STUDY ON THE EFFECT OF ENZYME TREATMENT CONDITIONS ON EXTRACTION EFFICIENCY AND MACADAMIA *(Macadamia integrifolia)* OIL QUALITY

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ABSTRACT

Macadamia nut oil is a source of healthy edible oils with a high content of unsaturated fatty acids and rich in bioactive compounds. Recent research has focused on developing new methods for extracting oil from macadamia nuts to enhance quality and improve oil extraction efficiency. In this study, we evaluated the enzyme treatment conditions to obtain higher oil yield and better oil quality from macadamia nuts. Specifically, we analyzed oils yield (%), peroxide value, acid and iodine value, saponification value, fatty acid composition, and sensory evaluation. The results showed that,among three enzymes tested (Viscozyme® L, Pectinex® Ultra SPL, Celluclast® 1.5L), Viscozyme® L was selected due to its superior oil extraction efficiency. Viscozyme® L enzyme demonstrated higheffectiveness when the particle fragment size was< 2 mm, combined with a treatment concentration of 0.5%, a treatment duration of 2 hours, and the addition of water at a rate of 30% (v/w – volume compared to weight of macadamia nut). Under these optimized conditions, the oil yield reached approximately 65 to 66%, a significant improvement compared to other treatment conditions yielding only about 15 to 20%. Furthermore, the acid and peroxide values were lower, and the oil exhibited favorable sensory characteristics. Notably, the fatty acid composition of the oil remainedunaffected by the enzymatic treatment. Consequently, this enzyme treatment method can be employed before pressing to increase the oil extraction yield and enhance the oil's quality, making it suitable for development and application in the functional food, cosmetic, and pharmaceutical industries.

Keywords: *Fatty acids, green technology, macadamia oil, functional foods, enzyme processing.*

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1. INTRODUCTION

In recent years, the cultivation of macadamia nuts has become popular in the Northwest and Central Highlands regions of Vietnam. Vietnamese macadamia nuts are now being exported to many countries worldwide, primarily as dried macadamia nuts and seeds. However, valued-added products like macadamia nutritional drinks, oil, cake, candies, and chocolate are imported mainly from abroad. Some companies have experimented with macadamia nut oil production in the Central Highlands, particularly in Dak Nong. Nevertheless, most production facilities currently utilize screw presses and employ high-temperature pressing methods. Unfortunately, this approach can lead to the decomposition of the nutritional quality and sensory value and potentially yielding substances harmful to consumers' health. Macadamia oil is a crucial product derived from macadamia nuts, known for itshigh content of monounsaturated fatty acids (approximately 80%) and the presence of bioactive compounds such as tocopherols, squalene, and phytosterol [1]. It is essential to note that the efficiency of oil recovery, the nutritional value, and the physicochemical properties of the oil are greatly influenced by the chosen oil extraction method [2].

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Currently, several methods for extracting oil from macadamia nuts are employed, including screw pressing, the use of solvents like hexane, ethanol, oil ether, and butanol, low-temperature hydraulic pressing, supercritical carbon dioxide, ultrasound, microwave, and enzymatic hydrolysis methods. Each of these methods has its own set of advantages and drawbacks. The solvent-based extraction method yields a high quantity of oil but often leads to environmental pollution and can adversely affect the quality of the oil. Consequently, various alternative extraction techniques have been researched to provide safe and environmentally friendly alternatives. One such method is supercritical CO_2, a modern approach known for its high oil extraction efficiency and eco-friendliness. However, this method can be costly, and the high pressure involved may reduce the presence of bioactive ingredients in the oil [3]. The traditional pressing method is characterized by its low cost and ease of implementation, resulting in oil with a bright color, characteristic aroma, and stable quality [4, 5]. However, this method can harm the bioactive components of macadamia oil when conducted at high temperatures. Therefore, the cold-pressing method is often considered one of the most advantageous and recommended. Nevertheless, oil extraction through the low-temperature pressing method yields low oil recovery efficiency, typically around 30 - 40%, approximately 25 - 50% lower than the solvent-based method [6].

