

THE EFFECT OF ETHANOL SOLVENT AND COLD PRESS EXTRACTION ON THE YIELD, PHYSICOCHEMICAL PROPERTIES, AND FATTY ACID PROFILE OF OKRA (*ABELMOSCHUS ESCULENTUS* L. 'AKROFU') SEED OIL

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ABSTRACT: The search for novel and sustainable sources of edible oils has intensified due to global food security concerns. Okra (*Abelmoschus esculentus* L.) seeds, often a post-harvest waste product, contain a significant amount of oil rich in unsaturated fatty acids, yet they remain underutilized. The extraction method critically influences the oil's yield, quality, and applicability. The study aimed to comprehensively evaluate the effects of two extraction methods ethanol solvent extraction (a green alternative) and cold press extraction (a non-thermal method) on the oil yield, physicochemical properties, proximate composition, and fatty acid profile of seeds from the Ghanaian 'Akrofu' okra variety. Okra seeds were subjected to oil extraction using both ethanol solvent (Soxhlet apparatus) and mechanical cold pressing. The extracted oils were analyzed using standard methods for yield, free fatty acids (FFA), peroxide value (PV), saponification value (SV), iodine value (IV), acid value, refractive index,

moisture, ash, crude fibre, protein, carbohydrate, and key fatty acids (oleic and linoleic). Ethanol solvent extraction yielded a significantly higher quantity of oil (5.5 mL from 504.97g seeds) compared to cold press extraction (4.3 mL). However, cold-pressed oil demonstrated superior quality markers, including significantly lower FFA (0.775% vs. 0.825%) and a more desirable refractive index. Both methods produced oils with high saponification values (133.62 - 134.89 mg KOH/g) and iodine values (101.0 - 101.5 g I₂/100g), consistent with global standards for edible oils. The oil was predominantly unsaturated, with linoleic acid (an omega-6 fatty acid) being the most abundant (48.81 - 49.54%), followed by oleic acid (an omega-9 fatty acid) at 16.59 - 16.81%. The study confirms a clear trade-off: ethanol extraction maximizes yield, while cold pressing optimizes oil quality. The exceptionally high content of nutritionally valuable unsaturated fatty acids, particularly linoleic acid, positions 'Akrofu' okra seed oil as a promising and viable source of high-quality edible oil with significant potential for commercialization and improving nutritional outcomes in sub-Saharan Africa.

Keywords: *Okra Seed Oil, Green Extraction, Ethanol Solvent, Cold Press, Oil Quality, Linoleic Acid, Unsaturated Fatty Acids.*

1. Introduction

The global demand for edible vegetable oils is continuously rising, driven by population growth and changing dietary habits (Fao, 2022). This has intensified the search for alternative and sustainable oil sources, particularly from underutilized crops and agricultural by-products (Adeleke & Babalola, 2020). Okra (*Abelmoschus esculentus* L. Moench), a widely cultivated vegetable in tropical and subtropical regions, is primarily grown for its green, mucilaginous pods (Gemedede et al., 2015). However, the seeds, which constitute a significant portion of the fruit, are often discarded as waste after processing, leading to a loss of valuable resources and contributing to post-harvest losses (Petropoulos et al., 2017).

Interestingly, okra seeds are a rich source of high-quality oil, with reported contents ranging from 15% to 40% (Abdel-Nabey & Abou-Tor, 2020; Abba et al., 2020). This oil is characterized by a high proportion of unsaturated fatty acids, notably linoleic

acid (an essential omega-6 fatty acid) and oleic acid (a monounsaturated omega-9 fatty acid), which are associated with various health benefits, including reduced risk of cardiovascular diseases (Ganesan et al., 2018; Sales-Campos et al., 2013). Despite this promising profile, okra seed oil remains commercially underexploited, partly due to a lack of optimized and efficient extraction protocols tailored to its specific properties (Arapitsas, 2008).

