

## Watermelon seeds and peels: fatty acid composition and cosmeceutical potential<sup>☆</sup>

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**Abstract** – Watermelon consumption results in generation of organic waste in the form of seeds and peels. We have evaluated the fatty acid profiles and antioxidant content of watermelon (Kinnaree cultivar) seed oil and peel wax. In addition, we assessed the potential use of these watermelon industry byproducts in the development of cosmeceuticals. The most abundant fatty acids in seed oil and peel wax were linoleic acid and arachidic acid, respectively. Fatty acids form an essential component in the cell membranes and have seen increased recognition in the cosmeceutical industry. Antioxidants also play a beneficial role in skincare in combating free-radicals resulting from sun damage and pollutants. The seed oil showed stronger antioxidant activity than the peel wax, as indicated by the DPPH radical scavenging ability of 0.894 mg  $\alpha$ -tocopherol equivalent/g dried seeds versus 0.036 mg  $\alpha$ -tocopherol equivalent/g dried peels. Therefore, the seed oil was formulated into skincare products, in the form of emulsions and nanoemulsions. The most effective formulae were stable at room temperature for seven days, or following repeated cycles of heating and cooling. This work demonstrates the potential for watermelon seed oil to be employed in skincare product formulations, which could maximize agricultural profit and minimize environmental waste.

**Keywords:** Watermelon / seeds / peels / antioxidant / nanoemulsion

**Résumé** – Graines et peaux de pastèque : composition en acides gras et potentiel cosmétique. La consommation de pastèques entraîne la production de déchets organiques sous forme de graines et de pelures. Nous avons évalué les profils d'acides gras et la teneur en antioxydants de l'huile de graines et de la cire de pelure de la pastèque (cultivar Kinnaree). De plus, nous avons évalué l'utilisation potentielle de ces sous-produits de l'industrie de la pastèque dans le développement de produits cosmétiques. Les acides gras les plus abondants dans l'huile de graines et la cire de pelure étaient l'acide linoléique et l'acide arachidique, respectivement. Les acides gras forment un composant essentiel des membranes cellulaires et sont de plus en plus reconnus dans l'industrie cosméceutique. Les antioxydants jouent également un rôle bénéfique dans les soins de la peau en combattant les radicaux libres résultant des dommages causés par le soleil et les polluants. L'huile de graines a montré une activité antioxydante plus forte que la cire issue de la peau, comme l'indique la capacité de piégeage des radicaux DPPH de 0,894 mg d'équivalent  $\alpha$ -tocophérol/g de graines séchées contre 0,036 mg d'équivalent  $\alpha$ -tocophérol/g de peaux séchées. Par conséquent, l'huile de graines a été formulée en produits de soins de la peau, sous forme d'émulsions et de nanoémulsions. Les formules les plus efficaces étaient stables à température ambiante pendant sept jours, ou après des cycles

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répétés de chauffage et de refroidissement. Ce travail démontre le potentiel de l'huile de pépins de pastèque dans les formulations de produits de soins de la peau, ce qui pourrait optimiser le profit agricole et réduire les déchets environnementaux.

**Mots clés** : Pastèque / pépins / pelures / antioxydant / nanoémulsion

## 1 Introduction

Watermelon (*Citrullus lanatus*) is a globally cultivated fruit, valued for its sweet flavor, high water content, and low-caloric value (Maoto *et al.*, 2019). In 2018, global watermelon usage stood at 166 million tons (FAOSTAT Statistical Database, Food and Agriculture Organization of the United Nations, 2020). However, while watermelon seeds are used in some regions of Asia for snacks and flour (Hu, 2005), and watermelon peels are sometimes pickled (Gusmini and Wehner, 2004) or used as a cooked vegetable (Terry, 2009), both resources are more frequently discarded generating huge quantities of undervalued seed and peel wastes. The oil content of watermelon seeds is in the range of 10–35%, varying depending on the genotype (Ziyada and Elhussien, 2008; Mahla *et al.*, 2018). Watermelon seed oil is reported to be a good source of essential fatty acids, carotenoids, tocopherols, thiamine, flavonoid, riboflavin, and other phenolic substances, quantities of which vary depending on watermelon variety and extraction method (Ouassor *et al.*, 2020). Linoleic acid is the most abundant fatty acid found in watermelon seed oil irrespective of variety. In addition, various bioactivities of the watermelon seed oil have been reported including: antioxidant-, anti-inflammatory-, cardio-protective- and antimicrobial activities (Eidangbe *et al.*, 2010; Madhavi *et al.*, 2012; Jorge *et al.*, 2015; Bello *et al.*, 2016; Thongtha *et al.*, 2017). Therefore, watermelon seed oil could be a good alternative source of plant-derived oil for consumption and pharmaceutical applications (Biswas, 2017). Watermelon rind represents another underutilized resource, components of which include carbohydrates, fiber and wax (Al-Sayed and Ahmed, 2013; Morais *et al.*, 2017). To date, limited information is known about the fatty acid profile of watermelon outer peels. Various efforts have been undertaken to utilize watermelon rind waste in food products, such as low glycemic cookies and cakes (Al-Sayed and Ahmed, 2013; Naknaen *et al.*, 2016). However, little information exists concerning formulation of lipids from either seeds or peels of the Kinnaree variety of watermelon for use in cosmeceutical products.