Exploring pretreatment methods before pressing is essential to enhance oil recovery efficiency in cold pressing. Some critical forms of treatment include crushing, reducing kernel size, and using heat or non-heat treatment to break or thin the cell wall, thereby facilitating the pressing process.

Employing the microwave method for treating raw materials before oil extraction is an effective and economically viable solution. Microwave treatment generates a high-frequency current that breaks the cell wallsthrough an impulse reaction. The rapid heating and vibration lead to cell rupture, effectively supporting the oil extraction. Before cold pressing (hydraulic), microwave pretreatment can boost oil extraction efficiency by up to 50% [5]. However, it's worth noting thatthis method may not be suitable for raw materials rich in biological compounds.

As the trend of consuming functional foods that support human health has increased in recent decades, it has become crucial to ensure that the extraction of macadamia nuts maintainsthe biologically active compounds and nutrients they contain. Macadamia nuts have ahigh content of unsaturated fatty acids, which benefit one's health. Using enzymes to support the extraction process is a suitable and environmentally friendly solution for oil extraction. Enzymatic pretreatments to enhance oil recovery and maintain quality in the extraction process have been the subject of prior investigations. De Aquino*et al.* (2019) reported that the enzyme method for extracting sunflower seed oil yielded similar results to the solvent-based extraction method [7]. They tested the Alcalase endo-protease enzyme to optimize oil extraction conditions from pine kernels [8]. In addition, Balvardi*et al.* (2015) suggested an environmentally friendly method using two enzymes, Alcalase 2.4L and Celluclast 1.5, to extract oil from wild almonds [9]. The primary purpose of using enzymes is to hydrolyze and aid in the breakdown of plant cell walls, thereby increasing the efficiency of oil extraction while preserving the quality and bioactive ingredients in macadamia nuts.

Nevertheless, there currently needs to be more references in the country regarding the applications of enzyme processing for extracting oil from macadamia nuts. In this study, we evaluate the enzyme treatment for oil extraction efficiency and the quality characteristics of macadamia nut oil andpropose an effective application process for macadamia nut oil extraction. We have comprehensively analyzed and compared the resulting macadamia oil's physicochemical properties, fatty acid composition, peroxide value, acid value, and organoleptic properties.

2. MATERIALS AND METHODS

2.1. Materials

Macadamia materials: Macadamia fruits (*Macadamia integrifolia*) were harvested from various farms in Dak Nong province in 2022 and 2023.

Preparation of macadamia nut samples: After harvesting, the fruits are gathered, and their outermost green skin is removed. Once peeled, the macadamia nutsundergo a drying process: initial drying for two days at room temperature (26 -28^{o}C).

Subsequent drying at 35°C for three days. Finally, they are placed at 50°C in a drying oven (SANYO DRYING OVEN, MOV-112, Capacity: 97 L, Sanyo Electric Co., Ltd., Japan) and dried until the desired dryness level is achieved. After this final stage, the husks are removed, leaving only the kernels, which are then used for research experiments.

In the current experiment, three enzymes were used, as follows:

Enzyme type	Commercial name	Source	Technical properties	Composition
Enzyme 1	Viscozyme® L	Cell Wall Degrading Enzyme Complex from *Aspergillus* sp. Lysing Enzyme from *Aspergillus* sp.	Storage temperature: 2-8°C; Appearance: transparent, liquid; Color: light brown; Products of Novozyme Corp; Activity: ≥100 FBGU/g; Density: ~1.2 g/mL at 25°C (lit.).	It contains a variety of carbohydrases, including arabanase, cellulase, glucanase, hemicellulase, and xylanase.
Enzyme 2	Pectinex® Ultra SPL	Pectinase from *Aspergillus aculeatus.*	Storage temperature: 2-8°C; Appearance: transparent, liquid; Color: light brown; Products of Novozyme Corp	Pectinase
Enzyme 3	Celluclast® 1.5L	Cellulase from *Trichoderma reesei* Generic name: 1,4-(1,3:1,4)- β-D-Glucan 4-glucano- hydrolase Celluclast® 1.5L.	Storage temperature: 2-8°C; Appearance: transparent, liquid; Color: light brown; Products of Novozyme Corp.	Cellulase

