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RESEARCH ARTICLE

Composition and Characterization of Refined Oil Compared with Its Crude Oil from Waste Obtained from *Mangifera indica*

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ABSTRACT:
Crude and refined seed kernel oil was extracted from *Alphanso* mango aiming the study of physico-chemical properties. The oil produced was refined through degumming, neutralization and bleaching process using local adsorbent (activated clay). The specific gravity, refractive index, acid value, saponification value and iodine value for both crude and refined mango seed kernel oil was determined. The solid form refined mango kernel fat resembles cocoa butter when compared with physical and chemical characteristics. They both are thus, used as a substitute for each other or just as extenders. Mango Fat can be used as edible oil apart from its general uses in manufacturing soap, cosmetic formulations and industrial applications.

KEYWORDS: seed kernel, mango, oil, soxhlet extraction, refining, degumming, bleaching etc.

INTRODUCTION:
Oil is more or less viscous organic liquid. Depending on their chemical composition; a distinction may be drawn between fatty, essential, mineral and silicone oil. Vegetable oils have numerous commercial value as oils and fats satisfy important nutritional requirement of human beings. Certain industrial wastes, such as fruits and vegetables are rich and cheaper source of fats and oils. Seed oils are important sources of nutritional oils, industrial and pharmaceutical[1]. Its content is important nutrients such as protein, starch, minerals, vitamins and fats[2]. The characteristics of oils from different sources depend mainly on their compositions and no oil from single source can be suitable for all the purposes[3]. Seed oils are known to deteriorate when processed inadequately with the principal decomposition reaction being oxidation. Heating is one of the most commonly used methods of food preparation in the home and industries and prolong use of oil for this purpose causes change in its physical and chemical properties[4].

Mango seed kernel oil is thick solid fat, pale yellow in colour. Mango Butter is a tropical butter that has a similar composition to Shea and Cocoa; however the fatty acid content profile is slightly different and enhances the spectrum of natural essential fatty acids, antioxidants and vitamins.

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Mango butter also reduces degeneration of skin cells and restores elasticity. Some dermatologists recommend mango butter for treatment of wrinkles. It has a protective effect against harmful UV radiations from sun. Mango seed kernel oil and its derivatives are used in cosmetic as a preservative since it has high content of stearic acid. It melts at body temperature or upon contact with skin and disperses smoothly, providing a protective, emollient layer.

Literature revealed that mango seed kernel contains about 10-13% percent oil[5]. This can be extracted by selection or combination of processes, such as hydrate presses, continuous screw presses and solvent extraction. However the most satisfactory approach is hot pressing using a hydraulic press, followed by solvent extraction.

In India, Maharashtra (Ratanagiri and Thane) is the major place where the king of fruits Alphanso mango is cultivated on large scale. The pulp of mango is used to prepared brine or in syrup, mango juice, nectar, jam, sauce, chutney, and pickle etc. After consumption or industrial processing of the mango, considerable amount of its seeds are discarded as waste and this can be create an economical imbalance[6].Therefore in the present paper attempt were made to extract and refined oil from the mango seed kernel. Refined seed kernel oil was compared with crude oil by physical and chemical characterization which is not reported yet.

EXPERIMENTAL:

Materials:

Chemicals and reagents

n-hexane, HCl, NaOH, NaCl, alcoholic potassium hydroxide, phenolphthalein indicator, ethyl alcohol, petroleum ether, sodium-thiosulphate, diethyl ether, CCl_4, Potassium iodide and glacial acetic acid. Throughout the experiments are done by using distilled water.

Source: Ripened Alphanso were purchased from the local market of Ratnagiri and Thane District from Maharashtra; India, During March to April; 2009 and 2010.

Methods:

Preparation of Alphanso mango seed kernel powder:

The mango seed kernel is enclosed in the hard cover which was separated manually. The kernel was sun dried in the open, until the casing splits and sheds the seeds. The kernels were further dried in the oven at 50°C[7] for 10-12 hrs to a constant weight in order to reduce its moisture content. The separation of thin cover from the kernel was carried out using tray to blow away the cover in order to achieve very high yield. Stainless steel grinder was used to craft kernels in fine powdered form and stored in brown bottle for prevention of its oxidation.