The skin is the largest organ of the human body. While the dermis is hydrophilic, the outer surface of the skin is hydrophobic, and presents a significant barrier affecting transdermal absorption of therapeutic agents. However, given the lipophilic nature of skin, lipid-based delivery systems provide an efficient method to bypass this constraint. Emulsions and nanoemulsions are both commonly used in cosmetic and pharmaceutical applications to deliver both hydrophilic and lipophilic active compounds across the barrier (Otto and Plessis, 2015). Emulsions offer better organoleptic properties than oily solutions being non-greasy, non-staining and washable. Moreover, the small particle size of emulsions promotes higher absorption of active ingredients compared to

oil-based solutions (Sakata *et al.*, 2014). Various studies indicate that nanoemulsions demonstrate improved transdermal drug permeation compared to conventional topical formulations (Elmataeeshy *et al.*, 2018; Shaker *et al.*, 2019). Nanoemulsions are isotropic, transparent (translucent) systems consisting of oil, water, and surfactant. They generally have a droplet diameter in the 10 to 100 nm range (Arora *et al.*, 2014). Nanoemulsions increase drug solubility, are easy to fabricate, and possess longer-term stability than conventional topical formulations, such as emulsions and ointments. The nano-range droplet size of nanoemulsions provides a relatively large surface area compared to conventional topical preparations and enhance the bioavailability of the drug. In addition, because less surfactant is required in nanoemulsions (5–20% w/w) compared to microemulsions, the chance of skin irritation is significantly decreased and production costs reduced (Maruno and da Rocha-Filho, 2009; Rinaldi *et al.*, 2017).

The aim of this study was to: (1) extract the lipid from watermelon seeds and peels (Kinnaree cultivar), (2) characterize their fatty acid compositions and (3) evaluate their antioxidant activities. A solvent extraction method performed at room temperature was employed to preserve the active ingredients. Formulation of extracted oil for topical use as emulsions and nanoemulsions were demonstrated; and the physical properties of each formulation were evaluated. This work highlights the significant potential of watermelon seed oil for use in the cosmeceutical industry. This could have the dual benefit of helping increase the value of byproducts of the watermelon industry and in decreasing the amount of agricultural waste from the watermelon consumption.

## 2 Materials and methods

### 2.1 Materials

Watermelon seeds and peels were provided by Nicha farm, Chonburi, Thailand. Tween 20, Tween 60, Tween 80 were purchased from P.C. Drug Center Co., Ltd., (Bangkok, Thailand). Span 20 and Span 80 were obtained from CT chemical (Bangkok, Thailand). Standard fatty acid methyl esters (FAMES), 2-2'-diphenyl-1-picrylhydrazyl (DPPH), low viscosity sodium carboxymethyl cellulose (SCMC), boron trifluoride and  $\alpha$ -tocopherol were supplied by Sigma-Aldrich (Saint-Louis, MO, USA). N-hexane, methanol and heptane were purchased from Merck (Darmstadt, Germany). All other chemicals and reagents were of analytical or chromatographic grade.

### 2.2 Extraction of watermelon seeds and peels

Seeds and outer peels were obtained from the Kinnaree watermelon grown in the Chonburi province, Thailand.

The whole seeds and outer peels were dried in a hot air oven (Memmert<sup>®</sup> CTC256, Buchenbach, Germany) at 60 °C until a stable weight was obtained. Both seeds and peels were then ground into a fine powder and kept at 4 °C with desiccant. Lipid extraction was performed by immersing the powdered seeds or peels in hexane at a ratio of 1 g:2 mL and 1 g:5 mL, respectively. The mixture was incubated at room temperature for 2, 6, 12 and 24 h prior to filtration using Whatman No.1 filter paper. The solvent was then removed from the filtrate using a SpeedVac concentrator (Thermo Fisher Scientific, Waltham, MA, USA). The oil yield was calculated using the equation;  $\% \text{ yield} = \text{weight of extracted oil or wax} / \text{dry weight of seeds or peels}$ .

