PHYSICOCHEMICAL PROPERTIES AND ASSESSMENT OF SKIN BARRIER FUNCTION AS AFFECTED BY COCOA FACIAL MASK COSMETIC

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ABSTRACT – *Utilization of cocoa products in the prevention of skin photoageing have been regularly reported. However, specific information concerning cocoa liquor incorporation in a development of facial mask cosmetic with regard to physicochemical stability and skin barrier function upon intervention of the so-called product remained unclear. This study aims to examine the stability of cocoa facial mask (CFM) formulation in different storage conditions as well as its durability in freeze-thaw cycles and the prospect of integrating CFM that offers a skin barrier function by using transepidermal water loss (TEWL) as a tool. CFM formulated from cocoa liquor as a key ingredient showed good physicochemical stability in different temperatures and conditions storage with no significant difference (p>0.05) throughout four weeks assessment both in accelerated stability and freeze-thaw cycles. The attributes, namely aspect, application/touch and odour apparently were not affected by temperature variations whereas its colour, pH and viscosity values remained unaltered. In term of transepidermal water loss (TEWL), emission of water from the skin of volunteers applied with CFM formulation, was significantly lower (p<0.05) than the placebo (24.48 ± 1.84% TEWL changes compared to placebo at only 2.39 ± 0.02%). These results, besides the estimation of physicochemical stability under ageing, can be useful in determining the best storage conditions for an effective skin barrier function of CFM.*

Key words: Cocoa facial mask, topical delivery, polyphenols

INTRODUCTION

The determinant step in the development of a cosmetic formulation involves stability study, with the objective of predicting physical, physicochemical, chemical and microbiological alterations that may occur since its manufacturing, until the end of its expiration date (Almeida *et al.*, 2013). Moreover, this study allows the evaluation of cosmetic product performance, safety, efficacy and literally contributes for its development time reduction which is highly required by the market and consumers. The stability test guides the development of cosmetic formulations, thus providing information for preparation improvements, in case of instability manifestations occurrence and/or incompatibility among ingredients (Almeida *et al.,* 2013; Nishikawa *et al.*, 2007). Skin surface has an acidic pH, also known as 'the acid mantle' (Lambers *et al.*, 2006). This acidic surface pH and its concomitant pH gradient over stratum corneum (SC), are important for a fine skin condition, hence controlling the presence of resident skin microflora whilst supporting essential physiological processes like the formation of an optimal structure of dermal lipid barrier and SC homeostasis (Fluhr and Elias, 2002; Rippke *et al.*, 2002; Parra and Paye, 2003).

The use of facial masks has been found since antiquity (Wilkinson and Moore, 1982). Nowadays, the interest regarding these topical formulations, is keep growing due to appealing attributed effects such as deep cleanness, tonification, astringency, moisture, emollience (softening and soothing) and tensor action (Draelos, 1999; Martine *et al.*, 1995; Nishikawa *et al.*, 2007). Facial masks may be used as vehicles involve in the permeation of active substances through the SC, indicating a cosmetic potentiality (Baumann and Lazarus, 2001; Lupo, 2001; Miyazaki *et al.*, 2002; Otieno *et al.*, 2006; Prakash *et al.*, 2007). Polyphenols particularly in the form of flavonoids, have been used in cosmetics for a long time. Phenolic compounds have been found to stimulate synthesisation of endogenous antioxidant molecules in skin cells, thus providing the skin with hydration and increase moisture retention to help revitalize dull-looking skin. (Martins *et al.*, 2020). Beneficial effects of flavonoids excessively found in cocoa liquor which is also known as chocolate liquor, unsweetened chocolate, cocoa mass or simply liquor, have been documented empirically by Katz *et al.* (2011). Its phenolic and flavonoid contents have been reported to be higher than any other phytochemical-rich foods (Sabahannur *et al.,* 2018). Chemical composition of cocoa liquor depends on cocoa varieties, fermentation and roasting conditions of cocoa bean processing (Wollgast and Anklam, 2000). Cocoa liquor does not contain any alcohol liquids, halal and safe to be consumed as well as for use as topical applications for the skin.

