

EFFECT OF PROCESSING ON THE PHYSICOCHEMICAL PROPERTIES AND FATTY ACID COMPOSITION OF (*Vigna angularis*) Adzuki Bean SEED OIL

¹Jide Alfred O., ²Aladejimokun A.O., ³Karimu O. A., ⁴Victor-Osanyinlusi Remi

¹Department- Science laboratory Technology, Rufus Giwa Polytechnic Owo. Ondo State, Nigeria.

²Department- Science laboratory Technology, Rufus Giwa Polytechnic Owo Ondo State, Nigeria.

³Department- Pharmaceutical Technology, Rufus Giwa Polytechnic Owo. Ondo State, Nigeria.

⁴Department- Pharmaceutical Technology, Rufus Giwa Polytechnic Owo. Ondo State, Nigeria.

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Abstract: Vegetable oils are valuable sources of energy and essential fatty acids, making them important components of human nutrition and various industrial products. Their quality is influenced by processing methods, particularly refining. Analyses of crude and processed oil extracted from Adzuki beans (*Vigna angularis*) seeds were carried out to establish the effect of processing on identity and quality characteristic of the oils. The crude oil was subjected to refining process: degumming, neutralisation, and bleaching. There was no remarkable difference in the Specific Gravity, refractive index and moisture content of the crude and processed oils. However, there is slight increase in Smoke Point (°C) (110 ± 0.12 - 115 ± 0.31), Flash Point (°C) (121 ± 0.50 - 131 ± 0.63), Fire Point (°C) (125 ± 0.20 - 138 ± 0.35) and a slight decrease in Colour (unit) (22.00 ± 0.20 - 15.00 ± 0.15) of the oil samples. Free fatty acid (% Linoleic) (0.58 ± 0.02 - 0.12 ± 0.01), Acid Value (mg/KOH/g oil) (1.18 ± 0.01 - 0.24 ± 0.05), Peroxide Value (meq Peroxide/kg) (12.05 ± 0.05 - 10.05 ± 0.01), Saponification Value (mg/KOH/g oil) (195.05 ± 0.05 - 190.05 ± 0.01) and increase in Iodine Value (105.00 ± 0.03 - 112.00 ± 0.23) while there is an increase in the value of Palmitic acid (22.05-23.05%), Stearic (56.05-56.91%), Lauric (1.05- 1.45%) Oleic (78.60-80.50%), Linoleic (18.50-21.48%), Linolenic (16.58-16.65%) as the crude oil was processed to bleached oil. The oil high proportion of unsaturated fatty acids, high iodine value and low free fatty acid content are all attribute that qualify oil to be safe for consumption. The oil quality will be further enhanced if it is processed to deodorisation.

Keywords: Extraction, Degumming, Fatty acid, Neutralisation, Physicochemical, *Vigna Angularis*, Seed oil.

1. INTRODUCTION

Currently, Nigerian markets are flooded with lot of vegetable oils of varying quality. crude oil is a term given to oil recovered from seeds, nuts or plants by solvent extraction or mechanical pressing without further processing. They are usually made up of neutral lipids which include free fatty acids, tri - di- and mono-acylglycerols, and polar lipids such as phospholipids (Amos-Tautau, 2013). They also contain a small amount of unsaponifiable matters such as tocopherols, phytosterols and hydrocarbons typically squalene (Amos-Tautau, 2013).

Many people relied on Vegetable oil as a source of many food because it helps in meeting global nutritional demand and also used for industrial purposes. Although there are various other plant sources where oil can be extracted, oils derived from soybeans, palm, rapeseed and sunflower are considered the most widely produced globally (Jide *et al.*, 2023). Oils and fat are found in fish, fruits, animal tissue and seeds (Taiwo and Alade-Meyin, 2015). The method used to extract