Extraction of oil from mango seed kernel powder:[8]

The kernel powder (100g) was placed in the thimble and about 300ml of *n*-hexane was poured into the round bottom flask. The apparatus was heated at 60°C and allowed to stay for 5hrs under continuous extraction using Soxhlet apparatus. At the end of the extraction, the resulting mixture *(miscella*) containing the oil was distilled off to recover solvent from the oil. The total yield obtained is expressed in percentage.

Refining of Extracted mango seed kernel oil:

I] Activation of Clay:[9]

The clay sample was obtained from the thane creek reservoir, kalwa town, Maharashtra, India. The clay was ground and then mixed with water. Impurities such as sand and stone were removed. To activate the clay, 2M HCl was added to the clay slurry and the mixture was boiled for 2hrs at about 100°C. The mixture was then washed with water to remove acid, dried and ground.

II] Degumming and Neutralization:[9-10]

The extracted oil was degummed repeatedly by addition of boiling water. The mixture was stirred for 2 minutes and allowed to stand in the separating funnel. Thereafter, the aqueous layer was removed. The procedure was repeated to ensure removal of most gums. For the neutralization, degummed oil was poured into a beaker and heated to 80°C, 0.1M NaOH was added and stirred to obtained uniform solution. Sodium chloride was added to help settle out the soap formed. This was transferred into a separating funnel and allowed to stand for 1hr; the soap formed was separated from the oil. Hot water was added again and again to the oil solution until the total soap remaining in solution was removed. This neutralized oil was used for the further analysis.

III] Bleaching:[10]

Neutralized oil was poured into a beaker and heated to 90°C. Activated clay (15% by weight of oil) was added. The mixture was stirred continuously for 30 minutes. The temperature was allowed to rise to 110°C for another 30 minutes. The content was filtered hot in an over at 70°C. Then oil was cool and used for the further analysis.

Characterization of Physical properties of crude and refined kernel oil:

Proximate analysis was carried out as described by the Association of Official Analytical Chemists[11]

Determination of Moister content (volatile matter):

The crude oil and refined oil (1g) was initially weighed separately and was kept in an oven at 80°C for 6 hrs. The weight of it was measured after every 2hrs. The procedure was repeated until a constant weight was obtained. After every 2 hours, these components were removed from the oven and placed in the desiccators for 30 minutes to cool. It was then removed and re-weighed. The percentage moisture in the oil was calculated for both the samples.

Determination of Melting point:

The melting point was determined by using standard Thiele's melting apparatus.

Determination of pH Value:

In crude oil and refined oil (0.5g), 50ml of hot distilled water was added and both samples were then cooled in a cold-water bath to 25°C. The pH electrode was standardized with buffer solution and the electrode immersed into the samples and the pH value was recorded.

Determination of Specific gravity:

Density bottle was used for determining the density of the oil. A clean and dry bottle of 25ml capacity was weighed (W_0) and then filled with the oil, stopper inserted and reweighed to give (W_1). The oil was substituted with water after washing and drying the bottle and weighed to give (W_2).

The expression for specific gravity is = $(W_1-W_0)/ (W_2-W_0)$ = Mass of the substance / Mass of an equal volume of water.

Determination of Refractive index:

Refractive index of crude oil and refined oil was carried out using the Abbe refractometer. This equipment was first standardized with water, to a refractive index of 1.33. Thereafter, few drops of the oil were transferred into the glass slide of the refractometer. Water at 30°C was circulated round the glass slide to keep its temperature uniform. Through the eyepiece of the refractometer, the dark portion viewed was adjusted to be in line with the intersection of the cross. At no parallax error, the pointer on the scale pointed to the refractive index. This was repeated and the mean value noted and recorded as the refractive index.

Determination of Viscosity:
A clean, dried viscometer with a flow time above 200 seconds for the fluid to be tested was elected. The sample was filtered through a sintered glass (fine mesh screen) to eliminate dust and other solid material in the liquid sample. The viscosity meter was charged with the sample by inverting the tube's thinner arm into the liquid sample and suction force was drawn up to the upper timing mark of the viscometer, after which the instrument was turned to its normal vertical position. The viscometer was placed into a holder and inserted to a constant temperature bath set at 37°C and allowed approximately 10 minutes for the sample to come to the bath temperature at 37°C. The suction force was then applied to the thinner arm to draw the sample slightly above the upper timing mark. The afflux time by timing the flow of the sample as it flow freely from the upper timing mark to the lower timing mark was recorded.

