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A Comparative Study of the Extraction and Characterisation of Oils from *Glycine max* L. (Soya Bean Seed), *Elaeis guineensis* (Palm Kernel Seed) and *Cocos nucifera* (Coconut) Using Ethanol and n-Hexane

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KU and AE designed the study, author UJ performed the statistical analysis, authors EM and OC wrote the protocol and the first draft of the manuscript. Authors OA and CI managed the analyses of the study. Authors OC, OI and EC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To extract and characterize oils from *Glycine max* L. (soya bean seed), *Elaeis guineensis* (palm kernel seed) and *Cocos nucifera* (coconut) using ethanol and n-Hexane.
Place and Duration of Study: Department of Industrial Chemistry, Renaissance University, Ugbawka, between December 2017 and July 2018.
Methodology: Using Soxhlet extraction method, the milled seed samples (soya bean, coconut and palm kernel) were bagged and extracted with accurately measured quantity of each solvent (n-

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Hexane and Ethanol). Physicochemical analysis which includes: Acid values, iodine value, free fatty acid (% oleic) and saponification value was carried out on the extracted oil.

Results: The percentage oil yield from ethanol extracts were 8.58% (8.58 g), 26.01% (26.01 g) and 31.21% (31.21 g) for soya bean, coconut and palm kernel respectively while that of n-Hexane extracts were 14.69% (14.69 g), 31.85% (31.85 g) and 45.28% (45.28 g), due to the fact that 100 g of the milled sample was used. Free fatty acid values were the same in n-Hexane and ethanol extracts of soya bean oil. These values were slightly higher in the coconut and palm kernel oil extracts using ethanol. Acid values were observed to be slightly higher in the ethanol extracted oils. The Saponification values were generally higher in ethanol extracted oils than in hexane extracted oils. There was maximum oil recovered with hexane than ethanol when the extraction process was carried out for 120 minutes.

Conclusion: In spite of the fact that n-Hexane gave a better extraction yield, ethanol appeared to be a better alternative for the extraction process because it is green, less toxic and safer to handle.

Keywords: Extraction; characterization; solvent; soxhlet; n-Hexane; ethanol.

1. INTRODUCTION

There have been an increase in the world production of oilseeds over the last thirty years [1], this appears to be related to the increasing demand for oilseed products and by-products as oilseeds are primarily grown for their oil and meal [2]. Oils from most edible oilseeds were used in the food industry. Though there is growing emphasis on industrial utilization as feedstock for several industries with about 80% of the world production of vegetable oils for human consumption, the remaining 20% utilization is between animal and chemical industries [3]. According to Takads [4], bio-oils from oilseeds are used as Straight Vegetable Oil (SVO) or as biodiesel (trans esterified oil) depending on type of engine and level of blend of the oil; soya bean oil is not an exception. This phenomenon has created a school of thought that it is better to use oilseeds as bio-fuel, which will lessen the competition for fossil fuels, which are not renewable [5]. Fossil fuels are not only costly in terms of price but are also costly to the environment as they degrade land, pollute water and cause a general destabilization of the ecosystem with global warming as an end result [6]. Furthermore, crude oil wields socio-political power that often dictates the pace of economic growth in specific locations, especially non-oil producing nations. Nevertheless, the petroleum industry requires a greater quantity of oil to meet its demand. In order to meet the required amounts needed by all industries, these fats and oils must be available in large quantities locally with an effective extraction process at an affordable cost [7].