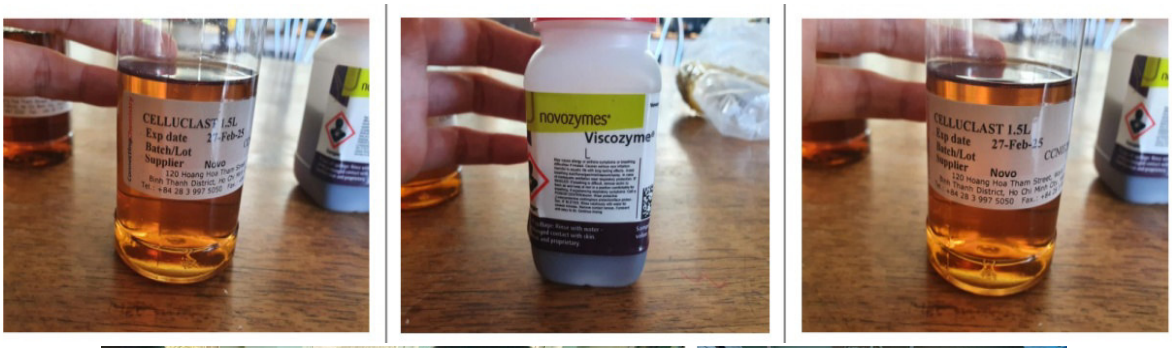

Figure 1. Preparation of materials

The macadamia oil pressing machine used in this study is ahydraulic oil press, which functions as a cold press oil machine. It has a batch capacity of 5 kg, equivalent to 20 kg/hour. The device offers a temperature range of 25 to 100ºC. Vina Nha Trang Mechanical Joint Stock Company provided this equipment, and additional equipment details can be found in figure 2.

The centrifuge device used is aHermle Z323 model with a 4 x 100 ml capacity. It is equipped with high-speed micro rotorscapable of reaching speeds up to 17.000 rpm or 26.810 xg. The device offers a temperature range from -20 to 40ºC. This centrifuge is manufactured in Germany; additionalequipment details are shown in figure 2.

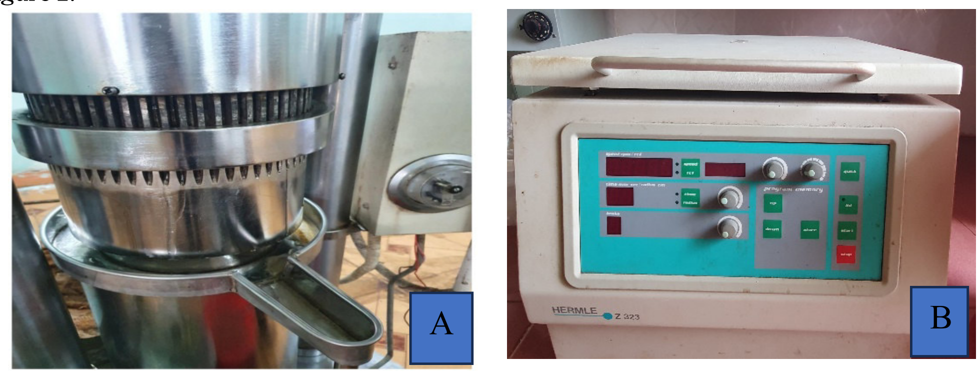

Figure 2. Hydraulic oil press (picture A) and centrifuge device (Picture B)

2.2. Research methods

2.2.1. Experimental design

To conduct experiments on the effect of enzyme treatment on extraction efficiency and the quality of macadamia oil and to determine optimal enzyme-assisted conditions on the oil extraction process, the experiments are set up according to figure 3 as follows:

Experiment 1: Effect of particle size and enzyme type on extraction efficiency and quality of macadamia oil

3 particle sizes: A1- 4 - 6 mm; A2- 2-4 mm; A3- <2 mm.

