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Characterization of Aromatherapy Massage Oils Prepared from Virgin Coconut Oil and Some Essential Oils

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Abstract The aim of this study was to characterize aromatherapy massage oils prepared from virgin coconut oil (VCO) and some essential oils. VCO extracted from fresh coconut endosperm by a centrifugation method, which was the most effective method to prepare VCO, was composed mainly of saturated fatty acids, in particular myristic acid. Three essential oils (lemon, eucalyptus and lavender oils) at concentrations of 1, 3 and 5% w/w were blended with the VCO to prepare massage oils. Physical and chemical properties as well as microbial analysis of the massage oils were assessed to evaluate quality characteristics of the preparations. Results showed that types and concentrations of essential oils used somewhat affected viscosity, refractive index and three chemical characteristics (acid, peroxide, and iodine values) associated with oxidative stability of the massage oils. Generally the rank order of acid, peroxide and iodine values of the freshly prepared massage oils appeared to be lemon oil > lavender oil > eucalyptus oil. The results of a accelerated storage stability study (45 °C, 4 months) clearly showed a dramatic increase in both acid and peroxide values of VCO and the blended massage oils compared to initial values, whereas the iodine values of these preparations decreased slightly. The plain VCO and the blended massage oils did not exhibit antimicrobial activity on the test microorganisms and were free from microbial contamination.

Keywords Virgin coconut oil · Lemon oil · Eucalyptus oil · Lavender oil · Essential oil · Acid value · Peroxide value · Iodine value · Refractive index · Viscosity

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Introduction

Coconut, *Cocos nucifera* L. (Arecaceae) [1], is a typical palm distributed over most of the islands and coasts of the tropical regions of the world [2]. Coconut palm is one of the major economic crops in Thailand. It produces an ovoid fruit consisting of husk (35%), shell (28%), meat (endosperm) (28%) and water (15%) [3]. It is generally recognized that the coconut provides many items of great value to man, such as a source of coconut meat, juice, milk and oil. In traditional folklore medicines of many cultures, coconut has been used to treat several health problems, including intestinal worm infections, skin diseases or lesions (rashes, cuts, injuries, and swellings), gastrointestinal diseases (e.g. diarrhea) [4–6]. Currently, there is a great deal of research and commercial interest in coconut products including coconut protein isolate, coconut skim milk, coconut flour

and coconut oil, and the latter, especially virgin coconut oil (VCO), has gained a lot of attention.

Coconut oil can be extracted from coconut meat using two different processes, namely the dry and the wet processes. In the dry process, the coconut meat is dried first by exposing to sunlight or very high temperatures for several days. However, upon exposure to sunlight and high temperature, the bioactive components (e.g. tocopherols and polyphenols) may be inactivated. The unrefined oil is extracted from the copra (dried coconut meat) by expression or prepress solvent-extraction methods [4]. Before consumption, the coconut oil needs to go through refining, bleaching and deodorization processes [7]. In the wet process, the oil is extracted from fresh coconut meat under mild temperature [4], resulting in the production of VCO, which retains more biologically active components.

Coconut oil has many applications. For example, a large percentage of coconut oil is used for edible proposes, such as in cooking (especially frying) and making margarine. It is a source of medium chain triglycerides which can be used as nutritional supplement for patients with malabsorption [8]. Coconut oil is used for the manufacture of chemical feedstocks, synthetic detergents, soaps and cosmetics [9, 10]. To reduce the use of mineral oils which can cause environmental damage, coconut oil has been selected as an alternative base oil for industrial lubricants [11]. In addition, the need for odorous essential oils in aromatherapy has led to a remarkable growth in the use of botanical ingredients including VCO.

In view of the ever expanding use of aromatherapy as a complimentary therapy, the development of safe, effective and high quality natural aromatherapy products is of great interest. One of the aromatherapy products commercially available is aromatherapy massage oil. The main ingredients of the massage oil are carrier oil(s) and essential oil(s) [12]. Carrier oils, also known as base oils or vegetable oils, are used to dilute essential oils prior to skin application to carry the essential oils into the skin. Among the different vegetable oils, VCO has shown high potential as carrier oil for aromatherapy [13]. Despite its widespread use, little scientific data about the characteristics of VCO as aromatherapy carrier oil have been published. The characterization of VCO and some of its aromatherapy massage oil products, therefore, was a major aim of this study. Initially, a small batch of VCO (starting with 100 mL of coconut milk) was prepared using three different methods, namely fermentation, refrigeration and centrifugation. The most suitable method was selected to produce a large scale production. Certain physical, chemical and microbiological properties of the preparations were measured to ascertain their quality characteristics. In this investigation, VCO was blended with three selected essential oils (lemon, eucalyptus and lavender oils) to produce the massage oil formulations. Effects of types and concentrations of essential oils on the aforementioned properties and accelerated stability study of the VCO and blended massage oils were also evaluated.

Materials and Methods

Materials

All the mature coconuts (12–14 months) used in the current study were obtained from the same healthy coconut plantation cultivated in Songkhla Province, Thailand. The coconuts were chosen with care and only those which had the same stage of maturity were used. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 were obtained from the American Type Culture Collection. *Bacillus subtilis* (clinical isolate) and *Candida albicans* (clinical isolate) were kindly provided by Songklanagarind Hospital, Songkhla, Thailand. Phenolphthalein Test Solution (TS) used for determination of acid and saponification values was from ScienceLab (TX, USA). Potassium hydroxide, hydrochloric acid and methanol were from JT Baker Inc. (NJ, USA). Alcohol, petroleum ether and chloroform were purchased from BDH Laboratory Supplies (Poole, UK). Iodobromide TS, potassium iodide TS, starch TS and sodium thiosulfate Volumetric Solution (VS) used for determination of iodine values were supplied by ScienceLab (TX, USA). Lemon oil (B.P.) (*Citrus limon*, L.), eucalyptus oil (B.P.) (*Eucalyptus globulus*, Labill.) and lavender oil (B.P.) (*Lavandula vera,* DC.) were purchased from Srichand United Dispensary Co., Ltd (distributor), Bangkok, Thailand. Karl Fisher reagent was obtained from Riedel-de Haën (Seelze, Germany). Gentamycin and ketoconazole were supplied by Fluka (Buchs, Switzerland). α-Tocopherol (97%) was obtained from Sigma-Aldrich Chemical Company, Inc. (MO, USA). Soybean–Casein Digest Medium and Mueller Hinton agar were supplied by Merck (Darmstadt, Germany). Sabouraud's dextrose agar was obtained from Difco (KS, USA). Sodium dihydrogen orthophosphate dihydrate, disodium hydrogen orthophosphate anhydrous and sodium chloride used to prepare phosphate buffer saline pH 7.2 (0.1 M) were purchased from Univar (NSW, Australia). All chemicals and solvents were pharmaceutical grade or analytical grade.

Methods

Preparation of Coconut Milk

The coconuts were opened within 24 h after harvesting. To obtain the fresh squeezed coconut milk (milky white

oil-in-water emulsion), the fresh endosperm of the mature coconut was grated and pressed using an electric grater and a mechanical press, respectively. This process was performed without addition of water.

Preparation of Virgin Coconut Oil

To separate oil from water in the coconut milk, the coconut milk was processed using three different procedures including fermentation, refrigeration and centrifugation. This study was conducted in small scale batches to determine the optimum extraction method for VCO production. The study was also performed to investigate the suitable conditions (i.e. fermentation temperature, cooling period) for the VCO preparation. The most suitable method which gave the highest yield of VCO was selected to prepare a pilot scale preparation.

Fermentation A sample (100 mL) of fresh coconut milk was fermented at different fermentation temperatures (30, 45 and 60 °C) for 24–30 h. After the fermentation, the two layers were formed, a kind of cream at the top and the watery layer underneath. The volume of the cream phase was measured and the cream was further separated from the water phase. The cream obtained was kept at room temperature for 2–3 days. After that, the VCO was separated from any residues by decantation. Then, the volume of the resulting VCO was measured.

