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Quality characteristics and volatile compounds of oil extracted from njangsa seed

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Abstract

Unconventional and underutilized plant oils are increasing popular and helping to meet demand for food, health and other industrial application. In recent years, the search for alternative sources that deliver healthful benefits, low-environmental impact and ethical responsibility have become a priority to both scientists and industry. Oil from njangsa seed (*Ricinodendron heudelotti*) was recovered using four selected enzymes—hemicellulase, protease, pectinase, and amylase in an enzyme-assisted aqueous extraction (EAE) and the recovery, quality indices and fatty acid composition was compared to solvent extraction using hexane (HE). Njangsa seed oil (NSO) recovered from the EAE ranged between 29 and 36% and were significantly ($p < 0.05$) lower than HE (46%), and the quality indices (free fatty acid, peroxide, para-anisidine, and thiobarbituric acid values) were consistently lower and better than HE-NSO. The fatty acid profile of EAE-NSO was qualitatively and quantitatively comparable to HE-NSO, with differences among the enzymes used. Alpha-eleostearic acid was the most abundant fatty acid and was consistently *ca* 41% among the samples. Among the volatile compounds analyzed on the polar and nonpolar columns, 13 (2 alcohols, 9 aldehydes, and 2 esters) were detected in EAE-NSO using hemicellulase, and 35 (4 aldehydes, 1 ester, and 30 hydrocarbons) in HE-NSO. The most abundant flavor compounds identified were pentanal, methylcyclopentane, cyclohexane, 3-methylpentane, (E,E)-2,4-nonadienal, (E)-2-heptenal, hexanal, 1-butanol, and butanal. This study suggests that the amount, quality, and content of oil and volatile compounds in NSO can be influenced by the choice of extractant.

KEYWORDS
enzyme-assisted aqueous extraction, njangsa seed, oil quality, polyunsaturated fatty acid, volatile compounds

INTRODUCTION

The distinctive flavor and aroma of many vegetable and seed oils, such as extra virgin olive oil, result from a complex mixture of phenolic and volatile compounds, belonging to several chemical classes that include alcohols, aldehydes, ketones, esters, hydrocarbons, acids, furans, terpenes, and other unidentified volatile compounds (Cecchi & Alfei, 2013). The volatile composition of such oils depends on the genetically determined enzymatic activity of the lipoxygenase process (Angerosa et al., 2004; Kalua et al., 2007) within each oil-bearing cultivated plant variety (Fregapane & Salvador, 2013). Volatile compounds can have appeasing odors and pleasant notes arising primarily from C6 aldehydes, alcohols, and their corresponding esters. In contrast, unpleasant aromas and odors may result from biochemical processes that involve the ripening cycle of fruit (Aparicio & Morales, 1998), sugar fermentation (Morales et al., 2017), amino acid conversion (de Cadiñanos et al., 2019), auto-oxidation (Bendini, 2012), and enzymatic activities of molds and anaerobic microorganisms (Bennett &

Inamdar, 2015). The volatile compounds profile of oils may also be influenced by several production and refining technologies. Such factors include the methods and conditions used for oil extraction and milling, the pedoclimate (temperature, water content, aeration) of the soil, irrigation management, and oil storage conditions (Fregapane & Salvador, 2013).

Oil is an essential component of foods and it is an important commodity in the development of industrial and pharmaceutical products (Díaz-Suárez et al., 2021; Kaseke et al., 2021; Lykke et al., 2021; Souza et al., 2019). Soybean, rapeseed, sunflower, and oil palm are extracted from their seeds, kernels, and fruits and have been the main sources of oil for commercial application. However, there is increasing demand and interest for novel sources of ingredients for food and to develop high value uses; including functional and nutraceutical applications. Njangsa (*Ricinodendron heudelotti*) is a plant indigenous to several West African countries and the seed is used in sauces, soups, and condiments due to its characteristic spicy flavor (Ezekwe et al., 2014). The role of njangsa seeds within and beyond the culinary arts is currently underexplored. To the best of our knowledge, the study herein represents the first investigation of the volatile compounds in njangsa seed oil (NSO).

Previous studies have shown that njangsa seeds contain about 45%–67% oil (Abaidoo-Ayin et al., 2017; Assanvo et al., 2015; Manga et al., 2000), and polyunsaturated fatty acids (PUFA) constitute ~75% of its total fatty acids (FAs). This is equal to or higher than most commercial vegetable oils (Díaz-Suárez et al., 2021; Kaseke et al., 2021; Souza et al., 2019). Some earlier studies have reported the inverse relationship between the consumption of foods rich in PUFA and health benefits (Riemersma, 2001). Of particular interest here is alpha eleostearic acid (α-ESA) (c9,t11,t13-octadecatrienoic acid), a long chain (LC)-PUFA. Alpha eleostearic acid, the most abundant FA in NSO (~53%), is an isomer of conjugated linolenic acid (CLnA) that has been linked to anticancer properties, reversal of oxidative stress, and positive effects on some cardiovascular health markers (Grossmann et al., 2009; Saha et al., 2012; Yeboah et al., 2011). Other FAs such as linolenic, oleic, and stearic acid, respectively, account for 27.9%, 6.4%, and 6.5%, of the total FAs in NSO (Assanvo et al., 2015; Manga et al., 2000). The seed is also a major source of phytosterols (Yeboah et al., 2011).

Most oil extraction is carried out by conventional pressing and direct solvent extraction usually with hexane (Lykke et al., 2021) with the extraction methods affecting quality characteristics of the oil. Milder extraction methods that do not involve the use of harsh solvents and other emerging techniques such as pulse electric field, supercritical fluid, microwave-assisted ultrasound-assisted extraction and green solvents such as water, ethanol, ethyl acetate, and ionic liquids, are gaining popularity (Díaz-Suárez et al., 2021; Kaseke et al., 2021; Souza et al., 2019). The use of severe heat treatment during conventional oil extraction does not only affect the quality of the oil but may also denature proteins present (Souza et al., 2019), reducing its value or render the protein unusable. Previous studies in our lab solely used hexane to extract oil from njangsa seed (Abaidoo-Ayin et al., 2017). The safety of using solvents and chances of residual hexane in the oil is of concern to personnel and consumers, respectively, while downstream processing and stringent regulations make the use and disposal of solvents costly. Therefore, alternative safe and environmentally friendly oil extraction methods are preferred.