Even though many researchers have worked on antioxidants and anti-ageing of cacao, very few researches were conducted on skin benefits pertaining to intervention of cocoa liquor/mass. In order to find possible sources for future novel antioxidants in cosmetic and pharmaceutical formulations, the present study was conducted to determine cocoa liquor-containing facial mask specifically for its skin barrier function using transepidermal water loss (TEWL) as a tool as well as physicochemical properties and stability in different temperatures and conditions storage. Therefore, the main objective of this study is to develop a cocoa facial mask formulation containing cocoa liquor with a view of determining its potentiality upon incorporation with other cosmetic grade materials, giving the skin an added protection in fighting free radicals from the environment.

MATERIALS AND METHODS

Materials

Cocoa liquor was acquired from Barry Callebaut Malaysia Sdn. Bhd. Ingredients which have made up the CFM formulation such as emulsifier, viscosity inducing agent, emollient, white clay powder and wetting agent were purchased from Gattefossé.

Methods

Formulation of CFM

Firstly, cocoa liquor was melted in an oven at 40°C. The ingredients in both phases were weighed according to Table 1. Each phase was mixed in a beaker using a homogenizer (Heindolph, Germany) at 1000 rpm for about 40.0 ± 5.0 min. Fresh CFM was left to rest for 24 hrs before further evaluation. After 24 hrs, the physicochemical properties of CFM were assessed.

Table 1: Composition of CFM formulation

Ingredients		Amount (%)
Oil Phase:		
	Cocoa Liquor	50
	Emulsifier	q.s.
	Emollient	q.s.
	Wetting agent	8
Non-oil Phase:		
	White Clay Powder	20
	Preservative	q.s

Stability Test

A number of opaque polyethylene bottles with each one comprising 100 g of sample, were stored at these following conditions, i.e., low temperatures (5.0 ± 1.0ºC), oven (45.0 ± 2.0°C), room temperature (28.0 ± 2.0°C), and room temperature protected in a dark space (24.0 ± 2.0°C) (Vieira *et al.*, 2009; Nishikawa *et al.*, 2007). The procedure was assessed as day 0 (after 24 hrs) in each condition. Subsequent sample was subjected to these physicochemical properties, namely aspect, application/touch, colour, odour, pH value and rheology.

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 hrs. Subsequently, the CFM was thawed at room temperature for another 24 hrs and later placed in an oven at higher temperature (40°C) for 24 hrs and again placed at room temperature for 24 hrs. The CFM was analysed for any sign of significant changes. This completes one cycle. The process was repeated for 4 cycles.

Physicochemical Properties

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 pH meter. The CFM sample was weighed (1 g) and dissolved in (9 mL) distilled water. Determination of viscosity using AR-G2 controlled stress rheometer (TA Instruments®, New Castle, USA), was linked to a computer system (TA Instruments Universal Analysis 2000 Software). Colour attribute was determined using spectrophotometer (Konica Minolta CM-5, Tokyo, Japan) and reported using (ΔE) calculated from “L”, “a”, “b” systems. The CFM was found to be comparable with pure cocoa liquor as well as selected commercial brand.

Study Protocol

Twelve volunteers (aged 20 to 35 years) were selected for the study. Informed consent was signed by each one of volunteers prior to commencement of the study. All volunteers were healthy females with no known dermatological diseases or allergy to substances in the formulations. The exclusion criteria were presence of any dermatitis and/or other skin or allergic diseases, smokers and any previous treatment of cheek skin with cosmetic formulations such as sunscreens, moisturizers or anti-ageing cosmetics, in two months duration prior to the study. Measurement of skin barrier function using TEWL as a tool were made weekly for 2 months. Approximately 500 mg of CFM was applied to the right cheek whereas a placebo (basic facial mask formulated with 70% white clay powder without addition of any cocoa liquor) to the left cheek twice weekly at home by the volunteers. The area around the eyes was omitted. Prior to skin measurement, the volunteers were asked to remain in the room for at least 15 min in order for the skin to become acclimatized to room temperature.

