Extraction and Characterization of Oil from Lima Beans Using 2^3 Full Factorial Designs

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Abstract

A study on the extraction and characterization of oil from lima beans with the application of 2^3 full factorial designs has been carried out. The extraction was done by solvent extraction using n-hexane as solvent with soxhlet apparatus. The 2^3 full factorial designs involve varying three parameters, volume of the solvent, time and mass of the sample for two levels each. The results showed that the extraction: volume (X_1) and mass of the sample (X_3) have a positive effect on the yield but time (X_2) has a negative effect on the entire process. The oil obtained was then subjected into physical and chemical analysis to determine the features, or qualities. It was found that the oil was light yellow in color with a characteristic odor at the extract and has melting point, boiling point, pH value and refractive index of 88^{o}C, 89^{o}C, 6.03 and 1.57, respectively. Also, saponification value, acid value and iodine value are 134mgKOH/g, 5.036mg/KOH, and 6.77g, respectively. The 2^3 full factorial designs and the mathematical model applied form a first order regression equation as: $Y = 0.8294 + 0.1094X_1 – 0.0231X_2 + 0.0844X_3 – 0.0006X_{12} + 0.0194X_{13} + 0.4581X_{23} – 0.3719X_{123}$, which was obtained showing the individual effect of volume, time and mass and the interaction of the entire process.

***Keywords**: solvent extraction, saponification, acid value, iodine value, refractive index.*

Introduction

The market of oil and fat is gradually expanding, probably at a rate slightly faster than the increase in population, and the demand for both domestic and industrial use is met by extracting the oil from plant and animal fats.

Farmers and small business owners wondered if it is possible and profitable to add value to their seeds and nuts by extracting the oil but it is not easy because there are so many variables. The expansion of trade naturally puts pressure on the commodity and in the first instance the increase in demand can be met by the simple expedient of growing more crops (McIntosh and Miller 2001).

Crops that contribute most significantly to the market of oil and fat are groundnut, soya bean, cotton seed, etc., and other series of beans such as lima beans, though very low in fat and with smaller beans, but rich in fiber and health beneficial (Bachmann 2004). Lima beans *(Phaseolus lunatus)*, also referred to as sieva beans or butter beans, are in the family Fabaceae. Man has cultivated edible beans for thousands of years, they are widely planted and useful for home gardens. Early varieties were tough and required string removal and long cooking to soften them (Wright 1993). Before the late 19th century, most lima beans were raised for shelled, dried beans, not fresh green beans.

In Australia, lima beans are principally used as green baby lima beans canned in brine either alone or as a component of mixes of 3-4 beans for use in salads. They are also retailed directly to food consumers as raw beans in small 200g-1kg packages. Currently, up to 1,000 t/year are imported, no lima beans are commercially produced in Australia despite several years of research demonstrating the

feasibility of such production (Redden *et al.* 1996, Redden 1998).

Lima beans, as well as the oil, though low in fat of about 0.71%, are very rich in the best sort of fiber – soluble fiber that helps to eliminate cholesterol from the body. The oil from lima beans is a good source of potassium, iron, copper and manganese. It is low-sodium oil that helps to reduce blood pressure. It supplies high-quality protein, which provides a healthy alternative to meet other animal proteins.

Lima beans are used industrially in the production of margarine and salad oil. The oil from lima beans is also used domestically for cooking but the large portion of it is used in the edible fat industries. However, it is very expensive, unlike other seed oil which is common and rich in fat and could bring an increase in the potential market. Thus the demand for oil from lima beans is very low because it is expensive and it is produced on a not too large scale (Ensminger *et al.* 1996).

Most oil processing in the US is done on a large industrial scale, the process uses proprietary information. Small-scale oil extraction is more commonplace in some parts of the country. But due to the economic imbalance and the fluctuations in the exchange rate, engineers and scientists are coming out with new technologies of getting oil from seed with ease by the use of simple equipments at a cheaper rate (Wood 1988).

Crude vegetable oils and fats are being extracted by the following methods: hydraulic pressing method, mechanical pressing method and solvent extraction method.