## 2.3 Determination of fatty acid composition

### 2.3.1 Preparation of fatty acid methyl esters (FAMES)

FAMES were prepared using the methods reported by Yurchenko *et al.* (2016) with slight modification. Watermelon seed oil (100  $\mu\text{L}$ ) was mixed with 800  $\mu\text{L}$  of 0.5 M methanolic NaOH and heated at 80 °C for 7 min. After cooling down to room temperature, 900  $\mu\text{L}$  of 20% methanolic boron trifluoride was added to the reaction mixture and incubated at 80 °C for a further 2 min. Heptane (400  $\mu\text{L}$ ) was added to the mixture prior to incubation at 100 °C for 1 min. FAMES were separated from the reaction mixture by addition of 1500  $\mu\text{L}$  saturated NaCl, and following phase separation the upper phase was collected. FAMES were stored at -20 °C until use. For extraction of peel wax, a similar FAME extraction procedure was employed; however, peel wax was dissolved in 100  $\mu\text{L}$  heptane prior to heating in 800  $\mu\text{L}$  of 0.5 M methanolic NaOH at 80 °C for 7 min.

### 2.3.2 Analysis of FAMES by gas chromatography

FAME standards were used for both qualitative and quantitative analysis of the fatty acid composition of seed oil and peel wax. Standards used for this analysis were caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), linolenic acid (C18:3), behenic acid (C22:0), erucic acid (C22:1), and lignoceric acid (C24:0). For quantitative analysis, 6 concentrations (1, 10, 20, 40, 60, and 100 mg/mL) of the mixed standards were analyzed, to allow construction of a standard curve of each fatty acid. FAMES (standards or extracted samples) were analyzed using a 6890-gas chromatograph (Agilent Technologies, Palo Alto, CA) connected to a HP-FFAP column (30 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu\text{m}$ ). One microliter of each sample was injected in to the instrument using spitless injection at a temperature of 200 °C. Helium was used as a carrier gas. The oven temperature was programmed to range between 150 °C and 220 °C, with the temperature increasing at a rate of 4 °C/min, followed by constant heating at 220 °C for 20 min. Eluted substances were detected by a flame ionization detector. All data were acquired using the Agilent ChemStation software.

## 2.4 Antioxidant activities of watermelon seed oil extract

Potential antioxidant activities were assessed *in vitro* by measuring the quenching efficacy of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals (Molyneux, 2004). In brief, known concentrations of extracted seed oil or peel wax, in heptane (50  $\mu\text{L}$ ), were mixed with 100  $\mu\text{L}$  of 0.2 mM DPPH in methanol in a 96-well plate. The plate was then incubated at room temperature, in the dark, for 30 min. Following incubation, absorbance was measured at 517 nm using a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA). The % DPPH inhibition was calculated using the equation;  $\% \text{ inhibition} = (A_{\text{Control}} - A_{\text{Sample}}) / (A_{\text{Control}}) \times 100$ ; where A represents absorbance at 517 nm. Solutions of  $\alpha$ -tocopherol at 10, 25, 50, 75 and 100  $\mu\text{g/mL}$  were used as positive controls. Half maximal inhibitory concentration (IC50) values were determined from the graphed values of percentage of DPPH quenching, versus sample concentrations, using Microsoft Excel 2016 (Microsoft, Redmond, WA, USA). All experiments were performed in triplicate. Statistical analysis was carried out employing the one-way analysis of variance (ANOVA) and Tukey's post tests using a Minitab 18 software (Minitab Inc., State College, PA, USA).

## 2.5 Formulation of watermelon seed oil extract

Potential dosage forms of watermelon seed oil extract in both emulsion and nanoemulsion forms were prepared using biphasic systems as outlined below. The liquid phases were evaluated for physical properties and stability.

### 2.5.1 Preparation of emulsions

Emulsions were formulated using the beaker method as indicated in Table 1. The major components of the final watermelon seed oil extract emulsion consisted of the seed oil, surfactant (Tween 80 and Span 80), water and pharmaceutical excipients such as stiffening agent (Stearyl alcohol and glyceryl monostearate), humectant (propylene glycol), and suspending agent (SCMC).

Briefly, the emulsions were prepared as follows: the oil phase (see Tab. 1 upper panel) containing surfactant and watermelon seed oil extract was heated in a water bath to 72 °C, and the water phase (see Tab. 1 lower panel) heated in parallel to 75 °C. Then, both clear solutions were thoroughly mixed. The resulting emulsion formulations were kept in a tightly sealed glass container, at room temperature, until use. Following storage, emulsion formulations were evaluated for their pH, stability and their morphology assessed by microscopy.