Enzyme-assisted extraction (EAE) has received considerable attention and offers several advantages over both pressing and solvent extraction including; safe and milder operation, environmentally friendly and lower capital investment (Kaseke et al., 2021; Mechqoq et al., 2021). EAE have been used to simultaneously produce oil and usable fractions including protein (Souza et al., 2019). Other studies have shown improved oil recovery (20%–30%) in mustard seeds treated with cellulolytic enzymes and increased cellular degradation and oil recovery in olive and pomegranate seeds pre-extracted with enzymes (Kaseke et al., 2021), and an increase in soybean oil extractability (Cheng et al., 2019). EAE is based on the ability of the enzymes to hydrolyze cell wall structures, protein network of the cotyledon, and oleosin-based membranes that surround the oil-bearing materials (Kumar et al., 2017; Rosenthal et al., 2001; Souza et al., 2019). Oil recovery in EAE is dependent on the quantitative process parameters such as enzyme type, mean particle size, and type of enzyme, with smaller particle size enhancing oil extraction (Díaz-Suárez et al., 2021; Kaseke et al., 2021; Rosenthal et al., 2001; Souza et al., 2019).

The aim of this study was to investigate the influence of four selected enzymes; hemicellulase, amylase, pectinase, and protease on the extraction yield, quality characteristics, and the volatile components of oil from njangsa seed compared to hexane extraction.

MATERIALS AND METHODS

Njangsa seed obtained from the South Western region of Cameroon in 2017 were dried in an oven at 100°C for 3 h and ground to less than 0.4 mm particle size flour in an Oster tabletop blender (Boca Raton, FL). Hemicellulase (from *Aspergillus niger*, 19,000–23,000 ACH/g), protease (from *Aspergillus oryzae*, 45,000–70,000 CFA/g), pectinase (from *Aspergillus* species, 1100 PE/g) and amylase (from *Bacillus amyloliquefaciens*) were obtained from Enzyme Development Corporation (New York City, NY). Hexane, hydrochloric acid, methanol, and 4-bromofluorobenzene solution (25,000 μg/ml in methanol) were purchased from Fisher

Scientific (Pittsburg, PA). Fatty acid methyl ester (FAME) component mix was obtained from Supelco Inc. (Bellefonte, PA). Sodium sulfate, sodium hydroxide, sodium methoxide, and C7–C40 saturated alkanes standard (certified reference material, 1000 μg/ml each component in hexane) were obtained from Sigma-Aldrich (St. Louis, MO).

Enzyme-assisted aqueous extraction

NSO was extracted by homogenizing 25 g of milled njangsa seed in 150 ml distilled water and 2% dosage (based on the weight of the flour) of one of the following enzymes: hemicellulase, protease, pectinase, and amylase. The pH of the slurry was adjusted to the optimal pH of each enzyme (hemicellulase: pH 5.0, protease: pH 4.0, pectinase: pH 4.0, and amylase: pH 5.0) with 0.5 N NaOH or 0.5 N HCl. The reaction mixture was incubated and maintained at the optimum temperature of the enzyme (hemicellulase: 55°C, protease: 37°C, pectinase: 40°C, and amylase: 70°C) in a water bath (Amerex Instruments, Inc., Concord, CA) with constant shaking at 120 rpm for 24 h. Thereafter the slurry was centrifuged at 4000 rpm, for 20 min at 20°C in an Eppendorf centrifuge 5810 (Hauppauge, NY). The upper layer was decanted into a separatory funnel and the oil phase was passed through a column of anhydrous sodium sulfate to remove any residual moisture. A control experiment without any of the enzymes (flour + water) was run in tandem. All experiments were carried out in triplicate. NSO was stored in amber vials at −20°C until further analysis. Percent recovery was calculated as [(mass of extracted oil/mass of dried flour) × 100].

Solvent extraction

The selected parameters for the solvent extraction were based on results from optimization studies. To 25 g of njangsa seed flour was added 200 ml hexane and stirred at 400 rpm for 1 h at 23°C. The filtrate from the slurry was passed through a column of anhydrous Na_2SO_4. The oil was evaporated to dryness with a rotary evaporator under vacuum and stored in amber vials at −20°C.

Oil quality characteristics

Pooled samples from the extraction were used to evaluate the physicochemical properties of the oil. Peroxide value (PV), saponification value (SV), and free fatty acid (FFA) content of NSO was determined titrimetrically per AOCS official methods; Cd 8b-90, Cd 3-25, and Ca 5a-40, respectively. Thiobarbituric acid (TBA) value was measured at 530–532 nm and ρ-anisidine value (ρ-AV) at 320 nm spectrophotometrically (Beckman Coulter DU 720, Sharon Hill, PA) as described in AOCS official method Cd 19-90 and Cd 18-90, respectively.

Fatty acid composition

The method described by Yirankinyuki et al. (2018) was followed in the FA analysis. In brief, 50 mg NSO was weighed into screw-capped test tubes. To this 50 μl of heptadecanoic acid internal standard and 2 ml 0.5 N sodium methoxide in methanol were added and incubated at 50°C. After 30 min of incubation, 1 ml water and 2 ml hexane were added to the tube and vortexed. The hexane layer containing the FAME was passed through a column of anhydrous Na_2SO_4 and analyzed on a SP-2380 capillary column (60 m × 0.25 mm ID, 0.2 μm film thickness) on a 6850 series GC–MS (Agilent Technologies, Newark) interfaced with a 5973-mass spectrometry detector. FAME component mix was run in tandem. A gradient temperature program comprising of an initial temperature of 70°C, increased to 190°C at 30°C/min after injection, then ramped to 210°C at a rate of 1°C/min followed by holding for 10 min provided separation of the major FAs in 45 min. FAMEs were identified by comparing their relative and absolute retention times to those of the standard, and FA composition was reported as relative percent of total peak area.

