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Enzyme Assisted Aqueous Extraction of Oil from Fig-Leaf Gourd Seed (*Cucurbita ficifolia*) and Physico-Chemical and Quality Characteristics of the Oil

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Abstract

The aim of this study was to investigate the effect of enzymatic treatment in enzyme-assisted aqueous extraction of oil from Fig-leaf gourd seeds (*Cucurbita ficifolia*). Two commercial enzymes (Viscozyme L and Alcalase 2.4L) were screened for their effectiveness in releasing oil from the seeds. Several process parameters including enzyme concentration, temperature and duration for enzyme incubation were investigated in term of their effect on oil yield. Application of Alcalase 2.L resulted in significantly higher oil yield (23.33%), than that with Viscozyme L (18.75%) and the control (without enzyme) (14%). The highest oil yield was obtained under the following conditions: enzyme concentration 1.5% (v/w of dried matter) and incubation at 50°C for 2h with constant shaking. While the enzymes enhanced the oil extraction, the oil yield was still significantly lower than that obtained by Soxhlet method. The oil extracted from fig-leaf gourd seeds contained flavonoids (15.834 mg/100g), carotenoids (54.083 mg/100g), and exhibited radical scavenging activity (33.42%). The oil was of good quality with the peroxide value of 1.92 meqO_2/kg and free fatty acid of 2.4%. Fatty acid composition of the oil exhibited the predominant of linoleic acid (53.24%) along with oleic acid (19.6%) and palmitic acid (16.73%). Total unsaturated fatty acid in oil was 73.52% thus the fig-leaf gourd seed oil may be considered as a potential source of nutraceuticals.

Keywords: *Antioxidant; Cucurbita ficifolia; Enzyme Assisted Aqueous Extraction; Oil Quality; Oil Yield*

Introduction

Pumpkin belongs to the *Cucurbitaceae* family and is cultivated around the world. It is a leafy green vegetable, valuable for its edible seeds, fruits and greens. The genus *Cucurbita* contain 27 species, which are commonly known as squashes, pumpkins, gourds, or marrows [1]. Pumpkin fruits are variable in size, color, shape, and weight. The mature fruit is sweet and used to make confectionary and beverages, while immature fruit is used for cooking as a vegetable. Pumpkin seeds constitute about 3.0% in weight of fresh fruit (32% in dry basis) and are considered as an excellent source of protein (25.2 - 37%) and oil (37.8 - 45.4%). The pumpkin seed contains a good source of potassium, phosphorus and magnesium, and also moderate amounts of other trace minerals including calcium, sodium, manganese, iron, zinc, and copper [2].

Edible oil from pumpkin seeds is rich in vitamins A, B and E, zinc, selenium, and phytosterols. The four fatty acids presented in significant quantities are palmitic, stearic, oleic, and linoleic acids [3]. Linoleic acid may promote blood clotting, inhibit inflammatory response and enhances the immune system, while oleic acid contributes to lowering the risk of cardiovascular disease. These fats maintain to lower dangerous LDL cholesterol levels and discourage arterial plaque build-up. Pumpkin seed oil has been used traditionally as medicine in many countries. It's applied in therapy of small disorders of the prostate gland and urinary bladder caused by hyperplasia. Pumpkin seeds oil is also rich in biological substances especially anti-oxidative activity such as tocopherol, carotenoids, especially β- carotene, lutein and other compounds which protective oxygen cell against the damage effects of reactive oxygen species and prevent several cancer diseases due to the ability to inhibit the action of oxidants [4-7].

There are five cultivated species of *Cucurbita* which are economically important such as *C. pepo* (marrow), *C. maxima* (winter squash), *C. turbaniformis and C. moschata and C. ficifolia* (fig-leaf gourd) [8]. Fig-leaf gourd (*Cucurbita ficifolia*), also known as Thai gourd, Malabar gourd, H'mong pumpkin and black-seed squash, is found at high altitudes in tropical areas [9]. Its leaf look alike fig leaf and fruits are as large as watermelon. Young fruits are used as a cooked vegetable like pumpkin while the mature flesh is prepared for candy or jam and fermented for alcoholic beverage. The most nutritional part of *Cucurbita ficifolia* is its fat- and protein-rich seeds which are eaten whole, roasted or toasted, and may be ground and used in different stews. These may also be used as vermifuge [10].