The ability of a particular oilseed to fit into the growing industries depends on its utilization potential, rate of production, availability and ease of the processing technology. Thus while some oilseeds are being largely utilized in the oil processing industries, quite a number of oilseeds are under-exploited. Soya bean (*Glycine max* L.), coconut (*Cocos nucifera*) and palm kernel (*Elaeis guineensis*) are important oilseed crops in the world, they are rich in protein and oil 14-20% in soya bean, about 45% in palm kernel, and 30% in coconut [7]. Oil can be extracted from these seeds by using traditional methods of extraction (on a very small scale), mechanical expression (hydraulic and screw presses) which can be manual, semi-automated or automated, and solvent extraction (e.g. hexane, fluid carbon dioxide) or a combination of two of these methods. The efficiency of traditional methods of extracting the oil is low (less than 50%), labour intensive, time consuming and possibly compromising quality and safety standards [8]. Mechanical pressing of oilseeds is the most common method of edible oil extraction used in the world [9]. The main reason for this is that it provides a non-contaminated, protein-rich low-fat deoiled cake, an important byproduct in many developing nations at a relatively low cost. However, mechanical presses do not have high extraction efficiencies, even when using hydrothermally treated oilseeds. Extraction efficiencies seldom exceed 80%, compared to over 98% achieved by the solvent extraction method [10]. Solvent extraction is capable of removing virtually all of the oil available in the cells of the oilseed though the equipment required is generally too expensive to acquire and there is the inherent danger of fire explosion due to the chemicals used in the extraction process [11]. The process of solvent extraction, however, is faster and less expensive compared to mechanical extraction. Conventionally, soya beans are subjected to solvent extraction because of the low oil content (about 18-20%) and relatively lower oil recoveries in mechanical expression systems. Solvent extraction, in general, implies the removal of miscellanea from an insoluble solid phase by dissolution in a liquid solvent [4]. Literature on the exact quantities of oil extracted per seed conditioning and kind of solvent apart from hexane is unknown. Hexane however, is classified as being hazardous to working staff and based on this assertion has been banned by the government of the United States of America. It is therefore important to determine the extraction rates for other suitable solvents such as ethanol, which are available at relatively cheaper prices [2].

Increasing the extraction of oil to meet the rising demand for vegetable oils in different industries requires a suitable solvent which is readily available in the country at a relatively cheaper cost [5]. This study therefore, sought to provide a fair idea on a possible solvent replacement using ethanol which is available, has ability to extract oil from vegetable seeds and it may be produced from a large variety of biological materials using simple technology over hexane, which is considered hazardous, expensive and occasionally scarce based on demand and cost of petroleum [12].

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The samples used in this study- soya bean seed, palm kernel seed and coconut were seasonal crops. These seeds were readily available in the dry season [13,14,15]. The samples were bought from Ogbete Main Market Enugu, Nigeria in December, 2017. The samples were washed with distilled water and oven dried at a temperature of 80°C for 5 hrs. The samples were further milled until fine particle size was achieved. A 100 g of the milled seed sample (soya bean, coconut and palm kernel) were bagged and used for the extraction process.

2.2 Solvent Extraction Process

Solvent extraction was done, using Soxhlet extraction apparatus [16]. Exactly 150 ml of each solvent (n-Hexane and Ethanol) was measured into 250 ml flat bottom flask and heated at a constant temperature of 70°C using n-Hexane and 80°C using Ethanol to reflux. The heat caused the solvent to vapourise through the thimble containing the sample as the solvent boiled in the flask; the vapour was trapped and cooled by the condenser above the thimble. The cooling turned the vapour into warm liquid which hydrolyzed the sample in the thimble. When the thimble was filled with the drops of the warm solvent from the condenser, the solvent (which contained traces of the oil) was poured out into the flat bottom flask beneath the thimble containing either n-Hexane or Ethanol automatically through a siphon arm. The process was continued for the duration of 2 hrs for each sample. At the end of each extraction process, the milled sample was removed from the thimble and the extraction process repeated, but this time for a different sample seed. The oil was poured into a weighed empty beaker and placed in a steam bath at a controlled temperature of 65°C. At the end of each extraction, the weight of the extract was multiplied by 100 and divided by the original mass of the sample (100 g) to give the percentage oil yield.

2.3 Determination of Oil Quality

Several indices are used for the determination of oil quality. For the purpose of this experiment, free fatty acid, iodine value, acid value and saponification value were determined.