Measurement of TEWL

The instrument used to measure stratum corneum (SC) barrier function was Tewameter® TM 300 (Courage & Khazaka Electronic, Germany). The water vapour released from a given surface on the basis of Fick's law of diffusion was measured as:

$$dm / dt = -D * A * d0 / d1 \quad \text{(Eq. 1)}$$

where dm / dt is diffusion current; A is water to skin ratio coefficient; d0 is variation in concentration; d1 is space travelled and D is coefficient of diffusion of the water in the air. The diffusion current dm/dt indicates the amount of water vapour, which is transported within the time unit through the given surface. The diffusion current d0/d1 determines the quantity of vapour, which travels vertically over the skin surface, with reference to the space travelled. This law is valid only within a homogeneous diffusion area, which can be obtained by means of a cylinder open at both ends. The Tewameter was equipped with a probe consisting of a handle and a cylindrical part open at both ends (internal diameter: 10 mm; height: 20 mm). The cylindrical part contained a pair of sensors. The humidity that evaporated from the skin surface travelled through the cylindrical part of the probe. The saturation gradient was indirectly measured by the pair of sensors (temperature and humidity) and then processed by means of a microcomputer. The TEWL values were expressed in grams per square metre per hour ($g/m^2/h$).

RESULTS AND DISCUSSIONS

Formulation of CFM

The CFM formulation which is mainly comprised of cocoa liquor, was homogeneously mixed with other cosmetic grade ingredients (Table 1) at high shear rate in order to obtain a fine formulation of cocoa cream clay mask.

Physicochemical Properties on Accelerated Stability Test and Freeze-thaw Cycles

The results of stability test and freeze-thaw cycles obtained from the 4 weeks assessment on physicochemical properties pertaining to aspect, odour, application/touch, pH, colour and viscosity values, were shown in Tables 2 and 3,

Table 2: Evaluation of the physicochemical properties in the accelerated stability study of CFM

	Storage Condition															
	G				**E45°**				**T.A.**				**T.A. (dark space)**			
	Week															
Parameter	**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
Application/Touch	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Odour	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Colour	25.31 ±0.35^{a}	25.39 ±0.05^{a}	26.29 ±0.01^{a}	24.78 ±0.04^{a}	25.68 ±0.05^{a}	24.72 ±0.02^{a}	25.78 ±0.01^{a}	24.74 ±0.02^{a}	25.66 ±0.04^{a}	24.89 ±0.05^{a}	26.05 ±0.03^{a}	26.12 ±0.03^{a}	25.47 ±0.03^{a}	24.97 ±0.02^{a}	25.97 ±0.03^{a}	25.55 ±0.02^{a}
pH	5.76 ±0.03^{a}	5.75 ±0.03^{a}	5.62 ±0.010^{a}	5.36 ±0.18^{a}	5.40 ±0.05^{a}	5.51 ±0.17^{a}	5.44 ±0.04^{a}	5.37 ±0.11^{a}	5.43 ±0.03^{a}	5.42 ±0.02^{a}	5.44 ±0.02^{a}	5.45 ±0.03^{a}	5.34 ±0.03^{a}	5.36 ±0.05^{a}	5.48 ±0.03^{a}	5.33 ±0.04^{a}
Viscosity (Pa.s)	25.46 ±0.61^{a}	25.35 ±1.10^{a}	25.80 ±2.72^{a}	24.48 ±3.51^{a}	25.16 ±0.32^{a}	24.79 ±3.81^{a}	24.84 ±2.70^{a}	24.14 ±2.75^{a}	25.04 ±2.28^{a}	25.80 ±3.71^{a}	24.98 ±2.94^{a}	25.11 ±3.53^{a}	25.57 ±4.91^{a}	24.86 ±1.58^{a}	25.10 ±4.07^{a}	25.53 ±3.45^{a}

Note: *Means in the same row followed by different letters were significantly difference at p<0.05
G – Low Temperature (5.0 ± 1.0ºC); E45º – Oven (45.0 ± 2.0°C); T.A. − Room Temperature (28.0 ± 2.0°C); T.A. (dark) − Room Temperature (dark space) (24.0 ± 2.0°C)
Aspect: N – Normal
Application/Touch: A − Pleasant touch, easy skin application (spreadability)
Odour: N – Normal

