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Extraction and Physico-chemical Assessments of Groundnut Oil-Bixa Orellana Dye Leachates

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Abstract: *Natural colorants are currently appreciated as desired natural products. Even though achiote (*B. Orellana*) is a valuable crop, it is still currently underutilized. Annatto extract retains a natural, nontoxic food colourant that is generally approved and used. The mechanical absorption of the bixin pigment from* Bixa Orellana *seeds has been demonstrated as a direct additive to groundnut/peanut oil. The outcomes show that it is possible to efficiently extract this natural dye from Bixa Orellana seeds which have a proximate profile of moisture content (6.47 ± 0.03) %, ash content (4.67 ± 0.01) %, carbohydrate (41.83 ± 0.24) %, fiber (27.51 ± 0.01) %, and crude lipid (2.21 ± 0.02) in a medium of groundnut oil. The quality of bixa leached groundnut oil was assessed through its physicochemical functionalities (colour, pH, specific gravity, free fatty acid, peroxide value, iodine value, saponification value, acid value, viscosity, and refractive indices. The percentage differences established before and after extraction were -243.48% (Specific gravity), 51.98% (Free fatty acid), 44.89% (Iodine value), 77.35% (Peroxide value), 12.17% (Saponification value), 23.73% (Acid value),-29.42% (Viscosity) and -119.88% (Refractive index). These developments technically declare that nontoxic and natural bixin pigments can be employed in adding value to organic groundnut or peanut oil.*

Keywords—Bixa orellana seed; bixin; leachates; extraction and groundnut oil.

1.0 INTRODUCTION

Bixa Orellana L. (family: Bixaceae), often known as annatto or bixa seed, is a tiny tree native to tropical Central and South America but now widely scattered across the tropics. It's best known for producing the natural color annatto, which is recovered from the fruit [1]. The shrub produces pink flowers as well as bright red spiky fruits with red seeds. The fruits get dry and harden into brown capsules as they age. This is a 2-8 m tall evergreen shrub with a trunk of about 10 cm in diameter, with a slightly bitter orange sap and twigs green with minute reddish scales. The leaves are spirally coordinated, simple, ovate, and measure 7.5-24 by 4-16 cm, deeply cordate at the base. It is longly acuminate at the apex, dark green or green above, brownish-green or grey beneath. Flowering panicles with a scaly pedicel that thickens at the apex and 5-6 bulky glands; sepals 4-5, obovate and free with 1 to 1.2 cm of elongation, protected by reddish-brown scales.Petals are 4-7 cm, obovate at 2-3 by 1-2 cm, purplish, pinkish, or whitish-tinged. When mature, they are globular or largely lengthened ovoid pods, 2-4 x 2-3.5 cm, compact with two thickly covered valves with long greenish-brown, green, or red bristles; the seeds are many, obovoid [2]. The name 'Bixa' originated from a South American local name [3]. It retains a high economic value in America (the United States) since annatto colorants manufactured from it are considered "free from certification" by the Food and Drug Administration [4]. Conventional dishes such as rice, cochinita pibil, and chicken adopt achiote as an agent of condiment and colorant in butter, cheese, popcorn, beverages, and bread. Bolivia, India, Peru, Brazil, Mexico, Colombia, Jamaica, Puerto Rico, Ecuador, and the Dominican Republic are the top achiote producers [5].

The seeds of the inedible fruit are gathered and can be leached by soaking the seeds with abrasion in water. It is offered as a solution or granules for culinary purposes and is used to color food products such as cheeses, seafood, and salad oil as water or oil-soluble annatto extracts are available [6] [7]. To leach the color from the pulp, solvents such as vegetable oil, lipids, alkali, and alcoholic solutions have been utilized [8]. Processes for separating and concentrating the dye include acid precipitation, recrystallization, and spray drying in either oil or water-soluble forms. Bixin ($C_{25}H_{30}O_4$), the dicarboxylic acid of mono-methyl ester carotenoid [9], is the major component of the seed coat (80%). The two stable forms of bixin were first isolated in 1913 and were originally referred to as alpha (α) and beta (β). Similarly, later research revealed that the beta and alpha forms were bixin's Cis and Trans isomers [10].