Enzyme assisted aqueous extraction (EAAE) is a promising technique for recovery of oilseed oils and protein, where water is used as an extraction solvent and enzymes as a vector to promote the oil release from solid matrix, with many benefits compared to the conventional extraction. EAAE is effective for oil extraction in case of coconut, soybean, and corn germ oil as the recovery rates range of 90-98%. The technique's benefits rely on it's simple, easy operation, economically viable, environmentally- friendly and reduce the energy consumption [11].

Application of commercial enzymes in oil extraction brings improvement of yield and quality, obtaining oils with functional properties and defatted meal that can be processed further for value-added products [12]. Moreover, the extracted oil does not need further refining for instant consumption. The application of enzyme in oil extraction includes three aspects, which include understanding of microstructure of oil-bearing material for it's pretreatment; designing various enzyme-assisted processes, and recovering oil from an emulsion system [13].

Oil droplets are always surrounded with protein as a major constituent of cells. The component of cell wall is cellulose, hemicellulose and pectin. The cotyledon cell, which contains a large amount of oils and proteins, was wrapped in the cell wall and obstruct releasing of oils and proteins. Therefore, the hydrolysis of fruit and oilseed cell walls usually involved with mainly pectinases, cellulases, and hemicellulases [11]. The criteria of enzyme selection depend on the position of the oil within the cellular structure and the chemical nature of the compound around it [14]. Time, pH, temperature, solid to liquid ratio, and particle size of the ground seeds affect the oil and protein extraction rate. Thus, optimization of these parameters necessary to achieve high efficiency of oil and protein extraction [15].

When oil is extracted into an aqueous enzymatic phase. It forms an emulsion that is difficult to separate due to the added stability of other cellular components, especially protein [16]. There are several common methods such as ultrasound treatment, heating treatment, ethanol treatment, the phase inversion method, freezing–thawing, and enzymatic treatment, which are widely used in cream demulsification. In case of enzymatic treatment, adding of proteolytic enzymes to the emulsion promote hydrolysis of the lipophilic protein surrounding lipid, and enabling removal of lipid [17].

Aim of the Study

The overall goal of this study was to evaluate effect of hydrolytic enzymes in extraction of oil from fig-leaf gourd seeds. Specifically the influence of enzyme concentration, incubation temperature, duration was investigated. The oil was evaluated for its physico-chemical characteristics and antioxidant activity.

Materials and Methods

Materials and chemicals

Fig-leaf gourd seeds (*Cucurbita ficifolia*) were supplied from a local seed supplier (Thien Lam Co. Ltd, Tuyen Quang Province, Vietnam). The undamaged seeds were shelled and dried at low temperature (60°C) for a period of time. The moisture content of the dried seeds was 4.84% by checking on an infrared moisture analyzer. The dried seeds were crushed into powder by using a blender (passed a sieve No.35). The seed powder was kept in zip-bags at cold conditions until extraction.

Two types of commercial enzymes were used in this study: Viscozyme-L and Alcalase 2.4L FG, both brought from a local agent of Novozymes. Viscozyme L, produced from *Aspergillus aculeatus*, is a multi-enzyme complex containing a wide range of carbohydrases. This enzyme has optimum temperature of 25 - 55°C and pH range of 3.3 - 5.5. Alcalase 2.4L, which is an endo-proteases of the serine type, was produced from *Bacillus licheniformis.* The Alcalase 2.4L is active at pH 6.5 - 8.5 and temperature of 45 - 65°C.

Folin-Ciocalteu reagent (Merck, Germany), Rutin, beta-carotene, DPPH (Sigma Aldrich) and all chemicals/solvents used in this study including hexane, methanol, CH_3COOK, $AlCl_3$, Acetic acid glacial, saturated Potassium Iodide (KI) solution, phenolphthalein 1%, KOH solution 0.1N, $Na_2S_2O_3$ 0,01N, Chloroform, Ethanol 95% were of analytical grade and purchased from local distributors. Some solutions were prepared fresh before testing.