3 enzymes: Viscozyme® L, Pectinex® Ultra SPL, Celluclast® 1.5L.

In this experiment, the macadamia nutsare treated with enzymes at a fixed concentration of 0.5%. The pretreatment time is set at 1 hour, and the pressing is conducted at approximately 25ºC. Each treatment involves 1 kg of milled macadamia nut.

Experiment 2: Effect of enzyme concentration

In the experiment, four different enzyme concentration levels of 0.3%, 0.4%, 0.5%, and 0.6% are compared with a control without enzymes. All treatments use macadamia nut powder with less than 2 mm particle size. Each treatment involves processing 1 kg of milled macadamia nuts. This setup aims to analyze the impact of varying enzyme concentrations on the macadamia oil extraction process when working with fine nut powder.

Experiment 3: Evaluating the impact of enzyme treatment time and water ratio

In this experiment, macadamia nut material, milled to a size of <2 mm, is subjected to soaking and treatment with 0.5% Viscozyme® L enzyme. This treatment varies in reaction time, set at three intervals of 1 hour, 2 hours, or 3 hours. Additionally, water is added at three different levels of 20%, 30%, and 40%, depending on the treatment. Each treatment involves processing 1 kg of milled macadamia nut. The aim is to assess the effects of enzyme treatment time and water ratio on the macadamia nut material.

Experiment 4: Investigating the impact of pressing temperature

In this experiment, the pressing temperature is varied within the ranges of 25 - 40°C (precisely 25, 30, 35, and 40°C). Macadamia nut material, milled to a size smaller than 2 mm, is soaked and treated with 0.5% Viscozyme® L enzyme. Additionally, water is added at a consistent level of 30%. The reaction can proceedfor 2 hours, and each treatment involves 1 kg of milled macadamia nuts. The objective is to assess the influence of different pressing temperatures on the macadamia nut material.

2.2.2. Analyzing methods

The formula for calculatingoil recovery efficiency (Oil Yield%) is as follows:

Oil yield (OY, %) = (Material weight before pressing - Material weight after pressing) *100/Percent oil in the material.

Peroxide value: TCVN 6121: 2018 (ISO 3960: 2017) [10].

Acid value: TCVN 6127: 2010 (ISO 660: 2009) [11].

Saponification value: TCVN 6126: 2015 (ISO 3657: 2013) [12].

Iodine value: TCVN 6122: 2015 (ISO 3961: 2013) [13].

Oil content: AOAC 2003.05 and AOAC 2003.06 [14].

Fatty acid composition in macadamia oil: TCVN 9675: 1 (ISO12966-1) and TCVN 9675: 2 (ISO12966-2) [15].

Sensory evaluation: Employing the descriptive method, establish an assessment committee comprising five individuals with expertise in sensory evaluation. Develop sensory criteria using an illustrative table, conduct pre-assessment training for evaluation levels, outline assessment methods, and implement a scoring system. Calculate the average score from committee members using a scale of 1 to 4 (with 1 indicating the lowest and 4 signifying the highest preference or quality).

