ECRONICON OPEN ACCESS

EC NUTRITION
Research Article

Extraction of Oil from Fig-Leaf Gourd Seed (*Cucurbita ficifolia*) Using Enzyme-Assisted Three-Phase Partitioning Method and Evaluation of Quality Parameters of the Final Oil

Anh TN Le and Tuan Q Dang*

Department of Food Technology, International University-VNU-HoChiMinh City, Vietnam

***Corresponding Author:** Tuan Q Dang, Department of Food Technology, International University (VNU-HCMC), Thu Duc City, Ho Chi Minh City, Vietnam.

Received: March 29, 2022; **Published:** April 22, 2022

Abstract

This study was aimed at the effect of enzymatic treatment on the oil yield in enzyme assisted three-phase partitioning (EATPP) extraction of oil from fig-leaf gourd seeds (*Cucurbita ficifolia*). Two types of enzymes (cellulase/protease) were assessed for their effectiveness in oil release from the seeds. Several parameters in enzymatic treatment, such as enzyme concentration, incubation temperature, and treatment duration were studied. The optimum conditions were chosen based on the highest oil yield extracted by EATPP. In details, the yield of oil extracted by common Soxhlet method (control) and by TPP without enzyme (blank) were 46.63% and 31.17%, respectively. Meanwhile, for the Celluclast®, the highest oil yield of 37.50% was achieved in the conditions of 0.5% (v/w of dried matter) enzyme concentration, at 40°C within 2h of incubation; for Alcalase®, it was 41.0%, achieved in the conditions of 1.5% (v/w of dried matter) enzyme concentration, at 50°C within 3h of incubation. Apparently, the oil yield from Soxhlet extraction was significantly higher than from EATPP. However, the oils obtained by EATPP were of higher quality than that extracted by Soxhlet method, in terms of total phenolic content, total flavonoid content, radical scavenging activity, peroxide value and free fatty acid values. Application of enzymes in TPP process for fig-leaf gourd seed helped to increase the extraction efficiency and obtain oil of good quality.

Keywords: *Cucurbita ficifolia; Enzyme-Assisted Extraction; Oil Quality; Oil Yield; Three Phase Partitioning*

Introduction

Fig-leaf gourd *(Cucurbita ficifolia)* is species of squash that belongs to the genus *Cucurbita* of the cucumber family *Cucurbitaceae*, with other names in different localities such as Thai gourd, Malabar gourd, H'mong pumpkin (in Vietnam), and black-seed squash. Compared to four species of Cucurbita (*C. pepo, C. moschata, C. maxima, and C. mixta*), *C. ficifolia* has most different characteristics, such as black seeds and white flesh. The fig-leaf gourd seeds are an excellent source of protein (35.25% of the seed meal) and fat (49.89%). The seed meal contained 5.91% ash and 4.74% crude fiber [1]. Pumpkin seed protein of high-quality is utilized as nutritional supplements or functional agents in food formulations.

The oil from fig-leaf gourd seeds is rich in poly-unsaturated acids such as oleic and linoleic acid (14.0 and 60.0%, respectively), while palmitic acid accounts for much as 14.0%, and stearic acid even less [2]. The pumpkin seed oil is plentiful in bioactive compounds such as

tocopherols, sterols, β-carotene, and lutein that have along with some fatty acids [3,4]. Pumpkin seed oil has antimicrobial, anti-oxidant, anti-inflammatory, antihypercholesterolemic activity and therapeutic effects [5]. It is suitable to be consumed for high blood pressure, breast cancer, diabetic nephropathy, sexual problems [6], and serves as vermifuge [7]. The most critical health benefit attributed to pumpkin seed oil is preventing the growth and reducing the size of the prostate, improving bladder function and reducing urethral pressure [8].

Solvent extraction is accepted for plant oil production on the industrial scale using hexane as a solvent due to its convenience, solubility, non-corrosive nature and low cost. However, hexane solvent is known as hazardous and flammable air pollutant [9]. Alternatively, green methods for obtaining oil are under research, such as aqueous extraction, optionally assisted with enzyme, microwave or ultrasounds [10,11]. A novel extraction called three phase partitioning (TPP) method has been explored recently. It was studied for extraction of proteins [12] successfully on the laboratory scale. Three phase partitioning process can be aplicable for oil extraction [13-15]. Tert-butanol (t-butanol) is used preferably in TPP to other solvents such as methanol, ethanol, n-propanol, isopropanol, 1- octanol) an organic phase thanks to higher efficiency [16]. Furthermore, it was proved that it easily escapes from oil by evaporation technique at 50°C [17].