Melting profile

A Perkin-Elmer Model DSC 4000 (Norwalk, CT) equipped with an intercooler was used to assess the melting profile of NSO. Analyses were performed using a modification of the AOCS recommended procedure (Cj 1-94). The samples were held for 2 min at 25°C, followed by rapid heating to 80°C at 10°C/min and held for 10 min. The samples were then cooled to −80°C at 5°C/min and held for another 10 min. In the final step, samples were heated to 80°C at 5°C/min. Normal standardization was performed with cyclohexane (two thermal transitions; at −87.06 and 6.54°C), and indium (thermal transition at 156.6°C). The melting point and enthalpy changes of the samples were recorded.

Njangsa seed oil volatile compounds

The volatile compounds in hemicellulase-EAE and HE NSO were determined using a headspace sampler coupled to a GC with mass spectrometric detection (GC–MS). Oil samples (~0.25 g with 0.10 μl 4-bromofluorobenzene internal standard solution) were weighed into a 10-ml headspace vial fitted with a steel crimp cap and teflon/silicon septum. The samples were analyzed with an Intuvo (Agilent, Santa Clara,

CA) GC–MS equipped with a headspace autosampler. The autosampler parameters included 200°C oven temp, 210°C loop temp, and 250°C transfer line temp. The duration of injection was 0.5 min. Compounds were separated using a nonpolar Agilent (Alexandra, VA) HP-5MS UI column (30 m × 0.25 mm × 0.25 μm) and a polar Agilent (Alexandra, VA) VF-WAXms column (30 m × 0.25 mm × 0.25 μm). Helium was used as carrier gas at a constant flow rate of 1.2 ml/min. The ionization source and quadrupole temperature were set at 230 and 150°C, respectively. The temperature profile was used to separate components on either column included an initial column temp of 40°C that was held for 4 min and ramped (2°C/min) to 120°C before being ramped (10°C/min) to a final temp of 250°C and held for 4 min. Identification of volatile compounds was achieved by comparing their mass spectrum with standard spectrums in the National Institute of Standards and Technology (NIST) MS library. The list of identified volatile compounds was further narrowed by employing Kovats Retention Index (Kovats, 1958) values that were determined by normalization with n-alkane standards and correlated with the GC elution times for the volatile components. Kovats retention index values were compared with values cited by online chemical databases (National Center for Biotechnology Information, 2022; NIST Chemistry WebBook, 2022).

Statistical analysis and data visualization

Analyses were performed using the Statistical Package for Social Science (SPSS) version 24.0 (SPSS Inc., Chicago, IL). Differences between the means were analyzed using one-way analysis of variance. Post-hoc comparisons to evaluate pairwise differences among group means were conducted with the use of Tukey HSD tests. Alpha value was set at ($p \leq 0.05$).

Graphical representation of the data was displayed using heatmaps to show relationships between the extraction method and volatile compounds determined on the two columns. The jupyter notebook interface was used to create heatmaps using python. The heatmaps show individual values contained in a matrix and represented as colors. The sequential color ramp was used to distinguish between values (area percent), where lighter colors correspond to smaller values and darker shades to larger values.

RESULTS AND DISCUSSION

Oil recovery: Hexane extraction and enzyme-assisted aqueous extraction

Investigation of the volatile compound profile of njangsa seed oil was initiated by first determining the utility of selected enzymes as an alternative to solvent extraction. In our experiments (Table 1), 46 ± 0.1% of NSO was recovered by hexane extraction. This value is comparable to the 44.1% reported by Abaidoo-Ayin et al. (2017) but lower than the 52.2% reported by Soxhlet extraction (Yirankinyuki et al., 2018). When pectinase, protease, amylase, or hemicellulase was used in EAE, 29 ± 0.4%, 31 ± 0.0%, 35 ± 0.2%, and 36 ± 0.1% of NSO was recovered, respectively (Tables 1 and 2). Lower oil recoveries from EAE can be attributed to partial hydrolysis of structural polysaccharides, cell wall constituents, and lipid body limiting the permeability of the cell structure. Other studies reported both lower and higher oil recovery during EAE compared to solvent-assisted extraction (Díaz-Suárez et al., 2021; Kaseke et al., 2021; Souza et al., 2019). The differences are attributed to the formation of emulsions, incomplete degradation of the cell wall, and restricted diffusion of oil from the matrix in EAE. In the absence of any of these enzymes (control), 19.24% NSO was recovered (Table 1). The lower oil recovery in the control (in the absence of any cell wall hydrolyzing enzyme) shows that, while the reduced particle size (with grinding) allowed some oil to diffuse, water alone could not facilitate further cell wall degradation. Plant cell walls are composed of cellulose, hemicellulose, lignin, and protein, and lipid bodies enveloped in a lipoprotein layer (Najafian et al., 2009; Souza et al., 2019). The higher oil recovery recorded when hemicellulase was the enzyme of choice suggests that the secondary cell wall of njangsa seeds may have high concentrations of hemicellulose compared to pectin and other lignocellulosic polymers.

To hydrolyze these lipoprotein and lignocellulosic polymers, lignocellulolytic and proteolytic enzymes such as hemicellulases, proteases and pectinases are suitable since enzymes are specific for the reaction they catalyze. The nature of these linear- and branched-chained polysaccharides and, their linkages are uniquely accessible by these specific enzymes that disrupt the cell wall and oil-bearing structures. Therefore, enzyme selection is an important step in the EAE process, and it may be interesting to know if the selected enzymes impart the secondary cell wall (i.e., pectin, hemicellulose, cellulose) of njangsa seeds. A protease was included in this screening due to the microfibrils of cellulose cross-linked with protein present in most plants, and the hydrolysis of these proteins may facilitate oil release.