### 2.5.2 Preparation of nanoemulsions

Watermelon seed oil extract nanoemulsions were prepared using ultrasonication with a Vibra Cell VCX 750 sonicator (Sonics Materials Inc., USA). Briefly, watermelon seed oil extract, surfactant, and water were taken in a beaker and mixed under continuous stirring for 30 min. Tween 20, Tween 60, Tween 80, Span 20, and Span 80 were employed as surfactants. The oil concentration to surfactant concentration ratio was

**Table 1.** The composition of watermelon seed oil extract emulsions.

Compositions	F1	F2	F3	F4	F5	F6
Oil phase						
Span 80 (%w/v)	6	6	6	6	6	6
Watermelon seed oil extract (%w/v)	1	1	1	1	1	1
Stearyl alcohol	1	–	1	1	–	–
Glyceryl monostearate	–	1	–	–	1	1
Water phase						
Tween 80 (%w/v)	6	6	6	6	6	6
Propylene glycol (%w/v)	10	10	10	10	10	10
SCMC (%w/v)	–	–	0.5	1	0.5	1
Water qs to (ml)	100	100	100	100	100	100

1:10, 1:15 and 1:20 in a final volume of 20 ml. The mixture was sonicated for 5 min at 40% amplitude in an ice bath resulting in the formation of nanoemulsions.

Correctly prepared nanoemulsion formulations selected for further study were transparent and clear. Cloudy or milky/white solutions were excluded from this study. Nanoemulsions was analyzed to ascertain pH, droplet size, and stability.

## 2.6 Evaluation of formulations

### 2.6.1 Determination of emulsion and nanoemulsion pH

Emulsion and nanoemulsion pH were measured in triplicate, at 25 °C, using a pH meter (Mettler Toledo MP 220, Greifensee, Switzerland).

### 2.6.2 Microscopic analysis of emulsion structure

Emulsions were stained with Ponceau 4R aqueous solution, and their morphology analyzed using a light microscope (Axio Lab. A1, Carl Zeiss, Germany) connected to an EOS 80D camera (Canon, Japan). All images were captured using a Zeiss Axiovision LE software (Carl Zeiss, Germany).

### 2.6.3 Determination of nanoemulsion droplet size

Nanoemulsion droplet size was determined by dynamic light scattering using a Zetasizer Nano ZS particle and molecular size analyzer (Malvern Instrument Ltd., Worcestershire, UK). The nanoemulsion was diluted 1 in 200 with distilled water in a microcentrifuge tube (1.5 ml) (Eppendorf, NY, USA). The prepared samples were analyzed to determine droplet size, polydispersity index (PDI), and zeta potential. Triplicate dilutions of each sample were analyzed.

### 2.6.4 Assessment of emulsion stability

Prepared emulsion formulations were stored in a clear glass bottle, with a tightly sealed rubberized cap for stability testing. This study evaluated the stability of both emulsions and nanoemulsions under two different conditions: (1) storage at 25 °C for seven days to replicate regular storage, (2) six cycles of a heating-cooling cycle to assess the effect of extreme temperature changes. The heating-cooling cycle consisted of storing the samples in a hot air oven (Memmert<sup>®</sup> CTC256,

Buchenbach, Germany) at 40 °C for 48 h followed by a cooling period in a refrigerator (Panasonic<sup>®</sup> NR-BU303 SSTH, Osaka, Japan) at 4 °C for 48 h. Physicochemical characteristics (droplet size, PDI, and pH) were evaluated before and after the stability studies ( $n=3$ ). The statistical analysis was performed using a paired t-test (Minitab Inc., State College, PA, USA). Emulsion and nanoemulsions which displayed instability in the form of turbidity, creaming, cracking, and phase separation were excluded from study. All preparations were observed visually for any turbidity or phase separation. Emulsion creaming was also measured, creaming being defined as the migration of the dispersed phase, under the influence of buoyance, *i.e.* phase separation. The creaming index (% CI) was calculated as the following:  $\% CI = (CC/CT) \times 100$ ; where CC is the total height of the cream layer/upper phase and CT is the total volume height.