In this work, solvent extraction method will be used, which involves the counter current flow of solvent and oil bearing material in the extraction vessel. Solvent extraction, though being a complex operation, constitutes the most efficient method for the recovery of oil from any oil bearing material and yields high quality oil. The common solvents are hexane, benzene, and methylene chloride, which are derivatives of petroleum.

Extraction of oil from lima beans is done in this work by using the 2^3 factorial design methods to improve upon its yield, where two factors of two levels and three variables of treatment were considered. The factors include solvent type and concentration factor, which varied in accordance with the level of treatment.

Thus it has become necessary to extract oil from lima beans using 2^3 factorial design methods to improve upon its yield and characterize it to reveal the predominant constituent of lima beans that may have some other medical importance as well.

Extraction Method

The process of extraction was carried out by a direct method of extraction in which both the sample and the solvent were mixed together into a round bottom flask.

The mass of the sample was weighed constant for the first four runs but the volume of the solvent and the time varied for the first two and the last two runs. Four Soxhlet apparatuses were used at a row.

For the first apparatus, 15g of sample and 300ml of solvent were mixed together in a flask of the extractor for 3 hours. For the second apparatus, 15g of sample and 150ml of solvent were mixed together for 2 hours. For the third apparatus, 15g of sample and 300ml of solvent were mixed together for 3 hours. Also for the fourth apparatus, 15g of sample and 150ml of solvent were mixed together for 2 hours. The extractors were then connected to glass tubes in the middle, then to the condenser above it and then placed on heating mantle set at 50^oC and the time was set at 3 hours for the first and second apparatus and 2 hours for the third and forth apparatus. At the end of each time interval, the middle glass tubes were disconnected and the round bottom flasks containing the solvent, oil and the sample were removed and then allowed to cool to room temperature. Each of these samples was then filtered to separate the residue. Filtrate, solvent and oil were placed in a beaker for the solvent to evaporate by exposing it to atmosphere to save time instead of recovering the solvent. The oil and the beaker were weighed as w_1 after which the oils were poured together and the beakers were washed, dried and weighed as w_2 to determine the mass of the oil. The oil percentage was calculated as follows:

weight of oil + beaker = w_1,
weight of beaker = w_2,
% yield of oil
= ((15g – (w_1 - w_2))/15g)*100%.

The whole process was repeated for another constant mass of 10g for the second four runs until the complete extraction was effected. The pure oil obtained was removed and dried on a hot plate set at 100^{o}C for 15 minutes to remove some of the solvent left in the oil.

Characterization Method

Physical Analysis

Boiling Point: This was determined by putting the oil in a beaker and inserting a thermometer into it, it was heated to bubble on a heating mantle and the temperature was immediately recorded as its boiling point.

pH: The pH electrode was lowered into the buffer solution for the standardization of the pH meter. The calibrated control was adjusted and the meter indicated the exact pH. The electrode was rinsed with water and then with a portion of the sample oil and then immersed into the oil sample for about 3 minutes, the pH and the temperature of the oil were noted.

Refractive Index: A few drops of the oil were placed on the face of the prism of the refractometer and allowed to gently spread, closed and tightened for some time for the oil and the prism to attain a steady temperature. The refractive index was then read from the demarcation line after adjusting to where it coincides with the diagonal crossing.

The specific gravity, the viscosity, and the density of the oil were not characterized due to the very low yield of oil as it was indicated in the literature survey that lima beans contain 0.71% of fat.

Chemical Analysis

Acid Value: Acid value of lima oil was determined as Acid value = 2·FFA, where the free fatty acids (FFA) value of oil is given by:

$$\text{FFA} = \frac{\text{volume of base x } 2.82}{\text{sample weight}}.$$

Saponification Value: 0.5g of lima oil was weighed into asconical flask and 25ml of KOH (molecular weight = 56.1) was added to it. The conical flask was attached to reflux condenser and allowed to boil in a water bath for thirty minutes with occasional stirring. Immediately after 30 minutes, 1 mL of phenolphthalein solution was added to the mixture and was titrated while hot against 0.5M HCl acid solution. The volume of 0.5M HCl used was noted as "*a* ml", blank determination was also carried out with the same method using distilled water, and the second volume used was also noted as "*b* ml". Thus,

$$\text{Saponification value} = \frac{(b-a) \text{ x } N \text{ x } 56.1}{\text{sample weight}},$$

where N is the normality of HCl solution.