Quality characteristics and thermal behavior of njangsa seed oil

The quality indices of NSO indicate the presence of both primary and secondary oxidation (Table 1). The values for FFA% of the oils extracted by EAE, hexane extraction or 20°C water (control) were 3.09%–4.14%,

TABLE 1 Yield, quality characteristics, and thermal properties of solvent and enzyme-assisted aqueous extracted NSO

Parameter	Solvent-extraction	Enzyme-assisted aqueous extraction				Control
		Hemicellulase	Protease	Pectinase	Amylase	
Yield (%)	46.43 ± 0.05^{a}	36.12 ± 0.07^{b}	31.28 ± 0.04^{d}	28.58 ± 0.38^{e}	34.51 ± 0.23^{c}	19.24 ± 0.27^{f}
SV (mg KOH/g of oil)	208.60 ± 0.19^{a}	199.58 ± 0.12^{e}	198.42 ± 0.12^{f}	200.63 ± 0.30^{d}	203.47 ± 0.12^{c}	204.54 ± 0.20^{b}
AV (mg KOH/g of oil)	10.17 ± 0.02^{a}	8.26 ± 0.02^{c}	8.22 ± 0.02^{c}	6.17 ± 0.02^{e}	6.36 ± 0.02^{d}	8.59 ± 0.01^{b}
PV (mEq/kg of oil)	11.04 ± 0.01^{a}	10.08 ± 0.01^{bc}	10.00 ± 1.00^{c}	10.14 ± 0.01^{b}	10.12 ± 0.02^{b}	9.99 ± 0.02^{c}
FFA (%)	5.09 ± 0.06^{a}	4.14 ± 0.01^{c}	4.13 ± 0.02^{c}	3.09 ± 0.01^{d}	3.09 ± 0.00^{d}	4.29 ± 0.01^{b}
TBA	0.56 ± 0.01^{b}	0.25 ± 0.01^{c}	0.24 ± 0.01^{d}	0.20 ± 0.01^{e}	0.26 ± 0.01^{c}	0.78 ± 0.10^{a}
p-AV	0.99 ± 0.01^{a}	0.74 ± 0.01^{c}	0.68 ± 0.10^{d}	0.63 ± 0.01^{e}	0.72 ± 0.01^{c}	0.91 ± 0.01^{b}

Note: Values are means ± SD; mean values in the same row followed by different superscript are significantly different.

TABLE 2 Melting profile of NSO

Extraction	Peak a		Peak b		Total enthalpy $\sum\Delta H_m$ (J/g)
	Temp (°C)	ΔH_m (J/g)	Temp (°C)	ΔH_m (J/g)	
Hexane	−12.46 ± 0.03^{c}	−24.10 ± 0.72^{c}	2.58 ± 0.13^{a}	−3.08 ± 0.08^{b}	−27.19 ± 0.78^{c}
Hemicellulase	−12.20 ± 0.20^{bc}	−7.45 ± 1.02^{a}	2.20 ± 0.04^{ab}	−0.88 ± 0.16^{a}	−7.29 ± 0.14^{a}
Protease	−11.49 ± 0.06^{a}	−15.42 ± 1.03^{b}	2.07 ± 0.10^{ab}	−0.36 ± 0.07^{a}	−15.78 ± 0.87^{ab}
Pectinase	−12.27 ± 0.10^{c}	−17.15 ± 1.82^{b}	1.39 ± 0.18^{b}	−0.72 ± 0.09^{a}	−17.87 ± 1.81^{b}
Amylase	−13.24 ± 0.07^{d}	−6.43 ± 0.56^{a}	2.73 ± 0.46^{a}	−10.95 ± 1.33^{c}	−17.37 ± 0.77^{ab}
Control	−11.91 ± 0.27^{ab}	−5.47 ± 0.16^{a}	1.72 ± 0.86^{ab}	−0.72 ± 0.53^{a}	−6.19 ± 0.69^{a}

Note: Values are means ± SD; mean values in the same row followed by different superscript are significantly different.

5.09%, and 4.29%, respectively. Very little information is available in the literature relative to the chemical properties of NSO. However, data reported by Ogbuagu et al. (2019) report FFA value for Soxhlet-extracted oil of 6.32%, which is 1.2 to 2.0 times larger than our experimentally determined values. Conversely, Abaidoo-Ayin et al. (2017) reported a value of only 0.33% FFA which corroborates the finding by Yirankinyuki et al. (2018) of 0.2% FFA. All reported and experimental values for FFA% were determined by titration. While some of the literature cited values are low, the experimentally determined FFA% values for NSO are consistent with the acceptable standard range (1.61%–13.06%).

High saponification value (SV) justifies the usage of fats and oil for soap production (Zhou et al., 2019) and is inversely proportional to the mean molecular weight of the glycerides in the oil. Puangsri et al. (2005) reported significant differences ($p > 0.05$) between the SV of EAE and solvent-extracted papaya seed oils. The same was observed for NSO. The SV for EAE oils samples ranged from 198.42 to 203.47 mg KOH/g of oil which is within the codex standard permissibility level limits of 190–209 mg KOH/g of oil (Puangsri et al., 2005). The hexane extracted (HE) oil (SV = 208.60 mg KOH/g of oil) was near the upper limit of the CODEX Standard permissibility level and the control (204.54 mg KOH/g of oil) was only slightly higher than the SV for the EAE oil.

Secondary oxidation parameters of the oils are also presented in Tables 1 and 2. The specific extinctions at 320 nm (p-AV) ranged from 0.63 to 0.74 for the EAE-NSO and increased to 0.99 and 0.91 for HE-NSO and the control, respectively. Specific extinctions at 530–532 nm (TBA) ranged from 0.20 to 0.26 for the EAE oils and, once again, increased to 0.56 and 0.78 mg of malonaldehyde/kg, respectively, for the EAE-NSOs and the control. The EAE-NSOs were significantly ($p < 0.05$) lower than HE-NSO and control. An oil with a p-AV below 10 was considered good quality (Marina et al., 2009) and <2 (Subramanian et al., 2000).