Three phases formed in TPP mainly separate oil in upper organic phase, proteins in the middle precipitate phase and some hydrophilic components in bottom phase simultaneously [14]. The TPP process includes incubation step and extraction step. Incubation consists of mixing oil-source material with salt solution for a period of time. Extraction includes slow addition of t-butanol, following by phase separation (centrifugation). After centrifugation, oil dissolved in t-butanol forming the upper phase, proteins precipitated with salts in the middle phase, while the bottom phase contains bio-molecules soluble in water, such as carbohydrates, minerals, soluble fibers, etc [18]. In oil seed, triglycerides and free fatty acid molecules are covered with the phospholipids embedded with some structural protein. The mature embryos of oil seeds contain largely oils and protein. TPP applied to the oil seeds to make dissolution of oil in upper organic phase and precipitates protein having in embryos of oil seed easily take more amount of oil [19].

Enzyme-assisted three phase partitioning (EATPP) is an advanced technique which is a combination of enzyme-assisted extraction and TPP. The oil-contained material is pretreated with enzyme preparation followed by regular TPP process. Enzymatic treatment using Protizyme (a type of protease) helped increase efficiency of TPP for extraction of oil from soybean [14]. In plant materials, release of oils and proteins from the cotyledon cells is blocked by their cell walls; therefore, hydrolysis of cell walls helps releasing oil. It usually involves with mainly pectinases, cellulases, and hemi-cellulases. The criteria of enzyme selection depended on the position of the oil within the cellular structure and the chemical nature of the compound around it [20,21].

This study was aimed at evaluation of the potential for improvement of oil yield extracted from fig-leaf gourd seeds by applying hydrolytic enzymes in combination to three-phase partitioning method. The effect of enzyme concentration, temperature, duration for enzymatic treatment and types of enzymes in EATPP process were investigated and discussed. The final oil extracted by EATPP was compared to that by Soxhlet method, in terms of extraction yield and some quality parameters such as total flavonoid, total phenolic content, free radical scavenging activity, peroxide values, and free fatty acid values.

Materials and Methods

Materials and chemicals

A certain amount of fig-leaf gourd seeds (*Cucurbita ficifolia*) was purchased from a local supplier. The material was cleaned by removing soils and broken seeds, then put under drying at low temperature (60°C) for 48h. The final moisture content of the seeds achieved 5.0%. The seeds were shelled to remove shells, ground and sieved into powder (US Mesh No. 35), which was kept in zip-bags and stored in a desiccator.

Folin-Ciocalteu reagent (Merck, Germany), Gallic acid, Rutin hydrate, DPPH (Sigma Aldrich) were purchased from local agents. All other solvents and chemicals such as t-butanol, hexane, methanol, chloroform, ammonium sulphate, aluminum chloride, potassium iodide, potassium acetate (Himedia-India), were of analytical grade and purchased from local chemical distributors. Both commercial protease (Alcalase®) and commercial cellulase (Celluclast®) were bought from an agent of Novozyme in Vietnam. Alcalase® was produced from *Bacillus licheniformis*, having optimum temperature from 45 - 65°C; pH range of 7.0 - 9.0. Celluclast® was produced by a selected strain of the fungus *Trichoderma reesei*, having optimum temperature range 25 - 60°C and pH range 3.3 - 5.5.

Extraction procedure

Extraction of oil from the fig-leaf gourd seed powder by EATPP was done through steps as follows.

Dispersion and pH adjustment: A certain amount of the seed powder was dispersed in distilled water in a 50-mL falcon (solid-to-liquid ratio 1:6) by gentle stirring to make a slurry. The slurry's pH is adjusted to the desired (optimal) level the optimal level for each type of enzyme by using solution of 1N NaOH or 1N HCl.

Enzymatic hydrolysis: A certain amount (volume) of the enzyme (Cellulase/Protease) was added to the slurry at different concentrations, based on seed powder weight (v/w) in gentle stirring. The samples were incubated at different temperature levels for different durations of time.

TPP: Ammonium sulphate [$(NH_4)_2SO_4$] was added into the slurry (40% w/v of the aqueous phase) and vortexed gently. Followed that, a certain amount of t-butanol was added into the slurry slowly (150% of the aqueous phase). The slurry was placed in water-bath at 30°C for 60 minutes, stirred gently by a magnetic stirrer in every 5 minutes.

Centrifugation: After 60 minutes of incubation, the mixture was kept without stirring for another 60 minutes to facilitate separation of oil. Finally, the slurry was centrifuged at 4°C, 8000 rpm for 15 minutes, resulting in three phases.

Oil collection: The organic phase was collected by using a glass Pasteur pipette. Then, the residue was dried in a vacuum evaporation at 50°C to remove solvent and collect oil. The final oil extracted by cellulase-EATPP and protease-EATPP were measured and evaluated for it's physico-chemical properties, compared to the oils by Soxhlet and TPP without enzyme.

Control sample by Soxhlet extraction: An amount of 20g of the seed powder was put into a thimble and extracted in a Soxhlet apparatus using 250 mL hexane as a solvent. The extraction was done for 4h.

Experimental design

Effect of enzyme concentration on the oil yield

The procedure was carried out as described above. For both protease and cellulase, the enzyme concentrations were chosen at levels from 0 to 2.0%. Other conditions were fixed as follows: incubation time for 60 minutes, incubation temperature at 50°C.