Refrigeration A sample (100 mL) of fresh coconut milk was cooled at 4 °C for 2, 4, 6, 8 and 24 h in order to separate it into two phases; an upper cream phase and a lower watery phase. The volume of the cream phase was determined and the cream was further separated from the water phase. The cream obtained was subjected to mild heating (50 °C) in a thermostat oven for 1, 2, 4, 6 and 24 h. Then, the VCO was separated from any residues by decantation. The volume of the resulting VCO was measured.

Centrifugation A sample (100 mL) of fresh coconut milk was cooled to 4 °C for 0, 0.5, 1, 1.5, 2 and 4 h. After the cooling process, the sample was centrifuged (Sorvall RC-5B Plus refrigerated centrifuge, Pegasus Scientific Inc., USA) at 9,000 rpm at 10 °C for 5 min resulting in the formation of two layers; a creamy layer at the top, and a watery layer underneath. After separation from the water phase, the oily layer obtained was subjected to mild heating (50 °C) for 15 min and subsequently centrifuged at 9,000 rpm at 25 °C for 5 min to obtain VCO. Next, the VCO was decanted from the residues at the bottom of the centrifuge tube. The measurement of the coconut oil volume was carried out.

Chemical Characteristics of Virgin Coconut Oil

Determination of Acid, Saponification, Iodine and Peroxide Values Acid, saponification, iodine and peroxide values of the VCO obtained (small scale batch) were analyzed chemically according to the official protocols described in the section of Fats and Fixed Oils of USP 26 and NF21 [14].

Determination of Ester Value The ester value is the number of mg of potassium hydroxide that is required to saponify the esters in 1.0 g of the substance. The ester value is a measure of the combined acids present in the substance. It is determined by subtraction of the acid value from the saponification value.

Determination of Fatty Acid Composition The fatty acid determination of the VCO (small scale batch) was divided into two steps: the first step was preparation of fatty acid methyl esters and the second step was GC-MS analysis.

Preparation of Fatty Acid Methyl Ester The preparation of fatty acid methyl ester was carried out according to the published guideline by Jham et al. [15]. A 50 μL sample of VCO was hydrolyzed with 1 mL of 0.5 M potassium hydroxide in methanol at 100 °C for 5 min in screw-cap test tubes. Then, the hydrolysis mixture was esterified with 400 μL of hydrochloric acid in methanol (4:1 v/v). The mixture was heated in an oil bath at 100 °C for 15 min and allowed to cool to room temperature. After which 2 mL of distilled water was added and the mixture was extracted with 2 × 3 mL of petroleum ether. The upper layer (petroleum ether) was dried over anhydrous sodium sulfate and evaporated. Finally, the obtained fatty acid methyl ester was dissolved in 500 μL of chloroform and further analyzed by Gas Chromatography–Mass Spectrometry (GC–MS).

Gas Chromatography–Mass Spectrometry Analysis A GC-MS was performed on a Hewlett-Packard gas chromatograph (HP model 5890 Series, Palo Alto, USA) equipped with HP 5972 Mass Selective detector; an electron ionization was used. An aliquot of the test solution (1.0 μL) was injected into the fused silica capillary column Stabilwax (30 m × 0.25 mm i.d., 0.25 μm film thickness, Restek, Bellefonte, PA, USA). The injection port temperature was maintained at 225 °C. The transferline temperature was 240 °C. Carrier gas was helium with a flow rate of 1.0 mL/min. The column oven temperature was programmed as follows: 100 °C (4 min), 100–240 °C (3 °C/min), 240 °C (10 min).

The components were identified by matching their mass spectral data with the Wiley 275.L Mass Spectra Database

Library. The percentage compositions of fatty acids were computed from GC peak areas and calculated as a percentage of the total.

Determination of Water Content Of Virgin Coconut Oil

The water content in the VCO was measured by a Karl-Fisher titrator (Mettler DL 18, Mettler, Switzerland). Karl-Fisher reagent was standardized with sodium tartrate dihydrate. A sample of 1.0 mL of VCO was dissolved in a solvent mixture (1:1 v/v anhydrous methanol-chloroform) and was then titrated with the standardized Karl-Fisher reagent.

Pilot Scale Production of VCO

The centrifugation method was selected to prepare a pilot scale production of VCO, since it provided the highest yield of VCO. Samples (1.5, 3.5 and 6.5 L) of fresh coconut milk were cooled to 4 °C for 2 h. After the cooling process, the sample was centrifuged at 9,000 rpm at 10 °C for 10 min resulting in the formation of two layers; a creamy layer at the top, and a watery layer underneath. After separation from the water phase, the creamy layer obtained was subjected to mild heating (50 °C) until the oil was separated and subsequently centrifuged at 9,000 rpm at 25 °C for 10 min to obtain VCO. The VCO was then decanted from the residues at the bottom of the centrifuge tube.

Water content, refractive index and viscosity of VCO from each batch were determined. To prepare aromatherapy massage oils, VCO from all three batches were blended together and stored in air-tight light resistant containers at room temperature.

Preparation of Aromatherapy Massage Oils

In the current study, the carrier oil was the VCO and the selected essential oils were lemon, eucalyptus and lavender oils at three different concentrations, 1, 3 and 5% w/w. The antioxidant used in the current study was α-tocopherol (0.5% w/w). The VCO and the essential oil with or without the antioxidant were mixed together and stored in air-tight-light resistant containers at room temperature. Three samples of each formulation type were prepared. The containers were best kept as full as possible in order to exclude the action of air.

Physical Characteristics of Massage Oils

Viscosity Measurement The viscosity of freshly prepared massage oils and VCO was measured with a Brookfield bob-cup viscometer (LV type, Brookfield, UK) at 30 rpm at room temperature (32 ± 1 °C). The formulation was placed in the sample cup and allowed to stand until it reached room temperature before the viscosity was measured. The viscosity measurement of the test materials was performed again after 6-month storage at room temperature.

Refractive Index Measurement The refractive indices of freshly prepared massage oils and the VCO were determined with a Refractometer (ABBE '60' Refractometer, Bellingham & Stanley Ltd, UK) at room temperature (32 ± 1 °C). The refractive index of the test materials was determined again after 6 months of storage at room temperature.

Chemical Characteristics of Massage Oils

The chemical properties of the massage oils and the VCO were determined by measurement of three important indicators associated with oxidation and rancidity, namely acid value, peroxide value and iodine value. The details of the experiments are described in the section of Fats and Fixed Oils of USP 26 and NF21 [14].

Storage Stability Study

The stability of VCO and blended massage oils was determined by storing the samples of VCO and massage oils under accelerated temperature (45 °C), 70–75% R.H. for 4 months. Three samples of each formulation (40 g) were placed into well-filled air tight glass bottle and protected from light. The level of oxidative deterioration was investigated by measurement of the acid, iodine and peroxide values. Determination of these three indicators was carried out according to the official methods of the USP 26 and NF21 [14].

Antimicrobial Assay

The test bacteria used in this study were *Staphylococcus aureus* ATCC 25923 (gram+), *Escherichia coli* ATCC 25922 (gram−), *Pseudomonas aeruginosa* ATCC 27853 (gram−) and *Bacillus subtilis* (gram+), and the fungus used was *Candida albicans*. All strains, except *Candida albicans*, were cultured on Mueller Hinton agar (MHA) at 35–37 °C for 24 h. *Candida albicans* was cultured on Sabouraud's dextrose agar (SDA) at 35–37 °C for 24–48 h. Sterile normal saline was added to the slant and the suspension was transfer to a sterile tube. Then, the turbidity of the resulting suspension was adjusted to 25% transmittance at 540 nm with sterile normal saline.