vegetable oil, such as virgin or cold-press extraction, significantly influences its quality because these methods preserve the natural composition of the oil without the use of excessive heat or chemical treatment. Crude vegetable oils contain naturally occurring substances, including gums, phospholipids, pigments, tocopherols, and free fatty acids, which affect their quality, stability, colour, and edibility. Consequently, refining processes such as centrifugation, settling, filtration, and water washing are often employed to remove these impurities and improve the quality and shelf life of the oil (Chow, 2008; Chabiri et al., 2009). Adzuki bean (*Vigna Angularis*) has been cultivated thousands of years in East Asian countries, particularly China, Japan, and Korea, where it is widely utilized in the preparation of traditional foods, desserts, snacks, and confectionery products. In contrast, its cultivation and utilization in Western countries have historically been limited, although interest in the crop has increased in recent years due to its nutritional and functional properties (listari, 2014). It is Grown in more than 30 countries of the world and is regarded as an important legume crop because of its high nutritional value. The seeds are rich in starch, digestible proteins, vitamins, and essential minerals, making them a valuable food source (Srishti and Ekta, 2019). Adzuki bean (*Vigna angularis*) occurs in numerous varieties that differ in seed size, colour, maturity period, and adaptability to different climatic conditions and growing regions. In many Asian countries, particularly China, Japan, and Korea, the beans are widely used in the preparation of various food products, including cakes, rice jelly, beverages, and ice cream (Listari, 2014). Despite their extensive use as food, the potential of adzuki beans as a source of edible oil and other value-added products has received limited attention. Consequently, information on the physicochemical characteristics and fatty acid composition of oils obtained from processed adzuki bean seeds remains scarce.

Despite the increasing nutritional and industrial importance of adzuki bean (*Vigna angularis*), information on the effects of oil refining processes on its physicochemical properties and fatty acid composition remains limited. Refining operations such as degumming, neutralization, and bleaching are essential for improving the quality, stability, and acceptability of edible oils; however, these processes may also alter the chemical composition and nutritional quality of the oil. Therefore, evaluating the changes that occur during each stage of refining is necessary to determine the suitability of adzuki bean oil for food and industrial applications and to provide scientific information that will promote its effective utilization.

2. MATERIALS AND METHOD

Sample Preparation

The seed (*Vigna Angularis*) used for this work were purchased from market at Owo, in Ondo State, Nigeria. They were prepared for used by decoating, sun drying and milling to powdery form and kept in air tight bottle.

Materials

The materials used in this study included adzuki bean (*Vigna angularis*) seeds, n-hexane, phosphoric acid (50%), sodium hydroxide (NaOH), sodium chloride (NaCl), bleaching earth, distilled water, filter paper (Whatman No. 1), and fatty acid methyl ester (FAME) standards. All chemicals and reagents used were of analytical grade.

OIL EXTRACTION

The powdered seed were subjected to soxhlet extraction using hexane as solvent which was recovered through simple distillation. The enough oil required for analysis was obtained and stored in an air tight bottle for further analysis. This was modified from (AOAC, 2019; Gharby, 2022).

REFINING PROCESS

The crude oil extracted from adzuki bean (*Vigna angularis*) seeds was refined sequentially by degumming, neutralization, and bleaching using a modified chemical refining procedure (Gharby, 2022)

DEGUMMING PROCESS

About 450 cm³ of the extracted oil was heated to temperature of 70°C followed by addition of 0.6cm³ of 50% Phosphoric acid and the mixture was then vigorously stirred for 10 minutes. Thereafter 10cm³ of heated water to 80°C was added and whole mixture was agitated for 10 minutes and allowed to stand undisturbed for one hour. The mixture then Separated into two layers i.e oil and gum. The Gum was drained Off while the oil obtain was termed degummed oil (modified from Gharby, 2022; Salunkle et al., 1992)

NEUTRALISATION

About 200 cm³ of degummed oil was heated to 70°C with continuous stirring. Sodium hydroxide solution was gradually added, followed by saturated sodium chloride solution. The mixture was stirred at 90°C for 30 min, transferred to a separating funnel, and allowed to stand for 6 h. The soapstock was discarded, and the oil was washed repeatedly with hot water, dried in an oven, and cooled in a desiccator. The neutral oil was dried in an oven and later cooled in a dessicator (modified from Gharby, 2022; Salunkle et al, 1992).