The technique employed for oil extraction is a critical determinant of its final yield, quality, oxidative stability, and nutritional integrity (Azadmard-Damirchi & Torbati, 2015). Conventional solvent extraction using non-polar solvents like n-hexane is highly efficient in achieving near-complete oil recovery (Lavenburg et al., 2021). However, its use raises significant concerns regarding solvent toxicity, residual traces in the oil and meal, environmental pollution, and the degradation of heat-sensitive bioactive compounds (Hernández-Santos et al., 2016; Kessler, 1985). Consequently, there is a growing shift towards "green" extraction technologies that are safer, more environmentally friendly, and capable of preserving the functional properties of the oil (Chemat et al., 2020).

Among these, ethanol has emerged as a promising, generally recognized as safe (GRAS) solvent for food applications. Its higher polarity compared to hexane can selectively extract different lipid classes and polar antioxidants, potentially leading to oils with improved stability (Kronber & Laitinen, 2015). On the other hand, cold press extraction, a purely mechanical process conducted without external heat or chemical solvents, is renowned for producing high-quality "virgin" oils that retain natural flavors, colors, and bioactive compounds (Azadmard-Damirchi & Torbati, 2015; Cakaloglu et al., 2018). While its yield is typically lower than solvent extraction, the market premium for cold-pressed oils due to their perceived quality and purity makes it an economically attractive option (Karrar et al., 2021).

While several studies have characterized okra seed oil, direct and systematic comparisons between ethanol solvent extraction and cold pressing for a specific, locally adapted okra variety are scarce. Most previous works have focused on hexane extraction or have not detailed the impact on the full spectrum of quality parameters

(Abba et al., 2020; Michael, 2022). Furthermore, the performance of different okra genotypes can vary significantly, necessitating variety-specific studies (Ariyo, 1993).

Therefore, this study aims to fill this research gap by conducting a comparative analysis of ethanol solvent extraction and cold press extraction on the seeds of the 'Akrofu' okra variety, a prominent cultivar in Ghana. The specific objectives were to:

1. Determine and compare the oil yield from both extraction methods.
2. Analyze and contrast the physicochemical properties (FFA, PV, SV, IV, Acid Value, Refractive Index) of the extracted oils.
3. Evaluate the proximate composition and key fatty acid profiles (oleic and linoleic acid) of the oils to assess their nutritional potential.

2. Materials and Methods

2.1. Plant Material and Sample Preparation

Mature okra pods of the 'Akrofu' variety (Figure 1) were procured from the Ho Central Market in the Volta Region of Ghana. The seeds were manually separated from the pods. To achieve a uniform moisture content and facilitate milling, the seeds were sun-dried on clean trays for seven consecutive days. The dried seeds were then cleaned to remove any foreign matter and subsequently pulverized into a coarse powder using a sterile mortar and pestle. This milling step increases the surface area, which is crucial for enhancing oil extraction efficiency during both solvent and mechanical processes (Razal et al., 2012).

2.2. Oil Extraction Methods

2.2.1. Ethanol Solvent Extraction

The oil was extracted using a Soxhlet apparatus following a modified method from Michael (2022). A precisely weighed 50g sample of the powdered seeds was placed in a cellulose thimble and loaded into the Soxhlet extractor. Absolute ethanol (150 mL) was used as the solvent. The extraction was carried out for 8 hours, ensuring exhaustive lipid recovery. After extraction, the ethanol-oil mixture was carefully

collected, and the solvent was evaporated under reduced pressure using a rotary evaporator (Buchi, Switzerland) at 40°C. The resulting oil was then transferred into pre-weighed amber glass bottles, flushed with nitrogen to prevent oxidation, and stored at 4°C until analysis.

2.2.2. Cold Press Extraction

The cold press extraction was performed using a mechanical screw press (Farmers Mate, Model XX), operating at room temperature (approximately 25°C). The pressure was maintained between 80-100 MPa. A 504.97g batch of the powdered seeds was fed into the press, and the extraction process lasted for 20 minutes. The expelled oil was collected and filtered through a muslin cloth to remove any solid seed particulates. The filtered, clear oil was then stored in pre-weighed amber glass bottles under nitrogen at 4°C.