Effect of incubation temperature

The effect of temperature of enzymatic treatment was investigated by determining the oil yield of the EATPP process at 40, 50 and 60°C. Other factors were fixed as following: incubation time for 60 minutes and the enzyme concentration as found earlier.

Effect of enzymatic treatment duration

The effect of enzymatic incubation duration on the oil yield was investigated by determining the oil yield of extraction for 1, 2, 3, and up to 4h. The appropriate enzyme concentration and treatment temperature were found in the previous experiments.

Physico-chemical analysis

Extraction yield of oil

The extraction yield of oil was expressed as a percentage of the sample weight:

$$\% \text{ yield of oil extracted} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100$$

The yield of oil extracted by Soxhlet method (standard method) was calculated in the same way.

Total phenolic content in oil

Total phenolic content (TPC) in oil sample was determined by a method described elsewhere [22]. Briefly, 1 mL of oil was extracted with methanol. 0.1 mL of the solution was taken to test tube with cap, covered with the aluminum foil. 0.6 mL of Folin-Ciocalteu reagent was added and then, 1.5 mL of Na_2CO_3 20% and 6 mL of distilled water, all in the tube. The mixture was gently vortexed for few seconds and incubated in dark for 2h. The absorbance was measured at 765 nm using UV-Vis spectrophotometer (V730, Jasco, Japan). A standard Gallic acid curve was constructed by preparing the dilutions (2.0, 4.0, 6.0, 8.0 and 10.0 mg/L) in of the standard in methanol. The quantity of TPC was expressed in a unit of milli-gram of Gallic acid equivalent per 100 grams (mg GAE/100g).

Total flavonoid content in oil

Total flavonoid content (TFC) in oil sample was determined by a spectrophotometric method with some modification. Briefly, 0.5g of extracted oil sample was mixed with 1.5 mL of methanol 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. The tubes were vortexed gently for a minute and kept at room temperature for 30 minutes. The absorbance of the reaction mixture was read at 415 nm. A standard curve was prepared by measuring absorbance of the diluted solutions of rutin (used as standard) in 80% methanol at concentrations of 0, 25, 50, 75, and 100 mg/L. Total flavonoid content was estimated from the standard curve and expressed as mg of Rutin Equivalent per gram (mg RE/g) oil sample [23,24].

DPPH radical scavenging activity of oil

Antioxidant activity of oil was determined by a test for free radical scavenging capacity, using a stable free radical agent 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described in the literature [25]. Briefly, a 0.1 mM solution of DPPH in methanol was prepared and then 1.5 mL of this solution was mixed with 1.5 mL of each sample extract (as obtained in 2.7) at concentrations of 100, 50, 25, 10 mg/L in methanol. After 30 min 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 extract was calculated as:

$$\% \text{ inhibition of DPPH activity} = \frac{A-B}{A} \times 100$$

A: The absorbance of the black (pure DPPH).

B: The absorbance of the sample.

Free fatty acid values in oil

Free fatty acid (FFA) values of the fig-leaf gourd seed oil samples were determined by titration method (AOCS Ca5a-40) [26]. In the presence of phenolphthalein, neutralization of free fatty acids present in 2g of oil is performed using KOH solution until the solution turned pink and stable for more than seconds. The FFA value is expressed as % of oleic acid as follows:

$$Free\ fatty\ acid\ = \frac{Volume\ of\ titration\ (ml) x\ Normality\ of\ KOH\ x\ 28.2}{weight\ of\ sample\ (g)}$$

Peroxide values in oil

Peroxide values (PVs) were determined by a standard method (AOCS Cd8-53) [26] by titration with the sodium thiosulfate solution. The PVs are expressed as milli-equivalent of active oxygen per kilogram of oil (meq O_2/kg), calculated by:

$$Peroxide\ Value\ \left(\frac{mEq}{kg}\right) = \frac{V_{Na_2S_2O_3}\ used\ at\ endpoint\ \times Normality\ of\ Na_2S_2O_3 \times 1000}{weight\ of\ oil\ (g)}$$

Statistical analysis

All treatments were conducted in triplicate. Analysis of variance (ANOVA) with Tukey's test was carried out for significant difference between means at a significance level $P < 0.05$, using standard software SPSS ver 20.0.

Results and Discussion

Effect of enzyme concentration on the oil yield

According to figure 1, the effect of both types of enzyme on oil yield was significant ($p < 0.01$). However, the impact of each enzyme on the seed material was different.

