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Enzyme-Assisted Aqueous Extraction of Cashew Nut (*Anacardium occidentale L.)* Oil

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***Abstract*— Enzyme-assisted aqueous extraction method was applied to extract oil from cashew nut (Anacardium occidentale L.). The commercial enzyme (Viscozyme cassava C) was tested for effectiveness in releasing oil during the aqueous extraction. The effect of several parameters such as material/water ratio, enzyme concentration and duration for enzyme incubation on the oil yield was investigated. The conditions for maximum oil release were found with the material/water ratio of 1:9, enzyme concentration of 1% (v/w E/S), and in 3 h of enzyme incubation at 50oC with constant shaking. The maximum oil yield obtained at those conditions (38.88 % raw material) was significantly (p <0.05) higher than that of the control (without enzyme) (35.92 %), and it represented 86.28 % recovery of the total oil in seed. No hexane and other organic solvents were needed for this process. The cashew nut oil by enzyme-assisted aqueous extraction was relatively stable. Both peroxide value and free fatty acid value were lower than those in the oil obtained by Soxhlet method. Total un-saturated fatty acid in the cashew nut oil was about 84.43 %, in which the most abundant was oleic acid (65.0 %), followed by linoleic acid (18.53%). Cashew nut oil is a good dietary source of un-saturated fatty acids.**

***Keywords*— Cashew nut oil; enzyme-assisted aqueous extraction; fatty acid profile; free fatty acid; peroxide value**

I. INTRODUCTION

The cashew trees (*Anacardium occidentale*) grow in the tropics and subtropics, particularly in Brazil, India, Africa, and South East Asian countries including Vietnam, and have spread to parts of tropical South and Central America [1]. In the world, the cashew industry occupies the third place in production of edible nuts in 2000. India, Brazil, Nigeria and Tanzania were four major regions of cashew processing [2]. Recently, Vietnam became a leading country in processing and export of cashew kernels. Vietnam cashew kernels are exported mainly to the United States, China, European countries, Australia and New Zealand [3].

Cashew nut contains large amount of good quality oil (47.0 %), protein (21.0 %), moisture (5.9 %), carbohydrates (22.0 %), vitamins and minerals. The fat is abundant in unsaturated fatty acids that bring many health benefits for consumers [2]. Beside, cashew nuts also provide for body many essential vitamins, for instance, pyridoxine (Vitamin B-6), vitamin E and squalene. Vitamin E and squalene are potential antioxidants which support effects on cardiovascular health; squalene is also an important steroid precursor that has a role as an anticancer agent [4]. Phenolic compounds are important sources of bioactive compounds in the human diet [5]. The major phenolics found in cashew nuts are anacardic acids, cardanols, cardols, tocopherols and other minor phenolic constituents [6].

Cashew oil could be extracted by using organic solvents, high-pressure systems and all these traditional processes. Otherwise, enzymatic assisted aqueous extraction (EAAE) of oil is an emerging technology in the fat and oil industry. To compare with these methods; however, enzymatic assisted aqueous extraction has been many advantages [6]. It eliminates solvent consumption, which may lower investment costs and energy requirements [8], [9]. Some toxins or anti-nutritional compounds from oilseeds can also be removed [10].

Literature for previous studies in cashew nut oil extraction and enzyme assisted extraction of cashew oil is scarce. The purpose of this study was to investigate the possibility for application of enzyme for improvement of oil yield during processing with the evaluation of physicochemical characteristics (peroxide value, free fatty acid) and fatty acid composition of the oil obtained.

II. MATERIALS AND METHODS

A. Materials

Un-salty cashew nut kernels without the outer shells were purchased from Binh Phuoc province, Vietnam. All the reagents and chemicals were of analytical grade and provided from local agents. Viscozyme Cassava C (a type of commercial cellulose) was provided from Novozymes, Denmark.