The PV of the oils extracted by either EAE or 20°C water (control) were 10–10.14, 1, and 9.99 mEq/kg, respectively. The values of the EAE and control were statistically equivalent but statistically different from the value (11.04 mEq/kg) obtained from HE-NSO. Oils with PV within the range of 0–10 mEq/kg should have high resistance to lipolytic hydrolysis and oxidative deterioration, resulting in longer shelve life and the unlikelihood to become rancid (Cocks & Rede, 1966). Oils with a PV of 1–5 mEq/kg have longer shelf lives and a very slow to become oxidized (O'brien, 2009). Codex standard cites a PV limit of a 15 mEq/kg for virgin oils in general. Using these criteria, NSO with experimentally determined PV of 9.99–11.03 mEq/kg is likely to become oxidized if not sealed and stored properly.

As expected and compared with another study involving solvent and enzyme-extracted papaya seed oils (Puangsri et al., 2005), melting points as derived from DSC measurements of solvent and EAE NSO

TABLE 3 Fatty acid composition of solvent and enzyme-assisted aqueous extracted NSO

		Enzyme-assisted extraction				
FA (%)	Hexane-extraction	Hemicellulase	Protease	Pectinase	Amylase	Control
$C_{10:0}$ (capric)	0.11	0.10	0.11	0.10	0.10	0.11
$C_{14:0}$ (myristic)	0.84	0.86	0.76	0.77	0.84	0.80
$C_{16:0}$ (palmitic)	9.24	9.04	9.41	9.27	9.16	9.35
$C_{18:0}$ (stearic)	8.10	8.57	8.69	8.41	8.37	8.41
$C_{18:1}$ (oleic)	8.70	8.72	8.13	8.83	8.55	8.45
$C_{18:2}$ (linoleic)	31.78	31.43	31.55	31.46	31.64	32.18
$C_{18:3}$ (α-ELA)	40.92	41.08	41.06	40.86	41.06	40.67
$C_{20:0}$ (arachidic)	0.27	0.30	0.31	0.31	0.29	0.03
ΣSFA	18.56	18.8	19.26	18.86	18.76	18.70
ΣMUFA	8.70	8.72	8.13	8.83	8.55	8.45
ΣPUFA	72.70	72.02	72.61	72.31	72.69	72.85

were considerably similar (Table 2). The heating profile of all six treatments showed two major peaks: a and b. Peak a corresponds to the melting point of unsaturated triacylglycerols (TAG). The more saturated TAGs melted at higher temperatures (peak b), and this was more prominent for oils extracted with hexane and hemicellulase.

Fatty acid composition of njangsa seed oil

There were no practical differences in the FA composition of the enzyme-extracted, solvent-extracted and control NSOs (Table 3). α-ESA was the most abundant FA, and was consistently *ca* 41% among the samples. Other prominent FAs were linoleic (31.43%–32.78%), stearic (8.10%–8.69%), oleic (8.13%–8.83%), and palmitic acid (9.04%–9.41%). These values compare favorably with previous reports (Anjah & Oyun, 2009). Compared to commercial vegetable oils such as canola and soybean, NSO is superior in terms of PUFA content (72%–73%). Warner and Mounts (1993) reported PUFA contents of 30% and 60% for canola and soybean oils, respectively. These results show that NSO is a good source of PUFA. Conversely, oil from canola and soybean contain about 60 and 24% oleic acid, respectively. The oleic acid concentrations are significantly higher than that of EAE and solvent extracted NSO (8.7%).

Njangsa seed oil volatile compounds

The volatile compounds in NSO were determined for the first time in this study (Table 4, Figure 1) by analyzing hexane-extracted NSO and NSO obtained from the enzyme-assisted extraction (hemicellulase) that gave the highest oil yield using both polar and nonpolar column. The volatile compounds in NSO were determined by matching experimental GC–MS spectral peaks with standard spectrum peaks archived in the National Institute of Standards and Technology (NIST) MS library. The list of identified volatile compounds was further narrowed by employing Kovats Retention Index (Kovats, 1958) values that were determined by normalization with n-alkane standards and correlated with the GC elution times for the volatile components (Table 4). Kovats retention index values were compared with values cited by online chemical databases (National Center for Biotechnology Information, 2022; NIST Chemistry WebBook, 2022). The amount and type of volatile compounds in NSO varied between the hemicellulase-EAE and HE oils (Figures 1 and 2, and Table 4). Heat maps were generated to visualize the variation in volatile compounds from the two oil extracts, analyzed on polar and nonpolar columns (Figure 1). Lower values are pale, ranging to higher values that are darker in color (gradual changes within each bin in hue correspond to changes in area percent of volatile compounds).

Various classes of volatile compounds such as alcohols, aldehydes, esters and hydrocarbons were identified (Figure 2). The columns were both successful at separating the volatile compounds allowing for their identification, but the polarity of the column did not substantially influence on the type and classes of volatile compounds (Figures 1 and 2 and Table 4). There were some overlaps in the volatile compounds and classes that were separated and identified. A total of 44 volatile compounds (2 alcohols, 3 esters, 9 aldehydes, and 30 hydrocarbons) were identified when both polar and nonpolar columns were used. Among those volatile compounds, 13 (2 alcohols, 9 aldehydes, and 2 esters) were identified in hemicellulase-NSO and 35 (4 aldehydes, 1 ester, and 30 hydrocarbons) in the HE-NSO. The most abundant chemical groups were hydrocarbons, in HE-NSO, and

TABLE 4 Kovats retention index values (KI) and area percent of volatile compounds in njangsa oil identified by GC–MS using polar and nonpolar columns