2.3.1 Free fatty acid

Free fatty acid is the percentage by weight of a specified fatty acid (e.g. % oleic acid) [9]. High levels of free fatty acids especially linoleic acids are undesirable in finished oils because they can cause off-favours and shorten the shelf life of oils. The free fatty acid was determined according to the Official Method Ca 5a-40 of American Oil Chemists' Society AOCS (1993) [17]. About 0.5 g of the extracted oil sample was weighed into a 250 ml Erlenmeyer flask using an analytical balance before 20 ml of 95% neutralized ethanol was added to the flask. The solution was heated slightly to 20°C to aid the dissolution of the fat in the alcohol. 3 drops of phenolphthalein solution were added as indicator. The obtained yellowish solution was titrated with 0.1 M standard sodium hydroxide solution while shaking the solution vigorously. The colour of the solution turned pink and at the point when the pink colour persisted for 30s was termed the end point. The percentage of free fatty acid in the oil was calculated as follows:

$$\%\,Free\ Fatty\ Acid\ = \frac{T.V \times M \times 28.2}{Weight\ of\ sample}$$

where,

T.V = average volume of NaOH (ml),
M = Molarity of NaOH (0.1),

2.3.2 Iodine value

The iodine value was determined according to Official Method Cd 1-25 of AOCS [17]. 0.5 g of the filtered oil sample was weighed into a 500 ml flask. 15 ml of tetrachloride was added to the sample and swirled to ensure that the sample was completely dissolved in the tetrachloride. 25 mL of Wijs solution was dispensed into the flask containing the sample and swirled to ensure an intimate mixture. The flask with content was immediately kept in a dark place at a temperature of about 25°C – 30°C for 30 mins. The flask was removed and 20 mL of 10% potassium iodide (KI) solution added followed by an addition of 150 mL of distilled water. The solution was titrated with 0.1M Sodium thiosulphate ($Na_2S_2O_3$) solution while shaking it constantly and vigorously until the reddish colour had almost disappeared. 5 mL of 1% starch indicator was added and the titration continued until the blue-black colour just disappeared. A blank was preformed alongside.

$$Iodine\ Value = \frac{(T.\ V_2 -\ T.\ V_2) \times\ M\ \times\ 12.69}{Weight\ of\ sample\ used}$$

where,

$T.V_2$ = Titration of $Na_2S_2O_3$ blank (ml),
$T.V_1$ = Titration of $Na_2S_2O_3$ sample (ml),
M = Molarity of $Na_2S_2O_3$ solution (ml),

2.3.3 Acid value

Acid value is the number of milligrammes of potassium hydroxide necessary to neutralize the free acids in one gram of oil sample. The samples that contain virtually no free acids other than fatty acids, the acid value may be directly converted by means of a suitable factor to percent free fatty acids [18]. Where vegetable oils are used as lubrication products, the acid value can affect the properties of the lubrication oil, if larger quantities reach the oil sump.

The Acid value was determined according to the Official Method Ca 5a-40 of American Oil Chemists' Society (AOCS) (1993) [17]. About 0.5 g of the extracted oil sample was weighed into a 250 ml Erlenmeyer flask using an analytical balance. 20 ml of 95% neutralized ethanol was added to the flask. The solution was heated slightly at 20°C to aid the dissolution of the fat in the alcohol. 3 drops of phenolphthalein solution were added as indicator. The obtained yellowish solution was titrated with 0.1 M standard sodium hydroxide solution while shaking the solution vigorously. The colour of the solution turned pink and at the point when the pink colour persisted for 30s was termed the end point. The percentage of Acid in the oil was calculated as follows:

$$Acid\ Value = \frac{(A -\ B)\ \times (M)\ \times\ 56.1}{Weight\ of\ sample\ used}$$

where,

A =NaOH used in titration (ml)
B = NaOH used in the blank (ml)
M = Molarity of NaOH

2.3.4 Saponification value

The saponification value was determined using Official Method Cd 3- 25 AOCS (1993) [17]. About 0.5 g of the dried and filtered oil sample was weighed into a 250 ml Erlenmeyer flask before adding 50mls of 0.5M ethanolic KOH. The content was refluxed and allowed to stand for 15 mins. A blank was conducted simultaneously where all reagents were added with the exception of the oil sample. 1 ml of phenolphthalein indicator was added to the sample and the sample titrated with 0.5M hydrogen chloride (HCl) until the pink colour just disappeared and the volume of the HCl recorded.