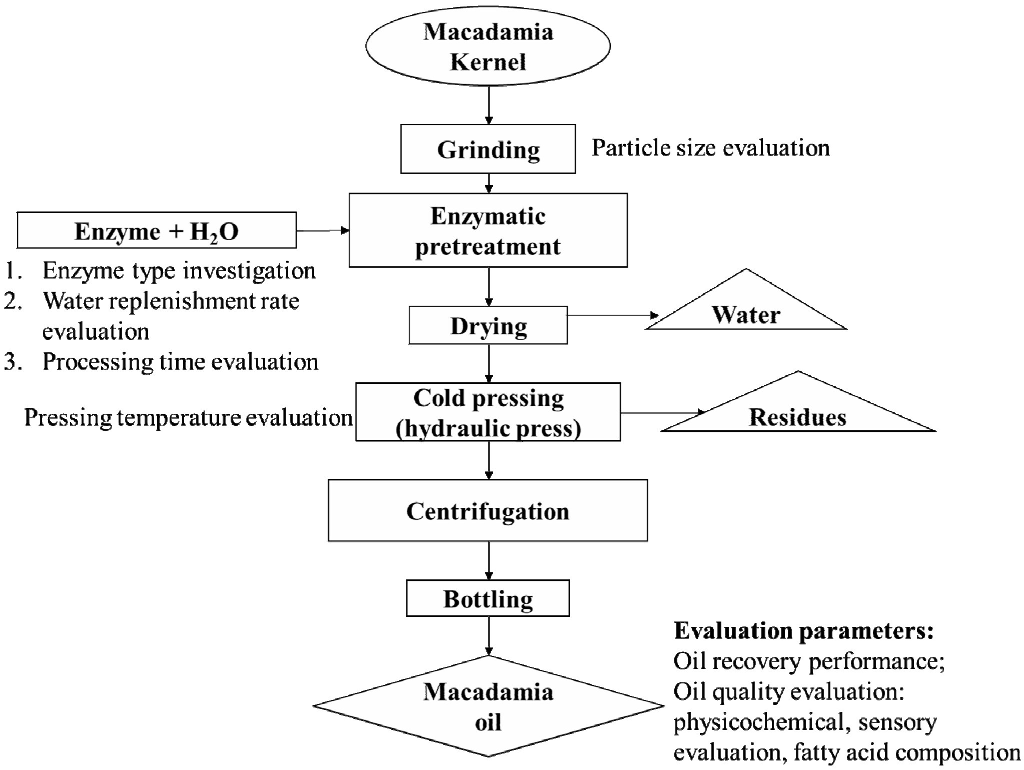

Figure 3. Diagram of the proposed macadamia oil extraction process

Statistical analysis

The experimental results were conducted with three replicates and subjected to statistical processing using IBM SPSS statistics 26.0 software for Windows (SPSS Inc., Chicago, USA). Significant differences between treatments were assessed through one-way analysis of variance (One-way ANOVA), with the Tukey test considering significance at $p < 0.05$. The data are expressed as the mean (Mean) of three replicates ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Effect of particle size and enzyme type on macadamia oil extraction efficiency and quality

Table 1 displays the results of evaluating the extraction efficiency of macadamia oil using different commercial enzymes and 03 distinct particle sizes through the hydraulic pressing method.

Table 1. Oil extraction efficiency by formulas (OY, %)

Factor A (particle size)	Factor B (type of enzyme)			
	B1 (Viscozyme® L)	B2 (Pectinex ultra spl)	B3 (Cellulast)	Control (CTL)
A1 (4 - 6 mm)	48.72 ^{Ca} ± 0.51	42.01 ^{Cb} ± 0.76	43.88 ^{Cb} ± 1.01	34.66 ^{Cc} ± 0.28
A2 (2 - 4 mm)	52.72 ^{Ba} ± 0.27	46.53 ^{Bc} ± 0.35	47.48 ^{Bb} ± 0.21	37.07 ^{Bd} ± 0.25
A3 (< 2 mm)	59.11 ^{Aa} ± 0.79	51.58 ^{Ab} ± 0.54	51.67 ^{Ab} ± 0.58	39.74 ^{Ac} ± 0.25
Medium	53.52 ^{a} ± 4.56	46.71 ^{b} ± 4.18	47.68 ^{b} ± 3.43	37.16 ^{c} ±2.21

A, B, C, D: Represent values with statistically significant differences ($p < 0.05$) in the same column; a, b, c, d: Represent values that have a statistically significant difference ($p < 0.05$) in the same row. *(Note: The difference in the Levene test is greater than 0.05, so the variance of the group of values is the same. The difference of F-test in both enzyme type, particle size, and enzyme*size is less than 0.05, thus showing a difference in oil extraction efficiency in different enzymes and particle sizes as well as between different enzymes and particle sizes).*

Table 1 illustrates the significant impact of enzyme type and particle size on the extraction efficiency of macadamia oil. The results showed that amongst tested enzymes, Viscozyme® L outperformed the others, yielding an average extraction efficiency of 53.52%, which was significantly higher (*$p<0.05$*) compared to Pectinex ultra spl (46.71%) and Cellulast (47.68%). In contrast, the control sample without enzymes yielded only 37.16% oil extraction efficiency.

