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Impact of Extraction Method on the Oil Yield, Physicochemical Properties, Fatty Acid Composition and Stability of Cashew Nut (*Anacardium occidentale* L.) Oil

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Abstract

The efficiency of different methods for extraction of oil from cashew nuts was investigated and compared in terms of oil yield, storage stability and quality. In the present study, cashew nut oil was extracted by 3 methods: cold-pressing, solvent extraction and enzyme assisted aqueous extraction (EAAE). The cold-pressing was conducted at pressure of 25 MPa using a custom-made device. The solvent extraction was done on Soxhlet apparatus for 6 h, using hexane as a solvent. The EAAE was carried out with commercial enzyme Viscozyme Cassava C. The three methods were compared for the extraction yields and several oil quality indicators, such as specific gravity, free fatty acid (FFA), peroxide values (PV), total phenolic (TPC), total flavonoid content (TFC), fatty acid composition and storage stability. The storage ability of oils was investigated by following the change in FFA and PV values in a period of 45 days at 60°C. The PVs, FFAs, TPC, TFC and the yields were significantly different among 3 methods but not the specific gravity. The oil obtained by cold-pressing was most stable against oxidization and rancidity, as it could be kept fresh for 12 months, while the EAAE and solvent extraction oils for 8 months, at room temperature conditions. The oils were rich in un-saturated fatty acid, with more than 65% mono- and polyunsaturated fatty acids. The fatty acid composition was not significantly different among the three methods.

Keywords: *Cashew Nut Oil; Cold-Pressing; Enzyme Assisted Aqueous Extraction; Storage Stability*

Introduction

Cashew (*Anacardium occidentale*) kernel (nut) is considered a sort of highly nutritious food, containing protein (19%), fat (44%), moisture (5%) and the rest as carbohydrates and minerals. The total lipid (oil) is a major part of cashew kernel, which makes up 40 - 47% in the whole seed [1,2]. Majority of the nut oil consists of monounsaturated fatty acid (MUFA, 58 - 62%), while polyunsaturated fatty acids (PUFA) comprise of 17 - 21%. Among unsaturated fatty acids (UFA) present in cashew nut, oleic (C18:1) and linoleic acid (C18:2) are the two prevalent ones. The primary saturated fatty acid is stearic (8 - 10%) and palmitic acid (10-12%) and total average content of saturated fatty acids (SFA) is about 20 - 22% [3,4]. Cashew nut oil (CNO) contains a fair amount of α-, γ-tocopherol and squalene, which are powerful antioxidants. Phytosterols, which are effective in reducing atherosclerotic risk and serum LDL cholesterol, are also present in CNO with β-Sitosterol as the most abundant sterol. CNO may contain different fat-soluble phenolics such as anacardic acids, cardanols, cardols and some carotenoids [4,5].

Vegetable oil is commonly extracted by using organic solvents such as hexane, acetone, petroleum ether, etc. The solvents are highly flammable and/or toxic. Also, solvent recovery is expensive and energy intensive; large volume of solvent and long extraction times is necessary to remove the oil from the seeds. Thus, the use of organic solvents is under close governmental control due to growing concerns about consumer safety and environmental impact [6].

Mechanical pressing of oil refers to a physical process for crushing oil glands in the plant materials to release the oil. "Cold-pressed oil" is defined as oils obtained from this method at temperature not exceeding 50°C. In cold pressing, the oil's biological properties such as nutrients, medicinal values, etc. are retained. Also, the product is free of residual solvents. In addition, this method reduces added water and the release of oil oxidation enzymes.

The oil yield achievable through cold pressing was between 34.5 - 35.9% of the total weight of the input material for pumpkin seed [7], flaxseed 36% [8] and hemp seed 26.7% [9].

Enzyme-assisted aqueous extraction (EAAE) has emerged as a potential alternative in vegetable oil processing, leading to elimination of solvent use, reduction in investment costs and energy requirements [10]. Oil was obtained in EAAE method from soybean by using cellulase and protease [11], from corn germ by using protease, cellulase and pectinase [12]. Recently, CNO was extracted under selected aqueous extraction conditions using commercial Viscozyme cassava C [13].

