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Research Article

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Effects of Extraction Solvents on the Yield and Physicochemical Properties of *Mangifera indica* L. Seed oil

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Abstract The extraction of oil from mango seeds was investigated using a Soxhlet Extractor. Mango seeds were collected, dried and grounded into powder. Hexane and ethanol were used to extract the oil at varying time of extraction of 4, 5, 6, 7 and 8 hours, respectively. Result shows that yield is time dependent, as increase in time of extraction results into increase in yield where the least time of 4hrs yielded an average of 4.00 % and 8hrs yielded an average of 13.06 % for hexane. While the use of ethanol at 4hr and 8hrs only yielded 1.99 % and 8.22 %.Thus the use of hexane achieved the maximum yield (13.06 %) of oil extracted. The effects of time and extraction solvent on the yield of oil was investigated by using a 2^2 factorial design and, result shows that factor 'A' which is time, produces the greatest effect (7.665) in the yield of mango than factors 'B' (3.335) being solvent and 'AB'(1.335) being interaction between solvent and time. The effect of the extraction solvent on the physicochemical properties was also investigated where the oil extracted with hexane was found to possess better overall quality than the ethanol extracts, acid value, saponification value, ester value, refractive index and specific gravity were 5.61mgKOH/gOil, 207 mgKOH/gOil, 201.39mgKOH/gOil, 1.443 and 0.909, respectively as against 30.30mgKOH/gOil, 205 mgKOH/gOil, 174.70mgKOH/gOil and 0.909, respectively of ethanol. The results didn't only show that hexane gives a higher yield of oil than ethanol but also revealed that hexane is a much better solvent for the extraction of mango seed oil.

Keywords *Mangifera indica*, seed oil, Extraction, Physicochemical characterization

Introduction

The mango fruit is a drupe or stone fruit which embeds a shell within its hardened endocarp. *Mangifera indica* tree belongs to the Anarcardiaceae family, a family of fruit bearing trees [1]. Mangoes are one of the most important fruits worldwide and they are grown in tropical and sub-tropical regions especially Asia [2]. The mango is original to the Indian subcontinent, South-east Asia and Africa. It is cultivated in many tropical regions and distributed widely in the world [1]. It is one of the most extensively exploited fruits for food, juice, flavour, fragrance and colour and is a common ingredient in new functional foods. Chutneys, jams, sauce, nectar, pickles are also made from mangoes. Various parts of the tree are used in medicines and its leaves are ritually used as floral decorations at weddings and religious ceremonies [3-4].

Mango trees (*Mangifera indica*) reach 35 - 40 m in height, with a crown radius of 10 m. The leaves are evergreen, alternate, simple, 15 - 35 cm long and 6 - 16 cm broad; when the leaves are young they are orange-pink, rapidly changing to a dark glossy red, and then dark green as they mature. The fruit takes from 3 - 6 months to ripen. The ripe fruit is variable in size and colour, and may be yellow, orange, red or green when ripe, depending on the cultivar [2]. Nearly half of the world's mangoes are produced in India, but the country accounts for a small percentage of the international trade because India consumes most of its own production [5]. While India is the largest producer of Mangoes in the world China and Thailand are the second and third largest producers, respectively [6-7]. The fruit pulp is high in dietary fiber, vitamin C and vitamin A [8]. It contains essential vitamins and dietary minerals at good levels. The mango peel also contains essential nutrients. According to mango varieties, the seed (kernel and endocarp) represents from 10 % - 25 % of the whole fruit weight. The kernel inside the seed represents from 45 % - 75% of the seed and about 20% of the whole fruit [2]. The kernel can be ground into kernel powder for oil extraction. The oil is semi-solid at room temperatures, but melts on contact with skin, making it appealing for baby creams, heat-care balms, hair products and other moisturizing products [9]. Also, the confectionary industry has developed an interest in the possibility of using mango kernel oil as a substitute for Cocoa butter which is very expensive [10].