Table 3: Evaluation of the physicochemical properties in the freeze-thaw cycle of CFM

	Freeze-thaw Cycle			
Parameter	**1**	**2**	**3**	**4**
Aspect	N	N	N	N
Application/Touch	A	A	A	A
Odour	N	N	N	N
Colour	26.97 ± 0.06 ^{a}	27.41 ± 0.1 ^{a}	27.37 ± 0.2 ^{a}	27.42 ± 0.1 ^{a}
pH	5.67 ± 0.04 ^{a}	5.48 ± 0.02 ^{a}	5.53 ± 0.01 ^{a}	5.58 ± 0.01 ^{a}
Viscosity (Pa.s)	24.91 ± 1.58 ^{a}	24.82 ± 2.37 ^{a}	24.82 ± 3.35 ^{a}	25.55 ± 2.79 ^{a}

Note: *Means in the same row followed by different letters were significantly difference at p<0.05
Aspect: N – Normal
Application/Touch: A − Pleasant touch, easy skin application (spreadability)
Odour: N – Normal

respectively. The “aspect” attribute of all storage conditions either for accelerated stability (Table 2) or freeze-thaw cycles (Table 3), were found to be fairly consistent throughout the whole week of assessment whereas pleasant touch with acceptable adherence and spreadability were literally perceived from the “application/touch” attribute. Since product colorant was originally contributed from the cocoa liquor itself, no significant difference (p>0.05) in term of colour intensity was observed from the accelerated stability test (Table 2) as well as freeze-thaw cycles (Table 3). Moreover, pH values ranging from 5.33 ± 0.04 to 5.76 ± 0.03 (Table 2) and 5.48 ± 0.02 to 5.67 ± 0.04 (Table 3) were not significantly difference (p>0.05) over time in all kind of different temperatures and storage conditions. This is in line with a relatively large number of reports concerning pH values between 5.0 and 6.0, that have been in great consideration for facial care product due to substantial biophysical results regarding barrier function, moisturization and scaling parameters (Lambers *et al.*, 2006). Viscosity of CFM sample showed no significant difference (p>0.05) throughout the entire assessments as shown in Tables 3 and 4. Viscosity of commercial brand which was recorded at 25.17 ± 2.16 Pa.s, has been found to be closed to the CFM viscosity. All CFM samples were stable at all kind of temperatures and even at extreme conditions (freeze-thaw cycles) for a month without having any issue in term of phase separation.

TEWL Effects

Gradual water permeation through skin barrier which is known as TEWL involves certain amount of uncontrolled water loss to the environment due to the water vapour pressure gradient on both internal and external sides of the skin barrier. Such barrier function has been affected most by external and internal factors in terms of physical stressors, climatic conditions, and systemic diseases (Elias, 2012). TEWL of increasing trend indicates defective barrier function. Today, TEWL has been validated as one of non-invasive approaches in the integrity assessment of SC barrier function as well as firming efficacy of extract formulation *in vivo* (Darlenski *et al.*, 2009). TEWL changes ranging from 10-20% have been associated with the water binding capacity of SC to be considered as healthy skin condition (Visscher *et al.*, 2011). Many studies have reported on the efficiency of antioxidant approaches regarding its prevention against damage to SC lipids and proteins which are in direct contact with invasive pro-oxidative climatic conditions. Consumption of high-flavanol cocoa improves skin hydration, and decreases TEWL (Heinrich *et al.*, 2006). According to Gasser *et al.* (2008), parameters of connective tissues such as glucosaminoglycans and collagen are highly influenced by cocoa polyphenols and have been proven to be comparable or better than commercially available anti-ageing cream. Intervention of antioxidant-containing formulations of *Citrus grandis* (pomelo), *Cucumis melo* (muskmelon), *Prunus armeniaca* (apricot), *Prunus persica* (peach) and *Vitis vinifera* (grape), were amongst that effectively measured in the restoration of SC barrier permeability and prevention against UV-induced photodamage (Prasad, 2014).