## 3 Results and discussion

### 3.1 Extraction of watermelon seed oil and peel wax

Fresh watermelons (Kinnaree cultivar) were utilized for extraction of seed oil and peel wax (Figs. 1A and 1E). To extract oil from watermelon seeds, the seeds were first dried at 60 °C to remove water, prior to extraction. The dried seeds measured approximately 5 mm, and were composed of outer seed peels and kernels. Whole dried seeds were ground to increase surface area to optimize the extraction process (Figs. 1B and 1C). Following oil extraction, a light-yellow colored and odorless oil was obtained (Fig. 1D). To extract lipid from watermelon outer peels, peels were first dried at 60 °C and ground (Figs. 1F and 1G). Following solvent extraction and evaporation, a dark green, soft-solid wax was obtained (Fig. 1H). The yields of both seed oil and peel wax following hexane extraction are shown in Table 2. Extraction times of 2–24 h were tested, with increased extraction times leading to higher yields of both seed oil and peel wax. Extraction for 24 h provided the maximum yield of fatty acids and was used as standard in our extraction procedures. Extractions were performed at room temperature to preserve active compounds and prevent oxidation. As a result of our reluctance to use higher temperatures, the oil yield of watermelon seed oil in our study were lower than those from previously published reports (Ziyada and Elhussien, 2008; Mahla *et al.*, 2018).



**Fig. 1.** Images of (A) Kinnaree watermelon seeds *in situ* within a halved fruit; (B) dried seeds; (C) ground seeds; (D) oil extracted from the seeds; (E) outer peel of Kinnaree watermelon; (F) dried peels; (G) ground peels and (H) peel wax.

**Table 2.** Extraction yield (%yield) of the extracted seed oil and peel wax (g/100 g of dried seeds and dried peels).

Extraction time (h)	% yield (mean $\pm$ SD)	
	Seeds	Peels
2	4.466 $\pm$ 4.274	0.187 $\pm$ 0.265
6	5.167 $\pm$ 5.061	0.585 $\pm$ 1.676
12	7.098 $\pm$ 1.889	1.155 $\pm$ 1.096
24	7.721 $\pm$ 4.609	1.422 $\pm$ 0.746

### 3.2 Fatty acid composition in watermelon seed oil and peel wax

Fatty acids were separated by gas chromatography and the analytes within the gas stream identified and quantified using a flame ionization detector (FID). Methyl esters of 14 common fatty acids found in seed oils were used as analytical standards. The elution order of the FAMES was as follows: caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), linolenic acid (C18:3), behenic acid (C22:0), erucic acid (C22:1), and lignoceric acid (C24:0), respectively (Fig. 2A). The seed oil contained 8 fatty acids each of 14–20 carbon atoms. Four of these were saturated fatty acids; myristic acid, palmitic acid, stearic acid and arachidic acid. Two fatty acids were classified as mono-unsaturated fatty acids; palmitoleic acid and oleic acid. And the other two fatty acids were poly-unsaturated fatty acids; linoleic acid, and linolenic acid (Fig. 2B). The watermelon peel wax contained a smaller number of fatty acids with 14–20 carbon atoms. These were myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and arachidic acid (Fig. 2C).

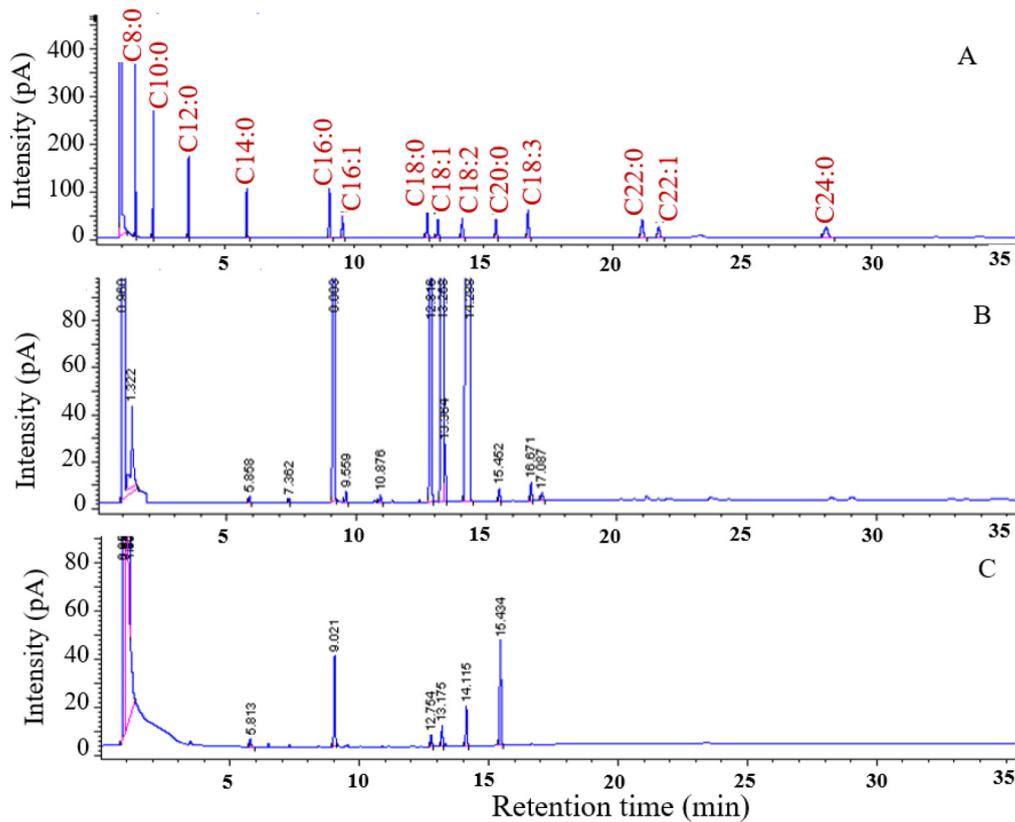
Based on the proportional concentration of each fatty acid (% of total fatty acid) estimated from the area under the