The fruit of the Mango is never totally consumed or processed because the seed is protected by a hardened shell (endocarp). This seed is majorly disposed of after consumption but could also be subjected to industrial processing [11]. Due to the large utilization of mango fruits, more than one million tons of mango seeds are being produced as waste annually [2]. If these seeds could be utilized one way or another, it would eliminate wastage and also birth the production of new products. The usefulness of the whole Mango kernel, especially the oil, in comparison with mango juice, is yet to gain rural and industrial attraction in African countries including Nigeria. This underutilization could be partly due to the limited knowledge of the composition of the kernel oil and its toxicology status [12] hence, this research work is necessary so as to provide more knowledge on the required experimental conditions for optimum production of mango kernel oil and on the properties of the oil. Therefore, this work is not only aimed at extracting oil from mango seeds by varying experimental conditions and characterizing the oil, but to determine the effect of solvent extraction on the physiochemical properties of the extracted oil.

2. Materials and Methods

2.1. Raw materials and reagents

Ripe mangoes were obtained from Oba Market, Benin City in Oredo Local Government Area of Edo state, Nigeria, hexane and ethanol were obtained from Charlec Laboratory, Nigeria Ltd.

2.2. Methods

Flowchart of the process is presented in Figure 1

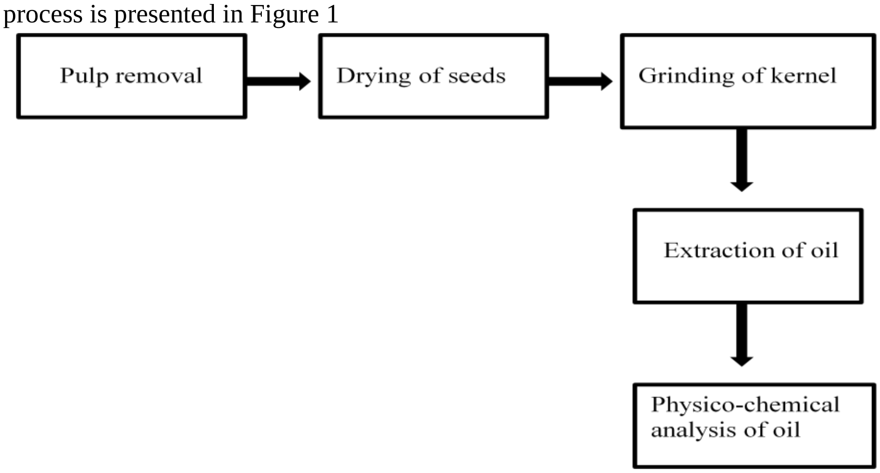

Figure 1: Flowchart process of oil extraction from mango seeds

2.2.1. Removal of pulp: The mango pulps were peeled out and the seed kernel were dried gradually.

2.2.2. Drying: The seed kernels were dried in a drying oven at 100 ºC for 8 hours. The seeds were then cracked open and the kernels were removed. However, further drying was carried out on kernels that were still moist.

2.2.3. Grinding: All the kernels were first subjected to preliminary grinding using a mortar and pestle. Then the reduced kernels were further ground using a kitchen blender to make the particle size of the kernel powder less than 2000 microns. The kernel powder was then stored in a plastic container in a cool and dry place.

2.2.4. Extraction of oil: Oil was extracted from the kernel using a soxhlet apparatus and a heating mantle. The soxhlet apparatus is made up of a condenser, an extractor and a flask (round bottom or flat bottom) which is being heated. The kernel powder encapsulated in filter paper was placed in the extractor. The organic solvent used for extraction was placed in the flask. The solvent vaporizes and rises to the condenser where it is condensed. The condensed organic solvent then drips on the kernel powder. The kernel powder upon contact with hot organic solvent begins to secret oil which is soluble in the organic solvent. When the oil and solvent mixture has risen high enough (higher than the siphon tube), the mixture flows through the siphon tube back into the flask. The oil is then recovered from the organic solvent by heating the mixture with a condenser coupled to the flask. The organic solvent vaporizes and is then condensed back into another container.

2.2.5. Effect of time and choice of solvent on oil yield

Process condition such as time and choice of solvent were carried out to determine their effects on the yield of oil from the kernel powder. The experiment was run 10 times at different times of 4,5,6,7, and 8 hours, respectively using both Hexane and Ethanol as different solvents according to the method described by Willams [13].

2.2.6. Physico-chemical analysis of the mango oil

2.2.6.1 Specific gravity

The specific gravity (SG): This is a dimensionless unit defined as the ratio of density of the mango oil to the density of water at a specified temperature. This was done by measuring the density of mango oil in reference to the density of distilled water at 20^0C using a specific gravity bottle. First the specific gravity bottle was weighed while empty. It was then filled with mango oil and weighed again. Then the difference in weights was divided by an equal volume of water to obtain the specific gravity of the mango oil [14].