Purpose of the Study

The main purpose of this study was to evaluate the efficiency of CNO extraction in cold-pressing, EAAE, and solvent methods, and to investigate the storage stability and quality of the oil produced from the methods.

Materials and Methods

Materials

Cashew kernels of guaranteed quality (un-salty, without the outer shells, but having silky skin) were bought from a local producer in vacuum package. The kernels were ground with a lab hammer grinder machine into powder of 350 - 380 µm and stored at 4°C.

All chemicals and solvents used for the experiment were of analytical grade and purchased from local agents. Standard Quercetin (Sigma-Aldrich), Gallic acid, Folin-Ciocalteu reagent (Merck-Germany), commercial cellulase Viscozyme Cassava C from Novozyme (Denmark) were purchased from local agents.

Enzyme assisted aqueous extraction of cashew nut oil

4g of ground cashew nut was dispersed with distilled water in a material-to-water ratio (1:9) (w/v) to make a slurry and shaken in falcon (50 mL), then Viscozyme Cassava C enzyme was added (1% v/w E/S) into the slurry, and pH adjusted to 5.5 - 6.0. The samples were shaken for 3h at 50°C. At the end of treatment, the enzyme was deactivated by heating at 90°C for 5 minutes. The oil layer was taken out after centrifugation at 13,000 x g for 30 minutes and then evaporated at 60°C for 4h to remove residual moisture [13].

Cold pressing cashew nut oil

The procedure was modified from Concha., *et al* [14]. 30g of ground cashew nut (moisture content about 4 - 5%) was cold-pressed by using a custom-made device, at a pressure range of 20 - 25 MPa and for 7 - 10 min per run.

Solvent extraction

20g of ground cashew kernels was subjected to extraction in a Soxhlet apparatus with hexane for 6h.

2.5 Physico-chemical analysis

The oil yield was calculated as percentage of the raw materials (cashew kernel powder). The specific gravity (SG) of oil was determined by using a pycnometer bottle. Peroxide value (PV) was measured by using Iodometric titration method (AOCS Cd8-53), expressed in meqO2/kg. Free fatty acid (FFA) values in oil were determined by titration method (AOCS Ca 5a-40), defined as the mg of KOH required to neutralize the fatty acids present in 1g of sample [15].

Total phenolic content

The total phenolic contents (TPC) in CNO samples were determined according to the method reported elsewhere [16]. Briefly, 1 mL of oil was extracted with 3 mL methanol and this step was repeated thrice. The methanol phase was collected, combined and adjusted to a volume of 10 mL. The portion of 100 µL of oil extract was mixed with 0.5 mL of fresh Folin-Ciocalteu reagent, 1.5 mL of sodium carbonate solution (20%) and 6 mL of deionized water. The mixture was incubated in a 20 mL capped test tube at room temperature for 90 min with constant shaking. Blue color development was measured at 765 nm using UV-VIS spectrophotometer (model Genesys 10S). The total phenolic content was expressed as mg Gallic Acid Equivalent/100g (mgGAE/100g) of oil.

Total flavonoid content

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method with Quercetin standard solutions, prepared in methanol [17]. Flavonoids were extracted with a mixture of sodium nitrite, aluminum chloride and sodium hydroxide solutions, and measured at 510 nm by using UV-Vis spectrophotometer. The results were expressed as mg quercetin equivalent per 100 g of oil (mg QCE/100g).

Fatty acid profile

The CNO was converted into fatty acid methyl esters (FAMEs) with boron trifluoride in methanol, then extracted with hexane. The hexane layer which contains the FAMEs was transferred to small tube and store at -20°C for analysis. Methyl esters of fatty acids were analyzed by gas chromatography method GC-ISO/CD 5509:94 as described elsewhere [13]. Specifically, FAMEs solution was pumped to a GC-2010 plus Shimadzu with flame ionization detector (FID) at 250°C. The machine was equipped with an Agilent column DB-FFAP (30m, 0.25 mm internal diameter, 0.25 µm film thickness). Nitrogen was used as the carrier gas at a pressure of 14 psi. The oven temperature was programmed from 70°C to 230°C and then held at 230°C. FAMEs were identified by comparing their relative and absolute retention times to those of authentic standards and reported as percentages.