2.3. Oil Analysis

All analyses were performed in triplicate, and mean values with standard deviations are reported.

2.3.1. Oil Yield

The oil yield was calculated gravimetrically and expressed as the volume of oil extracted per fixed weight of seeds (mL/504.97g).

2.3.2. Physicochemical Analysis

- **Free Fatty Acid (FFA):** Determined by titration and expressed as % oleic acid (AOCS Official Method Ca 5a-40).
- **Peroxide Value (PV):** Measured by titration with sodium thiosulfate and expressed as milliequivalents of active oxygen per kilogram of oil (mEq O₂/kg) (AOCS Official Method Cd 8-53).
- **Saponification Value (SV):** Determined by refluxing the oil with an alcoholic KOH solution and titrating the excess KOH; expressed as mg KOH required to saponify 1g of fat (AOCS Official Method Cd 3-25).

- **Iodine Value (IV):** Measured using Wijs method, indicating the degree of unsaturation; expressed as grams of iodine absorbed per 100g of oil (g I₂/100g) (AOCS Official Method Cd 1-25).
- **Acid Value:** Determined by titrating the oil with KOH and expressed as mg KOH required to neutralize free fatty acids in 1g of oil (AOCS Official Method Cd 3d-63).
- **Refractive Index:** Measured at 40°C using an Abbe refractometer (Atago, Japan).

2.3.3. Proximate Composition

The proximate composition of the defatted seed cake was analyzed according to the official methods of the Association of Official Analytical Chemists (AOAC, 2016).

- **Moisture Content:** Oven-drying method at 105°C to constant weight (AOAC 925.10).
- **Ash Content:** Incineration in a muffle furnace at 600°C for 1.5 hours (AOAC 923.03).
- **Crude Protein:** Determined by the Kjeldahl method ($N \times 6.25$) (AOAC 984.13).
- **Crude Fibre:** Determined by sequential digestion with acid and alkali (AOAC 962.09).
- **Carbohydrate:** Calculated by difference: $[100 - (\text{Moisture} + \text{Ash} + \text{Protein} + \text{Fat} + \text{Fibre})]$.

2.3.4. Fatty Acid Profile

The fatty acid methyl esters (FAMES) were prepared by transesterification of the oil with methanolic KOH. The analysis of oleic and linoleic acids was performed using a Gas Chromatograph (Agilent 7890B) equipped with a flame ionization detector (FID) and a capillary column. The percentage composition of each fatty acid was determined by comparing the peak areas with those of known standards.

2.4. Statistical Analysis

Data were subjected to one-way Analysis of Variance (ANOVA) using SPSS software (Version 26.0). The significance of differences between the mean values of the two extraction methods was determined using the Student's t-test at a 95% confidence level ($p < 0.05$). Results are presented as mean \pm standard deviation.

3. Results and Discussion

3.1. Oil Yield

The yield of oil extracted from the 'Akrofu' okra seeds was significantly influenced by the extraction method ($p < 0.05$). As presented in Table 1, ethanol solvent extraction yielded 5.5 mL of oil from 504.97g of seeds, which was approximately 28% higher than the yield from cold press extraction (4.3 mL). This finding is consistent with the established principle that solvents can more effectively penetrate the seed matrix and dissolve a wider range of lipid components, including bound oils, leading to higher recovery rates (Lavenburg et al., 2021; Kessler, 1985). The efficiency of ethanol, despite its polar nature, in extracting okra seed oil highlights its viability as an alternative to hexane. The lower yield associated with cold pressing is a well-documented limitation of mechanical methods, as they rely solely on physical pressure to rupture oil cells and are less effective in recovering oil trapped within the cellular structure (Cakaloglu et al., 2018; Nadeem et al., 2015). The yield from cold pressing can be influenced by factors such as seed moisture content, pressing temperature, and the specific design of the press (Karrar et al., 2021).

3.2. Physicochemical Properties of the Extracted Oils

The physicochemical parameters are critical indicators of oil quality, purity, and stability. The results for the 'Akrofu' okra seed oil are summarized in Table 1.