$$Saponification\ Value = \frac{(T.\ V_2 - T.\ V_2) \times\ M \times 65.1}{Weight\ of\ sample\ used}$$

where,

$T.V_2$ = 0.5M HCl required to titrate blank (ml),
T.V1 = 0.5M HCl required to titrate sample (ml),
M = Molarity of HCl solution (ml)

3. RESULTS AND DISCUSSION

3.1 Oil Extraction Yield

Comparing oil extracts in Table 1 and Fig. 1, there were significant differences between mean yields from the solvents n-Hexane and Ethanol. From Table 1, it can be deduced that at p value of 0.05, there were significant differences in the mean yield of the soya beans, coconut and palm kernel oil extracted. The p-values were 0.002, 0.001 and 0.00 for soya beans, coconut and palm kernel oils respectively. The average percentage oil yield using n-hexane were

14.69%, 31.85% and 45.28% for soya bean, coconut and palm kernel respectively while the percentage oil yield for ethanol were 8.58%, 26.01% and 31.21% for soya bean, coconut and palm kernel respectively. N-hexane yielded relatively higher oil in all the samples compared with ethanol. Palm kernel yielded the highest amount of oil closely followed by coconut, and soya bean having the lowest oil yield. This is because hexane is a very non-polar solvent which easily extracts the non-polar seed oils [18]; while ethanol which has both polar and non-polar parts does not extract as much oil. A polar solvent dissolves polar solutes easily and mixes poorly with non-polar solutes [19]. Thus ethanol can only dissolve oil because it has a non-polar part. The low oil yield observed with ethanol may also be ascribed to a lower selectivity of ethanol for the oil than other compounds such as phosphatides, polyphenols which could be present in the oil seeds [20]. The low extraction of oil with ethanol can also be attributed to the solubility of the seed oils in ethanol. The oil yield of ethanol could be improved by increasing the solvent oil ratio of ethanol and the surface area of ethanol in contact with oil seeds during oil extraction according to Devesh et al. [21].

Comparing the oil yields of the seeds in Table 2, it was observed that palm kernel had the highest oil yield, followed by coconut and soya beans. This could be due to the differences in the composition of the seeds.

3.2 Free Fatty Acid Value

Free fatty acid values were the same in n-Hexane and ethanol extracts of soya bean oil. Soya bean oil contains low fatty acids compared to coconut and palm kernel oils [2]. The fatty acid values were higher in the coconut and palm kernel oil extracts using ethanol. 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 extraction solvent, will yield oil with high acid content. The acid value of both extracts agrees with the findings of Nkafamiya et al. and Balaji et al. [22,23].

3.3 Acid Value

Acid values were observed to be higher in the ethanol extracted oils which was expected as the fatty acid values were high, probably due to the hydrolysis of the oils through the solvent. However, the overall low acid value of the oils implies a good stability of the oils and also which suggests low levels of hydrolytic and lipolytic activities in the oils. The lower the acid value of oil, the few fatty acid it contains which makes it less exposed to rancidity [23,24].

3.4 Saponification Value

In combination with acid values, saponification values are useful in providing information as to the quantity, type of glycerides and mean weight of the acids in a given sample of oil. Saponification is only of interest if the oil is for industrial purposes, as it has no nutritional significance [2]. These values are generally higher in ethanol extracted oils compared to n-hexane extracted oils. The larger the saponification number, the better the soap making ability of the oil [25]. The saponification value gives an indication that the extracted oil sample is not only suitable for the food industry but is also suitable for other industries (e.g. soap manufacture) [2]. The high saponification value may also be attributed to the nature of the oils and the metallic ions present among other factors [26,27].