Oil EAAE procedure

Certain quantity of ground seed was mixed with distilled water at a ratio of 1:6 w/v in a Falcon tube to form a thick paste. The pH was adjusted with 0.5N NaOH or 0.5 HCl to the desired and maintained at the optimal level for each enzyme. Then, a certain amount of the enzyme (Viscozyme L or Alcalase 2.4L) was added to the mixture at different concentrations (v/w d.m. of the seed weight) with gentle stirring for even distribution of enzyme.

After that, the sample was incubated at desired temperature for a specified time period in a shaking water bath. Meanwhile the shaking in the water bath was maintained at a rate of 120 rpm.

Then, the sample was heated to 90°C for 7 - 10 minutes (in hot water) to inactivate enzymatic activities. After enzyme inactivation, the sample was cooled down to 30°C, centrifuged at 7000 rpm for 15 minutes, resulting in phase separation into oil, aqueous oil emulsion and the meal at the bottom of the centrifugal tube. Using a micro pipette to remove oil first, follow by the oil emulsion and aqueous phase.

The yield of oil was defined as a recovery rate to the weight of dry seed materials and was calculated as follows.

$$\text{Percentage yield of oil} = \frac{\text{weight of oil}}{\textit{weight of sample}} \text{x } 100\%$$

Effect of enzyme concentrations on oil yield

For Viscozyme L, the extraction was carried out at the enzyme concentration levels of 0.0, 0.5, 1.0 and 1.5%. Other conditions were fixed such as incubation time (2h), temperature (50°C).

For Alcalase 2.4L, the extraction was conducted at the enzyme concentration levels of 0.0 - 2.0%. Other conditions were fixed such as incubation time (2h), temperature (50°C).

The conditions resulted in highest yield of oil were determined and selected.

Effect of extraction temperature

For Viscozyme L, the extraction was carried out at different temperatures of 30, 40, 50, and 60°C. Meanwhile for Alcalase 2.4L, the extraction was conducted at 40, 50, 60 and 70°C. Other factors were fixed such as incubation time (2h) and enzyme concentration as selected before.

The temperature resulted in highest yield of oil was determined and selected.

Effect of enzymatic treatment duration

The extraction was carried out for different enzyme incubation durations (1h, 2h, 3h) with the appropriate enzyme concentration level and temperature as selected.

Physico-chemical analysis

DPPH free radical scavenging activity

The free radical scavenging activity of the oil was determined by using 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method as described in the literature [18]. Briefly, 0.5g oil was extracted in 1.5 mL methanol (gentle vortex). After centrifugation, the supernatant was received, and the residue was re-extracted two times as above. The three portions of supernatant were combined and adjusted to 5 mL volume. A 0.1 mM solution of DPPH in methanol was prepared and then 1.5 mL of this solution are mixed with 1.5 mL of the oil extract solution at concentrations of 100, 50, 25, 10 mg/L in methanol. After 30 minutes incubation in the dark, the decrease in the solution absorbance was measured at 517 nm by a spectrophotometer. The percentage inhibition of DPPH by the extracts was calculated as follows:

$$\% \text{ Inhibition} = \frac{A-B}{A} \times 100$$

Where A = The absorbance of pure DPPH without sample; B = The absorbance of the DPPH solution after reaction with sample.

Total flavonoid content

Total flavonoid content (TFC) of the oil sample was determined by aluminum tri-chloride spectrophotometric method as reported [19]. Briefly, 0.5 mL of oil sample was mixed in 1.5 mL of ethanol 95%. Then the solution was added with 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and finally adjusted to 5 mL using distilled water before being vortexed. The tubes were kept at room temperature for 30 minutes and absorbance of the reaction mixture was read at 415 nm. Total flavonoid content was expressed as mg of Rutin Equivalent per gram oil sample (mg RE/100g). A standard curve was prepared by measuring absorbance at concentrations of 0, 25, 50, 75, 100 mg/L.