Figure 1. Bixa orellana pod with seed

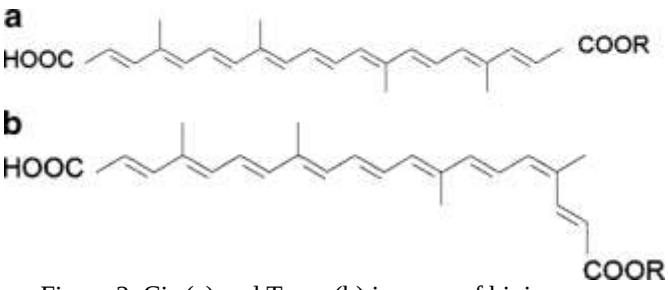

Figure 2. Cis (a) and Trans (b) isomers of bixin

Bixin is reactive and permeable in oils at a concentration of 0.1 percent by weight, as norbixin's sodium and potassium salts are very soluble in water [11]. Bixin's coloration varies with pH, shifting from yellow-orange to pink at lower pH levels, while pH does not affect the color's persistence [12]. Bixin maintains its stability at temperatures below 100 degrees Celsius but becomes unstable at temperatures over 125 degrees Celsius and when exposed to light. Cis-bixin is orange in color and unreactive in vegetable oil, but heating converts it to the red isomer trans-bixin, which is oil saturated [13].Norbixin and bixin are both available in the cis state in nature, but norbixin is synthesized when another methyl group of bixin is saponified under alkaline settings, and also with proteins to produce a peach-red color. A sufficient amount of alkali induces the pigmentation to develop a water-soluble alkali metal salt. In an acidic condition, the saponified form of the molecule is transformed and precipitated into the insoluble dicarboxylic form [14]. The main methods for converting unstable cisbixin to the more stable trans-bixin form are light and heat. When bixin is burned in its soluble state, it produces a yellow pigment known as ($C_{17}H_{20}O_4$). Water solubility is offered by the carboxylic acid component of the molecule, whereas oil solubility is provided by the ester form [15]. Annatto is widely considered to be a microbiologically clean product that is resistant to microbial invasion [16]. It is sold by the percentage of bixin (cis) content, or "points." The color of the seeds actively depends on the plant variety, growing area, manufacturing methods, climate or weather conditions, and seed storage conditions [17]. The components of annatto coloring compounds have been investigated with high performance and advanced liquid chromatography [18].

Rising temperatures, UV light intensity, availability of metallic ions, and molecular oxygen all contribute to annatto oxidation color degradation through oxidation [19]. According to several studies, the most effective agent for producing annatto color removal is light, followed by benzoyl peroxide. Hence, the oxidation of annatto is thought to be ineffective when it interacts with the air. However, the presence of light enhances antioxidants such as ascorbyl palmitate, which protect annatto from losing its color [19]. Peanut oil is a vegetable oil obtained from peanuts, often known as groundnut oil or Arachis oil. It retains a neutral and mild flavor with a deeper peanut aroma which is scented when produced using roasted peanuts. It is frequently used in African, American, Indian, Chinese, Southeast Asian, and Chinese cuisines for regular cooking and in frying for taste enhancement. It is frequently used in African, American, Indian, Chinese, and Southeast Asian cuisines, for regular cooking and in frying for taste enhancement. Peanut oil is abundant in monounsaturated fat with low saturated fat, which may help to reduce cholesterol and avoid heart disease [20]. Groundnut oil contains a low proportion of saturated fats, a higher fraction of polyunsaturated and monounsaturated fats, as well as vital vitamins and antioxidant qualities [21]. For consumers and general technical applications, oil quality and stability are critical. Hence, a rationale for investigating the chemical, physical, and nutritional properties of the extract medium with natural plant additives such as bisphenols, carotenoids, tocopherols, and bixin. Recent and similar studies in this direction include:

1. Exploiting B.Orellana as a famous form of natural colouring and flowering seeds with aqueous extraction [22].
2. B. Orelana seed oil acts as a co-solvent with CO_2, thereby increasing the solubility to more than ten times that of pure bixen, which could be very useful in pharmaceuticals [23].
3. Bioactive chemicals extracted from annatto seeds: extraction, evaluation, and optimization [24].
4. Using response surface methods, artificial neural networks were used to mimic the process of microwave assisted natural dye extraction from the seeds of bixa Orellana (Annatto) [25]. and
5. Annatto seed powder reveals some antioxidant indices with water and fat holding capacity [26].

Meanwhile, there is no specific approach to bixa orellana extraction in edible groundnut oil with its physicochemical implications.

2.0 METHODOLOGY

Mature seeds were carefully harvested beside the petrochemical laboratory, National Research Institute for Chemical Technology Zaria. The groundnut oil was purchased from a local market in the Zaria metropolis. The chemicals were of analar grade.

2.1 Proximate factors of Bixa seed powder

The seed powder was determined for moisture composition, fixed mineral residue as ash, fiber, carbohydrate and lipids according to AOAC methods [27], [28].

2.2 Physicochemical characterization of raw groundnut oil
Colour, specific gravity, viscosity, free fatty acid, refractive index, peroxide, iodine, saponification, and acid values were all determined according to standard methods [29].

2.3 Extraction of bixa leachates
Extractions of dyestuff from 20 g of the seed per 250 mL of the oil sample were carried out below 70°C, aided by mechanical abrasion for 60 min for selective leaching of cis- and trans- bixin only [30], [31].

2.4 Bixa leachate characterization.
The leached oil sample was re-characterized for parameters.

Figure 3. Groundnut oil- bixa leachates mixture

Figure 4. Natural groundnut oil

3.0 RESULTS AND DISCUSSION

Table 1. Proximate Evaluation of bixa seed

Parameter (per 100g)	Value
Moisture content (%)	4.97 ± 0.03
Ash content (%)	5.74 ± 0.01
Carbohydrate (%)	15.52 ± 0.24
Fiber content (%)	48.22 ± 0.01
Crude lipid (%)	7.11 ± 0.02

The proximate compositions of the bixa seed under the working conditions were its moisture, ash, total carbohydrate, fiber, and crude lipid content [32]. A significant level of moisture, ash, lipid, carbohydrate, and fiber were estimated from B. Orellana seeds (Table 1). The percentage moisture component is observed to be low and this indicates that it can be stored over a long period with less susceptibility to microbial attack. [33]. The ash content implies that the plant seed contains a higher mineral content and this may be the reason for its use in culinary and nutritional applications [34]. The crude lipid property of the seeds is validated by the present result and it indicates that a Bixa Orellana seed retains significant crude fats that still support its culinary uses.

This affirmation was subsequently balanced by the absence of very low antinutritional components like saponins, glycosides, and tannins [35], [36].
The seed's crude fiber represents the insoluble residues by acidic and alkaline hydrolysis, which estimates the available insoluble lignin and cellulose. The total sugar fraction in terms of carbohydrate was also established significantly [37].
The identified physicochemical indicators in table 2 were all evaluated according to the respective standard methods under the working conditions before and after extraction. The change in colour before and after extraction declares the homogeneity of the bixin dye from the seed with the unsaturated fatty acid portion of the groundnut oil [38]. Specific gravity is an important technical measure that compares the amount of an analyte to the number of water molecules at the same volume and temperature. It's especially important as it enables non-invasive access to molecular activity [39]. According to research, the specific gravities of various unsaturated fats vary depending on their molecular weights, which are influenced by refining procedures. [40]. Hence, a significant difference of-243.48% was recorded before and after extraction. The weight fraction of a specific triglyceride is known as a free fatty acid. Unsaturated available fatty acids in crude fat predict the amount of oil that will be lost during fatty acid removal refining stages [41].
A significant number of triglycerides, particularly linoleic acids, are unfavorable in refined oils..