B. Enzyme assisted extraction method

1) Effect of water ratio

Cashew nut (8 g) was ground to become thick paste and dispersed into distilled water at different ratios (1:5; 1:7; 1:9; 1:11 w/v) to make slurry and homogenized in a 50 mL Falcon. After that, enzyme (1% v/w E/S) was added to the mixture and pH was adjusted to 5.5- 6 by 3M HCl. The sample was incubated and shaken at 50^{o}C for 2h. At the end of treatment, the enzyme was deactivated at 90^{o}C in 5 min. The oil was recovered as an upper layer after centrifugation at 13,000g for 30 min. The upper oil layer was removed with a Pasteur pipette. Then, the oil was dehydrated by evaporation at 105^{o}C for 20 min. At high temperature, emulsion became solid, Pasteur pipette was used to take oil out of the mixture. The remaining white emulsion-interface was also removed and centrifuged for 15 min at 10,000g. Mass of total oil was measured by combining additional oil and the upper oil layer.

A value of g total oil/100g cashew was taken as % recovery of oil when calculating the oil recovery under various conditions in aqueous extraction [11].

% oil recovery =

$$\left(\frac{\text{weight of oil extractedx100}}{\text{total weight of oil estimated by Soxhlet method}}\right)$$

2) Effect of enzyme concentration

The procedure was continued the same as the previous experiment in different enzyme concentrations (0.5, 1 and 1.5% v/w E/S) during a 2h extraction with the optimum water ratio.

3) Effect of incubation time

The procedure was continued the same as in the previous experiments in different duration of time (1, 2, 3 and 4h) with optimum seed to water and enzyme concentration which had been found before. All treatments were performed in triplicate.

C. Physicochemical characteristic

Peroxide value and free fatty acid values were evaluated by AOCS standard methods [12].

D. Fatty acid analysis

The extracted oil was converted into fatty acid methyl esters (FAMEs) and analyzed by gas chromatography. After methyl esterification process, methyl esters were diluted and pumped to a GC-2010 plus Shimadzu with flame ionization detector (FID) at 250^{o}C and an Agilent DB-FFAP column (30m, 0.25mm internal diameter, 0.25 µm film thickness). The carrier gas was Nitrogen at a 14 psi pressure. The oven temperature was automated from 70^{o}C, increased to 230^{o}C and then kept at 230^{o}C.

E. Statistical analysis

Analysis of variance (ANOVA) was performed using a standard statistical software SPSS. A probability value at $p < 0.05$ was considered statistically significant. Data were presented as mean values ± standard deviation derived from triplicate determination.

III. Results and Discussion

A. Moisture content

Moisture content of cashew was determined by using an infrared moisture analyzer. Cashew nuts contained 4.6±0.1 % of bound water. The moisture content in material was low (< 5%).

B. Oil content in cashew nut by Soxhlet extraction

By Soxhlet extraction, the amount of cashew nut oil was determined as about 45.06% that quite higher than previously report value of 25.85 % oil by Idah et al. [2].

C. Effects of different parameters on the total oil extraction

1) Water effect

Fig. 1 showed that the maximum oil yield (36.17%) was obtained at seeds/water ratio 1:9 and it had significant difference ($p<0.05$) when comparing to others. The oil yield increased gradually from seeds/water ratio 1:5 to 1: 9; however, it decreased at seeds/water ratio 1:11. It meant that cashew nut oil yield did not increase following water ratio. Too much water may dilute the enzyme concentration and caused a negative effect on the enzyme efficiency.

Some previous studies reported that the optimal material/water ratio of rice bran was 1:4 w/v [13], coconut was 1:4 w/v [7], peanut was 1:2 w/v [11], sunflower seed [14] and sesame was 1:6 w/v [15]. The difference in optional material/water ratios may be result from different type of seeds had different physical properties or different enzymes used during process.

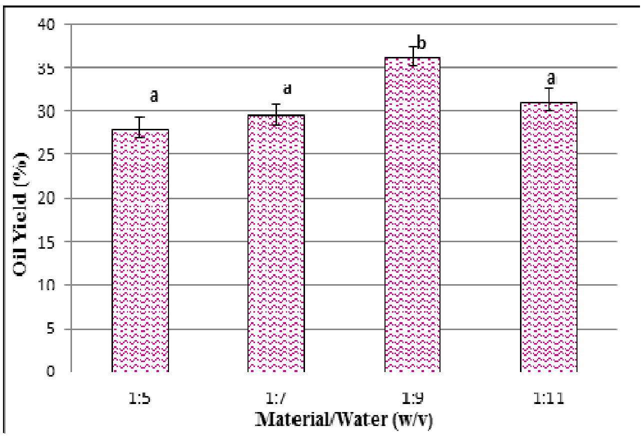

Fig.1 Effect of material/water ratio on aqueous enzymatic oil extraction from cashew nut.