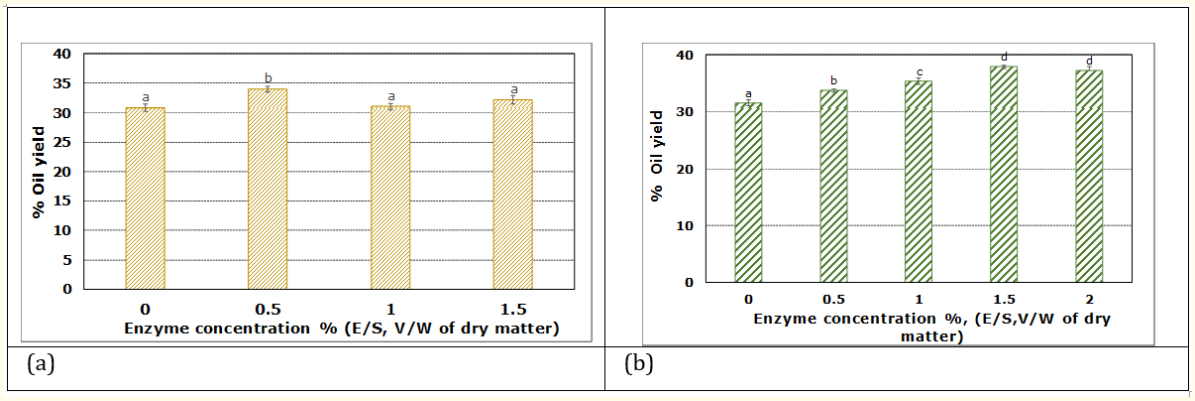

Figure 1: *Effect of cellulase (a) and protease (b) concentration on the oil yield.*

As for Celluclast®, at concentration of 0.5%, the enzyme was most effective with the oil yield of 34.0%, compared to 30.8% without enzyme. The higher oil extraction efficiency can be explained by the enzymatic action that improve oil yield by degrading the seed cell wall [27]. Applying more enzyme, at 1.0 and 1.5%, did not bring a significant increase in oil yield (31.0 and 32.2%, respectively). Similar trend was observed elsewhere, the oil yield tended to decrease at higher enzyme concentrations of 1% and 1.5%. This was possibly due to the fact that high concentration of enzyme may create excess amount of undesirable components, such as soluble polysaccharide fragments, resulted in limitation of oil release. The oil yield increased up to certain enzyme concentration then followed by steady or decreased rate due to the saturation of the substrates [11].

As for Alcalase®, the highest oil yield (37.8%) was achieved at enzyme concentration of 1.5%. It was significantly different from the control sample without enzyme (31.5%). The oil yield became steady when the enzyme concentration increased to 2.0%, again, it may be due to saturation of substrates. In a previous study, Gaur., *et al*. [14] reported that pretreatment of soybean with Protizyme brought a significant effect to soybean oil extraction by increasing from 90% (without enzyme) to 98% (with Protizyme). The hydrolysis of protein in oil-seed material breaks down protein molecules in cotyledon cells to liberate oil, because theses oil bodies are covered with the phospholipids attached to some structural proteins [16].

Effect of incubation temperature on the oil yield

As for celluclast®, there was significant difference in oil yields from the effect of enzyme treatment temperature (Figure 2). The highest oil yield (35.17%) was obtained at 40°C, which was significantly than the yield at 60°C (33.17%), but not different from the oil yield (34.33%) at 50°C. Thus, the temperature of 40°C was chosen as the most effective temperature for celluclast® acting with fig-leaf gourd seed material. The oil yield decreased when temperature increased gradually to 60°C. It might be explained that enzyme activity decreased dramatically at 60°C, leading to lower oil yield. For flaxseeds, three types of enzymes including cellulase, protease and pectinase were applied in combination at 40°C for extracting oil with the highest yield by EATPP [9].

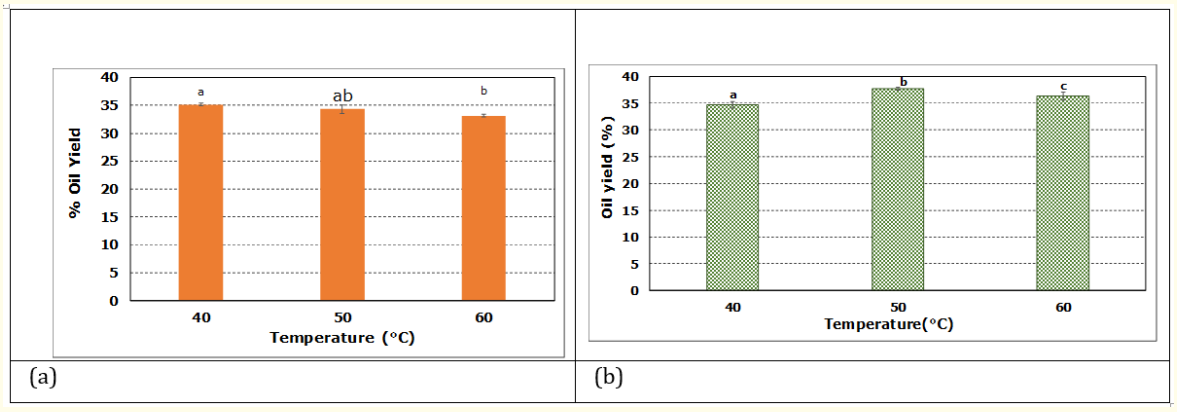

Figure 2: *Effect of incubation temperature of cellulase (a) and protease (b) on oil yield.*

For Alcalase®, the effect of incubation temperature in pretreatment of protease on the oil yield from fig-leaf gourd seeds was significant. The yield peaked up at 50°C (37.67%) before declined slightly to 36.33% at 60°C. It might be explained that due to enzyme activity decrease at elevated temperature of 60°C. So, the best choice of incubation temperature for Alcalase® in application for fig-leaf gourd seeds was 50°C (Figure 2). Similar result was reported in a study in EATPP using Protizyme (a type of protease) for oil extraction from

different materials. Incubation of Protizyme at 50°C resulted in the yield 98%, 87%, and 79% (of the intrinsic oil) in soybean, rice bran, and mango kernel, respectively.