Table 2. Physicochemical characteristics of natural and bixa leached groundnut oil

Characteristic	Natural groundnut oil	Bixa leached groundnut oil (20g/250ml)	% difference
Colour (Lovibond)	Light yellow (20)	Brick red (2)	-
Specific gravity (g/ml)	0.92	3.16	-243.48
Free fatty acid (%)	97.25	46.70	51.98
Iodine value (g/100g)	96.48	53.17	44.89
Peroxide value (Meq KOH/g)	2.34	0.53	77.35
Saponification value (mg KOH/g)	191.52	168.21	12.17
Acid value (mg/KOH)	0.59	0.45	23.73
Viscosity ($\times 10^{-4} m^2/s$)	0.6530	0.8451	-29.42
Refractive index	1.4631	3.2170	-119.88

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A significant number of triglycerides, particularly linoleic acids, are unfavorable in refined oils as they can generate off-flavors and reduce oil shelf life. In other words, the FFA content is a direct predictor of its overall quality, with a high concentration of fatty acids lowering the smoke point of oil, causing it to 'pop' during cooking [42]. In processed plant oils, lower FFA is technically recommended for man in terms of consumption [43]. The percentage difference between the FFA before (97.25%) and after (46.70%) extraction shows that the obtained oil has been positively impacted for subsequent use. Iodine value confirms the function of oil and fat unsaturation, which is stated in proportion to the level of absorbed iodine per 100 gram sample, especially during oxidation, which consumes the double bonds and results in a reduction in iodine. It's a sign of double bonds in the oil's molecular structure, which affects the oil's long-term storage and stability. Oils with a high level of iodine number are polyunsaturated, suggesting a high degree of unsaturation, and are sought after by oil processors, whilst oils with a lower iodine number are of inferior quality. [44].The results show a value of 96.48g/100g before and 53.17g/100g after extraction, with a percentage difference of 44.89%. This is an indication that the fatty acid present is unsaturated and a measure of fat or oil stability and resistance to oxidation [44].

The number of hydroperoxides, which is the index of the initial oxidation by-product of an oil sample, is determined by the peroxide value, which is a signal of the initial stage of oil deterioration. [45]. An initial level of 2.34 and a final level of 0.53 MeqKOH/g after extraction with a percentage difference of 77.35% were achieved. This implies that groundnut oil remains much more stable against rancidity, as a peroxide value greater than 10 units is considered rancid [46].

The saponification assessment represents the form of triglycerides that can be esterified into glycerol. It is useful in

determining the number, type, and mean weight of acids in a particular sample of oil when used in conjunction with acid values. It is only of interest if the oil is for industrial purposes, as it has no nutritional significance. However, because each fat has a fixed fatty acid composition within biological fluctuation, determining the saponification value is a fair way of defining the sample of the oil. [47].

The results reveal a higher initial saponification value of 191.52 and a final 168.21 mgKOH/g after extraction with a 12.17 percentage difference. The milligram of potassium hydroxide necessary to nullify the fatty acid in a gram of oil is referred to as the acid value. Its measurement is frequently used as a general indicator of the oil's quality and edibility. This is attributable to a rise in acidity, which is accompanied by the formation of unpleasant flavors and odors. [48].

Therefore, the natural groundnut oil sample declares an initial acid value of 0.59 and a final 0.45 mg/KOH after extraction with bixin dye leachate.