Determination of Solid fat index:
It is determined by AOCS method 1998[12]. The solid fat index (SFI) of mango seed fat describes the amount of solid fat remaining at defined temperatures. The solid fat indices of the oil phase blend must be in the specific range. Also it is an empirical value that is derived from expansion of a fat as a chilled sample is warmed. In the process of melting, previously crystallized parts of the sample become liquefied. Since the fat molecules in a liquid state are less efficiently arranged in space compared to closely packed crystalline regions, liquid fat takes up more volume. Therefore, the degree of expansion is related to the change in the solid content. Dilatometry does not directly measure the solids content of fat at any given time, rather it measures the change in volume compared to the starting point. SFI measurements depend on consistent operator skill and judgment for accuracy and reproducibility. An entire series of SFI determined by following calculations.

CALCULATIONS:

1. SFI at temperature *T* is:

(Total dilation) – [(thermal expansion) × (60 – *T*)]

Where, *T* = observed temperature.

2. Thermal expansion of sample per degree C in ml/kg is:

$$\frac{R(60) - R(37) - Vc\,(37)}{W \times (60 - 37)}$$

Where, *V*c (*T*) = volume corrections for expansion of glass and water at *T* ,
R (*T*) = dilatometer reading at *T*, *W* = weight of sample.
3. Total dilation between *T* and 60° in milliliters/kilogram

Characterization of chemical properties of crude and refined kernel oil:
Determinations for peroxide, iodine, and saponification values, unsaponifiable matter and free fatty acid contents were carried out using standard analytical methods (13).

Table 1: Characterization of physical properties of mango seed kernel oil (fat)

Properties	Mango seed kernel Crude oil	Mango seed kernel Refined oil
Appearance	Semi solid soft fat	Semi solid soft fat
Colour	Pale yellow	White
Odour	Neutral odour	Slight fatty odour
Solubility	Insoluble in water	Insoluble in water
% Oil content	10.2	10.2
%Moister content (volatile matter)	0.53	0.5
Melting point °C	29.2	29
pH (10g/I)	Slightly acidic at 28°C	Neutral at 28°C
Specific gravity g/ml 28°C	0.910+-0.03	0.910+-0.03
Refractive Index	2.5	2.3
Viscosity	42 mPas at 37°C	44 mPas at 37°C
Solid fat index (%)		
20°C	55.5	55
25°C	53.2	53
30°C	52.8	52.1
35°C	0	0

Determination of Saponification Value:
Crude and refined sample of kernel oil (2g) were weighed into a conical flask separately. After adding 25ml of 0.1N ethanolic potassium hydroxide, the content was constantly stirred for 1hr followed by reflux. By using phenolphthalein as indicator, it was titrated with 0.5M HCl till the until the solution changes to colourless. The same procedure was repeated for the blank. The expression for saponification value (S.V.) is given by: S.V = 56.1N (V_0-V_1)/M, where V_0 = the volume of the solution used for blank test; V_I = the volume of the solution used for determination; N = Actual normality of the HCl used; M = Mass of the sample.

Determination of Unsaponification value:
Fat sample (5g) along with 50ml of alcoholic potassium hydroxide solution was refluxed for 1 hr or until the saponification is complete. Saponified mixture was transfer to a separating funnel washed with ethyl alcohol followed by cold water. The temperature of mixture was approximately 20 to 25ºC. Petroleum ether (50 ml) was added with vigorously shaking to allow the separate layers. The lower soap layer was transfer into the separating funnel and the ether extraction was repeated for another 3 times using 50 ml portions of petroleum ether. Residue was dissolved in 50 ml of warm ethanol, neutralized and the procedure was repeated for the saponication value and it was titrated with 0.02N NaOH.