Effect of enzyme incubation duration

The effect of enzymatic treatment duration on the oil yield was significant for both Celluclast® and Alcalase® (Figure 3). As for Celluclast®, the yield was highest (37.50%) after 2h of incubation and then decreased after 4h of incubation (33.17%). Therefore, a duration of 2h was considered suitable for treatment of fig-leaf gourd seeds with Celluclast®. Longer time for enzyme incubation may lead to decrease in oil yield due to the depletion of substrates and/or product inhibition of enzymes [28].

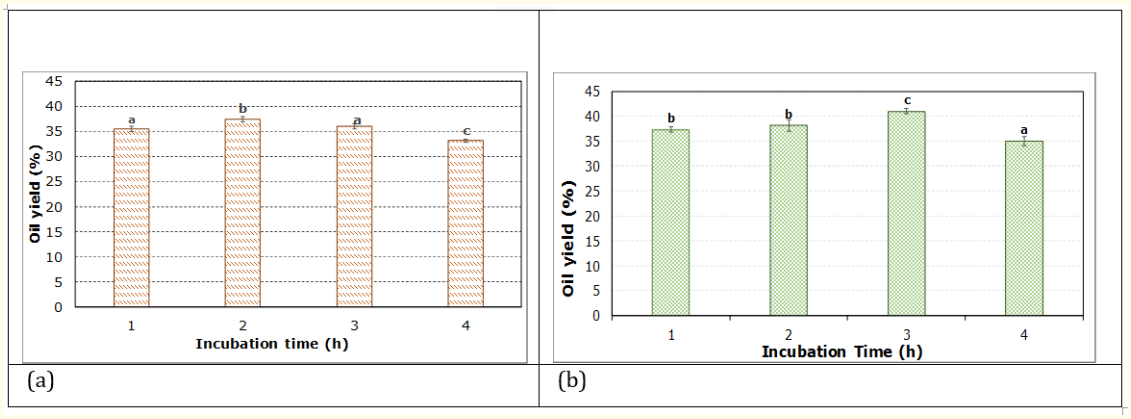

Figure 3: *Effect of enzyme incubation duration for cellulase (a) and protease (b) on oil yield.*

As for Alcalase®, the enzyme may need longer time for action. There was a gradual increase in the oil yield with treatment duration, reaching the highest value (41.0%) after 3h of enzyme treatment. Longer time of treatment for 4h resulted in lower oil yield (35.0%). The similar trend was observed elsewhere [28]. The duration of 3h was observed as sufficient for treatment of mango kernel with Protizyme (protease) in the EATPP process [14].

Oil yield and physiochemical properties of the seed oil

The oil yield extracted by EATPP and Soxhlet methods

Results in table 1 showed that the oil yield extracted by TPP with both cellulase (0.5%) and protease (1.5%) were higher that that by TPP without enzyme (blank), but lower than Soxhlet method (control). It was observed that the protease performed better than the cellulase, as the oil yield was 41.0 and 37.5%, respectively. Meanwhile the yield of oil extracted by TPP without enzyme and Soxhlet method was 31.2 and 46.8%, respectively. The oil yield by EATPP with Celluclast® and Alcalase® made up 80% and 87.5%, respectively, compared to the oil yield by Soxhlet method. About 35.3% of fig-leaf gourd seed dry mass consist of protein, but only 5.2% carbohydrates [1], so apparently protease would be of more important role than cellulase in releasing oil. Soxhlet method is considered as a standard method for oil content in seeds. In this study, the yield by Soxhlet was comparable to the values for pumpkin seeds, such as 39.7-44.5% in *C. pepo* [29], 43.69% in *C. maxima* [30]. In a previous research [2], the *C. ficifolia* seed oil content determined by a Soxhlet apparatus was 43.5% (w/w material), and the oil yield by supercritical fluid extraction reached about 92.0% of the yield by Soxhlet after 120 min

according to their kinetics study. Tan., *et al*. [9] reported the yield of flaxseed oil by EATPP as 71.56% of the intrinsic oil (by Soxhlet), while the impact of a cellulase on the yield increase was significant. In another research, Kulkarni., *et al*. [31] reported that the yield for EATPP (with accellerase® 1500) extraction of flaxseed oil was 63.15% of the total oil.