Furthermore, the results in table 1 highlight a substantial difference in oil extraction efficiency based on particle size, with a cleartrend of increased efficiency as the particle size decreases. Specifically, particle sizes smaller than 2 mm consistently performed better than larger flakes in all formulations. When the particle size was < 2 mm, the highest oil extraction efficiency was achieved, with 59.11% when treated with Viscozyme® L, 51.58% with Pectinex ultra spl, 51.67% with Cellulast, and 39.74% with CTL (without enzymes). It is evident that all three types effectively contribute to the breakdown of the cell wall of oil-containing granules. Viscozyme® L enzyme exhibited the highest initial efficiency, while there was no significant difference between Cellulast and Pectinex Ultra spl enzymes.

Initial evaluation showed that pretreating the seeds with Viscozyme® L enzyme and using macadamia particles smaller than 2 mm resulted in optimal support for hydraulic macadamia oil pressing, leading to a substantial improvement in oil extraction efficiency, approximately 20% higher compared to non-enzymatic treatments.

The higher oil extraction efficiency of Viscozyme® L enzyme can be explained as it contains a variety of carbohydrases such as arabanase, cellulase, glucanase, hemicellulase, and xylanase. These enzymes work synergistically to facilitate the release of oil constituents from the cell by breaking down hemicellulose, cellulose, and pectin molecules, ultimately breaking down cell wall components.

The use of enzymes to enhance oil extraction and the preservation of active ingredients has been extensively researched, proposed, and applied. However, it's essential to recognize that each type of oil-containing seed possesses a unique composition and structure within the oil-containing cell wall. Therefore, to optimize the extraction process for each specific type of oil-containing seed, the selection of enzymes and suitable conditions must be tailored accordingly. Typically, the components surrounding the oil include pectin, cellulose, hemicellulose, protein, and more. This is why mixed enzymes like Viscozyme® L proved more effective in supporting oil separation from seeds. The results of our experiment align with previous studies that have compared the impact of enzyme treatment on oil recovery from sunflower seeds. Specifically, Latif and Anwar (2009) conducted a similar comparison, evaluating the efficiency of 05 different enzymes (Protex 7L, Alcalase 2.4L, Viscozyme® L, Natuzyme, and Kemzyme). Viscozyme® L exhibited the highest oil extraction efficiency at 39.7 ± 0.4%, significantly greater than the non-enzymatic treatment (18.3 ± 0.3%) [16].