The susceptibility of the microbial to the test materials (massage oils containing 5% w/w essential oils and VCO) was determined using the well-diffusion method as

described by Shadomy et al. [16] with minor modifications. A sterile cotton swab was dipped in the inoculum (suspension) and the excess was removed by rotating the swab several times against the inside wall of the tube above the fluid level. The surface of MHA or SDA plates was inoculated with bacteria or fungus by streaking the swab over the surface. Streaking was repeated 3 times. After each time, the plate was rotated by 60°. In each of these plates, six wells were cut out using a sterile cork border (5 mm diameter). The space between each well was 15–20 mm and the space between the well and the edge of the plate was at least 15 mm. Solutions of the test substances (100 μL) were carefully filled into each well and the plates were then incubated at 35–37 °C for 16–18 h. The positive controls were gentamicin and ketoconazole. In addition, three pure essential oils were used as reference compounds and positive controls. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone (clear zone). The inhibition zones were recorded at 16–18 h after incubation at 35–37 °C. Three samples of each formulation were tested for their antimicrobial activity. The antimicrobial assay was carried out with freshly prepared formulations and with formulations stored at room temperature for 6 months.

Microbial Count

The microbial count of VCO and the massage oil formulations containing 5% w/w essential oils was determined.

Total Aerobic Bacteria Count The total aerobic bacteria count was performed following standard procedures as described in USP 26 & NF21 [14]. The test was carried out by mixing 5 mL of the test material with 95 mL of phosphate buffer saline pH 7.2, which resulted in a 10^{-1} dilution. Ten-fold serial dilutions were made in the same dilution to a 10^{-6} dilution. This gave the number of colonies from 30 to 300 colonies per plate. Subsequently, 1 mL of each dilution was pipetted into the plate, using two plates for each dilution. After that, 15–20 mL of Soybean-Casein Digest Medium (50 °C) was poured into the plate. The dilutions and the culture medium were mixed together and allowed to solidify. Plates were inverted and incubated at 35–37 °C for 24–48 h. After the incubation, the number of colonies was recorded for each plate. Arithmetic mean counts were obtained from each item having from 30 to 300 colonies per plate. Three samples of each formulation were tested for their total aerobic bacteria count. The test was carried out with freshly prepared formulations and with stored formulations at room temperature for 6 months.

Total Mold and Yeast Count The total mold and yeast count was determined using standard procedures as described in the USP 26 & NF21 [14]. The experiment was performed in the same way as previously described in the total aerobic bacterial count. However, SDA was used as the culture medium and the plates were incubated at 20–25 °C for 5–7 days. Three samples of each formulation were tested for their total mold and yeast count. The test was carried out with freshly prepared formulations and with formulations stored at room temperature for 6 months.

Statistical Analysis

Results were expressed as means ± standard deviation (SD). Statistical analysis (paired t test, Student's t test, One-way ANOVA) was determined using Minitab release version 14 (Minitab Inc., State College, USA). Differences at $p < 0.05$ were considered to be significant.

Results and Discussion

Preparation of Virgin Coconut Oil (Small Scale Batch)

In the current study, the VCO was obtained from fresh coconut meat since it has been reported that the coconut oil produced from dried copra is often of poor quality [4]. Furthermore, there are losses of the oil caused by microbial spoilage and insects during the drying or storage stages. Losses also occur due to incomplete oil recovery from the copra cake [4]. The use of the wet milling process should result in a better quality of coconut oil and the quantitative recovery of the oil should be increased. Three methods namely, fermentation, refrigeration and centrifugation were employed in order to investigate the suitable method for the oil extraction. At this stage, only a small amount of the coconut milk (100 mL) was used. In the case of the fermentation process, it was observed that at the experimental temperatures, the coconut milk separated into two layers which were a cream phase (the oil-rich phase) and a water phase. Only the cream phase was collected and kept at room temperature for 3 days. After the storage period, it was found that the cream had further separated into four fractions. From top to bottom, the fractions were free oil, cream, water and sediment products. The VCO obtained was clear and colorless. As seen from Table 1, the fermentation temperature of 45 °C gave the highest amount of the oil when compared to the other two fermentation temperatures, 30 and 60 °C ($p < 0.05$, ANOVA). Moreover, at 45 °C, the coconut oil had a very pleasant odor.

Using the refrigeration process to prepare the coconut oil was not successful in this study. When the coconut milk was cooled at 4 °C with different cooling periods from 2 to 24 h, only a small amount of water was separated. Moreover, the coconut oil was not separated after the cream phase was heated at 50 °C for 1–24 hours.

Table 1 Effect of fermentation temperature on the yield of virgin coconut oil (VCO) (mean ± SD, $n = 6$)

Temperature (°C)	Yield of virgin coconut oil (mL/100 mL of coconut milk)
30	10.25 ± 0.88
45	18.17 ± 0.98
60	12.00 ± 1.90

n number of sample

Table 2 Effect of cooling period on the yield of virgin coconut oil prepared by centrifugation method (means ± SD, $n = 6$)

Cooling period (h)	Yield of virgin coconut oil (mL/100 mL of coconut milk)
0.0	15.83 ± 1.60
0.5	25.00 ± 1.41
1.0	24.67 ± 1.97
1.5	28.00 ± 0.89
2.0	29.83 ± 0.47
4.0	27.00 ± 1.41

n number of sample

In the centrifugation method, it was observed that the coconut milk was separated into two layers after centrifugation at 10 °C from 0 to 4 h. These layers were cream phase and water phase which contained sediment products. Only the cream phase was collected and subjected to mild heating at 50 °C until the oil was separated. After a further centrifugation at 25 °C for 5 min, four fractions were observed: free oil, cream, water and sediment products. The VCO was separated from the other three unwanted layers by decantation. The cooling period seemed to affect the yield of VCO. It was found that the cooling period of 2 h tended to give the highest amount of VCO production (Table 2). The VCO obtained was clear and colorless with a faint odor of coconut. The VCO was stored in well-filled, air-tight glass containers and protected from light during storage.

Chemical Properties of Virgin Coconut Oil Obtained by Small Scale Production

Fatty acid compositions of VCO prepared by fermentation and centrifugation methods are given in Table 3. According to GC-MS analysis, the medium chain fatty acids with 12 to 18 carbon atoms were found in the VCO. Unlike other studies [3, 17, 18], no short chain fractions (caprylic and capric acids) were detected in the VCO prepared in this study. It was found that the VCO contained only small amounts of unsaturated fatty acids, octadecanoic acid and linoleic acid. Most of the fatty acids in the VCO were saturated indicating that the oil had excellent resistance to oxidative rancidity. With GC-MS library identification, the major fatty acids in the VCO prepared by both methods were myristic acid (35–38%), lauric acid (22–30%) and palmitic acid (23–24%). It was noted that the content of lauric acid in this study was lower than that reported in the published literature, which was about 50% [3, 17–19]. This variation is probably due to the differences in soil types, weather conditions, geological location and extraction method. Lauric acid has been reported to have antiviral, antibacterial, anticaries, antiplaque and antiprotozoal properties [20–22].

Table 3 Fatty acid constitutions and water content of virgin coconut oil prepared by fermentation and centrifugation methods

Method	Fatty acid in virgin coconut oil	Retention time (min)	Ratio of fatty acid (%)	Water content (% w/v; mean ± SD, $n = 3$)
Fermentation	C12:0, Lauric acid	13.264	22.3	1.93 ± 0.04
	C14:0, Myristic acid	19.597	34.6	
	C16:0, Palmitic acid	25.780	22.7	
	C18:0, Stearic acid	31.422	4.8	
	C18:1, Octadecanoic acid	32.003	12.8	
	C18:2, Linoleic acid	33.005	2.9	
Centrifugation	C12:0, Lauric acid	13.201	30.3	1.62 ± 0.04
	C14:0, Myristic acid	19.574	37.8	
	C16:0, Palmitic acid	25.777	23.9	
	C18:0, Stearic acid	N/A	N/A	
	C18:1, Octadecanoic acid	31.999	8.0	
	C18:2, Linoleic acid	N/A	N/A	

n number of sample (water content determination)

As shown in Table 3, the water content of VCO prepared by the centrifugation method was significantly lower than that found in the VCO produced by the fermentation method (Student's t test, $p < 0.05$). However, the water content of the VCO from these two methods was higher than the water content limit of 0.2% w/v set by the Thai Industrial Standards Institute, TIS 203-2520 (1977). Thus, the reduction of water content in the VCO was required in the pilot scale production.