Total carotenoid content

Total carotenoid content (TCC) in oil was determined following the method by Cenkowski., *et al* [20]. Briefly, 0.1g of extracted oil was diluted with 10 mL hexane, mixed well and then measured at 460 nm with a UV-Vis spectrophotometer against a blank of pure hexane. The standard curve is prepared with 0.1g of β-carotene diluted up to 100 mL with hexane. Aliquots are taken from this solution and diluted to five different concentrations. The TCC were expressed as mg β-carotene equivalents in 100g oil.

Peroxide value in oil

Peroxide values (PVs) present the level of rancidity in oil. The peroxide values were determined by a standard method (AOCS Cd8-53) [21] by titration with the sodium thiosulfate solution until the yellow color of the reactant has approximately been disappeared. Five drops of starch solution (1%) was added, which gave a light blue color. The mixture was titrated again until the light blue color discharged.

The PVs expressed as milli-equivalent of active oxygen per kilogram of oil (meq O_2/kg), calculated by:

Peroxide values = $(V_1 - V_0) \times T \times 1000 /$ m sample (g)

V_1: Volume of thiosulfate solution required to titrate the sample (mL).

V_0: Volume of thiosulfate solution required to titrate the blank determination (mL).

T: Normality of the sodium thiosulfate solution.

m: Mass of the sample (g).

Free fatty acid values of oil

The content of free fatty acids (as oleic acid) of sample oils was determined by titration (AOCS Ca5a-40) [21]. The amount of KOH solution required to neutralize free fatty acids present in 2g of oil was determined by titration with the presence of phenolphthalein indicator, until the indicator turned pink and stable for more than 10 seconds:

$$\%FFA\ as\ oleic\ acid = \frac{(A - B) \times N \times 282}{W} x100$$

Where: A= mL of standard alkali used in the titration; B= mL of standard alkali used in titrating the blank; N= normality of standard alkali; W= grams of sample.

Fatty acid profile of oil

The method was modified from GC-ISO/CD 5509:94. Fat was extracted and converted into fatty acid methyl esters (FAMEs) and analyzed by gas chromatography. Methyl esters were diluted and the solution was injected to Shimadzu GC-2010+ with flame ionization detector (FID) at 250°C and GC separation was carried out on a capillary column (Agilent DB-FFAP, 30m x 0.25 mm internal diameter x 0.25 µm film thickness). The carrier gas was Nitrogen at a pressure of 14 psi. The oven temperature was automated from 70°C, increased to 230°C and held at 230°C.

Statistical analysis

Statistical analysis was performed by using the standard SPSS program (Version 20.0). The significant difference in sample means was evaluated by using one-way ANOVA, followed by Turkey's test ($p < 0.05$). All the treatments were performed in triplicate and the data were expressed at mean ± standard deviation.

Results and Discussion

Effect enzyme concentration on oil yield

Figure 1a showed the effect of concentration of Viscozyme L on oil yield. It can be seen that 0.5% of Viscozyme L concentration was observed as the best for oil yield, which obtained the highest oil yield (18.76%) compared to control (without enzyme) (13.83%) and higher than with 1.0 and 1.5% concentration (16.33 and 16.08%, respectively).

In a report published previously [22], the oil yield of sunflower seed by enzyme aqueous extraction method was about 39.7% (of the total oil) by using Viscozyme L (concentration not known). In this study, 0.5% concentration of Viscozyme L was the optimum condition with the oil obtained is 18.75% (of seed weight).

The higher oil extraction yield (compared to the control) revealed the impact of enzymatic action that improved oil release by degrading the seed cell wall [23]. However, the oil yield decreased at higher enzyme concentrations (1.0, 1.5%). This was possibly due to the high