2) Enzyme concentration

As shown in Fig. 2, the oil yield at the enzyme concentration of 1% (v/w E/S), was significantly higher than that at 0.5% enzyme concentration. However, when the enzyme concentration was increased to 1.5%, the oil yield was decreased.

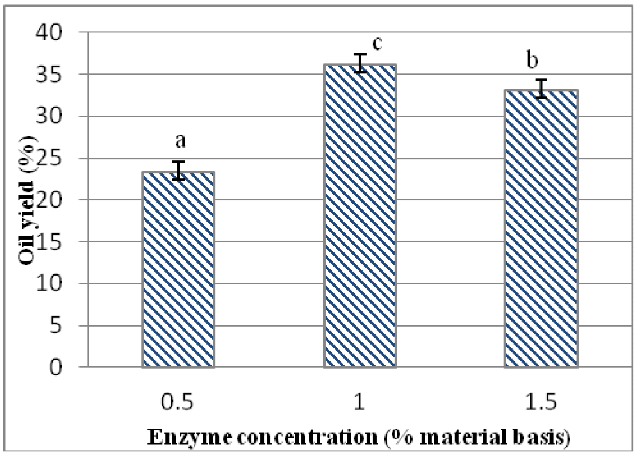

Fig.2 Effect of enzyme concentration on the oil yield

Latif and Anwar [14] showed that the oil yield of sunflower seed by an enzyme aqueous assisted extraction method was about 39.7 %, by using Viscozyme L. Karlovic et al. [16] previously reported that an aqueous enzymatic process with Celluclast - kind of Cellulase, induced an 80.0% recovery of corn oil from corn germ. In this study, by using 1% of Vicozyme Cassava C from *Trichoderma ressei* with optimum conditions, the oil recovery was about 80.26 %, based on the assumption of 100% oil yield from cashew nut extracted with hexan. Karlovic et al. [16] and Singh et al. [17] showed that the most abundant carbohydrate polymers in corn germ were arabioxylans. Thus, enzyme preparations which combined xylanase and cellulase activities may be the most effective [18].

3) *Duration for enzyme incubation*

The extraction yield of oil increased with increasing time of incubation and reach maximum (39.38%) at 3 h and then the oil yield did not change at 4 h as indicated in Fig. 3. Optimum temperature for incubation was 50^{o}C, since it was an optimal condition for Viscozyme cassava C application as stated by the supplier.

Latif and Anwar [14] reported that oil yield of sunflower seeds was nearly 40.0% during 2h of EAAE method by using Viscozyme L.

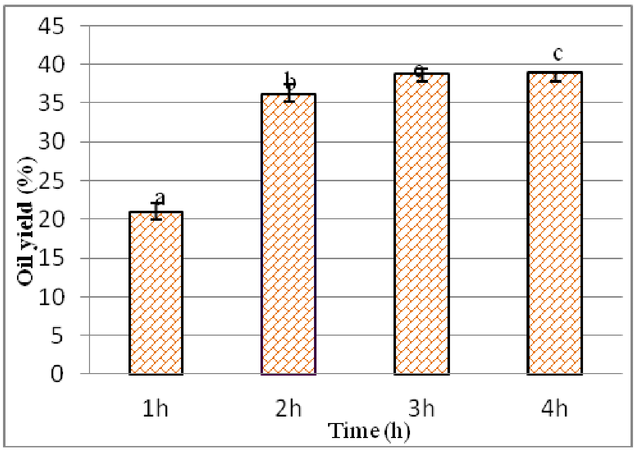

Fig. 3 Effect of incubation time on aqueous enzymatic oil extraction from cashew nut.

4) *Evaluation of total oil yield in optimal conditions*

There was a significant difference between the control sample (aqueous extraction without enzyme) and the optimum sample (oil was extracted by EAAE method).

The oil yield was increased from 35.92±1.26% when the enzymes was applied. The positive effect in aqueous enzyme-assisted extraction can be explained by the better solubilisation of proteins, which possibly causes a breakdown in the protein network characteristic of the cotyledon cells, and in the protein (oleosin) based membranes that surround the lipid bodies, in that way rescuing the oil [14].