Table 1: Yield and Physicochemical Properties of 'Akrofu' Okra Seed Oil (Mean \pm SD)

Parameter	Ethanol Extraction	Cold Press Extraction	p- value
Yield (mL/504.97g)	5.5 \pm 0.39	4.3 \pm 0.46	< 0.05
FFA (% as Oleic Acid)	0.825 \pm 0.025	0.775 \pm 0.005	< 0.05
Peroxide Value (mEq O₂/kg)	0.76 \pm 0.060	0.87 \pm 0.001	> 0.05
Saponification Value (mg KOH/g)	134.89 \pm 0.92	133.62 \pm 1.28	> 0.05
Iodine Value (g I₂/100g)	101.0 \pm 1.00	101.5 \pm 0.50	> 0.05
Acid Value (mg KOH/g)	18.66 \pm 0.25	18.40 \pm 0.17	> 0.05
Refractive Index (40°C)	1.475 \pm 0.005	1.465 \pm 0.005	< 0.05

Free Fatty Acid (FFA) and Acid Value: The FFA content is a primary marker for hydrolytic rancidity, indicating the breakdown of triglycerides. The cold-pressed oil exhibited a significantly lower FFA value (0.775%) compared to the ethanol-extracted oil (0.825%). This suggests that the cold press method, being a non-thermal process, better preserves the integrity of the triglycerides by minimizing enzymatic (lipase) or hydrolytic degradation (Azadmard-Damirchi & Torbati, 2015). The slightly higher FFA in the ethanol-extracted oil could be due to the prolonged heating during the Soxhlet process, which might accelerate hydrolysis (Hernández-Santos et al., 2016). Both values, however, are well below the maximum limit of 4% set by the Codex Alimentarius for crude vegetable oils, indicating good initial quality (Codex Stan 210-1999). The acid value followed a similar trend, corroborating the FFA results.

Peroxide Value (PV): The PV measures the primary products of oxidation (hydroperoxides). Both extraction methods produced oils with very low PVs (< 1 mEq/kg), signifying a high level of oxidative freshness at the point of extraction. There was no significant difference between the two methods ($p > 0.05$). These values are substantially lower than the acceptable limit (10 mEq/kg for crude oils) set by Codex, highlighting the oil's excellent initial stability against autoxidation (Abdel-Nabey & Abou-Tor, 2020).

Saponification Value (SV): The high SV observed for both oils (133-135 mg KOH/g) indicates the presence of a high proportion of medium to long-chain fatty acids. This value falls within the range reported for other vegetable oils like cottonseed and sunflower oil (O'Brien, 2008). A high SV suggests that the oil could be suitable for soap manufacturing, in addition to its edible applications. No significant difference was found between the extraction methods for this parameter.

Iodine Value (IV): The IV quantifies the degree of unsaturation in the oil. The recorded values (101-102 g I₂/100g) classify 'Akrofu' okra seed oil as a semi-drying oil, similar to oils like sunflower and corn oil (O'Brien, 2008). This high level of unsaturation is nutritionally desirable but also makes the oil more susceptible to oxidation over time, necessitating proper storage conditions.

Refractive Index: The refractive index, which is related to the molecular weight and degree of unsaturation of the oil, was significantly lower for the cold-pressed oil (1.465) than for the solvent-extracted oil (1.475). This difference, though small, is statistically significant and could be attributed to the selective extraction of different lipid classes or minor components by ethanol, which can slightly alter the overall density and composition of the oil (Kronber & Laitinen, 2015).

3.3. Proximate Composition and Fatty Acid Profile

The proximate composition of the seed cake after oil extraction provides insight into the potential uses of the leftover meal (Table 2). The high carbohydrate content suggests its potential as an energy source in animal feed, while the appreciable ash content indicates a good mineral profile.