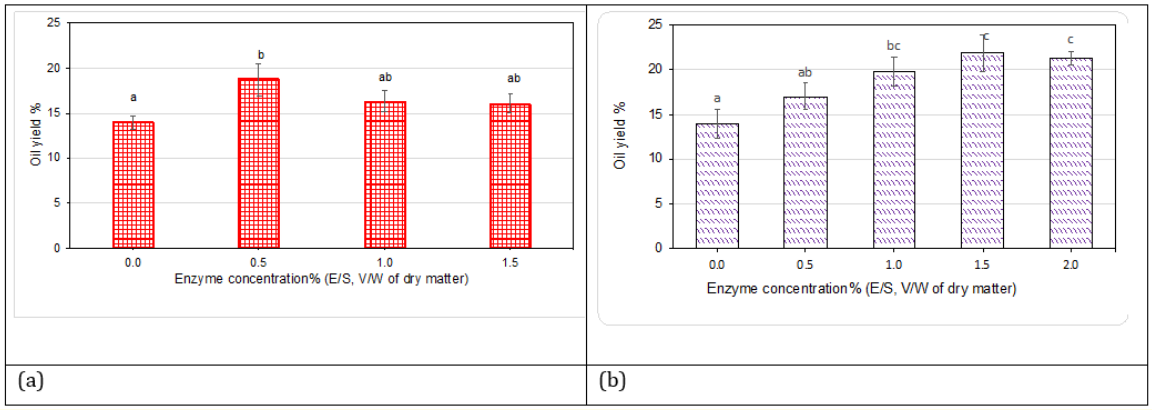

Figure 1: *Effect of enzyme concentration of Viscozyme L (a) and Alcalase 2.4L (b) on the oil yield.*

enzyme concentration may lead to extraction of undesirable components (such as soluble sugars) which in turn limit oil release [12]. The oil yield increased up to certain enzyme concentration then followed by steady or decreased rate due to the saturation of the substrates [24].

Figure 1b presents the effect of concentration of Alcalase 2.4L on the oil yield. The enzymatic treatment had significant impact on increase in the oil yield. The highest oil yield (21.83%) was obtained at enzyme concentration of 1.5%, compared to the control treatment (without enzyme) yield of 13.83%. As compared to Viscozyme L, Alcalase 2.4L required treatment at higher enzyme dose, and released oil at higher recovery rate.

A similar study [25], reported the highest oil yield from canola seed was 26.0%, by applying multifect CX 13L. As for sunflower seed, the oil yield obtained in enzyme-assisted aqueous extraction method was about 26.6% by using Alcalase 2.4L [22], which was quite similar to this study.

As mentioned, Alcalase 2.4L is a type of protease. Hydrolysis of seed protein possibly resulted in breakdown protein molecules in cotyledon cells that surround lipid body and liberation of oil. However, when the enzyme concentration was increased to 2.0% the oil yield was not changed much. Similar results have also been shown in the literature [24]. It was found that the oil yield was increase with the increasing of enzyme concentration and then became steady or decreased due to saturation of the substrates.

Effect of extraction temperature on the oil yield

Figure 2a showed the effect of temperature in extraction with Viscozyme L on the oil yield. The impact was significant at 50°C, which attained the highest pumpkin seed oil yield of 18.75%; however, the oil yield decreased when temperature was increased to 60°C. It might be due to enzyme inactivation at higher temperatures as at 60°C, which lead to lower the oil yield. In this study, the temperature of 50°C was chosen as the most effective temperature for extraction.

Figure 2b presents the effect of extraction temperature of Alcalase 2.4L on oil yield. It can be seen that the highest oil yield was obtained at 50°C and 60°C with the percentage of obtained oil was 21.83% and 21.92%. The oil yield decreased when temperature was