D. Determination of peroxide value

The peroxide value (PV) is an index of rancidity; it illustrates a poor resistance of the oils to per oxidation during storage. At day 0 and temperature 60^{o}C, the PV of cashew oil was found to be 2.63 meq/kg (Fig. 5) which was quite low compared to the value of 7.95 meq/kg reported by Evbuowman et al. [19]. Khojasteh and Solhnejad [20] showed that the PV in soybean oil is increased by increasing temperature from 20^{o}C to 180^{o}C. However, in that study, the PVs of soil bean oil were not significantly different among 20-100^{o}C. The PV of cashew nut oil extracted by EAAE method was higher when comparing to the PV of sunflower oil (1.37 meq/kg) [14] and sesame oil (1.2 meq/kg) [15].

The increase in PV was proportional to storage time. Data of PV obtained in Fig. 4 showed that the PVs of day 0, and day 14 were significantly different ($p<0.05$) and the PV of these days increased continuously by the time. Otherwise, there was no significant difference of PV between day 14 and day 21 ($p>0.05$). It may begin of quenching of free radicals or reduction of lipid oxidation.

The PV of cashew nut oil obtained by EAAE method was found to be slightly lower than the Soxhlet extraction method, during the course of 21 days of storage. It can be explained by milder operation with the EAAE method [15].

The PV of soybean oil storage at 60^{o}C change about from 0.5 to 89.2 meq O_2/kg after 16 days. In addition, the peroxide formation of sunflower at 60^{o}C was 1.5 at initial to 85.7 during 15 days [21]. Comparing to these results, we can see that the PV of cashew oil was not much difference about from 2 to 8 meq O_2/kg during 21 days. The cashew oil was relatively stable when comparing to soybean and sunflower oil because cashew nut oil contain some natural antioxidants.

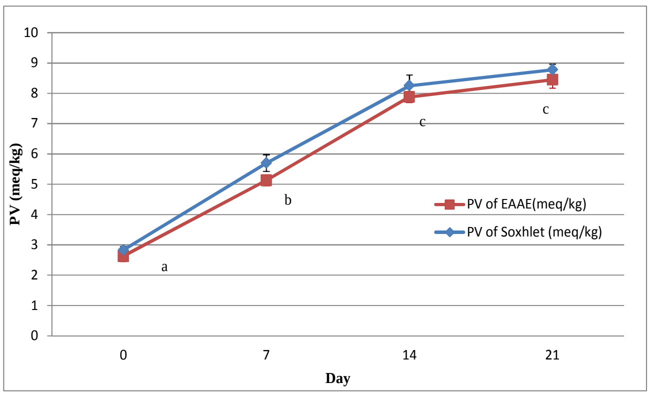

Fig.4 Peroxide value of cashew nut oil extracted by Soxhlet and EAAE method and stored at 60^{o}C

E. *Determination of free fatty acid*

Free fatty acid values (mg KOH/g) of the cashew nut oil taken by EAAE method and stored at 60°C were measured and calculated during a period of 21 days as showed in Fig. 5.

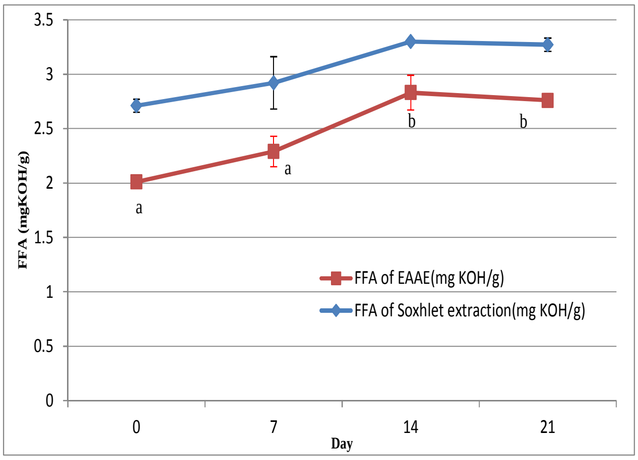

Fig. 5 Free fatty acid of cashew nut oil extracted by Soxhlet and EAAE method and stored at 60°C

F. *Determination of fatty acid profile*

Cashew nut oil includes 15.5% of saturated fatty acid (SFA), 84.43% unsaturated fatty acid (USFA) with a large proportion of mono-unsaturated fatty acid 65.52% (Table 1). Remarkably, oleic (65%) and linoleic acid (18.53%) were essential fatty acids which contained predominantly in cashew oil. Hence, cashew oil classes as oleic-linoleic oil. Beside, cashew oil also contains certain amount of palmitic (10.55 %) and small amount of stearic acid (3.77 %).