Calculations
Weight in g of the free fatty acids in the extract as oleic acid = 0.282 VN
Where, V = Volume in ml of standard sodium hydroxide solution,
N = Normality of standard sodium hydroxide solution
Unsaponifiable matter = 100 (A-B)/W, Where A = Weight in g of the residue,
B = Weight in g of the free fatty acids in the extract,
W = Weight in g of the sample

Determination of Free fatty acid:
Equal volume of diethyl ether and ethanol was added to 10g of crude and refined oil separately. The mixture was titrated with 0.1M NaOH using phenolphthalein indicator. Free fatty acid of crude and refined oil was dark pink colour was observed and the volume of 0.1M NaOH (V_0) was noted.
Free Fatty Acid (FFA) was calculated as: $V_0/W_0 \cdot 2.82 \cdot 100$,
Where 100ml of 0.lM NaOH = 2.83g of Oleic acid,
W_0 = sample weight

Determination of Iodine Value:
0.4g of the sample was weighed into a conical flask and 20ml of carbon tetra chloride was added to dissolve the oil. Then 25ml of Dam's reagent was added to the flask using a safety pipette in fume chamber. Stopper was then inserted and the content of the flask was vigorously swirled. The flask was then placed in the dark for 2 hours 30 minutes. At the end of this period, 20ml of 10% aqueous potassium iodide and 125ml of water were added using a measuring cylinder. The content was titrated with 0.1M sodium-thiosulphate solutions until the yellow colour almost disappeared. Few drops of 1% starch indicator was added and the titration continued by adding thiosulphate drop wise until blue coloration disappeared after vigorous shaking. The same procedure was used for blank test and other samples.
The iodine value (I.V) is given by the expression: I.V = 12.69C $(V_1-V_2)/M$,
Where C = Concentration of sodium thiosulphate used;
V_1 = Volume of sodium thiosulphate used for blank;
V_2 = Volume of sodium thiosulphate used for determination,
M = Mass of the sample.

Determination of Peroxide value (PV):
Peroxide value in the oil was determined by using glacial acetic acid, chloroform: 0.01 M sodium thiosulphate and potassium iodide (KI) 10% as the major solvents in 1.0 g of oil sample.

Table 2: Characterization of chemical properties of mango seed kernel oil (fat)

Properties	Mango seed kernel Crude oil	Mango seed kernel Refined oil
Saponification value (mg of KOH/g of oil)	173.3	171.1
Unsaponicable matter	3.45	3.45
Free fatty acid	1.11	1.0
Iodine value(g of 1/100g of oil)	45.55	45.55
Peroxide value(PV)	2.5	2.38
Acid value	2.22	2.0

Determination of Acid value (AV):
25ml of diethyl ether and 25ml of ethanol was mixed in a 250ml beaker. The resulting mixture was added to 10g of oil in a 250ml conical flask and few drops of phenolphthalein were added to the mixture. The mixture was titrated with 0.1M NaOH to the end point with consistent shaking for which a dark pink colour was observed and the volume of 0.1M NaOH (V_0) was noted. Free Fatty Acid (FFA) was calculated as: $V_0/W_0 \cdot 2.82 \cdot 100$, where 100ml of 0.lM NaOH = 2.83g of Oleic acid, W_0 = sample weight; then Acid Value = Free fatty acid·2.

RESULTS AND DISCUSSION:

The chemical properties of oil are amongst the most important properties that determines the present condition of the oil. The results obtained from percentage oil content are the yield of the final product depends on various factors like extraction process, physical parameters, and refining of crude oil. Extracted oil of mango seed kernel is a semi solid, pale yellow coloured fat, which turned colourless and odour less after refining and bleaching. Alphanso mango seed kernel contains 10.2 % oil content which falls within the range of the percentage oil content (10 – 12%) still it is depend on the variety of mango kernel. Though, something close to 100% yield has been expected, the mode of extraction is a very important parameter which affects the yield. Many researchers have used Soxhlet extraction method for extraction of mango kernel oil.

In comparison crude and refined mango seed kernel oil showed the percentage moisture content, 0.5% and 0.53%, melting point 29.2 ^oC, 29 ^oC respectively. pH value of the crude oil is slightly acidic but neutral in case of refined oil. This may be due to result of degumming and neutralization carried out during the refining process. Mango seed oil was insoluble in water, the specific gravity values for both crude and refined oil are obtained 0.910 +- 0.03 is equal. The refractive index analysis shows that there is little difference between the value obtained for crude oil, 2.5 and that of the refined oil, 2.38. It may be due to experimental error range that can be attributed to the presence of some impurities and other component of the crude oil mixture. Thus, the refractive index of both crude and refined mango kernel oil was in agreement with AOAC specification. Differences were observed between the value obtained for the viscosity of the crude and refined oil is 42 mPas and 44 mPas at 37^oC.