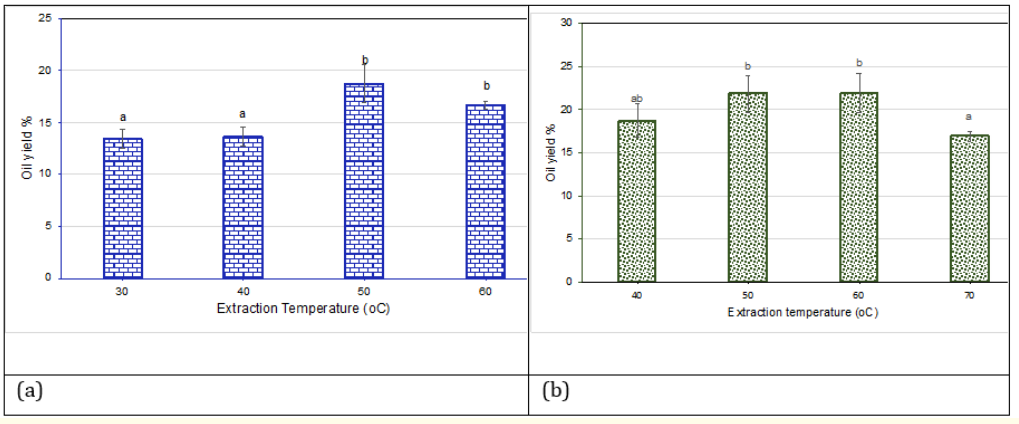

Figure 2: *Effect of temperature incubation with Viscozyme L (a) and Alcalase 2.4L (b) on the oil yield.*

increased to 70°C. Decease in enzyme activity at higher temperature (70°C) could be a reason for lower oil yield. In this study, the temperature of 50°C was chosen as the most effective temperature of extraction. Similarly, according to Zuniga., *et al*. [12], at temperature greater than 50°C, enzymatic hydrolysis began to decrease due to enzyme inactivation.

Effect of enzyme incubation duration on the oil yield

Figure 3a showed the effect of enzymatic treatment duration of Viscozyme L. It can be seen that the enzymatic treatment Viscozyme L in 2h resulted in the highest oil yield (18.75%), significantly higher to that from treatment in 3h (14.67%), but not significantly different to that in 1h (17.75%). Longer time of enzyme treatment may have negative impact on the oil yield. The oil yield decreased after a certain incubation period because the whole substrates have reacted with enzyme, leaving negligible substrates left for further enzymatic reaction to take place [13].

It is observed in figure 3b that treatment with Alcalase 2.4L for 2h resulted in highest oil yield (21.83%), which was significantly higher than that in 1h (17.83%). For 3h of treatment, the increase in oil yield was not significant. Similar trend was observed in a study of Gai., *et al*. [26], where the incubation time from 1.5 to 2.0h was also chosen as the optimum time for enzyme-assisted aqueous extraction of oil from *Forsythia suspense* seed.

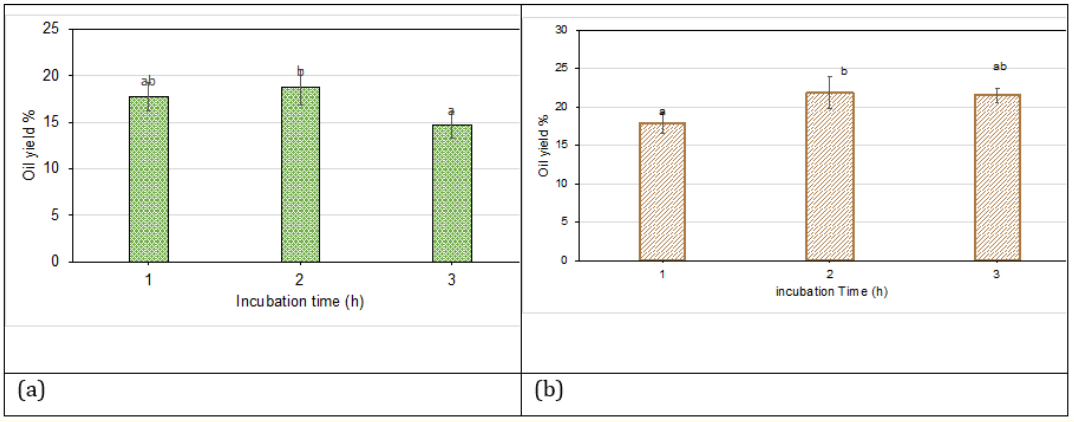

Figure 3: *Effect of enzymatic treatment duration with Viscozyme L (a) and Alcalase 2.4L (b) on the oil yield.*

In conclusion, the highest oil yield (23.33%) was obtained by using Alcalase 2.4L at concentration 1.5%, temperature 50°C for 2h. Meanwhile, the highest oil yield (18.75%) was attained by using Viscozyme L at concentration 0.5%, temperature 50°C for 2h. Both the enzyme showed the optimum temperature at 50°C and the appropriate incubation period of about 2h. Viscozyme L can be treated for 1h only. The yield obtained from Alcalase 2.4L was significantly higher than that obtained from Viscozyme L, however, treatment with Viscozyme L required lower dose of enzyme.