3.0 CONCLUSION

Bixa Orellana offers a range of potential for the food and pharmaceutical industries, beyond its currently established role as a colourant and additive for feed. Its seed proximate constituents are a direct source of carbohydrates, lipids, fiber, and minerals (insoluble ash content). Hence, the nontoxic and natural aesthetic capacity of bixin in groundnut oil has been established with the stated physicochemical indices. Apparently, the leached bixin in the groundnut oil reveals some significant modifications to the physicochemical nature of the oil. Hence, the leachate tends to be more valuable in technical nutritional applications. Similarly, it is also used worldwide in products like sugar, margarine, soft drinks, confectionery, ice cream and animal products.

The future work with annatto dye is to comparatively evaluate the physicochemical values of other edible oils.

Viscosity is a measurement of the internal cohesiveness of oil, better known as its resistance to flow. Oils with a low viscosity rate are known for their low cohesion ability, which illustrates a low barrier to flow. High viscosity sample oils are distinguished by molecules with a stronger cohesive ability to flow [49].

It is a crucial factor in calculating the oil's condition or its capacity to lubricate internal components, separate contact, and minimize friction. The causes of the increase may be due to oxidation, polymerization, carbon build-up (Soot), contaminants, anti-freeze, water ingress, and the addition of a wrong oil type and/or decreases in fuel dilution, sheering down of the viscosity index, thermal cracking, and overextended oil drain periods.

The initial point of 0.6530 and the final point of 0.8451 (X 10-4 m2/s) after bixin extraction at a percentage difference of-29.42% were estimated. These imply a high cohesive interaction between the triglyceride molecules and the bixin compound. The index of refraction within a medium represents a measurement of how light travels through it.

At typical conditions, the refractive factor is inversely related to the light that penetrates, leading to a corresponding significant shift in the balance of the light through the material [50]. However, in oil chemistry, it signifies the possibility of rancidity developing in the oil.

The higher the refractive index, the greater the risk of oxidative spoiling [50]. It's a crucial optical characteristic for analyzing light rays as they pass through a material's medium. The initial level of 1.4631 was mobilized to 3.2170 with a percentage difference of-119.88%.

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6.0 REFERENCES

1. Daniela de Araújo Vilar, Marina Suênia de Araujo Vilar, Túlio Flávio Accioly de Lima e Moura, Fernanda Nervo Raffin, Márcia Rosa de Oliveira, Camilo Flamarion de Oliveira Franco, Petrônio Filgueiras de Athayde-Filho, Margareth de Fátima Formiga Melo Diniz, José Maria Barbosa-Filho (2014). "Traditional Uses, Chemical Constituents, and Biological Activities of Bixa orellana L.: A Review",
The Scientific World Journal, vol. 2014, Article ID 857292, 11 pages, 2014. https://doi.org/10.1155/2014/857292

2. Fredrick AC, Chinasa EC, Ebube EC and Victor ON: Pharmacognostic study and antimalarial activity of the methanol extract and fractions of Bixa orellana L., (family Bixacaea) leaves (Annatto plant) on Albino mice.
Int J Pharmacognosy 2018; 5(8): 500-08. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5(8).500-08.

3. Donkin, R. (1974). Bixa Orellana: "The Eternal Shrub". Anthropos, 69(1/2), 33-56. Retrieved August 6, 2021, from http://www.jstor.org/stable/40458510

4. Bixa orellana. (n.d.). Definitions.net. https://www.definitions.net/definition/bixa+orellana.

5. Herbco (2021).Bixa orellana Annato plant overview. https://www.herbco.com/c-93-annatto.aspx

6. Wikipedia (2021). Annato. https://en.wikipedia.org/wiki/Annatto.

7. Annato Powder Extract, Water soluble colorant (2021). https://www.fromnaturewithlove.com/SOAP/product.asp?product_id=NCEXTANNATTOWSFR741

8. Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants (Basel, Switzerland), 6(4), 42. https://doi.org/10.3390/plants6040042

9. Curi-Borda, C. K., Linares-Pastén, J. A., Tat, T., Tarqui-Dueñas, R., Chino-Flores, N., Alvarado, J. A., & Bergenstahl, B. (2019). Multilayer Bixin Microcapsules: The Impact of Native Carbohydrates on the Microencapsulation Efficiency and Dispersion Stability. Foods (Basel, Switzerland), 8(3), 108. https://doi.org/10.3390/foods8030108

10. Michael Scotter (2009). The chemistry and analysis of annatto food colouring: a review. Food Additives and Contaminants, 2009, 26 (08), pp.1123-1145. 10.1080/02652030902942873hal-00573911

11. National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 6537492, Annatto. https://pubchem.ncbi.nlm.nih.gov/compound/Annatto.