Oil storage stability

The oven test method at 60°C was used to determine oil stability. Degree of oxidation was periodically assessed by measurement of peroxide values (PV) and free fatty acid (FFA) values. Oil samples were stored in an oven at 60°C, in the dark and sampled out for analysis after 0, 15, 30, 45 days of storage.

Statistical analysis

All treatments were conducted in triplicate. The results were subjected to analysis of variance (ANOVA) at $p < 0.05$ using standard statistical package SPSS, version 20.

Results and Discussion

Physico-chemical characteristics of CNO

Total oil yields

As shown in table 1, the total oil yields were calculated and expressed as percentage of sample weight (%, w/w). The yields were significantly different among the three methods ($p < 0.05$). The oil extraction yield by Soxhlet method using hexane was highest (45.04%) and the lowest was from the EAAE method (32.68%). A previous research [13] on CNO extraction showed that the yield of CNO from cashew kernels by EAAE method was about 39%, using Viscozyme Cassava C. The difference was possibly due to the source of cashew nut samples they investigated. Meanwhile, the oil yield by cold-pressing method at 25 MPa was about 40%. In general, cold-pressing method can produce higher oil yield than the aqueous extraction method. Compared to another study [9], oil of hempseeds was obtained by EAAE in the yield arrange of 28.4 - 32.8%, quite close to the results in this study.

No	Characteristics	CNO by cold-pressing	CNO by solvent method	CNO by EAAE
1	% oil yield	$39.12^{a} \pm 1.34$	$45.04^{b} \pm 0.86$	$32.68^{c} \pm 0.49$
2	Specific gravity	$0.9370^{a} \pm 0.0015$	$0.9538^{a} \pm 0.0117$	$0.9915^{b} \pm 0.0059$
3	TPC (mg GAE/100 g)	$73.02^{a} \pm 3.57$	$58.67^{b} \pm 0.03$	$25.62^{c} \pm 2.34$
4	TFC (mg QCE/100 g)	$17.39^{a} \pm 1.80$	$10.72^{b} \pm 0.11$	$9.49^{b} \pm 0.98$
5	PV (meq/kg)	$1.08^{a} \pm 0.13$	$3.30^{c} \pm 0.37$	$2.37^{b} \pm 0.12$
6	FFA (mg KOH/g)	$1.70^{a} \pm 0.11$	$2.35^{c} \pm 0.20$	$1.83^{b} \pm 0.08$

Table 1: *Physicochemical characteristics of oil extracted by cold-pressing, solvent method and EAAE.*

Data showed in mean ± standard deviation. Values in row with the same letter are not significantly different.

Specific gravity

Specific gravity (SG) is the ratio of the density of a respective substance to the density of water at 25°C. In table 1, SG values for oils from solvent extraction and cold-pressing were not different, but they were significantly different from that in EAAE ($p < 0.05$). The oil by enzymatic method was heavier that meant it is difficult to produce and refine. In addition, fatty acid composition of extracted oil by EAAE was also one factor that leads to difference in SG.

Total phenolic content

There was a significant difference in TPC in oils from the three methods. The TPC in cold-pressed oil was highest (73.02 mgGAE/100g) and almost 3 times higher than that in the EAAE oil. In solvent or EAAE methods, high process temperature (50 - 90°C) in combination with prolonged process duration (several hours) may impart negative effect on oil phenolic compounds so the TPC decreased. The TPC in cashew nut oils was found to be in range of 33.4 - 107.0 mgGAE /100g [18], quite close to the results in this study. It is worthy to note

that the TPC in raw cashew nut was about 137 mg GAE /100g [19]. Cashew nut oil contains a fair amount of TPC, higher than in many other seed oils such as in pumpkin seed oil (2.3 - 5.4 mgGAE/100g oil) [6]; virgin coconut oil (25 mg GAE/100g) [20] and virgin olive oil (15.5 - 44.4 mgCAE/mg) [21].

Total flavonoid content

There was a significant difference in TFC between cold-pressing and other methods (Table 1). The highest TFC was found in the cold-pressing oil (17.39 mg QE /100g). Cold-pressing is thus considered as a method that helps to preserve the content of bioactive compounds. It was reported that the extraction technique significantly affects the content of bioactive compounds in oil samples under exploration [6]. The TFC in fresh cashew nut kernel was about 1.99 mg/100g nut [22], showing that most of flavonoids are concentrated in the oil phase of cashew nut. There is almost no information on TFC in cashew nut oil available in the literature.