Furthermore, the oil yield obtained from EAAE method was significantly lower than that from Soxhlet method (45.67%). In comparison, the oil yield from Soxhlet method was comparable to that for pumpkin seed (44.61%) as reported by Jiao., *et al* [27]. Similarly, Latif and Anwar [28] reported the oil yield from EAAE for sesame oil, using Alcalase 2.4L and Viscozyme L was 24.8 and 21.4%, respectively, apparently lower than the yield obtained from Soxhlet method (50.2%). As for sunflower seed, the oil yield obtained by Sohxlet method and from EAAE method (using Alcalase 2.4L) was 45.5 and 26.6%, respectively [22]. It is necessary to note that although Soxhlet method provides higher oil yield than EAAE method, it is always accompanied with some issues such as long processing time, toxic solvent exposure to operators and environment.

Physiochemical properties of fig-leaf gourd seed oil and antioxidant activity

DPPH free radical scavenging activity of oil

The antioxidant activity, assessed by free radical scavenging test, was expressed as percentage of inhibition of free radical DPPH solution, was determined as 33.42%.

The result in this study was comparable to that of sesame seed oil (34.68%), lower than that of other seed oils such as: peanuts (42.65%), hazelnut (44.47%), flaxseed (47.78%) and pumpkin seed oil (52.21%) which is extracted by cold-press method according to Górnaś., *et al* [29]. A higher temperature or a longer time of processing in EAAE method compared to cold-press method may cause a loss of antioxidants leading to decrease in DPPH radical scavenging capacity.

Total flavonoid content

The TFC in oil obtained by EAAE method, expressed in micrograms of Rutin Equivalent per 100 gram of sample, was 15.83 mg RE/100g. For comparison, it was similar to that in canola oil (16.41 mg/100g), lower than in flaxseed oil (18.75 mg/100g) [30] and in avocado oil (19 mg RE/100g) [31]. The level of TCC was significantly lower that that in olive oil (156.0 mgRE/100g) [32]. The flavonoids are group of phenolic compounds, which display a wide range of pharmacological and biochemical properties, performing antioxidant action and playing a prevention role in cancer and heart disease [33].

Total carotenoid content

In this study, the TCC in fig-leaf pumpkin oil was 6.17 mg/100g, which is in a comparable range with other studies, as for *C. pepo* marrow seed oil (cold pressed), the values found 6.95 [34] or 5.59 [35]; Nevertheless, it was considerably lower than the TCC in Gac (*Momordica cochinchinensis* Spreng.) oil (166 mg/100g) obtained by EAAE method [36]. Carotenoids are valuable minor components in oil because of epidemiological evidence suggesting their strong antioxidant capacity, and protection against cancer and other degenerative diseases.

Peroxide values and free fatty acid values

Peroxide value is a indicator of lipid oxidative rancidity. The EAAE fig-leaf gourd seed oil in this study exhibited PV as 1.92 meqO_2/kg, which was consistent with that in EAAE oil from *C. pepo* pumpkin seed (1.8 meqO_2/kg) and lower when compared to oil from Soxhlet method (2.9 meqO_2/kg) [37]. In addition, PV of the fig-leaf gourd seed oil was higher than the PV of sesame seed oil (1.1 meqO_2/kg) and sunflower seed oil (1.25 meqO_2/kg) extracted by similar method [22,28]. Compared to the Codex Standard 19-1981 (Rev.2-1999), maximum peroxide level permitted of not more than 10 meqO_2/kg of oils, the fig-leaf pumpkin seed oil in this study was stable.

As for FFA, the level of free fatty acid in fig-leaf pumpkin seed oil was 2.40% in this study. In the literature, the FFA value reported for perilla seed oil was 2.21% [38], for EAAE oil from *C. pepo* pumpkin seed 2.46% [37], which were in a comparable range. However, it was higher than FFA of sesame seed oil (0.46%) and FFA of sunflower seed oil (0.66%), respectively [22,28]. % FFA value of 2.4% is equivalent to 4.8 acid value of oil. As approved Codex STAN 19-1981 for FFA, the acceptable level of acid value is less than 4 mg KOH/g, presenting the acid value of fig-leaf gourd oil in this study was slightly higher than the standard.