Owing to its being the highest amount of VCO produced, the VCO obtained from the centrifugation method was further characterized for some of its quality indicators, namely acid value, saponification value, ester value, iodine value and peroxide value.

It was found that the VCO had a low acid value of about 0.13, suggesting its low susceptibility to hydrolytic rancidity. The VCO had a saponification value of about 254.8, which was in close agreement with the value reported by Thieme [17]. Both acid and saponification values were within the specified limit of the Thai Industrial Standards Institute for VCO: acid value, 4; saponification value, 248–264. The ester value of the VCO was about 254.6. The VCO had a low iodine value of about 3.19, indicating the presence of few unsaturated bonds and hence low susceptibility to oxidative rancidity. Notably, the iodine value was lower than that reported by other publications (7–12) [17, 18, 23]. The peroxide value of 0.28 appeared to meet the published specification [24] and the requirement of the Thai Industrial Standards Institute.

It must be pointed out that in the current investigation, the methods used (USP methods) to determine these aforementioned indicators were quite different from those recommended by the Thai Industrial Standards Institute. The limits specified by this institute, therefore, are only a rough guideline for the VCO quality. Nevertheless, it could be concluded that the VCO prepared from fresh coconut by the centrifugation method was of superior quality. It was slow to oxidize and thus highly resistant to the development of rancidity, as indicated by the low value of the important quality parameters (acid, iodine and peroxide values). However, the water (moisture) content of the oil (>0.2%) did not meet the requirement of the Thai Industrial Standards Institute and this could shorten the shelf life of the coconut oil, or make the oil more susceptible to microbial degradation. In addition, moisture would lead to hydrolysis, resulting in increased free fatty acid content [17]. For this reason, reducing the amount of water in the VCO was necessary for larger scale preparation.

Pilot Scale Production

The information above provided us with the optimal way to extract VCO for massage application. The centrifugation method was selected to carry out pilot scale production because the highest amount of VCO was produced. In the current study, three batches of VCO were prepared. Refractive index, viscosity and % yield of each batch were measured. The results showed that an increase in the centrifugation time resulted in a much lower water content of the VCO (<0.25%). The VCO obtained was clear, colorless with natural coconut scent, and free from rancid odors. A similar yield (30%) was obtained even though the scale of production was increased from 1.5 to 3.5 L and to 6.5 L of coconut milk. The refractive index of each batch was about the same (1.4524) whereas the viscosity value ranged from 30.0 to 35.0 cps. The VCO from all three batches were blended together in order to prepare the aromatherapy massage oils.

Aromatherapy Massage Oils Prepared from Pilot Scale VCO

As previously mentioned the major ingredients of aromatherapy massage oils are carrier oils and essential oils [12]. Coconut oil, as a carrier oil, is generally recognized as safe (GRAS) by the U.S. Food and Drug Administration [3]. In addition to its non-irritancy, coconut oil has been reported to penetrate the skin very well, making the skin smoother and helps to reduce fine lines and wrinkles [3]. Furthermore, Masterjohn [25] demonstrated that coconut oil had anti-inflammatory properties, which could help to reduce the inflammation occurring in muscles. Also, the selected three essential oils, lemon, eucalyptus and lavender oils, have GRAS status granted by the Flavor and Extract Manufacturers Association (FEMA) [12]. Both lemon and eucalyptus oils are colorless, while lavender oil is pale yellow. Dermal LD_{50} of these three essential oils is more than 5 g/kg (rabbit) [12]. For aromatherapy use, lemon oil has been shown to possess several useful properties, such as treatment for poor circulation as well as stimulation of the brain, sense organs and parasympathetic nervous system [12]. Eucalyptus oil has also been used to treat several illnesses, including poor circulation, rheumatic pain and muscular problems [12]. Lavender oil, the most used essential oil of all in aromatherapy, has been used for treatment of headaches, exhaustion, muscular spasm, strains, cramps and rheumatic pain [12]. Furthermore, it has been found to absorb through the stratum corneum into the deeper layers of the skin (epidermis/dermis) and subsequently into the blood supply [26, 27]. Generally, these essential oils can relax the mind and reduce anxiety [12].

Apart from their benefits, skin irritation potential and/or possible damage caused by these three essential oils should be taken into account. Lavender at 10% has caused skin sensitization in humans and animals. However, little or no skin irritation has been observed. Lemon oil at 10% and

100% has caused skin allergy in dermatitis patients (0.5%). Eucalyptus oil at 10% has caused no skin irritation in human but an incidence of skin sensitization has been reported [12].

Since no chemical agents or high heat were involved in VCO extraction in the current investigation, it was assumed that all the nutrients and vitamins providing numerous benefits to the skin were preserved [8]. This makes VCO one of the most suitable carrier oils for essential oils in aromatherapy [13]. It has been demonstrated that VCO has positive benefits in aromatherapy massage oils, particularly in skin care. A perfect skin coverage of VCO results in slowing down transepidermal water loss and thus increasing hydration within the skin. Accordingly, skin dryness and roughness can subside [28].

The physical characteristics of the massages oils including appearance, viscosity and refractive index were investigated. The results showed that all prepared massage oils were clear and colorless. It was observed that the massage oil containing lemon oil had a pleasant coconut scent and a fresh odor reminiscent of lemon peel. In the case of eucalyptus oil, the massage oil had a pleasant coconut scent and a fresh rosy-citronella-like, citrusy odor of the essential oil. The VCO blended with lavender oil had a pleasant coconut scent and a sweet, floral, refreshing odor of the lavender oil. The increase in concentrations of essential oils from 1 to 5% w/w resulted in increasing the scent of massage oils. Furthermore, all the massage oils were found to be clear and colorless after they were stored at room temperature for 6 months.

Refractive Index and Viscosity of Massage Oils

The apparent viscosities and refractive indices of the massage oils are summarized in Table 4. Both measurements were performed when the massage oils were freshly prepared and repeated when they had been kept at room temperature for 6 months. The viscosities of VCO and its

Table 4 Apparent viscosity and refractive index of virgin coconut oil and blended massage oils after storage at room temperature (32 ± 1 °C) for 6 months (mean ± SD, $n = 3$)