BLEACHING PROCESS

Approximately 100 cm³ of neutralized oil was heated to 75°C with continuous stirring. Bleaching earth (1.0 g) was added, and the mixture was heated to 110°C for 45 min before filtration. The filtrate was collected as the bleached oil (modified from Gharby, 2022; Salunkhe et al., 2019) The mixture was then filtered and resulting oil is termed bleach oil.

PHYSICOCHEMICAL DETERMINATION OF OIL SAMPLE

The crude, degummed, neutralized, and bleached oil was analysed for physicochemical parameters. The moisture content and specific gravity was determined according to (AoAC 2010) while the refractive index was measured Using Abbey refractometer couple with thermometer The colour was determined using lovibond thermometer (model 520). The colour of crude was determined in half inch cell while that of degummed, neutralized and bleached oil were determined in Inch cell. The Colour was Calculated based on the expression (5R+Y) - B where R stands for red pigment, for yellow pigment and B for blue pigment (Abitojun and Oshodi, 2010) The flash, fire point were measured using Gollen-kamp Automatic Pensky martens, flash and fire Point tester with thermometer while the Smoke points was determined using Cleveland open Cup apparatus (AOAC, 2010). The free fatty acid Acid Value, Saponification Value, Peroxide value and Iodine Value were determined using method described by AOAC 2010)

FATTY ACID IDENTIFICATION

The Fatty methyl esters were analysed using an HP 6890 gas chromatography filled with flame Ionization detector and powered with HP Chemstation Rev 09.01 Software. The Carrier gas was Helium at Pressure of 19PSI. The same Sample (1.5 UL) was injected and the Separation was Carried out on an HP Capillary Column (HP INNOWAX, Cross linked PEG) 30.0m length, 0.32mm and 0.50µm film thickness. The oven temperature was held Initially at 60°C for 2minutes, increased from 180°C at 12°C/min to 320°C for 5minutes. The temperature of the Injection port and the detector were set at 250°C and 300°C respectively. The Peak were Identified by Comparison with standard fatty acid methyl esters (Arawande and Alademeyin, 2018)

Statistical Analysis

Statistical Significance test were Performed using SPSS at P <0.05 by means of one way analysis of Variance (ANOVA) followed by LSD Post hoc multiple Comparisons.

Table 1: PHYSICAL CHARACTERISTICS OF CRUDE, DEGUMMED, NEUTRALISED AND BLEACHED OIL (*Vigna angularis*) SEED OIL

Parameters	Crude	Degummed	Neutralised	Bleached oil
Specific Gravity	0.8142 ± 0.011	0.8141	0.8142	0.8142
Refractive Index	1.4626 ± 0.10	1.4621	0.4621	0.4621
Moisture Content	0.50 ± 0.12	0.50 ± 0.12	0.50 ± 0.12	0.50 ± 0.12
Flash Point	121 ± 0.50	124 ± 0.52	126 ± 0.54	131 ± 0.63
Smoke Point	110 ± 0.12	111 ± 0.13	112 ± 0.30	115 ± 0.31
Fire Point	125 ± 0.20	130 ± 0.25	133 ± 0.31	138 ± 0.35
Colour	22.00 ± 0.20	18.00 ± 0.20	16.00 ± 0.18	15.00 ± 0.15
Oil Content	45.00 ± 0.25			

Mean ± standard deviation of triplicate determination

Table 2: CHEMICAL PROPERTIES OF OIL EXTRACTED FROM (*Vigna angularis*) ADZUKI BEAN SEED OIL

Parameter	Crude	Degummed oil	Neutralized oil	Bleached oil
Free Fatty Acid %	0.58 ± 0.02	0.28 ± 0.01	0.12 ± 0.021	0.15 ± 0.01
Acid Value%	1.18 ± 0.01	0.57 ± 0.00	0.24 ± 0.02	0.30 ± 0.05
Peroxide Value (meq/koH)	12.05 ± 0.05	11.20 ± 0.03	10.05 ± 0.02	10.05 ± 0.01
Iodine value (wijs)	105.00 ± 0.03	108.00 ± 0.21	109.00 ± 0.22	112.00 ± 0.23
Saponification value	195.05 ± 0.05	193.05 ± 0.03	192.05 ± 0.02	190.05 ± 0.01