	Soxhlet	TPP (no enzyme)	EATPP (cellulase)	EATPP (protease)
Oil yield (%, w/w)	46.8^{a}	31.2^{b}	37.5^{c}	41.0^{d}
TPC (mg GAE/100g)	21.29^{a}	24.53^{ab}	26.84^{b}	25.79^{b}
TFC (mgRE/g)	12.50^{a}	16.88^{ab}	19.39^{c}	19.19^{c}
Antiox (% inhibition)	41.82^{a}	42.64^{ab}	48.77^{b}	45.55^{ab}
FFA (% oleic)	2.69^{a}	2.15^{b}	1.45^{c}	1.90^{d}
PV (meqCo2/kg)	8.51^{a}	7.29^{b}	4.73^{c}	5.64^{d}

Table 1: *Oil yield and physico-chemical properties of oil by different methods.*
(Note: the means in row with the same letter are not significantly different).

Total phenolic, total flavonoid content and antioxidant activity in oil

The TPC in oil extracted by Soxhlet was 21.29 mgGAE/100g, while that of oils by TPP method were 26.84, 25.79, and 24.53 mg GAE/100g with cellulase, protease and without enzyme, respectively (Table 1).

The TPC values in seed oils were quite different in the literature. Rezig., *et al*. [32] reported the TPC in *C. maxima* seed oil extracted by chloroform/methanol was 54.4 mg GAE/kg, while that in cold-pressed oil 23.3 mg GAE/kg. In other studies, TPC in *C. pepo* seed oil (cold-pressed) was 3.96 mgGAE/100g cold press oil [4], while Vujasinovic., *et al*. [33] reported the TPC in *C. pepo* oil cold-pressed from roasted seed was 19.6 mg GAE/kg. Teh and Birch [34] reported the TPC data for oil from Hemp seed (cold-pressing): 188.23; flax seed: 136.93; and Canola: 59.17 mgGAE/100g. The difference in TPC data may due to the fact that the oils were extracted from seeds by different methods, using different solvents and pre-treated differently.

The TFC in fig-leaf gourd seed oil by EATPP using cellulase, protease, and no enzyme were 19.39, 19.19, and 16.88 mgRE/100g, respectively. Meanwhile the TFC in oil by Soxhlet method was 12.50 mg RE/100g, which was significantly different from oils by EATPP (Table 1). The values were orderly comparable to the data from Teh and Birch [34] for Hemp seed oil (cold-pressing): 19.5; flax seed oil: 18.75; Canola: 16.41 mgLE/100g (luteolin as a standard). Dang and Nguyen [35] reported TFC data for (crude) cashew nut oil from cold-pressing and soxhlet as 17.39 and 10.72 mgQE/100g, respectively (quercetin as a standard). The TFC in fig-leaf gourd seed oil were much lower than in olive oil, which was in a range of 1.56 - 3.61 mg RE/g [36]. In general, the biologically active substances in pumpkin seed oil such as tocopherols, carotenoids are present in a larger extent [4].

Antioxidant activity of oil is attributed by a range of compounds, especially the phenolics present in oil. It is commonly evaluated by the test for DPPH scavenging capacity. In this study, the antioxidant activity, expressed as percentage of DPPH inhibition were 48.77, 45.55, and 42.64% for cellulase-EATPP, protease-EATPP and TPP-no enzyme, respectively (Table 1). The value for the oil by Soxhlet was significantly lower. It can be observed that enzymes helped to release more bioactive compounds to oil (and hence higher antioxidant capacity). It is correlated to the trends of TPC and TFC in oil as presented in table 1.

Siger., *et al*. [37] compared the % DPPH inhibition of seed oils, ranging from 11.1 to 65.3%, showing that hemp and pumpkin seed oil expressed highest DDPH scavenging capacity among the 9 types of oil. In another study, by comparing IC_{50} values, sunflower oil expressed highest antioxidant capacity among 12 types of commercial oils [38]. The phenolic content in oil may depend on the type of solvent for

extraction, as for *C. maxima* seed, the oil extracted with chloroform/methanol (rich in phenolics) was more effective in DPPH scavenging capacity than the poor phenolic oils extracted with hexane or pentane [32].

Free fatty acid and peroxide value in oil

PVs and FFA values in oils by cellulase-EATPP, protease-EATPP, no enzyme-TPP and Soxhlet methods were significantly different from each other. The values were: 4.73; 5.64; 7.29 and 8.51 meqO_2/kg; 1.45; 1.90; 2.15; 2.69%, respectively (Table 1). The PV and FFA values appeared in similar pattern, indicating that the oils extracted by TPP methods were of higher quality than the Soxhlet-extracted oil. Longer time of extraction (6h) at elevated temperature (70°C) for the Soxhlet method could be the reason leading to lower in quality of oil.

For *C. maxima* seed oil (extracted by Soxhlet with hexane) the PV and FFA were 0.85 meqO_2/kg and 0.27%; respective [30]. Nederal., *et al*. [39] reported the PV for *C. pepo* seed oils (by roasting, mechanical pressing) was lower than 6.0 meqO_2/kg and FFA value less than 1.0% oleic acid.