Formulations	Apparent viscosity (cps)		Refractive index	
	0 month	6 months	0 month	6 months
VCO[a]	33.5 ± 2.4	35.5 ± 2.1	1.4528 ± 0.0001	1.4526 ± 0.0004
1% w/w lemon oil in VCO	34.0 ± 0.0	34.7 ± 0.6	1.4535 ± 0.0003	1.4529 ± 0.0001
1% w/w lemon oil in VCO-E[b]	33.3 ± 1.2	34.3 ± 0.6	1.4537 ± 0.0001	1.4529 ± 0.0000[d]
3% w/w lemon oil in VCO	25.3 ± 0.6	31.0 ± 1.7[c]	1.4530 ± 0.0000	1.4529 ± 0.0000[d]
3% w/w lemon oil in VCO-E	26.3 ± 0.6	30.3 ± 0.6[c]	1.4537 ± 0.0000	1.4531 ± 0.0001[d]
5% w/w lemon oil in VCO	23.3 ± 0.6	26.3 ± 0.6[c]	1.4537 ± 0.0001	1.4531 ± 0.0001[d]
5% w/w lemon oil in VCO-E	23.7 ± 0.6	26.7 ± 1.2[c]	1.4539 ± 0.0002	1.4538 ± 0.0000
1% w/w eucalyptus oil in VCO	30.0 ± 0.0	36.3 ± 0.6[c]	1.4531 ± 0.0001	1.4525 ± 0.0000[d]
1% w/w eucalyptus oil in VCO-E	30.3 ± 0.6	37.0 ± 0.0[c]	1.4532 ± 0.0004	1.4529 ± 0.0001
3% w/w eucalyptus oil in VCO	32.7 ± 1.2	32.0 ± 1.0	1.4528 ± 0.0000	1.4530 ± 0.0000[d]
3% w/w eucalyptus oil in VCO-E	33.0 ± 1.0	32.7 ± 1.5	1.4529 ± 0.0001	1.4531 ± 0.0000
5% w/w eucalyptus oil in VCO	30.3 ± 0.6	30.3 ± 1.5	1.4529 ± 0.0001	1.4530 ± 0.0000
5% w/w eucalyptus oil in VCO-E	31.0 ± 0.0	31.3 ± 1.2	1.4535 ± 0.0004	1.4530 ± 0.0000
1% w/w lavender oil in VCO	34.7 ± 0.6	35.7 ± 0.6	1.4527 ± 0.0000	1.4527 ± 0.0003
1% w/w lavender oil in VCO-E	34.7 ± 0.6	35.0 ± 0.0	1.4530 ± 0.0000	1.4530 ± 0.0000
3% w/w lavender oil in VCO	34.0 ± 0.0	34.0 ± 1.7	1.4527 ± 0.0000	1.4523 ± 0.0002
3% w/w lavender oil in VCO-E	34.3 ± 0.6	34.3 ± 0.6	1.4527 ± 0.0000	1.4529 ± 0.0000[d]
5% w/w lavender oil in VCO	34.0 ± 1.0	33.7 ± 1.2	1.4529 ± 0.0002	1.4523 ± 0.0002
5% w/w lavender oil in VCO-E	34.0 ± 0.0	33.3 ± 2.9	1.4527 ± 0.0000	1.4530 ± 0.0000[d]

[a] VCO, virgin coconut oil from pilot scale production (three batches blended together)

[b] VCO-E, virgin coconut oil with the antioxidant (0.5% w/w α-tocopherol)

[c] For the same formulation, the viscosity of massage oils after 6-month storage was significantly different from that at 0 month (paired t test, $p < 0.05$)

[d] For the same formulation, the refractive index of massage oils after 6-month storage was significantly different from that at 0 month (paired t test, $p < 0.05$)

n number of sample

aromatherapy products were rather low. For the freshly prepared formulations, the viscosities of the massage oils were in the range of 23.3–34.7 cps. The viscosities varied with the compositions of the formulations. It can be seen that the viscosity of the massage oils containing lemon oil decreased significantly when the amount of the lemon oil was increased from 1 to 5% w/w (One-way ANOVA, $p < 0.05$). The viscosity of the formulation containing 5% w/w lemon oil was the lowest. The viscosity of the massage oil containing 3% w/w eucalyptus oil was significantly different from that of the formulations contained 1% w/w or 5% w/w of such oil (One-way ANOVA, $p < 0.05$). The viscosities of massage oils at three different concentrations of lavender oil were about the same, indicating that the viscosity of lavender massage oils was not affected by the concentrations used in the current investigation.

After 6 months of storage at room temperature (32 ± 1 °C), the viscosities of massage oil formulations containing lemon oil at 3 and 5% w/w, with or without the antioxidant, increased significantly (paired t test, $p < 0.05$). The viscosities of massage oils containing 3 and 5% w/w eucalyptus oil did not change whereas the viscosity of the 1% w/w concentration increased significantly (paired t test, $p < 0.05$). No significant changes in viscosities were observed in the VCO and the massage oils containing lavender oil at all concentrations (1–5% w/w) (paired t test, $p > 0.05$).

Massage usually involves the use of lubricating oils to help the aromatherapist's hands glide more evenly over the customer's skin. The viscosity of the massage oil directly relates to how easily the oil can be massaged onto the body. The massage oils containing lavender oil appeared to be the most suitable oil for body massage since its viscosities were appropriate and did not alter after 6 months of storage at room temperature. On the other hand, the viscosities of massage oils containing lemon oil increased during the storage. This could affect the spreadability and the flow of the products.

The refractive indices of all prepared massage oils at room temperature were about 1.453 (see Table 4). Refractive index measures the light refraction of oil. When they were freshly prepared, the refractive index of the massage oil containing lemon oil at 1% w/w was not significantly different from those of the massage oils containing lemon oil at 3 and 5% w/w (One-way ANOVA, $p > 0.05$). There were no significant differences of refractive indices in any of massage oils containing lemon oil plus the antioxidant (One-way ANOVA, $p > 0.05$). In the case of eucalyptus oil, the refractive index of the 1% w/w was significantly higher than that of the 3 and 5% w/w concentrations (One-way ANOVA, $p < 0.05$). No significant differences were observed when the antioxidant was added in these eucalyptus oil formulations (One-way ANOVA, $p > 0.05$). For lavender oil, the refractive index of the massage oil containing 1% w/w lavender oil was the highest when compared to that of the 3 and 5% w/w formulations.

After 6 months of storage at room temperature (32 ± 1 °C), significant changes in the refractive indices were found mostly in the massage oil formulations containing lemon oil (paired t test, $p < 0.05$). Similar to the viscosity, the refractive index of the VCO did not significantly change after a 6-month storage period at room temperature (paired t test, $p > 0.05$).

Chemical Characterization of Massage Oils

Chemical characteristics associated with oxidation and rancidity of the massage oils are shown in Table 5. According to the Thai Industrial Standards Institute [TIS 203-2520 (1977)], the specified limit for acid, peroxide and iodine values are 4, 3 and 7–11, respectively. It was found that the VCO (large scale) met the requirement of the Thai Industrial Standards Institute. Generally, blending of essential oils with the VCO resulted in an increase in these three indicators. Hence, the incorporation of essential oils into the VCO could increase the susceptibility to oxidation and rancidity. In general, the rank order of acid value, peroxide value and iodine value of the freshly prepared formulations appeared to be lemon oil > lavender oil > eucalyptus oil. Apart from the types of essential oils, amount (concentrations) of these essential oils seemed to affect the oxidation process. For example, in the case of acid value, it was found that the massage oil containing 1% lemon oil gave significant higher acid value than that of the 3 and 5% concentrations (One-way ANOVA, $p < 0.05$). However, no significant differences could be found in the presence of the antioxidant. In the case of eucalyptus oil, there were significant differences in the acid value of the eucalyptus massage oils at three different concentrations (ANOVA, $p < 0.05$). Overall, the highest acid value was obtained in the massage oil containing 1% w/w lemon oil whereas the lowest value seemed to observe in the formulation containing 3% w/w eucalyptus oil with the antioxidant.