3.5 Iodine Value

Iodine value is a measure of the unsaturation of fats and oils, and is expressed in terms of the number of centigrammes of iodine absorbed per gramme of the sample (% iodine absorbed) during oxidation, which consumes the double bonds resulting in a reduction in iodine. It is an indicator for double bindings in the molecular structure, which influences the long term stability properties of the oil (important for storage) [24]. Oils having high iodine number are polyunsaturated indicating the degree of unsaturation and are desired by oil processors, while a lower iodine number is indicative of lower quality [25]. The high iodine value indicates dehydrogenation. It is a measure of unsaturation in lipid, which again determines the degree of flow. The results of the iodine value in the ethanol extracted oils were high which may be due to the high content of unsaturated fatty acids in these oils, as observed by Abdullah et al. [28]. The high iodine values of the oils shows a good quality as regarding to its stability during storage.

Table 1. Oil extraction yields of the seeds per solvent

	Soya beans (mean± SD) 100 g	Coconut (mean± SD) 100 g	Palm kernel (mean± SD) 100 g
n- Hexane	14.62±0.09*	31.93± 0.11*	45.29± 0.02*
Ethanol	8.28± 0.43	26.16± 0.21	31.24± 0.03
p- value	0.002	0.001	0.00

**significant at p≤ 0.05*

Table 2. Comparing the oil yields of the seeds

Extracting agent	Soya beans	Coconut	Palm kernel
n- Hexane	14.62[a]	31.93[b]	45.29[c]
Ethanol	8.28[a]	26.16[b]	31.24[c]