Storage stability

Free fatty acid values

Figure 1 illustrates that FFA from the 3 methods increased in the first 15 days of storage. At day 0 and temperature 60°C, the FFA value (2.35 mg KOH/g fat) of the solvent method was significantly different from that in other two methods. The results were quite close to results from other authors [13]. In contrast, from day 15 to day 45 the levels of FFA in all CNO samples decreased significantly and continuously. However, there was insignificant difference between day 45 and day 0.

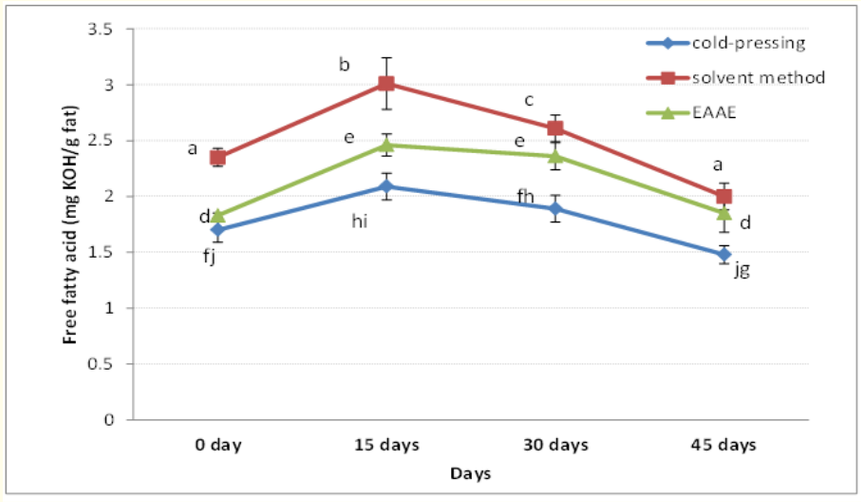

Figure 1: *Change in oil FFA values during storage.*

Peroxide value

The peroxide values (in meqO_2/kg) of the CNO samples during the course of storage of 45 days at 60°C are showed in figure 2. In general, PV in CNO from all methods increased continuously by days (time) in storage at 60°C. There was a significant difference in PV among the three methods and the PVs in cold-pressed oil were always lower than those of the others. At day 0, the PV in cold-pressed, solvent extraction and EAAE oil were 1.08, 3.30 and 2.37 meq O_2/kg, respectively. Comparing the PVs in EAAE oil at day 0 and day 15 of storage at 60°C, results were not much different from those obtained by other authors [13]. PV in CNO by Soxhlet extraction method was always highest among the three methods, partly due to long time (6h) exposure at high temperature resulting in oil rancidity.

The acceptable PV value level in virgin fats and oils is at most 15 meq O2/kg oil (Codex stan 19-1981), it was indicated that CNO by EAAE and solvent extraction methods can be kept at 60°C for 30 days, while the cold-pressed oil 45 days. Theoretically, if the temperature decreases by 10°C, the storage time can increase twice. It may imply that the EAAE and solvent extracted CNO can be stable in storage at room temperature for about 8 months, and the cold-pressed oil for 12 months.

Fatty acid profile

As shown in table 2, the CNO is largely made up of monounsaturated fatty acids, and the most abundant was oleic acid (C18:1, 60%). Linoleic acid (C18:2) was the most prevalent polyunsaturated fatty acid (19%). Linoleic acid is an omega-6 fatty acid and oleic acid is an