chromatographic peak, the most abundant fatty acids found in the seed oil was linoleic acid, which accounted for  $60.10 \pm 1.87\%$  of total fatty acid, followed by oleic acid ( $17.57 \pm 0.74\%$ ) and palmitic acid ( $12.08 \pm 3.62\%$ ), respectively (Fig. 3). Our results, obtained using hexane extraction of seed oil from the Kinnaree cultivar were in agreement with data from the previous literature assessing the relative amounts of these three major fatty acids from other cultivars using different extraction methods. For comparison, linoleic acid, oleic acid, and palmitic acid were previously reported to be in a range of 45.1–76.2%, 0.33–33.66%, and 4.30–16.2%, respectively (Biswas, 2017).

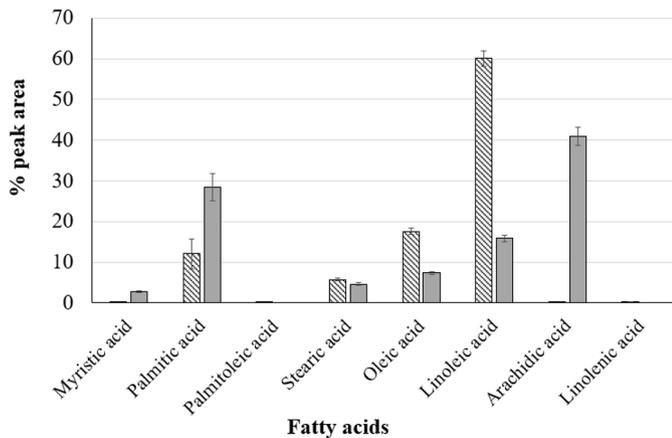
Our analysis of watermelon peel wax indicated that the top three most abundant fatty acids were arachidic acids ( $40.96 \pm 2.27\%$ ), palmitic acid ( $28.42 \pm 3.30\%$ ) and linoleic acid ( $15.86 \pm 0.77\%$ ) as shown in Figure 3. The high proportion of arachidic acid, a saturated long-chain fatty acid, in the hexane extracted peels, was not surprising given that the wax-like extract was highly viscous and semi-solid. However, a previous study using watermelons grown in Brazil indicated a different profile, in which the most abundant fatty acid was palmitic acid; while only a small quantity of arachidic acid was observed (Morais *et al.*, 2017). It is plausible that different varieties of watermelon possess greater variation in the fatty acid profiles of their peels than the seeds.

### 3.3 Antioxidant activity of watermelon seed oil and peel wax

The antioxidant activity of seed oil and peel wax was determined by the ability to scavenge DPPH radicals compared to a positive control ( $\alpha$ -tocopherol). The seed oil extract possessed a higher DPPH radical quenching ability than the peel wax, 0.894 mg  $\alpha$ -tocopherol equivalent/g dried seeds versus 0.036 mg  $\alpha$ -tocopherol equivalent/g dried peels. Antioxidant activity was also calculated in terms of IC<sub>50</sub> values. These also indicated stronger antioxidant activity in seed oil extracts than in peel wax. However, activities never exceeded that of the positive control ( $\alpha$ -tocopherol) (Tab. 3).



**Fig. 2.** Chromatograms of (A) standard FAMES; (B) FAMES of watermelon seed extract and (C) FAMES of watermelon outer peel extract.



**Fig. 3.** The percentage of fatty acids presented in watermelon seeds (▨) and peels (■) (as a % of total fatty acid).

As a result of higher antioxidant activity, seed oil was chosen for formulation of skin care products, in the form of emulsions and nanoemulsions.