Mean ± standard deviation of triplicate determination

Table 3: Fatty Acid Composition of Crude, Degummed, Neutralised and Bleached oil extracted from (*Vigna angularis*) Seed oil

Fatty Acid	Carbon no	Crude	Degummed	Neutralised	Bleached oil
Palmitic	16.0	22.05	22.30	22.45	23.05
Stearic	18.0	56.05	56.39	56.61	56.91
Lauric	12.0	1.05	1.21	1.32	1.45
Total Saturated Fatty Acid	-	79.15	79.90	80.16	91.4
Oleic	18.1	78.60	79.65	79.83	80.50
Mono Unsaturated Fatty Acid	-	76.60	79.65	79.83	80.50
Linoleic	18.2	18.50	19.20	20.30	21.48
Linolenic	18.3	16.58	16.60	16.65	16.60
Total Poly Unsaturated Fatty Acid	-	35.8	35.8	36.95	38.18 lol

3. RESULTS AND DISCUSSION

Table 1 shows the physical characteristics of crude, degummed, neutralized and bleached oils obtained from (*Vigna Angularis*) Anzuki bean seed oil. The oil content of the seed was found to be 45.00 ± 0.25 % and crude was light yellow with clear opacity and liquid at room temperature. The percentage oil yield was within the range of values (44.6 – 57.2 %) reported by (Atugwu et al., 2022) but lower compared to 71% reported for snake gourd seed oil (Etuk et al, 2022). So the percentage oil content of the seed (45.00%) indicates that the oil could be refined for edible and industrial use. The color of the extracted crude, degummed, neutralized and bleached oil was determined using Lovibond tintometer and was found to be 22.00, 18.00, 16.00 and 15.00 unit respectively. The phosphoric acid used for degumming, and the bleaching used in the bleaching process, are responsible for the progressive decrease in color from crude to bleached oil (Abitogun and Oshodi, 2010).

The low content of moisture in the oil sample is an indication that the seed can be stored and preserved for a long period of time without spoilage. The moisture percentage found in the oil (0.50% for all the samples) could be as a result of the traditional method of crude oil preparation. The refractive index and specific gravity are physical measures of adulteration of vegetable oils. There was no remarkable difference in the values of refractive indices which range between 0.4621-1.4626 and specific gravity ranging between 0.8141 – 0.8142 in the oil samples. The value of the refractive index of (0.4621-1.4626) falls within the range of edible oils as confirmed by Ilesanmi et al., 1990. The specific gravity of the oil sample was higher compared with 0.62 reported by (Arawande and Akinosotu, 2019) for castor seed oil. This could be due to the fact that the oils are from different sources.

The flash point (oc) for crude, degummed, neutralized and bleached oil were determined to be 121 ± 0.50 , 124 ± 0.52 , 124 ± 0.54 and 131 ± 0.63 respectively, smoke points for crude, degummed, neutralized and bleached oil were 110 ± 0.12 , 111 ± 0.13 and 112 ± 0.30 and 115 ± 0.31 respectively, while the fire points (oc) for crude, degummed, neutralized and bleached oil were determined to be 125 ± 0.20 , 130 ± 0.25 , 133 ± 0.31 and 138 ± 0.35 respectively. It was observed that the flash point increased from 121 ± 0.50 - 131 ± 0.63 , smoked point 110 ± 0.12 - 115 ± 0.31 , while fire point increased from 125 ± 0.20 - 138 ± 0.35 . The progressive increase in values of flash, smoke and fire points from crude to bleached oil samples might be as a result of removal of impurities such as volatile organic materials and the residual extraction solvent during oil processing (Nielsen 2002). The following values of flash, smoke and fire points of 121oc, 110oc and 125oc is lower than 298oc, 350oc and 147oc were reported for cashew nuts oil samples respectively by (Abitogun and Borokini, 2009).