Regarding oils extracted by EATPP, flaxseed oil exhibited PVs of 3.35 meqO_2/kg and FFA equivalent to 0.55% oleic acid [31], while Tan., *et al*. [9] reported FFA of 1.62% and PV of 3.9 meqO_2/kg for EATPP flaxseed oil (better than Soxhlet, comparable to cold-pressing).

FFA value in non-refined oils suitable for consumption as food should not exceed 2% oleic and 15 meqO_2/kg as stipulated by Codex Alimentarius Commission [39]. The EATPP-oils from fig-leaf gourd seed in this study satisfied this requirement. Lower peroxide values indicate good quality of oil with low level of oxidation and being rancid.

Conclusion and Recommendations

Recently, three-phase partitioning process has attracted interest of many researchers as an alternative method for recovery of plant oil. In combination with enzymes, enzyme-assisted three-phase partitioning method is one of modifications from TPP method, which aims to increase oil yield and quality of oil. Both Celluclast® (a type of commercial cellulase) and Alcalase® (a type of commercial protease) were effective in improving oil yield of extraction, when applied to fig-leaf gourd seeds (*Cucurbita ficifolia*). Celluclast® was best applied at concentration of 0.5% (v/w) at 40°C for 2h, which resulted in oil yield of 37.50% (w/w material). For Alcalase®, the best-chosen conditions were: 1.5% (v/w) enzyme concentration, 50°C for 3h, which resulted in the oil yield of 41.0%. Alcalase® was more effective than Celluclast®, however it required higher temperature and longer time for application. The Peroxide and FFA values in oil by protease-EATPP was higher than that by cellulase-EATPP, apparently because of lower temperature and shorter treatment time in the latter one. Further, the oil yields extracted by EATPP were higher than that by TPP (no enzyme) and lower than that by Soxhlet method. Nevertheless, TPC, TFC and antioxidant capacity of oils obtained by EATPP were higher, while the level of rancidity evaluated by peroxide and free fatty acid value were lower than that in oils by TPP (no enzyme) and Soxhlet method. In summary, application of enzymes in TPP process for fig-leaf gourd seed helped to increase the extraction efficiency and obtain oil of good quality.

It is recommended for further research in enzyme-assisted three-phase partitioning for oil extraction from fig-leaf gourd seed in application of different types of enzymes, enzyme combination and optimization of the process by using experimental design methodology.

Bibliography

1. Rodríguez-Miranda J., *et al*. "Physical properties of *Cucurbita ficifolia* seed and functional properties of whole and defatted meal". *International Journal of Food Processing Technology* 3.1 (2016): 27-35.

2. Bernardo-Gil MG., *et al*. "Supercritical fluid extraction of fig leaf gourd seeds oil: Fatty acids composition and extraction kinetics". *The Journal of Supercritical Fluids* 49.1 (2009): 32-36.

3. Veronezi CM and Jorge N. "Bioactive compounds in lipid fractions of pumpkin (*Cucurbita* sp) seeds for use in food". *Journal of Food Science* 77.6 (2012): C653-C657.

4. Akın G., *et al*. "Cold-pressed pumpkin seed (*Cucurbita pepo* L.) oils from the central Anatolia region of Turkey: Characterization of phytosterols, squalene, tocols, phenolic acids, carotenoids and fatty acid bioactive compounds". *Grasas Y Aceites* 69.1 (2018): e232.

5. Caili FU., *et al*. "A review on pharmacological activities and utilization technologies of pumpkin". *Plant Foods for Human Nutrition* 61.2 (2006): 70-77.

6. Yadav M., *et al*. "Medicinal and biological potential of pumpkin: an updated review". *Nutrition Research Reviews* 23.2 (2010): 184-190.

7. Ntui VO., *et al*. "Response of pumpkin (*Cucurbita ficifolia* L.) to some growth regulators". *Journal of Food Agriculture and Environment* 5.2 (2007): 211-214.

8. Muntean E., *et al*. "*Cucurbita maxima* Duch. as a medicinal plant". *Hop and Medicinal Plants, Year* XXI.1-2 (2013): 41-42.

9. Tan ZJ., *et al*. "Extraction of oil from flaxseed (*Linum usitatissimum* L.) using enzyme-assisted three-phase partitioning". *Applied Biochemistry and Biotechnology* 179.8 (2016): 1325-1335.

10. Jiao J., *et al*. "Microwave-assisted aqueous enzymatic extraction of oil from pumpkin seeds and evaluation of its physicochemical properties, fatty acid compositions and antioxidant activities". *Food Chemistry* 147 (2014): 17-24.

11. Jiang L., *et al*. "Aqueous enzymatic extraction of peanut oil and protein hydrolysates". *Food and Bioproducts Processing* 88.2-3 (2010): 233-238.