### 3.4 Analysis of emulsion properties and storage potential

Watermelon seed oil extract emulsions were stained with Ponceau 4R, a water-soluble dye and analyzed by light microscopy. The emulsions contained large globular droplets

**Table 3.** IC<sub>50</sub> values (mean ± SD) determined from DPPH radical scavenging assays. Different letters indicate significant differences among the samples (ANOVA  $P < 0.05$ ).

Sample	IC <sub>50</sub>
α-tocopherol	50.46 ± 1.37 μg/mL <sup>a</sup>
Seed oil extract	3653.29 ± 539.31 μg/mL <sup>b</sup>
Peel wax extract	8000 μg/mL <sup>c</sup>

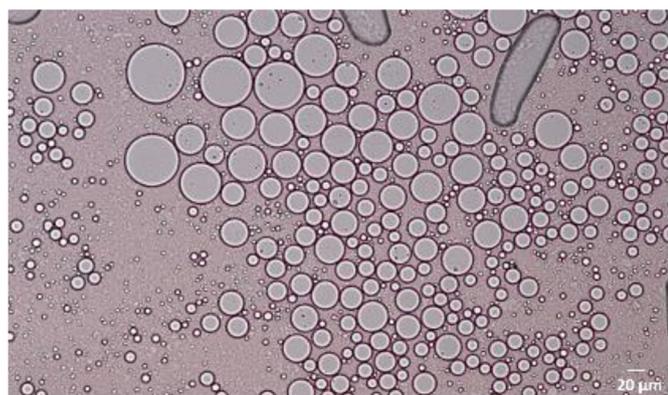
in the micrometer size range (Fig. 4). This confirmed that watermelon seed oil extract emulsions were an oil-in-water emulsion because the aqueous color solution was stained outside an oil droplet. Emulsion stability was tested following two conditions: 1) 7 days storage at room temperature (25 °C), and 2) following 6 heating and cooling cycles (the samples were heated in a hot air oven at 40 °C for 48 h followed by a cooling period in a refrigerator at 4 °C for 48 h). Six different emulsion formulations were tested, containing different amounts of stiffening agents and suspending agent (Tab. 1). Following storage at the indicated experimental conditions the pH of each formulation was assessed (Tab. 4). The pH values of all stored samples were lower than those of the freshly prepared samples. The decreasing pH values after 7 days of storage may be a result of free fatty acids that occurred after the hydrolysis of watermelon seed oil extract.

The different formulations displayed different physical properties depending on the amounts of stiffening agents

**Table 4.** The properties of watermelon seed-oil emulsions following storage.

Formulation	pH of freshly prepared	After 7 days		After 6 cycles	
		pH	%CI	pH	%CI
F1	6.82 ± 0.042	6.65 ± 0.038*	72.00 ± 9.14	N/A	N/A
F2	6.77 ± 0.021	6.54 ± 0.026*	83.33 ± 10.32	N/A	N/A
F3	7.03 ± 0.025	6.63 ± 0.015*	Undetectable	5.67 ± 0.091*	65.56 ± 12.62
F4	6.90 ± 0.006	6.62 ± 0.025*	Undetectable	5.59 ± 0.050*	Undetectable
F5	6.94 ± 0.015	6.42 ± 0.006*	16.67 ± 7.33	N/A	N/A
F6	6.77 ± 0.006	6.46 ± 0.015*	Undetectable	5.62 ± 0.040*	Undetectable

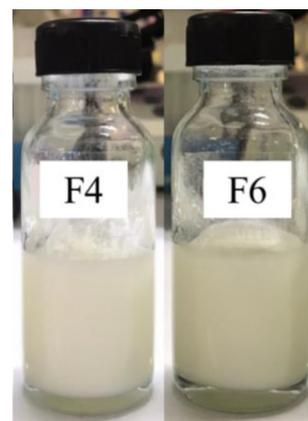
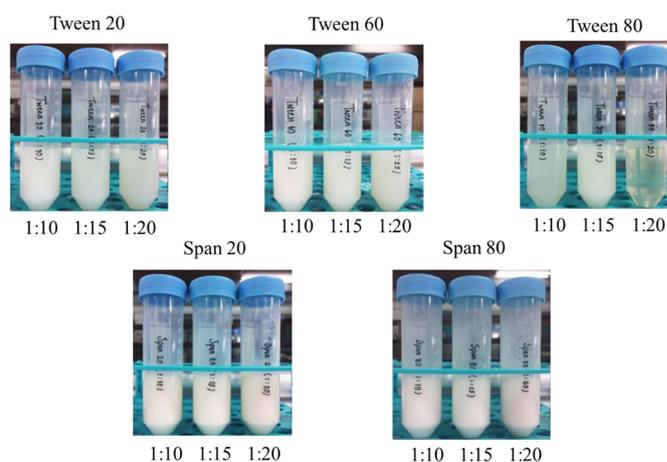
\*Asterisks indicate statistically significant differences ( $P < 0.05$ ) between the pH values of the samples before and after the stability tests.