Table 2 shows the chemical characteristics of crude, degummed, neutralized and bleached oil obtained from (*Vigna Angularis*) Adzuki bean seed oil. Free fatty acids are used to indicate the level of edibility and identify suitability for making soap in industry (Amos Tautau et al., 2013) and can also stimulate hydrolytic deterioration of oils to form off-flavor components. The free fatty acid (% oleic) of crude, degummed, neutralized and bleached oils are 0.58 ± 0.02 , 0.28 ± 0.01 , 0.12 ± 0.012 and 0.15 ± 0.01 The values are relatively low compared with values reported for plukenatia *Conophora* by Akintayo and Bayer, 2002). The allowable limit for free fatty acid for edible oil is 1.0-3.0% (Paul and Mittal, 1997). The acid value of crude, degummed, neutralized and bleached were 1.18 ± 0.01 , 0.57 ± 0.00 , 0.24 ± 0.02 and 0.30 ± 0.05 respectively. It was observed that free fatty acid and acid value decrease from crude to neutralized oil and slightly increased in bleached oil. This might be as a result of the uses of NaOH in neutralizing the oil sample which led to the reduction in free fatty acid, acid value and other impurities while there is a slight increase in free fatty acid and acid value for bleached oil as a result of the acidic nature of bleaching earth used for colour removal (Salunkhe et al, 1992). Iodine value shows the degree of unsaturation of the fat or oil (Omeje et al, 2019) and reflect the susceptibility of oil to oxidation. The higher the iodine value, the greater the degree of unsaturation and the greater the liquidity of the oil (Jacobs, 1999). The iodine value (Wijs) for crude, degummed, neutralised and bleached oils are 105.00 ± 0.03 , 108.00 ± 0.021 , 109.00 ± 0.22 and 112.00 ± 0.23 respectively. The iodine value of the oil sample was higher than 34.27/100g report for snake gourd tomatoes seed oil by (Adesina and Amos, 2013) and 29.00 ± 0.16 g/12/100g reported for African apple star by (Omeje et al, 2019). So the oil is more unsaturated fatty acid than saturated. Saponification value is the number of milligrams of potassium hydroxide required to neutralise the fatty acid liberated on complete hydrolysis of saponification of 1g of the oil. The saponification is used in checking oil adulteration. (Amira et al, 2014). The saponification values for crude, degummed, neutralised and bleached recorded 195.05 ± 0.05 , 193.05 ± 0.03 , 192.05 ± 0.02 and 190.05 ± 0.01 respectively. The value obtained is higher than 184.93 ± 3.47 mg/KOH/g reported for *Trichosanthes cucurmerina* seed oil (Etuk et al, 2022) and lower than 317.27 mg/KOH/g reported by Adesina and Amos, 2013) for palm kernel oil. The oil sample have high relative saponification value, which points to its application in the soap and cosmetic industries.

Peroxide value is an index of rancidity. It is a useful indication of oil quality since it shows how stable the oil is and how much fat is degrading. The peroxide value of the oil samples in this study were 12.05 ± 0.05 , 11.20 ± 0.03 , 10.05 ± 0.02 and 10.05 ± 0.01 for crude, degummed, neutralised and bleached oil respectively. The peroxide value of crude oil of 12.05 ± 0.05 is higher than 2.08 value of snake tomatoes as reported by (Alaedemeyin and Jide, 2023), while the value for degummed, neutralised and bleached oil were higher when compared with 2.03 ± 0.06 reported by (Etuk et al, 2022). However the peroxide value of the samples compare favourably with the maximum limit of 10.00 meq/KOH set by the CODEX Alimentarius Commission for vegetable oils (Abayeh et al, 1998).

Table 3: Shows the fatty acid composition of crude, degummed, neutralised and bleached oil extracted from *Vigna Angularis* seed. The fatty acids detected in the crude, degummed, neutralised and bleached oil are, stearic, lauric, palmitic, oleic, linoleic and linolenic acid. The individual amount (%) of fatty acid in the crude oil are, Palmitic 22.05, stearic 56.05, oleic 78.60, lauric 1.05, linoleic 18.50, and linolenic 16.58. The content (%) of the fatty acid in degummed oil are: Palmitic 22.05, stearic 56.05, lauric 1.05, oleic 78.60, linoleic 18.50 and linolenic 16.58 respectively. Furthermore, the following fatty acids are present in the neutralised oil palmitic 22.45, stearic 56.61, lauric 1.32, linoleic 80.16, and linolenic 79.respectively. Moreover, the amount of individual fatty acids present in the bleached oil are palmitic 23.05, stearic 56.91, lauric 1.45, oleic 91.4, linoleic 21.48 and linolenic 16.60 respectively.