	KI		Area percent (%)			
Volatile compound	Nonpolar	Polar	ENZ nonpolar	HEX nonpolar	ENZ polar	HEX polar
1-Butanol	655	1150	5.15		4.89	
1-Pentanol	762	1257	1.02		1.28	
2,4-Decadienal	1317	1767	1.00		0.71	
2,4-Decadienal, (E,E)-		1807			1.37	
2,4-Nonadienal	1212	1662	8.97	0.92	2.28	1.65
2,4-Nonadienal, (E,E)-		1697[b]			9.76	
2-Heptenal, (E)-	954	1323	8.75	0.92	9.43	1.56
2-Hexenal	850	1254	0.90		1.58	
3-Heptene, 4-propyl-	997[c]			0.29		0.40
4-Undecene, (E)-	1083	1108		1.03		0.69
5-Undecene	1038[a]			0.52		0.65
Butanal	587	883	4.42		3.31	
Cyclohexane	656	733		10.61		11.96
Cyclohexane, butyl-	1029	1070		0.94		1.11
Cyclohexane, pentyl-		1287		1.04		0.68
Cyclohexanone, 3-butyl-	1073[c]		0.00	0.83		0.49
Cyclopentane, hexyl-	1137	1174		0.72		0.50
Cyclopentane, methyl-	624	702		15.13		16.61
Decane	1000	1000		1.64		1.47
Decane, 2-methyl-	1064	1053		1.28		1.40
Decane, 3,7-dimethyl-	1128	1117[c]		1.36		0.34
Decane, 3-methyl-	1071	1061		1.47		0.42
Decane, 4-methyl-	1022	1050		1.28		1.14
Decane, 5-methyl-	1058	1046		0.95		0.94
Dodecane	1201[a]	1202		4.02		3.67
Formic acid, butyl ester	721	1024	1.68		1.79	
Formic acid, chloro-, (3,4,4-trimethyl-1,2-dioxetan-3-yl) methyl ester	458		3.03		0.93	
Hexanal	800	1086	5.20	0.42	4.57	1.81
Naphthalene, decahydro-	1049	1141		1.00		0.71
Naphthalene, decahydro-2-methyl-	1121	1240[c]		0.96		0.74
Nonane, 1-iodo-	1124	1152		0.54		0.76
Octane, 2,6-dimethyl-	1273	960		0.21		0.95
Oxalic acid, cyclohexylmethyl ethyl ester	1085[c]	1162[c]		0.38		0.36
Pentanal	698	986	42.23	3.88	44.25	11.91
Pentane, 3-methyl-	578			9.58		9.10
Trans-Decalin, 2-methyl-	1105	1184		0.96		0.63
Tridecane	1300	1300		4.02		3.67
Undecane	1102	1101		6.78		5.22
Undecane, 2-methyl-	1165	1156		1.62		1.05
Undecane, 3-methyl-	1171	1165		1.12		0.73
Undecane, 4-methyl-	1160	1152[c]		1.00		0.76
Undecane, 5-methyl-	1156	1148		0.89		1.14

(Continues)

TABLE 4 (Continued)

Volatile compound	KI		Area percent (%)			
	Nonpolar	Polar	ENZ nonpolar	HEX nonpolar	ENZ polar	HEX polar
Undecane, 6-ethyl-	1215			1.00		1.40
Undecane, 6-methyl-	1155	1145[c]		0.66		0.69
Sum			82.51	87.03	88.99	91.64

Note: KI were cross-referenced with values found in both PubChem and the most recent NIST databases unless otherwise annotated.
[a]Some published values were listed in the PubChem.
[b]Some published values were listed in the NIST database only.
[c]No prior published KI.