The percentages of USFA in cashew nut oil were reported by Toschi et al. [22] about 79.06 %, Venkatachalam and Sathe [23] about 78.88 %, Ryan [24] about 79.16 % and the U.S Department of Agriculture [25] about 80.26 %, which were lower than the value in the present study. Moreover, a trace amount of behenic acid (C22:0) was also reported in this study.

The fatty acid compositions of cashew, soybean and sesame oil are presented in Table 1 for comparison. The oleic acid (C18:1) in cashew was 65% that was quite high when comparing to soybean oil (17.7-28.5%) [26] and sesame oil (10.2%) [27]. Otherwise, linoleic (18.53%) in cashew oil was lower than in soybean oil (49.8-57.1%) [26] but still higher than in sesame oil (12.2%) [27].

TABLE I

FATTY ACID COMPOSITION OF CAHSEW NUT, SOYBEAN AND SESAME OIL

Fatty acid composition(%)	This study	[22]	[23]	[24]	[25]	Soy bean oil [26]	Sesame oil [27]
Capric (C10:0)	-	-	-	-	-	-	11.3
Lauric (C12:0)	-	-	-	-	-	0-0.1	2.8
Myristic (C14:0)	0.02	-	-	-	-	0-0.2	2.6
Tetradecannoic acid (C14:0)	0.02	-	0.03	0.07	0.04	-	-
Myrisoleic (C14:1)	-	-	-	-	-	-	9.9
Pentadecanoic (C15:0)	0.01	-	-	-	-	-	-
Palmitic (C16:0)	10.55	11.5	10.70	9.93	9.93	9.7-13.3	19.3
Palmitoleic (C16:1)	0.3	0.33	0.54	0.36	0.34	0-0.2	1.7
Margaric (C17:0)	0.12	0.13	0.12	0.14	0.12	-	2.2
Stearic (C18:0)	3.77	8.8	9.32	8.7	8.18	3-5.4	13.9
Oleic (C18:1)	65	61.44	61.14	57.24	59.67	17.7-28.5	10.2
Linoleic (C18:2)	18.53	17.09	16.88	20.80	19.74	49.8-57.1	12.2
Linolenic (C18:3)	0.16	0.2	0.32	0.23	0.67	5.5-9.5	
Arachidic (C20:0)	0.71	0.51	0.63	0.97	0.35	0.1	0.6
11- Eicosenoic (C20:1)	0.22	-	-	0.25	0.44	-	-
Eicosadienoic (C20:2)	-	-	-	-	-	0-0.1	-
Behenic acid (C22:0)	0.15	-	-	-	-	0.3-0.7	-
Erucic acid (C22:1)	0.22	-	-	0.28	-	0-0.3	-
Lignoceric acid (C24:0)	0.17	-	0.13	-	0.26	0-0.4	-
Total SFA	15.5	20.94	21.12	20.2	19.74	-	-
Total MUFA	65.52	61.77	61.68	58.13	60.36	-	-
Total PUFA	18.69	17.29	17.2	21.03	19.9	-	-

IV. CONCLUSIONS

At the optimal conditions, the highest oil yield was obtained about 38.88 %. The enzyme aqueous assisted extraction method was more effective method for gaining a higher oil recovery from cashew nut oil comparative to the control. This method may evidence to be an environment friendly alternative to Soxhlet extraction. Additionally, the free fatty acid value changed not much from 2.1 mg KOH/g to 2.76 mg KOH/g and had tendency to reduce during last seven days. The peroxide value increased continuously from 2.63 meq/kg to 8.45 meq/kg during 21 days. Peroxide value of cashew nut oil was more stable than soybean oil and sesame oil because of antioxidant components resulting in better oxidative stability of oil extraction. Moreover, unsaturated fatty acid on total oil which had percentages higher than saturated fatty acid. The high cost of enzyme and the low amount of oil yield was drawbacks of this method. The modified mixture of enzymes can break efficiently the cashew nut cell wall, so the oil yield could be enhanced [14].

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