It was observed that the amount (%) of fatty acids in the oil sample increase geometrically as the processing continues from one stage to another. This was established by the fact that more of the fatty acids are detected with the removal of impurities in each stage of processing. Also, oleic acid was observed to have the highest value followed by stearic, palmitic, linoleic and linolenic while lowest value is lauric acid in crude and degummed oil sample but are in trace amount in neutralised and bleached oil sample. The oil is suggested to be edible when completely refined owing to its high level of unsaturated fatty acid in conjunction with its low free fatty acid and acid values (Abitogun and Oshodi, 2010). Due to the large concentration of unsaturated fatty acid in the oil, the oil may not congeal at room temperature. The total amount (%) of saturated fatty acid found in the crude, degummed, neutralised and bleaching oil are 79.15, 79.90, 80.16 and 91.4 respectively with stearic acid having the highest value of 56.91 in the bleached oil. Early experiments show that palmitic and lauric acid raised plasma cholesterol, more than those found on similar intakes of palmitic acid. Indeed the cholesterol lowering effect was similar to that seen on oleic acid (Cox et al, 1996). However, the saturated fatty acid plays an important role in the structure of tissues as reported by (Gunstone et al, 2004). Moreover, the total amount (%) of monounsaturated fatty acids found in

crude, degummed, neutralised and bleached oil samples are 76.60, 79.65, 79.83 and 80.50 while the values (%) of polyunsaturated fatty acid present in the crude, degummed, neutralised and bleached oils samples are 35.08, 35.80, 36.95 and 38.18 respectively. There is a progressive increase in the saturated, monounsaturated and polyunsaturated fatty acid as well as the total fatty acid composition of *Vigna Angularis* seed oil during processing from crude to bleached oil. This might be as a result of effectiveness of the oil processing method in the removal of impurities during refining process. In monounsaturated fat, oleic acid (omega-9) was high in amounts (78.60 - 80.50) which is good for the construction of membranes, present in the epidermis (outer skin layer). Epidermis protect and work against body dehydration and also in the formation of hormones (Camilo et al., 2016). Because of the better quality products, oleic acid is used in various food industry (Amelita et al., 2016). Foods contain high percentage of unsaturated fat could lower blood levels of harmful cholesterol and aids in the absorption of vitamins A, D, E, and K (fat soluble vitamins) in the body (Camilo et al., 2016). Also, the quantity and composition of unsaturated fatty acid present in the edible vegetable oils determine their quality and digestion (El-Adawy and Taha, 2001). Therefore *Vigna Angularis* oil sample could be considered among vegetable oil of high nutritional value of its higher ratio of linoleic (omega-6 fatty acid) and oleic (omega-9) acid. All the fatty acid required by human body could be synthesised with exception Alpha linolenic acid and Linoleic Acid. These two fatty acids are known as essential fatty acid and can only be obtained through diet. They are required for proper growth and development, they also served as building blocks for the synthesis of other fatty acids (Francakova et al., 2015).

4. CONCLUSION AND RECOMMENDATION

The result of the study has shown that from the nutritional point of view, Adzuki beans oil (*Vigna angularis*) appears to have a beneficial effect on health and can also be considered to be a good source of oil suitable for food formulations as well as pharmaceutical, paint, soap and perfume industries due to its high level of unsaturation in the fatty acid contents compared to other seeds. If the oil is further processed to the deodorization stage, it will be of high quality and could supply essential fatty acids needed in the body when consumed. Further processing of the oil to the deodorization stage is recommended and the deodorised oil obtained should be assessed for physicochemical properties and fatty acid composition.

Authors' Contributions

Jide Alfred O. conceived the study and designed the research, and supervised the work. Victor-Osanyinlusi Remi and Bello carried out the analyses under the supervision of Jide Alfred O. Aladejimokun A.O. performed the data analysis. All authors read the manuscript again, contributed to its revision, and approved the final version for publication.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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