It is known that coconut oil is very resistant to the development of rancidity since it has a low content of oxidizable unsaturated fatty acids. In addition, VCO itself contains beneficial natural antioxidants, namely tocopherols which can protect the oil against atmospheric oxidation and rancidity [29]. In an attempt to increase the antioxidant protection, extra tocopherol was also added to the formulations. According to the Thai Industrial Standards Institute [TIS 203-2520 (1977)], both natural and synthetic tocopherols are recommended for use as antioxidants for

Table 5 Quality characteristics of virgin coconut oil and freshly prepared blended massage oils (mean ± SD, $n = 3$)

Formulations	Quality characteristics of blend oils		
	Acid value (mg of KOH/1 g oil)	Peroxide value (mequiv/1,000 g oil)	Iodine value (g of iodine/100 g oil)
VCO[a]	0.118 ± 0.040	0.160 ± 0.058	7.371 ± 0.620
1% w/w lemon oil in VCO	0.351 ± 0.026	0.277 ± 0.108	19.746 ± 0.257
1% w/w lemon oil in VCO-E[b]	0.231 ± 0.096	2.220 ± 0.699[d]	20.588 ± 0.561
3% w/w lemon oil in VCO	0.246 ± 0.019	1.946 ± 0.954	17.524 ± 0.341
3% w/w lemon oil in VCO-E	0.167 ± 0.045[c]	3.303 ± 1.676	20.730 ± 2.278
5% w/w lemon oil in VCO	0.211 ± 0.031	6.990 ± 3.848	23.485 ± 5.622
5% w/w lemon oil in VCO-E	0.227 ± 0.018	6.106 ± 0.492	24.546 ± 2.335
1% w/w eucalyptus oil in VCO	0.205 ± 0.025	0.976 ± 0.339	6.920 ± 0.672
1% w/w eucalyptus oil in VCO-E	0.211 ± 0.041	1.746 ± 0.095[d]	8.321 ± 1.142
3% w/w eucalyptus oil in VCO	0.093 ± 0.020	1.510 ± 0.489	8.310 ± 0.059
3% w/w eucalyptus oil in VCO-E	0.066 ± 0.012	2.859 ± 0.518[d]	8.879 ± 0.254[d]
5% w/w eucalyptus oil in VCO	0.148 ± 0.018	1.570 ± 0.453	11.158 ± 0.123
5% w/w eucalyptus oil in VCO-E	0.140 ± 0.028	2.722 ± 1.154	11.823 ± 0.223[d]
1% w/w lavender oil in VCO	0.123 ± 0.024	3.159 ± 0.240	9.079 ± 0.799
1% w/w lavender oil in VCO-E	0.111 ± 0.010	1.470 ± 0.500[c]	9.126 ± 0.202
3% w/w lavender oil in VCO	0.107 ± 0.021	0.812 ± 0.302	10.753 ± 1.031
3% w/w lavender oil in VCO-E	0.095 ± 0.013	1.672 ± 0.399[d]	11.681 ± 0.633
5% w/w lavender oil in VCO	0.254 ± 0.006	0.698 ± 0.539	12.481 ± 0.075
5% w/w lavender oil in VCO-E	0.170 ± 0.049[c]	1.405 ± 0.245[d]	13.409 ± 0.275[d]

[a] VCO, virgin coconut oil from pilot scale production (three batches blended together)

[b] VCO-E, virgin coconut oil with the antioxidant (0.5% w/w α-tocopherol)

[c] A significant reduction in the acid value or peroxide value was observed (Student's t test, $p < 0.05$) when compared with the formulation without tocopherol

[d] A significant increase in the peroxide value or iodine value was observed (Student's t test, $p < 0.05$) when compared with the formulation without tocopherol

n number of sample

coconut oil. When the formulations with and without the antioxidant were compared, it was found that the acid values of the formulations containing the antioxidant showed a tendency to reduce, although significant reduction was observed only in the massage oils containing lemon oil at 3% w/w or eucalyptus oil at 5% w/w (Table 5) (Student's t test, $p < 0.05$). The peroxide values of the preparations with the antioxidant were generally higher than those of the formulations without the antioxidant. A significant decrease in the peroxide value was found only in the massage oil containing lavender oil at 1% w/w (Student's t test, $p < 0.05$).

In the case of the iodine value, no significant differences were found in the formulations containing lemon oil at every concentration. For lavender oil, a significant increase in the iodine value was observed in the formulation containing the highest concentration of lavender oil (5% w/w) (Student's t test, $p < 0.05$). The significant increase in the iodine value was found in the formulations containing eucalyptus oil at the concentrations higher than 1% w/w (Student's t test, $p < 0.05$). The result suggests that the iodine value of the formulations is affected by types and concentrations of the essential oils. Generally, tocopherol at 0.5% w/w concentration did not seem to have significant inhibitory effects on the oxidation reaction as it was expected. Apart from increasing the amount of the antioxidant, it is likely that the addition of antioxidant-synergists into the formulations may enhance the effect of tocopherol. Further investigation using higher concentrations of tocopherol or certain antioxidant-synergists is recommended for future work.

Antimicrobial Properties of Massage Oils

As seen in Table 6, VCO and its massage oil formulations did not exhibit antibacterial or antifungal activities on the microorganisms tested. These findings are not in agreement with the results reported by Ogbolu et al. [30]. Using the agar-well diffusion technique, they found that VCO showed antifungal properties against *Candida* species,

particularly *C. albicans*. It has been reported that lauric acid and short chain fatty acids (caprylic and capric acids) show antimicrobial effects [22]. Although the VCO contained reasonable amounts of lauric acid, the antimicrobial effects were not detected. It might be speculated that these microorganisms may be more susceptible to short chain fatty acids which were not found in our VCO.

In addition to VCO, essential oils used in the current study, especially lavender oil and eucalyptus oil, have been reported to exhibit extensive antimicrobial activities [12], [31, 32]. In deed, it was found that pure lavender oil, eucalyptus oil and lemon oils were effective against the test bacteria (see Table 6). Nevertheless, the inhibitory properties of essential oils in combination with VCO were not observed in the current investigation. This is probably due to the fact that the amount of essential oils used may not be sufficient to inhibit the microbial growth. Furthermore, the antimicrobial effects of these essential oils may be diluted by adding VCO. Similarly, Donoyama et al. [33] found that undiluted tea tree oil exhibited antibacterial activity against *S. aureus* in vitro, but it was not effective when added to a jojoba base oil. After 6 months of storage at room temperature, the VCO and its massage oils were found not to show any antibacterial or antifungal activities on the test microorganism.

Microbial Counts in VCO and Blended Massage Oils

To ensure the safety of the products, a microbial count was performed. Aerobic bacteria, mold and yeast were not detected in the VCO and massage oil formulations. In addition, a similar result was observed after the preparations were stored at room temperature for 6 months. This result indicates that the preparations were free from microbial contamination and considered safe for customers.

Accelerated Storage Stability Studies

The storage stability of the VCO and the massage oil formulations was carried out at high temperature of 45 °C in order to accelerate oxidation development. Acid, iodine and peroxide values of these preparations were measured to examine possible rancidity. It was found that the development of rancidity of VCO and its massage oil products occurred during storage at elevated temperature as indicated by the significant increase in the acid value and peroxide value in most formulations (paired t test, $p < 0.05$) (Figs. 1, 2). For example, both acid and peroxide values of the massage oils containing lavender oil increased significantly after the formulations were stored at 45 °C for 4 months (paired t test, $p < 0.05$) (Figs. 1c, 2c). Similar to lavender oil, the massage oil formulations containing eucalyptus oil or lemon oil, which were stored at accelerated condition, exhibited higher acid and peroxide values than those of such freshly prepared formulations. However, no significant differences of these values were observed at some concentrations of these oils (paired t test, $p > 0.05$) (see Figs. 1a, b, 2a, b). Based on the aforementioned results, it can be summarized that lavender oil was

Table 6 Antimicrobial properties of virgin coconut oil and blended massage oils compared with positive controls (mean ± SD, $n = 3$)

Blended massage oils	Inhibition zone (mm)				
	S. aureus ATCC 25923	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*B. subtilis*	*C. albicans*
VCO[a]	–	–	–	–	–
5% w/w lemon oil in VCO	–	–	–	–	–
5% w/w lemon oil in VCO-E[b]	–	–	–	–	–
5% w/w eucalyptus oil in VCO	–	–	–	–	–
5% w/w eucalyptus oil in VCO-E	–	–	–	–	–
5% w/w lavender oil in VCO	–	–	–	–	–
5% w/w lavender oil in VCO-E	–	–	–	–	–
Lemon oil	18.67 ± 0.29	28.50 ± 0.70	21.07 ± 1.03	17.10 ± 0.36	–
Eucalyptus oil	–	27.37 ± 2.51	16.77 ± 1.46	–	–
Lavender oil	–	–	17.07 ± 1.60	14.12 ± 0.35	–
Gentamicin HCl (16 μg/mL)	25.6 ± 4.32	22.0 ± 3.0	16.5 ± 1.2	30.0 ± 2.25	n.d.
Ketoconazole (5 μg/mL)	n.d.	n.d.	n.d.	n.d.	35.0 ± 4.75