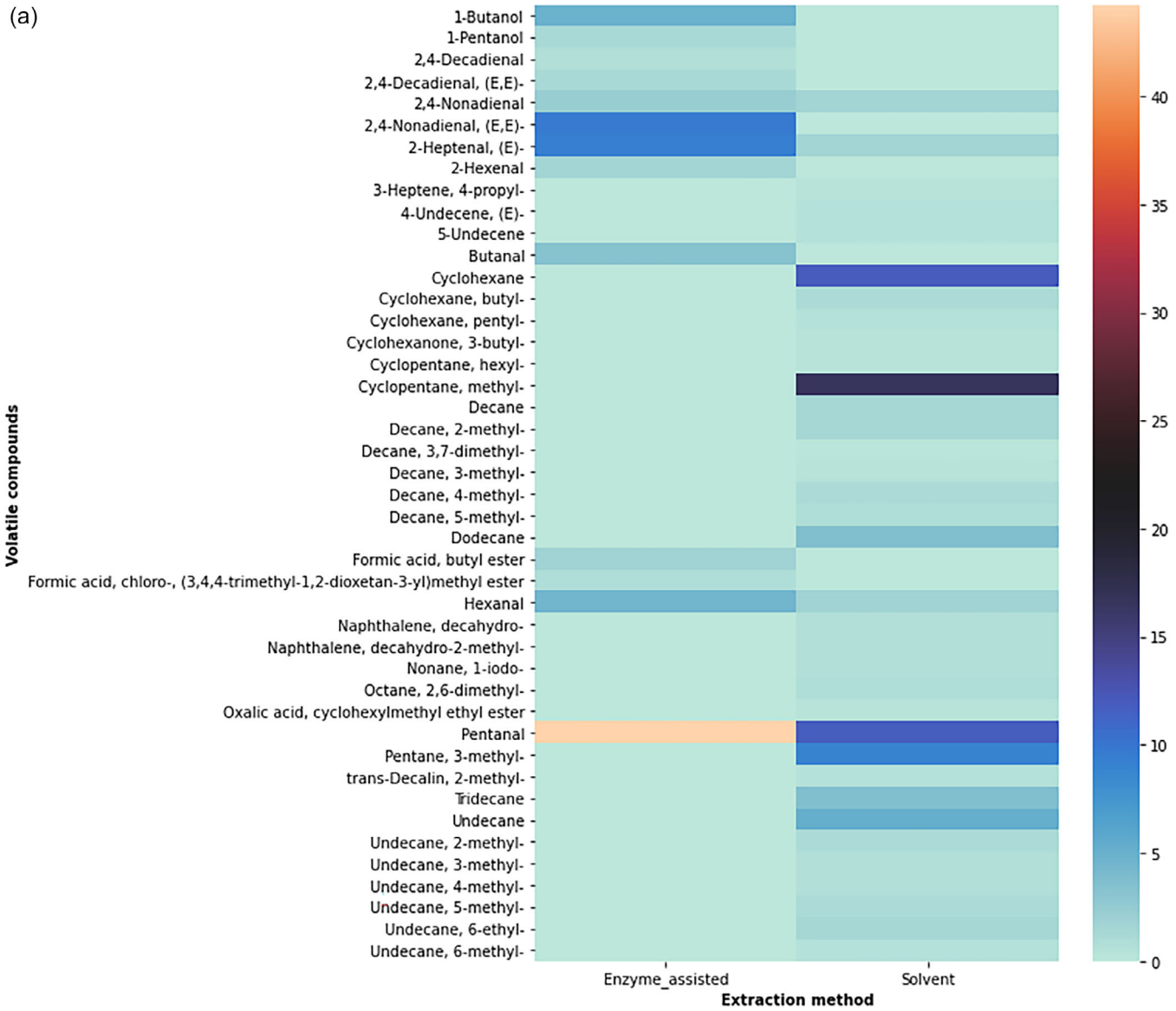

FIGURE 1 Heatmap developed by total peak area, as determined by GC–MS, of all identified volatile compounds within chemical groups for volatile compounds in oil obtained by the enzyme-assisted or hexane extractions of njangsa seed and analyzed on (a) polar and (b) nonpolar columns.

aldehydes in the hemicellulase-NSO accounting for 85.7% and 69.2% of the total number volatile compounds, respectively. A previous study on extra virgin olive oil detected a total of 32 volatile compounds. However, no hydrocarbons were detected (Overton & Manura, 1995), with aldehydes as the most prominent of the volatile

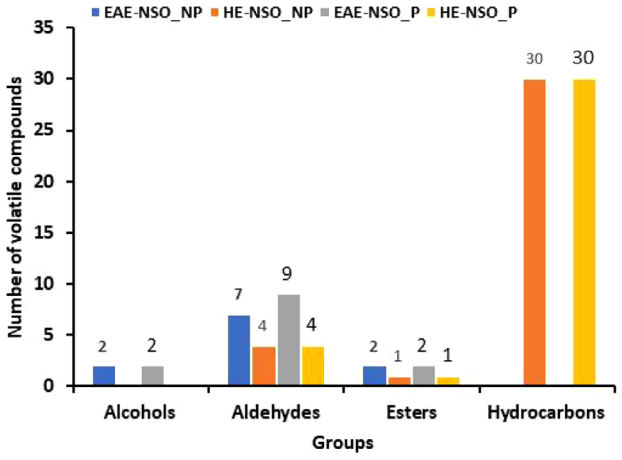

FIGURE 2 Number of volatile compounds identified in hemicellulase- and hexane-extracted njangsa seed oil analyzed on nonpolar (NP) and polar (P) columns.

compounds (41.6% of the volatile content) followed by alcohols.

The oil extracts shared no common alcohols, esters, or hydrocarbons; however, they contained 4 common aldehydes (2,4-decadienal, 2,4-nonadienal, hexanal, and pentanal). Aldehydes are the most abundant volatile compounds associated with stored/rancid oils and are considered the main secondary oxidation compounds formed during lipid oxidation (Beatriz & Hernández, 2013). The flavor profile for 2,4-decadienal and 2,4-nonadienal are both described as sweet and fatty (Kumazawa et al., 2010); whereas the flavor profile for hexanal and pentanal are described as fresh, green fatty, and grassy and as fermented bready, fruity, nutty, and berry, respectively (Durán-Guerrero et al., 2021). Two alcohols (1-butanol and 1-pentanol) were identified in EAE-NSO but none in HE-NSO compared to two esters in the EAE-

(b)