12. Kurmudle NN., *et al*. "Enzyme-assisted three phase partitioning: a novel approach for extraction of turmeric oleoresin". *Process Biochemistry* 46.1 (2011): 423-426.

13. Sharma A and Gupta MN. "Oil extraction from almond, apricot and rice bran by three-phase partitioning after ultrasonication". *European Journal of Lipid Science and Technology* 106.3 (2004): 183-186.

14. Gaur R., *et al*. "A novel process for extraction of edible oils: Enzyme assisted three phase partitioning (EATPP)". *Bioresource Technology* 98.3 (2007): 696-699.

15. Vidhate GS and Singhal RS. "Extraction of cocoa butter alternative from kokum (*Garcinia indica*) kernel by three phase partitioning". *Journal of Food Engineering* 117.4 (2013): 464-466.

16. Panadare DC and Rathod VK. "Three phase partitioning for extraction of oil: A review". *Trends in Food Science and Technology* 68 (2017): 145-151.

17. Shah S., *et al*. "Extraction of oil from *Jatropha curcas* L. seed kernels by enzyme assisted three phase partitioning". *Industrial Crops and Products* 20.3 (2004): 275-279.

18. Li Z., *et al*. "Lipid extraction from non-broken and high water content microalgae *Chlorella* spp. by three-phase partitioning". *Algal Research* 10 (2015): 218-223.

19. Boulard C., *et al*. "The structural organization of seed oil bodies could explain the contrasted oil extractability observed in two rapeseed genotypes". *Planta* 242.1 (2015): 53-68.

20. Liu JJ., *et al*. "Enzyme-assisted extraction processing from oilseeds: Principle, processing and application". *Innovative Food Science and Emerging Technologies* 35 (2016): 184-193.

21. Nadar SS., *et al*. "Enzyme assisted extraction of biomolecules as an approach to novel extraction technology: A review". *Food Research International* 108 (2018): 309-330.

22. 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.

23. Chang CC., *et al*. "Estimation of total flavonoid content in propolis by two complementary colorimetric methods". *Journal of Food and Drug Analysis* 10.3 (2002): 178-182.

24. Isla MI., *et al*. "Physico chemical and bioactive properties of honeys from Northwestern Argentina". *LWT-Food Science and Technology* 44.9 (2011): 1922-1930.

25. Shirazi OU., *et al*. "Determination of total phenolic, flavonoid content and free radical scavenging activities of common herbs and spices". *Journal of Pharmacognosy and Phytochemistry* 3.3 (2014): 104-108.

26. American Oil Chemists' Society. Official Methods and Recommended Practices of the American Oil Chemists' Society, 5th Edition, AOCS Press, Champaign (Method Cd 8-53; Ca 5a-40) (1997).

27. 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.

28. Zhang SB., *et al*. "Optimization of the aqueous enzymatic extraction of rapeseed oil and protein hydrolysates". *Journal of the American Oil Chemists' Society* 84.1 (2007): 97-105.

29. Nawirska-Olszańska A., *et al*. "Characteristics of antioxidant activity and composition of pumpkin seed oils in 12 cultivars". *Food Chemistry* 139.1-4 (2013): 155-161.

30. Alfawaz MA. "Chemical composition and oil characteristics of pumpkin (*Cucurbita maxima*) seed kernels". *Food Science and Agriculture* 2.1 (2004): 5-18.

31. Kulkarni NG., *et al*. "Extraction of flaxseed oil: a comparative study of three-phase partitioning and supercritical carbon dioxide using response surface methodology". *Food and Bioprocess Technology* 10.5 (2017): 940-948.

32. 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.

33. Vujasinovic V., *et al*. "The effect of roasting on the chemical composition and oxidative stability of pumpkin oil". *European Journal of Lipid Science and Technology* 114.5 (2012): 568-574.

34. Teh SS and Birch J. "Physicochemical and quality characteristics of cold-pressed hemp, flax and canola seed oils". *Journal of Food Composition and Analysis* 30.1 (2013): 26-31.

35. Dang TQ and Nguyen TK. "Impact of extraction method on the oil yield, physicochemical properties, fatty acid composition and stability of cashew nut (*Anacardium occidentale* L.) oil". *EC Nutrition* 14 (2019): 165-171.

36. Ebrahimi N., *et al*. "Spectrophotometric method for quantification of flavonoids in olive oil supplied from Tehran market of Iran". *Journal of Food Safety and Hygiene* 1.2 (2015): 59-62.

37. Siger A., *et al*. "The content and antioxidant activity of phenolic compounds in cold-pressed plant oils". *Journal of Food Lipids* 15.2 (2008): 137-149.

38. Xuan TD., *et al*. "An overview of chemical profiles, antioxidant and antimicrobial activities of commercial vegetable edible oils marketed in Japan". *Foods* 7.2 (2018): 21.

39. Neđeral S., *et al*. "Chemical composition and oxidative stability of roasted and cold pressed pumpkin seed oils". *Journal of the American Oil Chemists' Society* 89.9 (2012): 1763-1770.

Volume 17 Issue 5 May 2022