[a] VCO, virgin coconut oil from pilot scale production (three batches blended together)

[b] VCO-E, virgin coconut oil with the antioxidant (0.5% w/w α-tocopherol)

–, no inhibition zone was observed; *n.d.* not determined; *n* number of sample

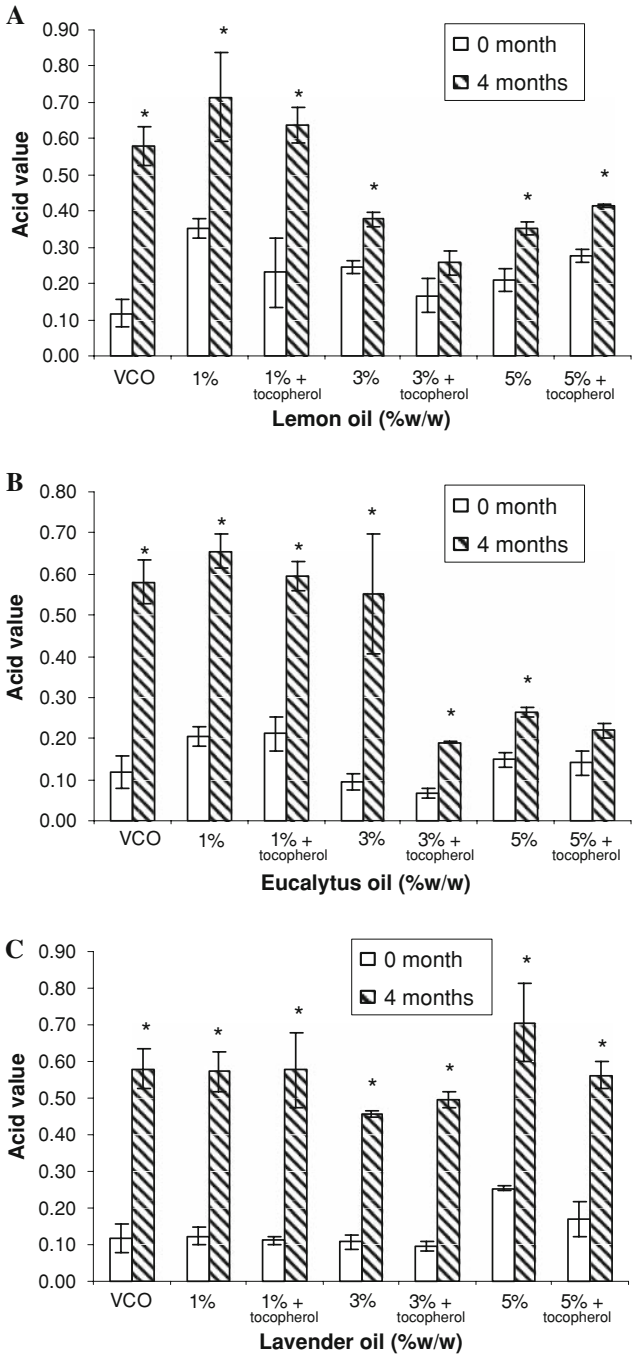

Fig. 1 Acid values of virgin coconut oil (VCO) and blended massage oils after 4 months at 45 °C; lemon oil (**a**), eucalyptus oil (**b**) and lavender oil (**c**). Each value represents mean ± S.D. ($n = 3$, n number of sample). *Acid value of the sample at 0 month is significantly different from that at 4-month storage at 45 °C (paired t test, $p < 0.05$)

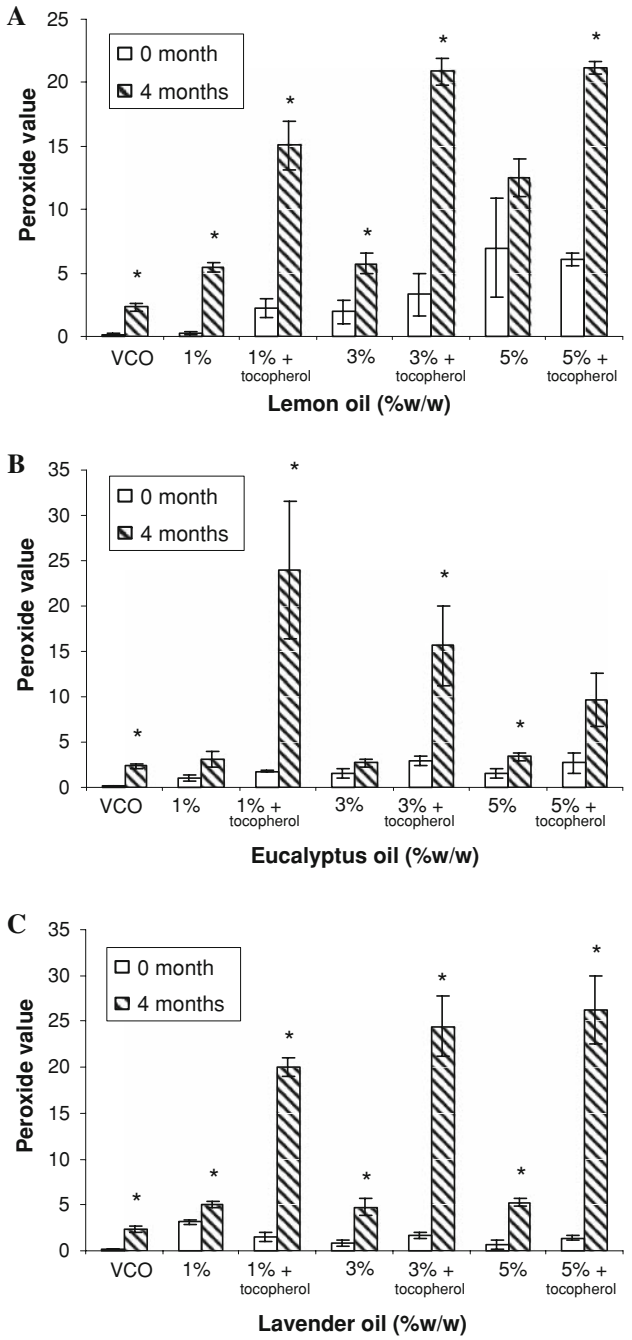

Fig. 2 Peroxide values of virgin coconut oil (VCO) and blended massage oils after 4 months at 45 °C; lemon oil (**a**), eucalyptus oil (**b**) and lavender oil (**c**). Each value represents mean ± S.D. ($n = 3$, n number of sample). *Peroxide value of the sample at 0 month is significantly different from that at 4-month storage at 45 °C (paired t test, $p < 0.05$)

highly susceptible to oxidation processes followed by lemon oil and eucalyptus oil. In contrast to the acid and the peroxide values, the iodine value of the VCO after 4 months of storage at accelerated temperature was significantly lower than the initial iodine value (paired t test $p < 0.05$) (Fig. 3). It is generally recognized that a decrease in the iodine value indicates a decrease in unsaturated fatty acids. Hence, it was likely that the reduction of these unsaturated acids occurred during the storage.