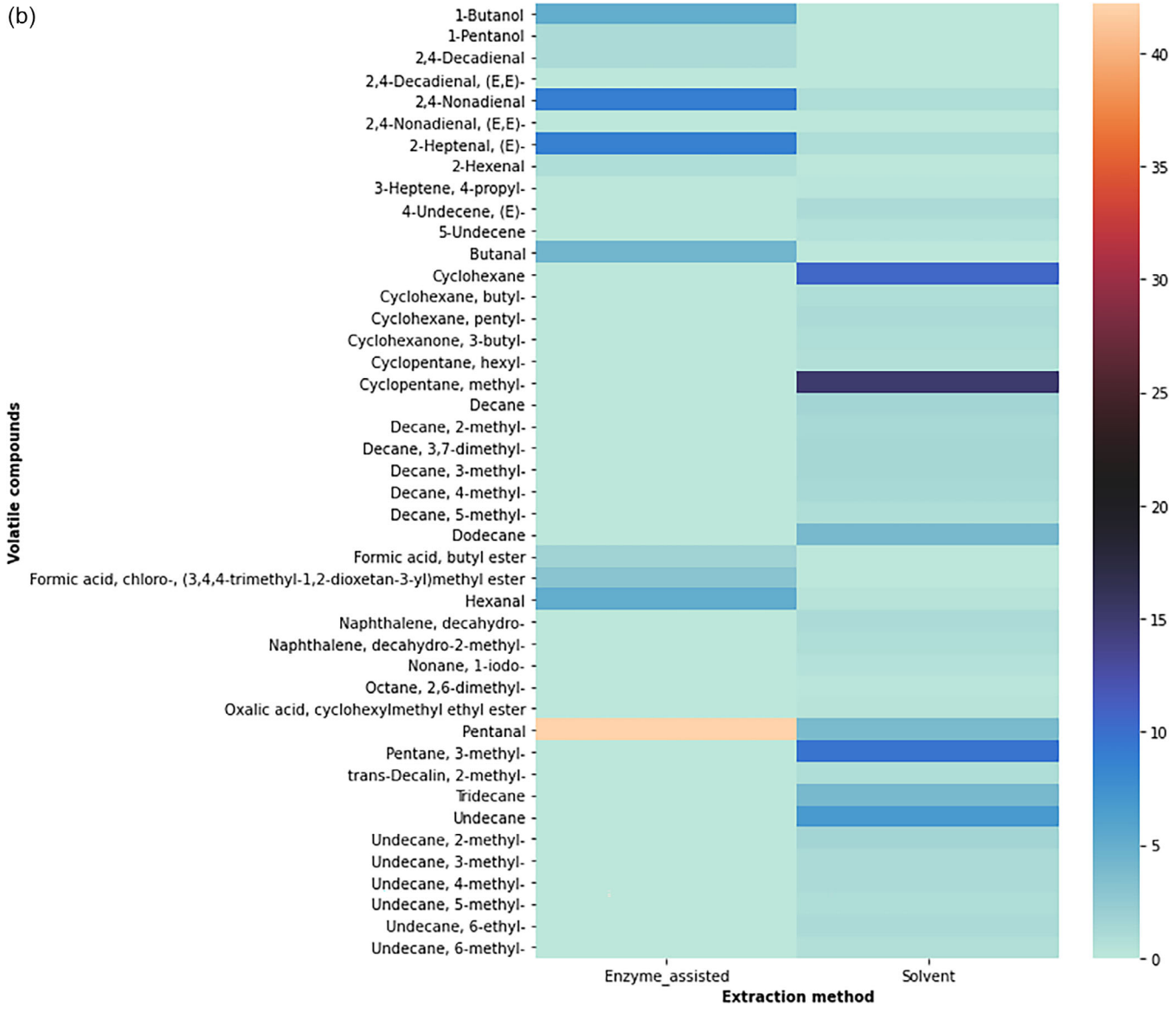

FIGURE 1 (Continued)

NSO and one ester in the HE-NSO. The EAE-NSO contained more aldehydes (9 vs. 4) but no hydrocarbons (0 vs. 30) compared to the HE-NSO. There were no positively identified acids or other classes of volatile compounds in either of the NSO extracts.

Many of the hydrocarbons identified in the HE-NSO are present in relatively low concentrations (Figure 1 and Table 4). It is also evident that compounds such as 1-pentanol and 2-hexenal in the EAE-NSO and 4-propyl-3-heptene, 5-undecene and the cyclohexylmethyl ethyl ester of oxalic acid were present in low concentrations. The most abundant compounds were pentanal, (E,E)-2,4-nonadienal, (E)-2-heptenal, and hexanal found in both oil extracts; methylcyclopentane, cyclohexane, and 3-methylpentane in the HE-NSO; and, 1-butanol and butanal in the EAE-NSO (Figure 1 and Table 4).

The significant amount of linoleic acid and eleostearic acid (Table 3) may account for the limited oxidative stability of NSO. Autoxidation of these FAs involve hydrogen abstraction on the doubly reactive allylic C-11, typically forming hexanal, pentanal, and heptanal. Therefore, oils with high linoleic and eleostearic acid content also tend to contain high amounts of these three saturated linear aldehydes (Beatriz & Hernández, 2013; Xu et al., 2017). In this study, all three volatiles were detected. The presence of 2,4-alkadienals (2,4-decadienal and 2,4-nonadienal) and alcohols (1-butanol and 1-pentanol) is evidence of secondary and tertiary oxidation (Grebenteuch et al., 2021). The concentration of these compounds varies with storage time and level of oxidation (Beatriz & Hernández, 2013; Xu et al., 2017). These oxidation products have also been determined in other edible oils, such as soybean, peanut, rapeseed, and linseed oils (Beatriz & Hernández, 2013; Guclu et al., 2016).

Further evaluation is needed to identify 10 compounds (S1) identified by GC–MS using polar and nonpolar columns but eliminated from Table 4 due to poor correlation to Kovats retention index values found in literature. These compounds could significantly affect the flavor profile of NSO as they account for as much as 7.03% of the area percent of the volatile compounds. Of particular interest is a family of six compounds erroneously identified as cyclohexane in the HE-NSO. These compounds have similar Kovats retention index values and likely represent another chemical family.

CONCLUSION

Differences were observed in the type, number, and quantity of volatile compounds generated with respect to extraction method. A total of 13 volatile compounds were identified in the hemicellulase-assisted oil extract compared with 35 in the HE oil. To our knowledge this is the first study to assess volatile compounds in NSO. The polarity of the column had limited influence on the analysis of the volatile compounds. Hemicellulase was more efficient in extracting oil from njangsa seeds compared with protease, pectinase, and amylase. Njangsa seeds are a good source of oil, rich in polyunsaturated FAs especially α-eleostearic acid and linoleic acid with potential health, nutritional and industrial application. Enzyme-assisted extracted oil exhibited better oil quality indices indicative of minimal oxidation compared with hexane extraction. This study provides baseline information for future research efforts that will focus on understanding the effect of environment on seed quality, sequential or simultaneous use of a combination of enzymes, and optimization of extraction conditions on oil recovery, quality and volatile profile, as well as insight into the health benefits of α-eleostearic acid from NSO. These results from this study suggest a niche market could be created for njangsa seed for consumers looking for natural products.

AUTHOR CONTRIBUTIONS

Alberta N. A. Aryee designed and directed the project. Immaculate T. Arrey performed the experiments, analyzed the data, and prepared the initial draft. Anh Nguyen and Papa Nii Asare-Okai carried out the FA and volatile compound analyses, respectively. Nii Adjetey Tawiah performed data integration and visualization. Samuel A. Besong and Gulnihal Ozbay reviewed the manuscript. Alberta N. A. Aryee, Victor T. Wyatt, and Kerby Jones contributed to the analyzing the data, interpretation of the results, writing—critical review and editing of the manuscript. All authors have read the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

ETHICS STATEMENT

No human or animal subjects were used it the course of this research.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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