Fatty acid composition

Table 1 presents the fatty acid profile of fig-leaf gourd seed oil and some other oil for comparison. It showed that saturated fatty acids were occupied 23.86% while total percentage of unsaturated fatty acid in the oil extracted by EAAE method reached 73.51%. The oil was rich in unsaturated fatty acid of which a majority comprises from poly-unsaturated fatty acids (53.34% of the total fatty acids). In addition, among the saturated fatty acids, palmitic acid is in majority, accounted for 16.73%, followed by stearic acid (6.9%). Oleic and linoleic acid are the most abundant mono-unsaturated and poly-unsaturated fatty acid (19.6 and 53.24%, respectively). The pattern of fatty acids in *C. ficifolia* seed oil is quite similar to that of oils of the *Cucurbita* seeds.

Fatty acids	EAAE fig-leaf gourd seed oil (EAAE) (This study)	*C. pepo* pumpkin seed oil (sohxlet) [27]	*C. Pepo* pumpkin seed oil (EAAE) [37]	*C. Pepo* pumpkin seed oil (cold press) [34]	Sesame seed oil (EAAE) [28]	Sunflower seed oil (EAAE) [22]
Palmitoleic acid (C16:1)	0.08	0.14	-	0.11	-	0.13
Palmitic acid (C16:0)	16.73	13.71	14	11.95	9.56	7.16
Margaric acid (C17:0)	0.083	0.07	-	-	-	-
Stearic acid (C18:0)	6.911	5.99	5.03	5.26	5.59	4.41
Oleic acid (C18:1), Omega9	19.6	24.63	24.6	27.55	38.65	27.64
Linoleic acid (C18:2), omega6	53.24	53.72	55.8	53.21	44.53	59.45
Gama-Linoleic acid (C18:3)-Omega3	0.105	0.18	0.3	0.4	0.85	0.15
Gondoic acid (C20:1)	0.482	-	-	0.11	0.41	0.14
Behenic acid(C22:0)	0.134	-	-	0.10	0.25	-
TSFA	23.86	21.33	19.03	17.57	15.4	11.57
TMUFA	20.17	24.77	24.6	27.80	38.65	27.91
TPUFA	53.34	53.90	56.1	53.63	45.48	59.6

***Table 1**: Fatty acid composition of some seed oil samples.*

In general, the *Cucurbita species* seed oils contain high amount of linoleic acid (range 53.0 - 56.0%), which is lower than that in sunflower seed oil, but higher than in sesame seed oil. On the other hand, sesame seed oil contains higher level of oleic acid than sunflower seed oil and *Cucurbita* species seed oils [22,28,34].

The fig-leaf gourd seed oil contains high amount of C18:1 and C18:2 acids, which are valuable types of omega-class fatty acids. However, they are not stable at high temperatures, which recommends it's possible use as the salad oil without heating or cooking treatment. Linoleic acid (C18:2, omega-6) is a type of essential fatty acids which is beneficial to reduce the risk of cardiovascular diseases by raising level of high-density lipoprotein, the "good" cholesterol.

Conclusion and Recommendations

The present study showed that Alcalase 2.4L was more effective in releasing oil from fig-leaf gourd seed than Viscozyme-L in term of the oil yield, which was up to 23.33%. The enzyme assisted aqueous extraction process requires mild conditions (of temperature, pH, mixing, etc.), short duration, simple recovery of oil and environment friendly. However, the yield of oil is considerably lower than that by Soxhlet method. It thus suggests to further investigation on measures (different enzyme types, material pre-treatment, process conditions, etc.) for increasing the extraction yield.

Analysis of physico-chemical properties of the oil revealed that oil was of good quality. The oil contains a range of antioxidants, specifically flavonoids and carotenoids and it thus exhibits antioxidative capacity. The oil contains high ratio of poly-unsaturated fatty acids (especially omega-6 18:2) and mono-unsaturated fatty acid (omega-9 18:1). It could be a valuable source of edible oil and functional food supplement.

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