2.2.6.2 Refractive Index

The refractive index of a substance measures how the substance affects light travelling through it. It is equal to the speed of light in a vacuum divided by the speed of light in that substance. When light travels between two materials with different refractive indexes, it bends at the boundary between them. The refractive index test was carried out using a programmable Refractometer. Here, the apparatus was standardized using pure distilled water whose refractive index at 20 ºC is 1.3330. This was followed by replacing the distilled water with a drop of the sample in the machine. After about 1-2 minute(s) the machine read off the refractive index.

2.2.6.3 Saponification Value

Saponification value is the number of mg of potassium hydroxide (KOH) required to saponify the esters in 1g of a sample, and to neutralize the free acids. It also indicates the amount of average molecular weight of triglycerides contained in the oil. This involves weighing 1g of oil into 250ml dry round bottom flask. 50ml of 0.5ml alcoholic potassium hydroxide was added to the oil. Porous beads were added to ensure uniform heating. The reflux condenser was setup and the contents of the round bottom flask was refluxed for about 1hr, after refluxing the mixture is allowed to cool and then titrated against standard hydrochloric acid and the titre values are recorded. Similarly,50ml of the same alcoholic KOH, blank (no oil added) was refluxed in a round bottom flask for 1hr, cooled and titrated against standard 0.5N HCl. The titre value was also recorded and the titre value obtained was used to determine the saponification value using Equation (1):

$$Saponification\ value = \frac{56.1 \times Z \times M\ of\ HCl}{Weight\ of\ sample} \quad (1)$$

where: Z = volume of HCL required to neutralize excess alkali (ml); M = strength of the KOH.

2.2.6.4 Acid value

Acid value (or "neutralization number" or "acid number" or "acidity") is the mass of potassium hydroxide (KOH) in milligrams that is required to neutralize one gram of chemical substance. The acid number is a measure of the amount of carboxylic acid groups in a chemical compound, such as a fatty acid, or in a mixture of other compounds. This involves weighing 1g of the oil sample into 250ml conical flask. Meanwhile 95% alcohol (neutral alcohol) was prepared by diluting methanol with sodium hydroxide (5ml NaOH + 95ml ethanol = 100ml neutral alcohol).50ml of neutral alcohol and 50ml benzene were added to the oil in the flask. The contents of the flask were shaken well to dissolve. The contents were then titrated against 0.1N potassium hydroxide solution using phenolphthalein as indicator. The end point was the appearance of a pale permanent pink colour and the titre value were recorded. The acid value is thus calculated using Equation (2):

$$Acid\ value = \frac{X \times M \times 56.1}{Weight\ of\ sample} \qquad (2)$$

where: X is the volume of KOH required to neutralize the oil solution; M is the strength of KOH

2.2.6.5 Ester Value

Ester value was obtained by subtracting the acid value from the saponification value. Ester value represents the number of milligrams of potassium hydroxide required to saponify the esters present in 1g of the oil.

3. Results and Discussion

Results

3.1 Determination of yield of oil

For the two extraction solvents, hexane and ethanol, the yield of oil is shown below. (Detailed calculations are shown in appendix C)

Table 1: Yield of oil from hexane extraction

Time (hrs)	Amount of oil extracted (g)	Average yield (%)
4	1.20	4.003
5	2.00	6.676
6	3.00	10.021
7	3.70	12.374
8	3.90	13.069

Table 2: Yield of oil from ethanol extraction

Time (Hrs)	Amount of oil extracted (g)	Average yield (%)
4	0.6	1.991
5	1	3.313
6	1.7	5.629
7	2.2	7.266
8	2.5	8.223

3.2 Factorial Experiment Design Using Manual Method

where A = Time (- = 4hours, + = 8hours)

B = Organic solvent (- = Hexane, + = Ethanol)

Table 3: Manual factorial results (Detailed calculation is shown in appendix C)

	Main Effects		Interaction Effects	
Run	**A**	**B**	**AB**	**Yield (%)**
1	-	-	+	4.00
5	+	-	-	13.00
6	-	+	-	2.00
10	+	+	+	8.33
Absolute value of effect	7.665	3.335	1.335	

From the above table, it is shown that factor '**A**' which is time produces the greatest effect in the yield of mango, 7.665.