12. Sathiya Mala, K., Prabhakara Rao, P., Prabhavathy, M. B., & Satyanarayana, A. (2015). Studies on application of annatto (Bixa orellena L.) dye formulations in dairy products. Journal of food science and technology, 52(2), 912–919. https://doi.org/10.1007/s13197-013-1038-3

13. Thomas Bechtold and Rita Mussak (2009).Handbook of Natural Colorants Edited .John Wiley & Sons, Ltd. ISBN: 978-0-470-51199-2

14. Alan Mortensen (2006). Carotenoids and other pigments as natural colorants. Pure Appl. Chem., Vol. 78, No. 8, pp. 1477–1491, 2006. doi:10.1351/pac200678081477

15. Keita H, Dos Santos CBR, Ramos MM, Padilha EC, Serafim RB, Castro AN, Amado JRR, da Silva GM, Ferreira IM, Giuliatti S, Carvalho JCT (2021).Assessment of the hypoglycemic effect of Bixin in alloxan-induced diabetic rats: in vivo and in silico studies.
Journal of biomolecular structure & dynamics 39, 1017-1028 [PubMed: 32028848]

16. Ryan Raman (2019). What Is Annatto? Uses, Benefits, and Side Effects.
https://www.healthline.com/nutrition/annatto

17. PennState Extension (2012). Seed and Seedling Biology. https://extension.psu.edu/seed-and-seedling-biology

18. ROUSEFF, R. L. (1988). High Performance Liquid Chromatographic Separation and Spectral Characterization of the Pigments in Turmeric and Annatto. Journal of Food Science, 53(6), 1823–1826. doi:10.1111/j.1365-2621.1988.tb0

19. Kiokias, S. (2003). Antioxidant properties of annatto carotenoids. Food Chemistry, 83(4), 523–529. doi:10.1016/s0308-8146(03)00148-1

20. Webmed (2021). Peanut Oil: Are There Health Benefits? https://www.webmd.com/diet/peanut-oil-health-benefits#1

21. Arya, S. S., Salve, A. R., & Chauhan, S. (2016). Peanuts as functional food: a review. Journal of food science and technology, 53(1), 31–41. https://doi.org/10.1007/s13197-015-2007-9

22. Mehta, A. A., Behera, L. K., Tandel, M. B., Jadeja, D. B., & Vashi, B. G. (2015). Efficacy of different oils used for the extraction of annatto colour from the seeds of Bixa orellana L. Journal of Applied and Natural Science, 7(2), 828-831. https://doi.org/10.31018/jans.v7i2.690

23. G. F. Silva1, Felix M. C. Gamarra2, A. L. Oliveira3 and F. A. Cabral2 (2008). Extraction of bixin from annatto seeds using supercritical carbon dioxide

24. Quiroz JQ, Torres AC, Ramirez LM, Garcia MS, Gomez GC, Rojas J. Optimization of the Microwave-Assisted Extraction Process of Bioactive Compounds from Annatto Seeds (Bixa orellana L.). Antioxidants (Basel). 2019;8(2):37. Published 2019 Feb 6. doi:10.3390/antiox8020037

25. Sinha, K., Chowdhury, S., Saha, P. Das, & Datta, S. (2013). Modeling of microwave-assisted extraction of natural dye from seeds of Bixa orellana (Annatto) using response surface methodology (RSM) and artificial neural network (ANN). Industrial crops and products, 41, 165-171. doi: 10.1016/j.indcrop.2012.04.004