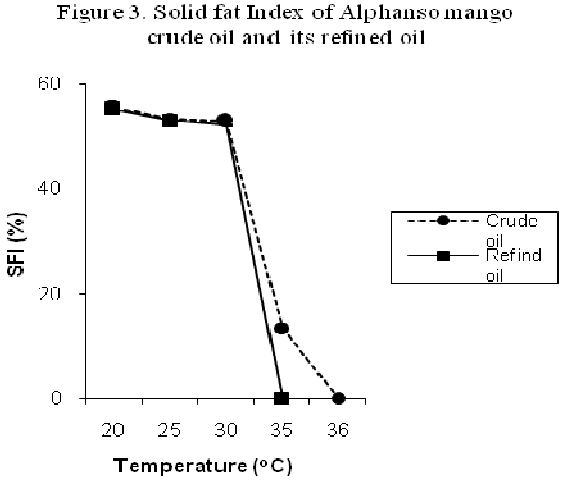

Figure 1. Solid fat index (%) of the crude and refined oil at 20 ^0C – 35 ^0C.

SFI values for fats that contain emulsifiers are not very accurate due to some dissolution of emulsifiers into the indicator at the fat/indicator boundary. A strong impetus therefore exists to adapt an alternative method. The solid fat index (SFI) of mango seed fat describes the amount of solid fat remaining at defined temperatures it is shown in fig. 1.

The chemical properties of oil are free fatty acid and peroxide values are valuable measures of oil quality. The iodine value is the measure of the degree of unsaturation of the oil. The low free fatty acids content is indicative of low enzymatic hydrolysis. This could be an advantage as oil with high free fatty acids develops off flavour during storage (14). The saponification value is high and this suggests the use of the oil in production of liquid soap, shampoos and lather shaving creams. Table 2 shows the results for the saponification value of the crude and refined oil that were found to be 173.3mg KOH/g of oil and 171.1mg KOH/g of oil respectively. It indicates that, for the crude oil, required more alkaline to enable it neutralize the available freely fatty acid librated by the oil, when compared with the refined oil.

The products formed during this oxidative process can be determined by chemical analysis and one of the frequently used tests employed to predict the quality of seed oils is the determination of peroxide value and iodine value. The average degree of un-saturation of the oil shows the amount of iodine which can be absorbed by unsaturated acids is higher 45.55 which equals to refined one. As a result of their agreement with standard, both the crude and refined oil could be classified as a non-drying oils (15), since their iodine values are lower than 100. Certainly, the oils are used extensively as lubricants and hydraulic brake fluids. The chemical properties analysis shown in the Table 2 indicates that the acid value of crude and refined oil is 2.22mg NaOH/g of oil and 2.0mg NaOH/g of oil respectively. The value is higher in crude oil due to free fatty acid present; while it less for the refined oil as a result of the chosen strength 0.1M of NaOH used in the treatment of the crude oil, which must have neutralized some of the free fatty acid present in it. In addition, both values fall within the range specified in literature. The extraction and use of vegetable oils has for centuries played an important role in the manufacture of a large number of industrial products and food items (16). This cram show to facilitate the mango seed is a excellent foundation of oil. Mango seed oil was obtained since the seed kernel with good yield (10.2%), allowing the possibility of economical exploitation.

The process quality assurance of this oil preserve is monitoring using iodine value and peroxide value. These satisfactory results achieved by solvent extraction process using laboratory Soxhlet apparatus. The mango seed oil produced in this research work was analyzed for specific gravity, Viscosity at 28°C, refractive index at 28°C, acid value, saponification value and iodine value. Most of the values comply with the standard specifications. The oil is of good quality and could be recommended suitable for industrial usage. High unsaponifiable matters content (3.45%) guarantees the use the oils in cosmetics industry. It may use alternative source of coca butter because it contains unsaturated fatty acid grater that saturated in refined oil. The observations in this study are useful in commercial processing of mango fruits and utilization of mango kernel fat and meal.

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