Table 4: Factorial Design of Experiment Using Minitab

Minitab Factorial Experiment results

Run Order	Blocks	Center Pt	Solvent	Time	Yield (% w/w)
1	1	1	Hexane	4	4
2	1	1	Hexane	8	13
3	1	1	Ethanol	4	2
4	1	1	Ethanol	8	8.333

Table 5: Absolute effect of main effects from Minitab

Term	Absolute effect from Minitab
Time (A)	7.665
Extraction solvent (B)	3.335
Time and Solvent (AB)	1.335

Table 6: Physicochemical Properties of extracted mango oil

Oil Sample	Acid value (mgKOH/ gOil)	Saponification value (mgKOH/gOil)	Ester value (mgKOH/gOil)	Refractive index	Specific gravity at 20 ºC
Extracted with Hexane	5.61	207.0	201.39	1.443	0.909
Extracted with Ethanol	30.30	205.0	174.70	1.451	0.900

Table 7: Comparison of mango oil yield with literature

Extraction Solvent (8hours)	Experiment yield (% w/w)	Literature yield (% w/w)
Hexane extract	13.00	13.00
Ethanol extract	8.33	6.96

Table 8: Comparison of Physicochemical properties of extracted oil with literature values

Oil Sample	Acid value (mgKOH/gOil)	Saponification value(mgKOH /gOil)	Ester value (mgKOH/gOil)	Refractive index	Specific gravity at 20 ºC
Hexane extract Experimental	5.61	207.00	201.39	1.443	0.909
Hexane extract Literature	5.35	207.5	202.15	1.433±0.02	0.900±0.03
Ethanol extract Experimental	30.30	205.00	174.95	1.451	0.900
Ethanol extract literature	27.55±0.55	206±13.8	178.45±13.25	1.444±0.01	0.903

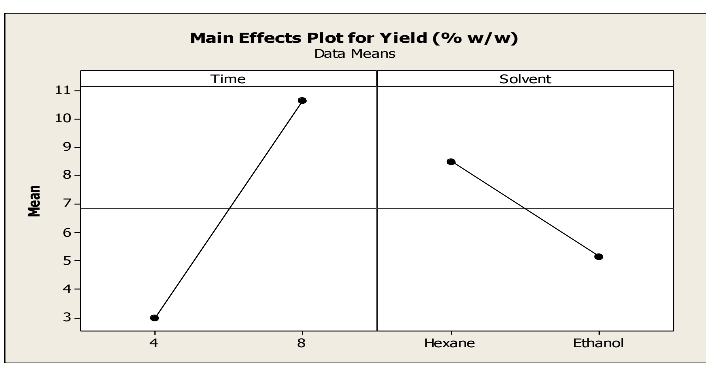

Figure 2: Main effects plot for mango oil yield

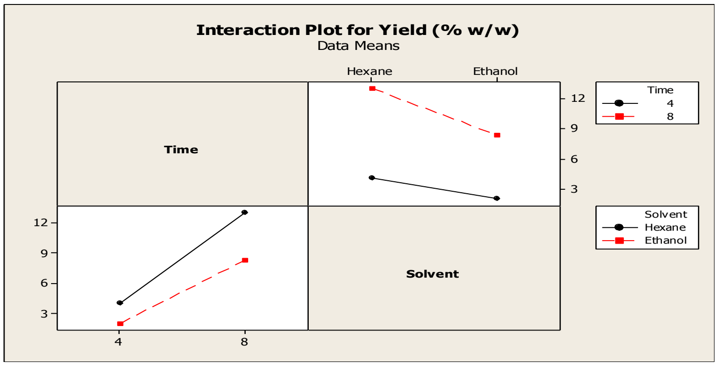

Figure 3: Full interaction plot matrix for mango oil yield

4. Discussion of Results

4.1. The Effects of the Extraction Solvent and Time on the oil Yield

The effect of extraction time on the yield is clearly shown in Table 1 and 2 using hexane and ethanol as extraction solvents, respectively. It was observed that the yield of oil increased as the time of extraction increases from 2 to 8 hours. This was so because enough time is required for the oil to break away from the kernel powder. The longer the time of extraction, the longer the kernel powder stays in contact with the extraction solvent. The rate of increase in yield is much faster at lower extraction times than at higher extraction times. This is so because as the time of extraction increases, the amount of oil that can be extracted from the kernel powder reduces until no more oil can be extracted.