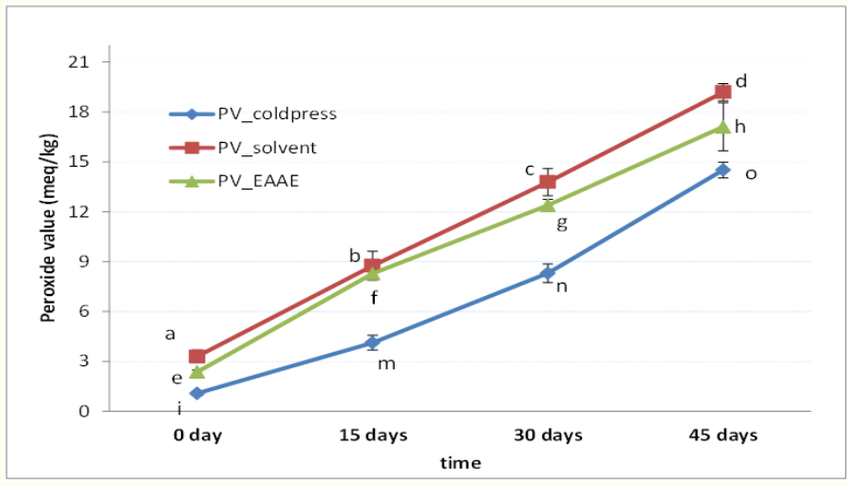

Figure 2: *Change in oil PVs during storage time.*

	CNO by EAAE (this study)	CNO by cold-pressing (this study)	CNO by solvent method (this study)	CNO by solvent method [2]	Olive nut [23]	Sesame oil [24]
Capric acid (C10:0)	-	-	-	-	-	11.3
Lauric acid (C12:0)	0.01	0.004	-	-	-	2.8
Myristic acid (C14:0)	0.02	0.02	0.02	-	-	2.6
Myrisoleic (C14:1)	-	-	-	-	-	9.9
Pentadecylic acid (C15:0)	0.01	0.01	0.01	-	-	-
Palmitic (C16:0)	9.66	8.78	9.16	11.50	11.1	19.3
Palmitoleic (C16:1)	0.35	0.33	0.29	0.33	0.6	1.7
Margaric acid (C17:0)	0.11	0.10	0.10	0.13	-	2.2
Stearic acid (C18:0)	9.28	7.68	8.31	8.80	2.0	13.9
Oleic acid (C18:1)	60.09	58.44	57.6	61.44	77.4	10.2
Linoleic (C18:2)	19.14	19.26	18.9	17.09	6.0	12.2
α-Linolenic acid (C18:3)	0.17	0.16	0.16	0.20	0.7	11
Arachidic acid (C20:0)	0.62	0.49	0.52	0.51	0.3	-
Gondoic acid (C20:1)	0.2	0.18	0.17	-	0.6	-
Behenic acid (C22:0)	0.12	0.11	0.11	-	0.1	-
Erucic acid (C22:1)	0.001	-	-	-	-	-
Lignoceric acid (C24:0)	0.14	0.11	0.12	-	-	-
Total SFA	19.97	17.30	18.35	20.94	13.6	52.1
Total MUFA	60.64	58.95	58.06	61.77	78.6	21.8
Total PUFA	19.31	19.42	19.06	17.29	6.8	23.2

Table 2: *Fatty acid composition (% of total) of oils extracted from cashew nut and the other plant seeds.*

Conclusion

In our research, CNO was obtained by cold-pressing, solvent extraction and EAAE. The results showed that cold-pressing method offered many advantages, including high extraction yield, high content of bioactive compounds in oil, minimum of degradation of oil and shorter extraction time. For the EAAE, under optimum conditions with added Viscozyme Cassava C, the oil yield of was lower than the other methods. However, the advantages of this method are in its solvent-free process and products without toxic organic solvent residues. The CNO by EAAE and solvent methods can be kept at 60°C for 30 days, while the cold-pressed oil can be kept for 45 days. It may imply that these oils can be kept fresh for 8 months, while the cold-pressed oil can be kept for about 12 months at room temperature. In general, CNO can be regarded as rich in unsaturated fatty acid (80%) and bioactive compounds (phenolics).

Bibliography

1. Venkatachalam M and Sathe SK. "Chemical composition of selected edible nut seeds". *Journal of Agricultural and Food Chemistry* 54.13 (2006): 4705-4714.

2. Alasalvar C and Shahidi F. "Natural antioxidants in tree nuts". *European Journal of Lipid Science and Technology* 111.11 (2009): 1056-1062.

3. Toschi TG., *et al*. "A study on cashew nut oil composition". *Journal of the American Oil Chemists' Society* 70.10 (1993): 1017-1020.