Iodine Value: 1g of oil was added to 10ml of CCl_4 and was dissolved in 10ml of w_{ij}'s solution. The mixture was initially moistened with potassium iodide solution and then allowed staying in a dark for 30 minutes. The solution was titrated with 0.1 M sodium thiosulphate using starch as indicator just before the end point (clear solution). The titre value was taken as "*s* ml". The same process was repeated for blank titration and was recorded as "*b* ml", so that

$$\text{Iodine value} = \frac{(b-s) \text{ x } M}{\text{sample weight}},$$

where M is the molarity of sodium thiosulphate.

Results and Discussion

Tables 1-4 show the results obtained from the extraction and characterization of oil from lima beans. During the extraction, certain parameters were varied to observe the net effect on the extraction processes: the volume of the solvent, the time and the mass of the samples were varied while the temperature and some other controllable parameters were kept constant.

As shown in Tables 1 and 2, numbers of runs were conducted for 3 hours and 2 hours each with different volumes of solvent and different masses of samples and each run was repeated two times making a total of 16 runs at constant temperature of 50^{o}C.

The first run was conducted two times with 300 ml of n-hexane solvent and 15g of sample for 3 hours, the yield was 1.00 and 0.98g, respectively, which implies that the yield of oil drops for the second time. For the 2nd run, with 150 ml of solvent and 15g of sample for 3 hours, each trial yielded 0.77 and 0.79g which is lower than the 1st run due to the decrease in the volume of the solvent, but for the same time interval and the same mass of sample this means that the use of more solvent results in the better yield of oil.

For the 3rd run, with 300 ml of solvent and 15g of sample for 2 hours, the yield was 1.15 and 1.04g, respectively, which implies that even with the same volume of solvent and the same mass of sample but for different time intervals compared with the first run the extraction yielded more oil. In this case, the time is also regarded as been affecting the rate of yield of oil.

For the 4th run, with 150 ml and 15g for 2 hours, the extraction yielded 0.80 and 0.78g, respectively. It can therefore be deduced from here that from the 1st four runs the highest yield of oil for the two time intervals is the run 3 where the volume of 300 ml and 15g of mass for 2 hours yielded 1.15 and 1.04g, respectively. From this result, it implies that the use of more solvent and more mass for short time yielded more oil at constant temperature.

Also, for the second 4 runs, the volumes of the solvent remain 300 ml and 150 ml for 3 and 2 hours but the sample reduces to 10g for each of the runs.

The 5th run, with 300 ml and 10g for 3 hours, yielded 0.85 and 0.83g, respectively. The 6th run, with 150 ml and 10g for 3 hours, yielded 0.62 and 0.61g, respectively. Also, the 7th run, with 300 ml and 10g for 2 hours, yielded 0.78 and 0.88g, respectively. Therefore, the 2nd level of the 7th run with more solvent for short time yielded 0.88g, more than the yield of 0.85g in the 1st level of the 5th run. The 8th run, with 150 ml and 10g for 2 hours, yielded 0.70 and 0.69g, respectively.

From these experimental results one could further realize that during the process of extraction, because of the small particle size, the interfacial area between the solid and the liquid increases, so more solvent will be able to diffuse into a greater part of the solid in the smallest distance possible for a shorter time interval, thereby intensifying the extraction process.

Therefore, the increase in the volume of the solvent increases the amount of extract for a shorter time interval. Also, the percentage of yield of oil reduces as the sample weight decreases.