**Fig. 4.** The morphology of emulsion droplets as stained by *Ponceau 4R* and imaged by light microscopy. The scale bar indicates 20 μm.

added during formulation. Increased concentrations of stearyl alcohol and glyceryl monostearate (stiffening agents) provided harder texture; while an increased concentration of SMC provided higher viscosity. Attempts at preparing a formulation without SMC did not result in a stable emulsion. The emulsion separated into an upper optically opaque droplet-rich layer (creaming layer) and a transparent lower layer, through gravitational separation thus deeming this formulation undesirable. The creaming layer was quantified as a percentage of total emulsion volume and could be decreased by addition of SMC. Higher SMC concentrations increased viscosity of the aqueous phase and improved emulsion stability by inhibiting droplet aggregation, in agreement with a previous study (Huan *et al.*, 2016). Formulations F4 and F6 containing high percentages of SMC were both stable after storage at room temperature for seven days and following subjection to heating-cooling cycles (Fig. 5). These data indicate that emulsions formulated using watermelon seed oil extract demonstrate sufficient stability for use in cosmeceuticals. Further optimization of pH and excipients such as adjustment agents, and preservatives would need to be optimized for use in skin products (Tiwari, 2007).

### 3.5 Analysis nanoemulsion properties and storage potential

Watermelon seed oil extract nanoemulsions consisted of a mixture of seed oil, surfactant, and water. Our studies

**Fig. 5.** Stable emulsion after the 7 days or 6 cycles test.**Fig. 6.** The appearance of all formulations following probe sonication.

indicated that the optimum ratio of oil and Tween 80 was 1:20. This resulted in a transparent and clear solution while other surfactants resulted in turbid and milky solutions unsuitable for our purposes (Fig. 6). The pH value, droplet size, and zeta potential of the optimal formulation are shown in Table 5. After stability tests under both conditions, the appearance of nanoemulsion was transparent and did not change as ascertained by visual inspection. In addition, the droplet size remained constant, lower than 20 nm, and the zeta potential did

**Table 5.** The pH value, droplet size, and zeta potential of the optimal formulation.

Sample	pH	Droplet size		Zeta potential
		Z-Ave (nm)	PDI	
Freshly prepared	7.41 ± 0.02	15.87 ± 1.81	0.410 ± 0.026	-7.99 ± 1.96
After 7 days	7.20 ± 0.03*	18.39 ± 1.57	0.496 ± 0.074	-7.15 ± 1.56
After 6 cycles	6.93 ± 0.01*	14.08 ± 2.40	0.263 ± 0.033	-7.61 ± 0.47

\*Asterisks indicate statistically significant differences ( $P < 0.05$ ) between the pH values, droplet size, and zeta potential of the samples before and after the stability tests.

not significantly change. These data indicated overall stability of the nanoemulsion formulation and indicated its potential for use as a cosmetic product. However, the pH values of the nanoemulsions decreased under both storage conditions ( $P < 0.05$ ), like the changes observed for the emulsion formulations. For consideration as a cosmeceutical, the formulation would need to be adjusted and stabilized at pH 5.5–6 in order to be suitable for the skin. Besides the addition of pH adjustment agents, pharmaceutical additives, coloring agents, flavoring agents, and preservatives could also be included in the final formulation (Tiwari, 2007).

## 4 Conclusions

This study reveals that watermelon (Kinnaree cultivar) seeds and peels are rich in various fatty acids which are suitable for use in cosmeceutical formulations. These two byproducts of the watermelon industry contained distinct profiles of essential fatty acids. Our comparative study indicated that seed oil possessed stronger antioxidant activity than that of peel wax. Therefore, seed oil should be more effective than peel wax as an additive in cosmeceutical products to reduce and prevent cellular damage, a major cause of skin aging. Two forms of cosmeceutical products, emulsions and nanoemulsions, were successfully formulated from watermelon seed oil extract. Stable emulsions were successfully formulated with either stearyl alcohol or glyceryl monostearate and 1% SCMC. Furthermore, stable nanoemulsions were developed using watermelon seed oil and Tween 80 at a ratio of 1:20, resulting in transparent and clear solutions. Our data suggest the potential use of watermelon seed oil as a component in cosmeceutical products. These findings would be beneficial for the future development of value-added products from watermelon seeds, which are considered to be widely available agricultural and food waste.

## Conflict of interest

The authors declare the absence of any conflicts of interest.

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