Comparing the yields from the two solvents, results showed that hexane achieved more yield than ethanol at the different extraction times. This is because hexane is a very non-polar solvent which easily extracts the non-polar mango oil; while Ethanol which has both polar and non-polar parts doesn't extract as much oil. A polar solvent dissolves polar solutes easily and doesn't mix with non-polar solutes well. Thus ethanol can only dissolve oil because it has a non-polar part. In all, hexane is still considered as a better extraction solvent because it has a lower boiling point (68^{o}C) than ethanol (78^{o}C). This implies that hexane needs lesser energy than ethanol to extract. Apart, hexane is easier to recover after the extraction. Since ethanol requires a higher temperature than hexane, suffice to note that higher temperature might damage the oil being extracted.

4.2. The Factorial Experiment Design for the effects of various factors on the oil yield

In determining the oil yield, a factorial experiment was designed using 4 runs (i.e. 2^2) where the 2 main effects are: (A = time) and (B = extraction solvent) was put into consideration at their highest and lowest values. (I.e. time - = 4hours and + = 8hours, extraction solvent - = Ethanol and + = Hexane). The absolute value of effect - main effects and interaction effects were determined and the result as presented in Table 3 shows that the factor A, time, as determined was the major factor affecting the oil yield with absolute value of 7.665. Thus, increase in time produces an increase in the yield of mango oil and also the factor B, extraction solvent, had a great effect on the oil yield with absolute effect of 3.335.

The manual calculation of the factorial experimental results was compared with the factorial experimental design calculation using a software called "Minitab," as shown in Tables 4 and 5 and the results were found to compare favourably well and the same, particularly with extraction carried out with hexane.

4.3. Physicochemical properties of mango oil

The acid value of mango oil is the mass of KOH in milligrams that is required to neutralize 1g of the mango oil. Basically, the acid value is used to quantify the amount of acid (free fatty acids, acid phosphates or amino acids) present in a sample. For oils, it is a measure of the free fatty acid content. Free fatty acids exist in oil because of the hydrolysis of fatty acid which is usually accelerated with heat and pressure. Result shown in Table 6 revealed that the Ethanol extract has a higher acid than the Hexane extracted oil. This is because more fatty acids hydrolyzed during the ethanol extraction than during the hexane extraction. Since the hydrolysis is accelerated by heat, the ethanol extraction which requires a higher temperature than that of hexane would cause more fatty acids to hydrolyze. Hence, using ethanol as an extraction solvent yields oil with high acid content. The acid value of both extracts agrees well with literature [3, 15].

Refractive index is the ratio of the speed of light in a vacuum to that in the oil under examination which is related to the degree of saturation and the ratio of *cis/trans* double bonds. It can also provide hints on the oxidative damage and for rapid sorting of fats and oils of suspected adulteration. The refractive index as shown in Table 6 increased from 1.443 to 1.451 for the hexane and ethanol extracts, respectively. it can be inferred that the refractive index of the ethanol extract was slightly higher than the hexane extract because the mango oil underwent adulteration due increase in heating temperature since the high boiling point of ethanol is higher (78 ºC). However, both refractive index values of the mango oil compared well with literature [3, 15].

The differential analyses of the saponification value of the two extracts were found to be of a negligible amount. It implies that the saponification value of mango oil is not dependent on the extraction solvent used. This high saponification value of the extracted oil makes a good candidate for the soap industry. Both saponification values of the mango oil fall within the literature range of values [3, 15].

Suffice to note that the ester values decreased from 201.39 to 174.95 for the hexane and ethanol extracts, respectively, and the higher the ester value, the more the palatability of the oil. Hence the hexane extracts would be more palatable. Both ester values and together with the specific gravity of both hexane and ethanol extracts were also found to fall within literature range [3, 15].

5. Conclusion

The use of soxhlet extractor for the extraction of oil from mango seed was found to depend majorly on two factors; extraction solvent and time of extraction. Although the time factor was the major determinant of the mango oil yield, however, the use of hexane gave a higher yield than ethanol. The acid value and refractive index of the mango oil from ethanol extraction were higher than those of the hexane extraction. The ester value from the hexane extract was also higher than that of the ethanol extract. Thus, this study has shown that hexane extraction gives a higher oil yield and better quality than ethanol extraction.

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