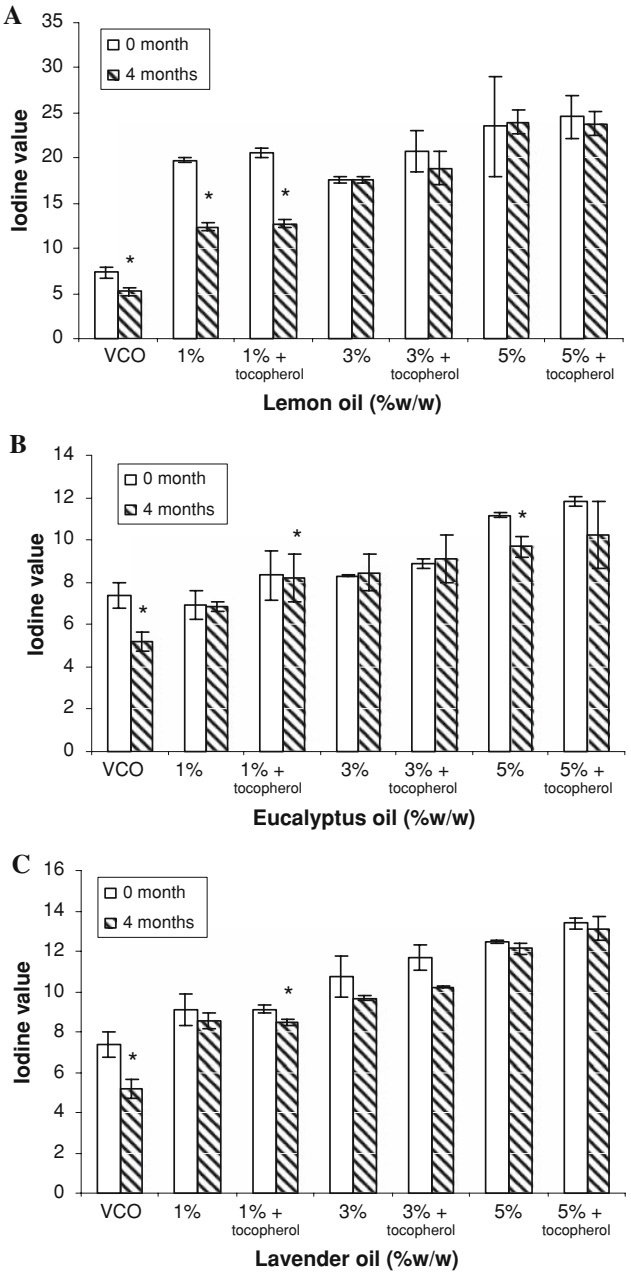

Fig. 3 Iodine values of virgin coconut oil (VCO) and blended massage oils after 4 months at 45 °C; lemon oil (**a**), eucalyptus oil (**b**) and lavender oil (**c**). Each value represents mean ± S.D. ($n = 3$, n number of sample). *Iodine value of the sample at 0 month is significantly different from that at 4-month storage at 45 °C (paired t test, $p < 0.05$)

Generally, the iodine value of the massage oils at the accelerated temperature was not significantly different from the initial value (paired t test, $p > 0.05$) (Fig. 3). Besides, the reduction of the iodine value was observed in some formulations. In the case of lemon oil, the formulations containing 1% w/w lemon oil with and without tocopherol showed a significant decrease in the iodine value (paired t test, $p < 0.05$) (Fig. 3a). However, there was no significant difference in the iodine value at higher concentrations of the oil (paired t test, $p > 0.05$). For eucalyptus oil, the iodine value showed significant reduction in the formulations containing 1% w/w concentration with tocopherol whereas no significant differences were found in the other formulations (Fig. 3b). In the case of lavender oil, the iodine value did not significantly change after the storage at accelerated temperature (paired t test, $p > 0.05$) (Fig. 3c). A significantly lower iodine value was found only in the formulation containing 1% w/w lavender oil with the antioxidant (paired t test, $p < 0.05$). It is interesting to note that at the end of storage period, the acid and peroxide values of the VCO and massage oils increased, whereas the iodine value showed a tendency to decrease. Similarly, Anwar et al. [34], who studied the effect of *Moringa oleifera* oil on the oxidative stability of some vegetable oils, reported that with the advance of the storage period (ambient temperature), the peroxide values of the investigated oils increased from the initial values, whereas the iodine values of the oils decreased. After 4 months of storage at 45 °C, the VCO and the massage oil formulations were still clear and colorless with aromatic scents of coconut oil and essential oils.

At accelerated condition of 45 °C, the positive effect of tocopherol was demonstrated to some extent by the acid value. Generally, the acid values of stored formulations containing tocopherol (0.5% w/w) tended to be lower than those of such formulations without antioxidant (Fig. 1). The significant reduction in the acid value was observed in the stored formulations containing 3% lemon oil or 3% eucalyptus oil in the presence of the antioxidant (Student's t test, $p < 0.05$). Contrary to the acid value, the peroxide values of the stored formulations containing tocopherol were significantly higher than those of such formulations without the antioxidant (Student's t test, $p < 0.05$) (Fig. 2). In generally, the iodine values of the stored formulations with tocopherol did not significantly differ from those of such formulations without tocopherol (Fig. 3).

The present oxidative stability data suggest that VCO and the aromatherapy massage oils should not be stored at high temperature. In order to gain more storage stability details, a further stability study using lowered storage temperatures (i.e. 4 °C, room temperature) and different storage periods needs to be performed to determine the effects of these parameters on the storage stability of the massage oil products.

Positive Remarks of VCO and Massage Oil Formulations

For tropical countries, especially Thailand, VCO can be used as an easily accessible source of vegetable base oil for aromatherapy. In the current study, VCO, which was

produced by the centrifugation process, seemed to meet the specifications of the Thai Industrial Standards Institute. VCO was a good source of saturated fatty acids such as myristic acid and lauric acid. The saturation of VCO and the low values of the important quality parameters obtained suggest its high stability and low susceptibility to becoming rancid. Using visual inspection, all the prepared massage oils exhibited good physical stability as shown by no color change and no precipitation during the study periods of 4 months or 6 months at 45 °C and at room temperature, respectively. In addition, there was no alteration in the aromatic scents of the massage oils during the storage periods. The apparent viscosities of freshly prepared aromatherapy products were rather low and thus only small pressure was required to rub the massage oils onto the skin. Due to their low viscosities, the massage oil preparations could be easily spread over the skin surface. It was revealed that the viscosities of some formulations, particularly the formulations containing lavender oil, did not alter after a 6-month storage period at room temperature. Importantly, both fresh and stored massage oil preparations were free from microbial contamination.

Conclusions

This study has revealed that the most appropriate method for VCO extraction was centrifugation as it gave the highest yield of VCO. The VCO obtained was clear, colorless with a pleasant coconut scent. Most of the fatty acids found in the VCO prepared by the centrifugation method were saturated and the predominant saturated fatty acid was myristic acid. All the prepared massage oils were clear and colorless with the characteristic odor of each essential oil (lemon oil, eucalyptus oil and lavender oil). The viscosity and refractive index of the massage oils was somewhat affected by the formulation composition. In an attempt to increase the oxidative stability of the formulations, additional antioxidant, 5% w/w tocopherol, was added into the massage oils. The results showed that the tocopherol did not seem to have any significant inhibitory effects on the oxidation reaction as it had been expected. Both types and concentrations of the essential oils influenced the oxidation and rancidity of the aromatherapy massage oils as indicated by the alteration of the three indicators; acid, peroxide and iodine values. Generally the rank order of acid value, peroxide value and iodine value of freshly prepared massage oils appeared to be lemon oil > lavender oil > eucalyptus oil. During storage at high temperature (45 °C) for a period of 4 months, both acid value and peroxide value of VCO and the massage oils were found to increase from the initial values whereas the iodine value of the preparations tended to decline. The massage oils containing lavender oil were found to be highly susceptible to the oxidation process followed by lemon oil and eucalyptus oil. Antimicrobial activity was not observed in the VCO and the massage oils. In addition, VCO and its massage oil products were considered safe for customers since they were free from microbial contamination.

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