensely Modified; Application: A-Pleasant touch, easy skin application (spreadability); D-Unpleasant touch, sticky, difficult to apply on skin (spreadability); MD –Very unpleasant touch, very sticky, compromises skin application; Odor: N-Normal; M-Modified; IM-Intensely Modified; Color: ∆E values; pH: pH values; Viscosity: values measured in Pa.s using rheometer.
4. Conclusion

In the present study, the formulation of cocoa facial mask was developed with long-term stability. FRAP and β-carotene recorded values of 252.31 ± 0.001 mmol Fe2+/g and 83.42 ± 0.03%, respectively. The EC50 of β-carotene linoleate bleaching (β-CB) reported at 2.92 ± 0.03 mg/mL. The data suggest presence of high antioxidants in cocoa facial mask indicating its potentiality in the development of cosmetics product.

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