The oil from lima beans was characterized according to the physical and chemical analysis as shown in Tables 1 and 2. The physical parameters determined are color, odor, boiling point, pH and refractive index. The color, which was determined by close observation, is light yellow, the boiling point and the melting point of the oil were taken as 88^{o}C and 89^{o}C, and the refractive index was 1.57 at 29^{o}C, which is greater than those of the groundnut oil and soybeans oil ranging from 1.460 to 1.470, and that of the walnut being equal to 1.473 at 250^{o}C. The use of refractive index, which is obtained by observing the propagation of light through a substance, is needed to detect adulterated oil. The pH value was gotten to be 6.03, which indicates the presence of an unsaturated fatty acid in the oil since the pH is almost acidic in nature. The acid value was also calculated as 5.036mg KOH/g, which is the measure of free fatty acid present in the oil. Some deterioration that took place during storage of either raw materials from which oil is obtained (i.e., the storage of lima beans) or oil after isolation results from the hydrolysis of triglycedic to yield free fatty acid. The iodine value gives total degree of unsaturated substance expressed in the percentage of iodine absorbed by the oil, therefore, it implies that 6.77g of iodine was absorbed by 100g of fat.

The whole analysis was carried out and the values were calculated without comparing them with any standard value since no previous study had ever being carried out on the extraction of oil from lima beans as the beans contain approximately little fat. This implies that all the values lie within the experimental values, therefore, these values could be used as standard values or as a reference for future studies on oil from lima beans.

From the factorial design analysis, a mathematical model was obtained from the entire extraction process, where 2^3 full factorial designs with two levels and three variables were used. The general form of the factorial mathematical model for the entire extraction process is given as follows:

$$Y = 0.8294 + 0.1094X_1 - 0.0231X_2 + 0.0844X_3 - 0.0006X_{12} + 0.0194X_{13} + 0.4581X_{23} - 0.3719X_{123}.$$

From the model equation, the following observations were made.

The volume of the solvent X_1 has a positive effect of 0.1094, the time X_2 has a negative effect of -0.0231, and the mass of the sample X_3 has a low positive effect of 0.0844 on the entire process of extraction. The interaction of the solvent volume and the time X_{12} has a negative effect of -0.0006 on the process, which is very low compared to the effect caused by the interaction of the solvent volume and the mass X_{13} of 0.0194 which is positive. The interaction of the time and the mass X_{23} has a very high positive effect of 0.4581 on the process. The interaction of the three parameters, volume, time and mass X_{123} produces a very high negative effect of -0.3718 on the process.

Therefore, from the model equation one can deduce that the percentage of yield of oil would increase for a shorter time interval as both the volume of the solvent and the mass of the sample increase during heating at 50^{o}C.

Conclusion

It can be concluded from this study that there are some active components present in lima beans as well as a lot of nutrients which are very good for body development. Also, lima beans contain very small quantity of oil, therefore, such oil cannot be used industrially because large quantity of lima beans will be needed in order to obtain the quantity of oil needed industrially as lima beans are grown in the southern part of America and are very scarce in Nigeria. The costs of getting the beans and the extraction will be very high, thereby making it to be very expensive. From the experimental results, the yield of oil obtained using hexane as a solvent showed that an increase in yield is achieved by increasing both the sample quantity and the volume of solvent. Concerning the physical properties of the oil, the odor analyzed gave a pleasant smell which implies that if a large quantity of oil could be gotten it would have been a better substitute for groundnut oil or any other seed oil used for cooking. The color of the oil is light yellow. Due to the low yield of oil, the density, the specific gravity and the viscosity could not be determined but the extract has a refractive index of 1.57, which means that if the oil is eventually gotten in large quantity, it can stay for some period without deterioration. The saponification value was recorded to be 134mgKOH/g and the acid value was found to be 5.036mgKOH/g. Other values, such as iodine value, pH, boiling point as well as melting point, were found to be 6.77g, 6.03, 89^{o}C and 88^{o}C, respectively.

References

Bachmann, J. 2004. Selling to restaurants: Business and marketing. ATTRA Publication #IP255, August. ATTRA (Appropriate Technology Transfer for Rural Areas) – National Sustainable Agriculture Information Service, National Center for Appropriate Technology (NCAT), Fayetteville, AR, USA.

Ensminger, A.H.; Ensminger, M.K.J.; et al. 1996. Food for health: A nutrition encyclopedia. Pegus Press, Clovis, CA, USA.