26. Cuong, T. V., & Chin, K. B. (2016). Effects of Annatto (Bixa orellana L.) Seeds Powder on Physicochemical Properties, Antioxidant and Antimicrobial Activities of Pork Patties during Refrigerated Storage. Korean Journal for Food Science of Animal Resources, 36(4), 476–486. https://doi.org/10.5851/kosfa.2016.36.4.476

27. AOAC, (2005). Official Methods of analysis 15th edn. Association of Official Analytical Chemists International. Gaithersburg Maryland, USA. (Pentaclethra marcropholla). Pakistan Journal of Nutrition, 4: 382-383.141-144.

28. AOAC, (2000). Official Methods of Analysis. 17th edition. .Association of Official Analytical Chemists.

29. Oils and fats (2015). Manual of methods of analysis of foods food safety and standards authority of india ministry of health and family welfare government of india new DELHI. https://old.fssai.gov.in/Portals/0/Pdf/Draft_Manuals/OILS_AND_FAT.pdf

30. M.A.S. Barrozo, K.G. Santos, F.G. Cunha, Mechanical extraction of natural dye extract from Bixa orellana seeds in spouted bed, Industrial Crops and Products, Volume 45, 2013, Pages 279-282, ISSN 0926-6690, https://doi.org/10.1016/j.indcrop.2012.12.052.

31. Math, R. G., Ramesh, G., Nagender, A., & Satyanarayana, A. (2016). Design and development of annatto (Bixa orellana L.) seed separator machine. Journal of food science and technology, 53(1), 703–711. https://doi.org/10.1007/s13197-015-2019-5

32. P.I. Dike, O.O. Ibojo, F.Y. Daramola, O.A. Conrad (2016). Phytochemical and proximate analysis of foliage and seed of Bixa orellana linn. International Journal of Pharmaceutical Sciences Review and Research 36(2):247-251

33. Zheng Y1, Yates M, Aung H, Cheng YS, Yu C, Guo H, Zhang R, Vandergheynst J, Jenkins BM, Influence of moisture content on microbial activity and silage quality during ensilage of food processing residues, Bioprocess Biosystem Engineering, 34(8), 2011, 987-95. doi: 10.1007/s00449-011-0549-4. 2011.

34. Salami, S.O., Afolayan, A.J. Evaluation of nutritional and elemental compositions of green and red cultivars of roselle: Hibiscus sabdariffa L.. Sci Rep 11, 1030 (2021). https://doi.org/10.1038/s41598-020-80433-8

35. Aneta Popova and Dasha Mihaylovaand (2019). Antinutrients in Plant-based Foods: A Review. The Open Biotechnology Journal 13(1):68-7.10.2174/1874070701913010068

36. M. Gulzar, A. Minnaar (2017). Chapter 12 - Underutilized Protein Resources From African Legumes, Editor(s): Sudarshan R. Nadathur, Janitha P.D. Wanasundara, Laurie Scanlin, Sustainable Protein Sources, Academic Press, 2017, Pages 197-208, ISBN 9780128027783, https://doi.org/10.1016/B978-0-12-802778-3.00012-3.

37. Dokumen.pub. (2021). Dietary Fiber: Properties, Recovery and Applications 0128164956, 9780128164952 - DOKUMEN.PUB. https://dokumen.pub/dietary-fiber-properties-recovery-and-applications-0128164956-9780128164952.html

38. Zhou Ying, Zhao Weiwei, Lai Yong, Zhang Baohong, Zhang Dangquan (2020). Edible Plant Oil: Global Status, Health Issues, and Perspectives .Frontiers in Plant Science .11.1315.https://www.frontiersin.org/article/10.3389/fpls.2020.01315. 10.3389/fpls.2020.01315 .ISSN=1664-462X

39. ThoughtCo. (2021). Specific Gravity: When Gravity Itself Isn't Specific Enough. https://www.thoughtco.com/specific-gravity-2699007

40. Isah Musa Fakai*, Ahmad Ibrahim Bagudo, Habiba Aminu and Maimuna Isaac (2016). Physico-Chemical Analysis and Antifungal Activity of Datura metel Seed Oil. International Research Journal of Pharmaceutical and Biosciences. Pri -ISSN: 2394 - 5826 http://www.irjpbs.com. E-ISSN: 2394 – 5834.