In this study, skin hydration effect upon application of placebo and the CFM formulation were carried out through an assessment of 2 months application to observe the maintenance as well as enhancement of such effect. The mean TEWL values for placebo group at 0 hr, 1st month and 2nd month were 17.99 ± 1.51, 18.20 ± 1.78 and 17.56 ± 0.45 g/h/m^2, respectively, or 2.39 ± 0.02% TEWL changes, whereas areas treated with the CFM formulation recorded mean TEWL of 18.30 ± 1.56, 14.30 ± 0.98 and 13.82 ± 1.35 g/h/m^2 for 0 hr, 1st month and 2nd month, respectively, or 24.48 ± 1.84% TEWL changes. Analysis of variance has detected significant difference in TEWL measurement data with probability p<0.05 for areas treated with the CFM formulation by 24.48 ± 1.84% TEWL changes compared to placebo at only 2.39 ± 0.02% (Figure 1). In fact, this was beyond the 10-20% TEWL changes range which has been associated with the water binding capacity of stratum corneum to be considered as healthy skin condition (Visscher *et al.*, 2011). In other words, emission of water from the skin of volunteers applied with CFM formulation was significantly lower (p<0.05) than the placebo, suggested effectiveness of the CFM formulation against lipid peroxidation on stratum corneum and also due to the potent antioxidants of CFM. Improvement of the skin barrier function upon CFM application suggesting its ability to reduce the water loss effect, thus improved skin hydration (Dal’Belo et al., 2006).

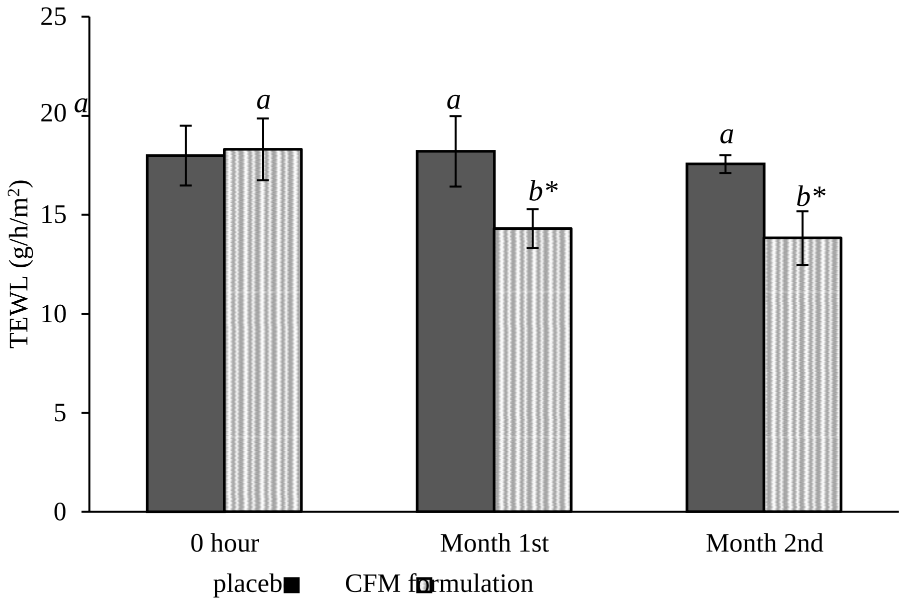

Figure 1. Average TEWL measurement observed from both placebo and the CFM formulation at 0 hr, month 1st and month 2nd
*(*Means in the horizontal bar followed by different letters were significantly difference at p<0.05)*

CONCLUSIONS

This study provides useful results to estimate physicochemical stability under the ageing of CFM formulation. This formulation showed stability for the organoleptic characteristics, pH as well as apparent viscosity values. It is recommended to keep the formulation under refrigeration (5.0 ± 1.0°C) to assure lower variations in other variables analysed, in accordance with the observed during the stability study. The non-invasive method in skin barrier function demonstrated from TEWL effect, reflected the overall prevention of skin alteration and early ageing. Therefore, it brings out the promising benefits of CFM as a natural cosmeceutical that offers proven biological activities.

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