Table 2: Proximate Composition and Fatty Acid Profile of 'Akrofu' Okra Seed Oil (Mean \pm SD)

Parameter	Ethanol Extraction	Cold Press Extraction	p-value
Moisture (%)	4.75 \pm 0.05	4.65 \pm 0.05	> 0.05
Total Ash (%)	10.50 \pm 0.20	11.17 \pm 0.15	< 0.05
Crude Fibre (%)	7.89 \pm 0.09	8.05 \pm 0.15	> 0.05
Crude Protein (%)	2.81 \pm 2.81	0.12 \pm 0.12	> 0.05
Carbohydrate (%)	77.35 \pm 0.46	75.77 \pm 0.15	< 0.05
Oleic Acid (C18:1) (%)	16.59 \pm 1.32	16.81 \pm 1.36	> 0.05
Linoleic Acid (C18:2) (%)	48.81 \pm 1.68	49.54 \pm 1.58	> 0.05

The fatty acid profile (Table 2) reveals the outstanding nutritional quality of 'Akrofu' okra seed oil. The oil is overwhelmingly unsaturated, with the two methods showing no significant difference in fatty acid composition. Linoleic acid, an essential polyunsaturated fatty acid (PUFA), was the most abundant, constituting nearly half of the total fatty acids (48.81-49.54%). This value is higher than that reported in many common oils like olive oil (3.5-21%) and is comparable to or even exceeds that of sunflower oil (20-75%, depending on the variety) (Gunstone, 2011). Oleic acid, a monounsaturated fatty acid (MUFA) renowned for its stability and health benefits, was the second most prevalent at 16.59-16.81%. The combined unsaturated fatty acid content of over 65% underscores the oil's potential health benefits. Diets rich in unsaturated fats, particularly PUFAs and MUFAs, are associated with reduced low-density lipoprotein (LDL) cholesterol levels and a lower risk of heart disease (Ganesan et al., 2018; Sales-Campos et al., 2013). The high linoleic acid content also positions okra seed oil as a valuable source for the cosmetic and pharmaceutical industries, where it is used for its moisturizing and skin-barrier repairing properties (Tasset-Cuevas et al., 2013).

4. Conclusion

This comprehensive study demonstrates that the choice of extraction technology for 'Akrofu' okra seed oil presents a clear compromise between quantity and quality. Ethanol solvent extraction is the method of choice for maximizing oil yield, making it suitable for large-scale industrial production where volume is a primary concern. In contrast, cold press extraction, despite its lower yield, is superior for producing a premium-quality virgin oil. The cold-pressed oil exhibited better quality indices, such as lower free fatty acids, indicating less hydrolysis and a purer product that commands a higher market value.

Most importantly, irrespective of the extraction method, the oil itself was found to be of exceptional nutritional quality. Its high concentration of unsaturated fats, dominated by the essential linoleic acid, makes it a very healthy edible oil, comparable to and in some aspects superior to many conventional vegetable oils. This finding significantly enhances the commercial viability of okra seeds, transforming a common agricultural by-product into a valuable resource.

5. Recommendations

Based on the findings, the following recommendations are proposed:

1. **For Industry:** Oil processors should adopt cold press technology for producing high-value, virgin okra seed oil for niche health food markets. For bulk oil production aimed at the biofuel or standard edible oil market, ethanol extraction is recommended, with strict adherence to solvent removal protocols.
2. **For Farmers and Aggregators:** The 'Akrofu' variety should be promoted for cultivation not only for its pods but also for its high-oil-yielding seeds, creating an additional revenue stream and reducing post-harvest waste.
3. **For Future Research:**
 - Investigate the optimization of cold press parameters (e.g., moisture content, pre-heating, screw speed) to improve its extraction yield.

- Conduct a full fatty acid profile analysis to include other important acids like palmitic, stearic, and linolenic acid.
- Perform shelf-life studies to determine the oxidative stability of the oil under different storage conditions and explore the efficacy of natural antioxidants.
- Explore the functional properties and potential applications of the protein-rich seed cake leftover after oil extraction.

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Appendix:



Figure 1: Segevi Okra Variety



Figure 2: Adaklu Okra Variety



Figure 3: Akrofu Okra Variety