41. Abdullahi, a., 2021. Free project on comparative physico-chemical analysis of groundnut, palm and shear nut oils. https://www.haskenews.com.ng/2021/04/free-project-on-comparative-physico.html.

42. Musa M. 1Sulaiman, A. U 2Bello; I. 3Itumoh J. E. 4Bello K.; 1Bello A. M.; 1Arzika, A. T.(2012). Physicochemical properties of some commercial groundnut oil products sold in sokoto metropolis, northwest nigeria. Journal of Biological Science and Bioconservation. Cenresin Publications www.cenresin.org. Volume 4, June 2012.ISSN 2277-0143

43. Okenwa-ani Chioma*, Okafor Alexander, Kanayochukwu Ugochi, Anieze Erasmus, Egbujor Melford, Chidebelu Innocencia, Ugwu Joshua, Okoye Ifeanyi and Edenta Chidi (2020). 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. Journal of Scientific Research & Reports 26(1): 104-112, 2020; Article no.JSRR.54516. ISSN: 2320-0227. 10.9734/JSRR/2020/v26i130218.

44. Esfarjani, F., Khoshtinat, K., Zargaraan, A., Mohammadi-Nasrabadi, F., Salmani, Y., Saghafi, Z., Hosseini, H., & Bahmaei, M. (2019). Evaluating the rancidity and quality of discarded oils in fast food restaurants. Food science & nutrition, 7(7), 2302–2311. https://doi.org/10.1002/fsn3.1072

45. J.P. Davis, K.M. Price, L.L. Dean, D.S. Sweigart, J.M. Cottonaro, T.H. Sanders (2016). Peanut Oil Stability and Physical Properties across a Range of Industrially Relevant Oleic Acid/Linoleic Acid Ratios. Peanut Science 1 January2016; 43 (1): 1–11. doi: https://doi.org/10.3146/0095-3679-43.1.1

46. Babatunde, O.A and Bello, G.S (2016). Comparative Assessment of Some Physicochemical Properties of Groundnut and Palm Oils Sold Within Kaduna Metropolis, Nigeria. OSR Journal of Applied Chemistry (IOSR-JAC) e-ISSN: 2278-5736.Volume 9, Issue 11 Ver. II PP 26-30 .www.iosrjournals.org

47. Robin Koon (2009).Understanding rancidity of nutritional lipids. Natural Product Insider. https://www.naturalproductsinsider.com/regulatory/understanding-rancidity-nutritional-lipids

48. E. Ike (2019). The study of viscosity-temperature dependence and activation energy for palm oil and soybean oil. GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 25, 2019: 209-217

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49. Davis, J.P., Sweigart, D.S., Price, K.M. et al. Refractive Index and Density Measurements of Peanut Oil for Determining Oleic and Linoleic Acid Contents. J Am Oil Chem Soc 90, 199–206 (2013). https://doi.org/10.1007/s11746-012-2153-4

50. Anwesa Sarkar, J. P. Pandey, Anupama Singh, Lakshmi Tiwari and Anil Kumar (2015). A novel method of using refractive index as a tool for finding the quality of aqueous enzymatic extracted algae oil. Pelagia Research Library Advances in Applied Science Research, 2015, 6(4):50-60. https://www.imedpub.com/articles/a-novel-method-of-using-refractive-index-as-a-tool-for-finding-the-quality-ofaqueous-enzymatic-extracted-algae-oil