[c] = Highest oil yield; [b] = Low oil yield; [a] = Lowest oil yield

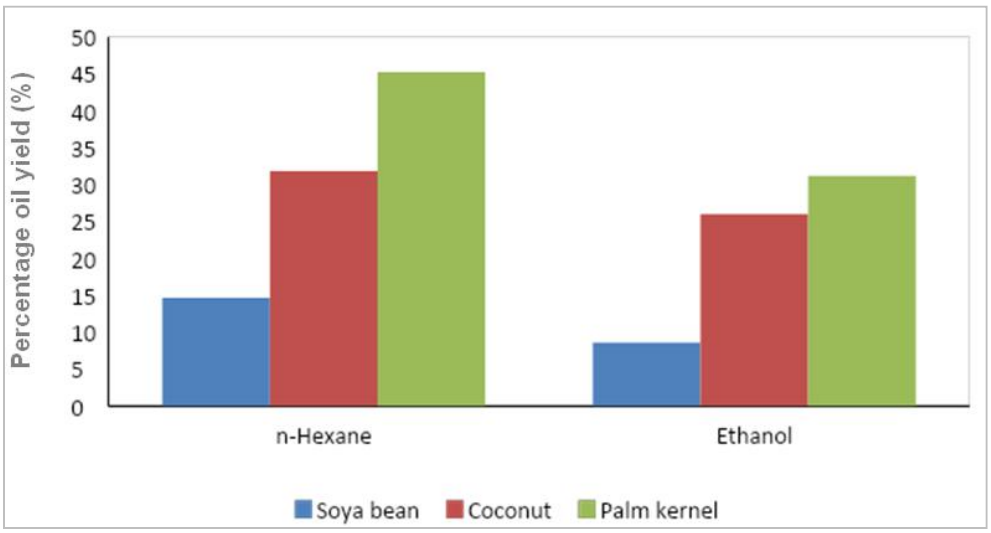

Fig. 1. Comparative chart of oil extraction yields of the seeds per solvent

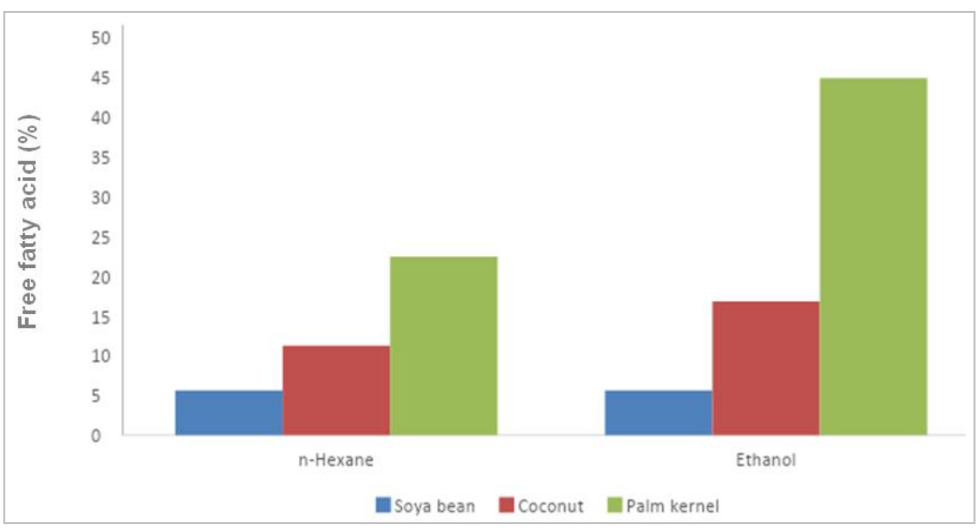

Fig. 2. Comparative chart of the free fatty acid value of the oils

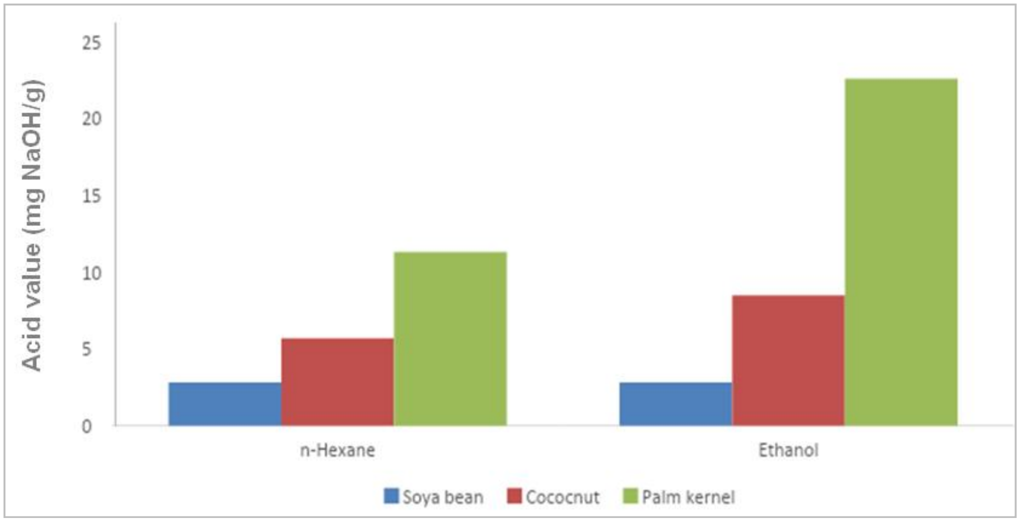

Fig. 3. Comparative chart of acid value of the oils

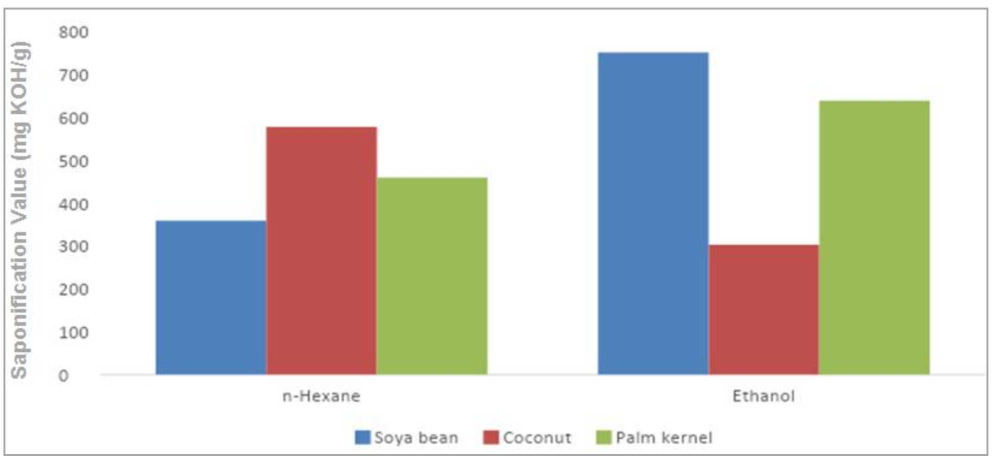

Fig. 4. Comparative chart of the saponification Value of the oils

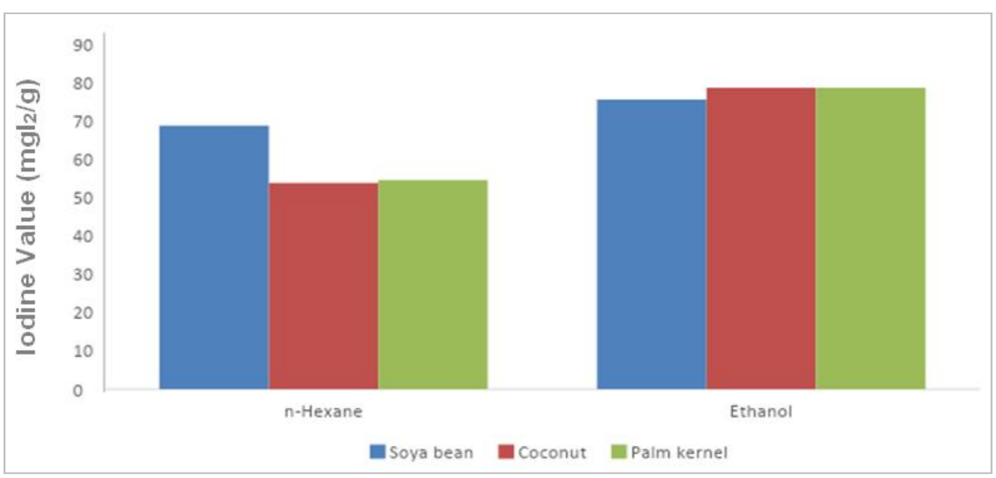

Fig. 5. Comparative chart of the iodine value of the oils

3.6 Oil Yields

For maximum oil yield, important parameters were taken into consideration:

3.6.1 Effect of extraction time

The extraction time is an important parameter for solvent extraction. It helps in deciding the optimum residence time required in an extractor. It is observed that extraction of oil increased with time for both the solvents. It is also observed that the rate of extraction is very high during the first 20 minutes of extraction and afterwards it tapers off. There is maximum oil recovered with hexane than ethanol when the extraction process was carried out for 120 minutes. The initial high rate of extraction may be due to quick solubility of the oil present at the solid surface and higher mass transfer driving force provided by low oil concentration in the fresh solvent. The later slower rate may be attributed to lower driving force due to increasing oil concentration in the solvent [29]. This result agrees with the findings of [21].

3.6.2 Effect of particle size

The extraction was carried out from the milled seeds of the palm kernel, coconut and soya bean. Lower extraction rate can be attributed to the fact that the bigger particles have less surface area directly exposed to the solvent as compared to the smaller size and the solvent has to penetrate into the core of the seed to leach the oil out of the seed which restricts the rate of oil extraction from the bigger seed particles. Thus the seeds used for this work had to be milled to very fine powdered particles for maximum yield of the oils.

3.6.3 Odour

The odour of the coconut oil was that of coconut smell, the palm kernel oil was that of palm kernel smell and the soya bean oil had relatively no smell. This result is due to the fact that the oils were not refined, bleached or deodorized.

4. CONCLUSION

The result of the solvent extraction of oils from Soya bean (*Glycine max* L.), coconut (*Cocos nucifera*) and palm kernel (*Elaeis guineensis*) oilseeds using ethanol and n-Hexane, showed that had a better extraction yield than ethanol. However, considering that there are remedies to improving the oil yield and amongst other factors, ethanol, could be a fair replacement for n-Hexane for oil extraction.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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