4. Ryan E., *et al*. "Fatty acid profile, tocopherol, squalene and phytosterol content of brazil, pecan, pine, pistachio and cashew nuts". *International Journal of Food Sciences and Nutrition* 57.3-4 (2006): 219-228.

5. Alasalvar C and BW Bolling. "Review of nut phytochemicals, fat-soluble bioactives, antioxidant components and health effects". *British Journal of Nutrition* 113.S2 (2015): S68-S78.

6. Rezig L., *et al*. "Cucurbita maxima Pumpkin Seed Oil: from the Chemical Properties to the Different Extracting Techniques". *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 46.2 (2018): 663-669.

7. Siegmund B and Murkovic M. "Changes in chemical composition of pumpkin seeds during the roasting process for production of pumpkin seed oil (Part 2: volatile compounds)". *Food Chemistry* 84.3 (2004): 367-374.

8. Choo WS., *et al*. "Physicochemical and quality characteristics of cold-pressed flaxseed oils". *Journal of Food Composition and Analysis* 20.3-4 (2007) 202-211.

9. Latif S and Anwar F. "Physicochemical studies of hemp (Cannabis sativa) seed oil using enzyme-assisted cold-pressing". *European Journal of Lipid Science and Technology* 111.10 (2009): 1042-1048.

10. Sharma A., *et al*. "Enzyme-assisted aqueous extraction of rice bran oil". *Journal of the American Oil Chemists' Society* 78.9 (2001): 949-951.

11. Lamsal BP., *et al*. "Flaking and extrusion as mechanical treatments for enzyme-assisted aqueous extraction of oil from soybeans". *Journal of the American Oil Chemists' Society* 83.11 (2006): 973-979.

12. Moreau RA., *et al*. "A comparison of commercial enzymes for the aqueous enzymatic extraction of corn oil from corn germ". *Journal of the American Oil Chemists' Society* 81.11 (2004): 1071-1075.

13. Nguyen PHN and Dang TQ. "Enzyme-assisted aqueous extraction of cashew nut (Anacardium occidentale L.) oil". *International Journal on Advanced Science, Engineering and Information Technology* 6.2 (2016): 175-179.

14. Concha J., *et al*. "Enzymatic pretreatment on rose-hip oil extraction: hydrolysis and pressing conditions". *Journal of the American Oil Chemists' Society* 81.6 (2004): 549-552.

15. AOCS. In: D. Firestone, (Ed.), official methods and recommended practices of the American oil chemists' society (5th edition). Champaign: AOCS (1997).

16. Parry J., *et al*. "Characterization of cold-pressed onion, parsley, cardamom, mullein, roasted pumpkin, and milk thistle seed oils". *Journal of the American Oil Chemists' Society* 83.10 (2006): 847-854.

17. Park YS., *et al*. "Antioxidants and proteins in ethylene-treated kiwifruits". *Food Chemistry* 107.2 (2008): 640-648.

18. Uslu N and Özcan MM. "Effect of microwave heating on phenolic compounds and fatty acid composition of cashew (Anacardium occidentale) nut and oil". *Journal of the Saudi Society of Agricultural Sciences* (2017).

19. Kornsteiner M., *et al*. "Tocopherols and total phenolics in 10 different nut types". *Food Chemistry* 98.2 (2006): 381-387.

20. Marina AM., *et al*. "Antioxidant capacity and phenolic acids of virgin coconut oil". *International Journal of Food Sciences and Nutrition* 60.2 (2009): 114-123.

21. Koski A., *et al*. "Oxidative stability and minor constituents of virgin olive oil and cold-pressed rapeseed oil". *European Food Research and Technology* 214.4 (2002): 294-298.

22. Bolling BW., *et al*. "The phytochemical composition and antioxidant actions of tree nuts". *Asia Pacific Journal of Clinical Nutrition* 19.1 (2010): 117-123.

23. Vognild E., *et al*. "Effects of dietary marine oils and olive oil on fatty acid composition, platelet membrane fluidity, platelet responses, and serum lipids in healthy humans". *Lipids* 33.4 (1998): 427-436.

24. Asghar A and Majeed MN. "Chemical characterization and fatty acid profile of different sesame verities in Pakistan". *American Journal of Scientific and Industrial Research* 4.6 (2013): 540-545.

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