McIntosh, M.; and Miller, C. 2001. A diet containing food rich in soluble and insoluble fiber improves glycemic control and reduces hyperlipidemia among patients with type 2 diabetes mellitus. Nutrition Reviews 59(2): 52-55, February.

Redden, R.; Wright, R.; and Tompkins, W. 1996. Lima beans as a new crop. Proc. First Australian New Crops Conference. Imrie, B.C. (Ed.); Bray, R.A.; Wood, I.M.; and Fletcher, R.J. (Assoc. Eds.). University of Queensland, Gatton College, Gatton, Queensland, Australia, 8-11 July 1996, vol. 2, pp. 63-72.

Redden, R. 1998. Lima beans. In: The new rural industries: A handbook for farmers and investors, Hyde. K. (Ed.), Rural Industries Research and Development Corporation (RIRDC), Canberra, ACT, Australia, pp. 347-50.

Wood, R. 1988. The whole foods encyclopedia. Prentice-Hall Press, New York, NY, USA.

Wright, R.M. 1993. Lima beans *(Phaseolus lunatus)* research. Proc. National Workshop for New Summer Grain Legumes. Redden, R.; and Fletcher, R. (Eds.). Queensland Department of Primary Industries (QDPI), Research and Development Corporation (RIRDC)/Grain Research and Development Corporation (GRDC), Toowoomba, QLD, Australia, August 1993, pp. 82-86.

Table 1. Percentage yield of oil.

Runs	Volume of solvent (ml)	Time (hr)	Mass of sample (g)	Yield of oil 1	2	%Yield of oil 1	2	Average % yield
1	300	3	15	1.00	0.98	6.67	6.53	6.60
2	150	3	15	0.77	0.79	5.13	5.27	5.20
3	300	2	15	1.15	1.04	7.67	6.93	7.30
4	150	2	15	0.80	0.78	5.33	5.20	5.27
5	300	3	10	0.85	0.83	8.50	8.30	8.40
6	150	3	10	0.62	0.61	6.20	6.10	6.15
7	300	2	10	0.78	0.88	7.80	8.80	8.30
8	150	2	10	0.70	0.69	7.00	6.90	6.95

The % yield of oil was calculated as follows:
Mass of sample = *A* (g), Mass of oil yield = *B* (g), % yield of oil = (*B* x 100)/*A*.

Table 2. Average yield of oil.

No. of runs	Volume of solvent (ml)	Mass of sample (g)	Time (hr)	Yield of oil 1	2	Average yield (g)	Average yield (g/hr)
1	300	3	15	1.00	0.98	0.990	0.330
2	150	3	15	0.77	0.79	0.780	0.260
3	300	2	15	1.15	1.04	1.095	0.548
4	150	2	15	0.80	0.78	0.790	0.395
5	300	3	10	0.85	0.83	0.840	0.280
6	150	3	10	0.62	0.61	0.615	0.205
7	300	2	10	0.78	0.88	0.830	0.415
8	150	2	10	0.70	0.69	0.695	0.348

Table 3a. Physical properties of lima beans oil.

S/No	Properties	Description
1	Colour	Light yellow
2	Odour	Pleasant
3	Specific gravity	_
4	Density	_
5	Boiling point	89^0C
6	Ph	6.03
7	Refractive index	1.57
8	Viscosity	_
9	Melting point	88^0C

Table 3b. Chemical properties of lima beans oil.

S/N	Properties	Description/value
1	Free fatty acid	-
2	Acid value	5.036mgKOH/g
3	Saponification value	134mgKOH/g
4	Iodine value	6.77g

Table 4. Results of the 2^3 full factorial designs.

Source of variance	Effect of the variance	*T* – test	*T* – value
B_0	0.8294	t_0	86.0300
B_1	0.1094	t_1	11.3460
B_2	-0.0231	t_2	-2.3989
B_3	0.0844	t_3	8.7526
B_{12}	-0.0006	t_{12}	-0.0648
B_{13}	0.0194	t_{13}	2.0099
B_{23}	0.4581	t_{23}	49.5200
B_{123}	-0.3719	t_{123}	-38.5800