Table 2. Physicochemical properties of macadamia oil

Physicochemical properties	Factor A (size)	Factor B (type of enzyme)			Mean	CTL
		B1 Viscozyme® L	B2 Pectinex Ultra spl	B3 Cellulat		
Acid number (mgKOH/g)	A1 (4 - 6 mm)	0.50 ± 0.02	0.53 ± 0.01	0.53 ± 0.01	0.52 ^{C} ± 0.02	0.55 ^{C} ± 0.03
	A2 (2 - 4 mm)	0.49 ± 0.01	0.51 ± 0.02	0.50 ± 0.02	0.50 ^{B} ± 0.01	
	A3 (< 2 mm)	0.47 ± 0.01	0.49 ± 0.01	0.48± 0.01	0.48 ^{A} ± 0.01	
	Mean	0.49 ^{A} ± 0.02	0.51 ^{B} ± 0.02	0.50 ^{B} ± 0.02		
Peroxide value (meq/kg)	A1 (4 - 6 mm)	1.32 ±0.05	1.48 ±0.04	1.46 ±0.04	1.42 ^{B} ±0.08	1.59 ^{C} ±0.01
	A2 (2 - 4 mm)	1.29 ±0.04	1.32 ±0.03	1.30 ±0.03	1.30 ^{A} ± 0.03	
	A3 (< 2 mm)	1.24 ±0.04	1.28 ±0.04	1.27 ±0.04	1.26 ^{A} ±0.04	
	Mean	1.28 ^{A} ±0.05	1.36 ^{B} ±0.09	1.34 ^{B} ±0.09		
Iodine value (gIod/100 g)	A1 (4 - 6 mm)	74.6 ± 0.72	74.8 ± 0.67	74.7 ± 0.53	74.5 ^{A} ± 0.57	74.4 ^{A} ± 0.07
	A2 (2 - 4 mm)	74.4 ± 0.74	74.7 ± 0.29	74.8 ± 0.84	74.7 ^{A} ± 0.60	
	A3 (< 2 mm)	75.1 ±0.51	75.0 ± 0.41	74.8 ±0.22	75.0 ^{A} ± 0.37	
	Mean	74.7 ^{A} ± 0.65	74.8 ^{A} ± 0.44	74.8 ^{A} ± 0.55		
Saponification value (mgKOH/g)	A1 (4 - 6 mm)	197 ±0.86	198 ± 0.35	197 ± 0.82	197 ^{A} ± 0.76	198 ^{A} ± 0.68
	A2 (2 - 4 mm)	196 ±0.93	197 ± 0.76	196 ± 0.74	196 ^{A} ± 0.74	
	A3 (< 2 mm)	197 ± 1.06	198 ± 0.69	198 ± 0.94	198 ^{A} ± 0.83	
		197 ^{A} ± 0.96	198 ^{A} ± 0.8	197 ^{A} ± 0.96		
Refractive value at 20^{o}C $^{(s/c)}$	A1 (4 - 6 mm)	1.466	1.466	1.466	1.466 ^{A}	1.466 ^{A}
	A2 (2 - 4 mm)	1.466	1.466	1.466	1.466 ^{A}	
	A3 (< 2 mm)	1.466	1.466	1.466	1.466 ^{A}	
		1.466 ^{A}	1.466 ^{A}	1.466 ^{A}		

A, B, C, D: Represent values with statistically significant differences (p<0.05) in the same column or in the same row. (*Note: The difference of F test in enzymes and particle size to acid value and peroxide value is less than 0.05, thus showing a statistically significant difference in acid value and peroxide value in different types. Different enzymes, different particle sizes. However, there was no difference between enzymes on acid value according to particle size).*

Furthermore, several quality parameters were analyzed to evaluate the impact of the enzyme and particle size on the quality of the macadamia nut oil, including the value of acid, peroxide value, iodine value, saponification value, and refractive value. The analytical results in table 2 show that particle size and the type of enzyme used influence the acid value and peroxide value. At the same time, they do not significantly affect the iodine value, saponification value, or refractive value. As particle size decreases, there is a noticeable reduction in the acid value and peroxide value, indicating an improvement in the quality parameters of the oil. This suggests that smaller particle sizes result in better oil quality.

Additionally, among the three enzymes tested, Viscozyme® L positively affected oil quality, with lower acid and peroxide values compared to the other two enzymes and even the control sample. In contrast, there was no significant difference between Cellulast enzymes and Pectinex Ultra spl enzymes. The particle size is one of the main factors influencing both oil pressing efficiency and the quality of macadamia oil. Uniformly ground particle size contributes to higher quality and improved performance during pressing. Smaller particle sizes lead to more significant destruction of oil-containing cells and the easy release of oil in its free form. However, it’s worth noting that the crushed powder particles are tiny, they can tend to clump during enzyme treatment, and the resulting powder may not be porous enough during the drying process. This, in turn, can reduce the efficiency of the oil extraction process [17]. Moreover, if the seeds are crushed into tiny particles, the greater surface area exposed to oxygen and the microflora can negatively impact the quality of the oil. This is especially true if no prompt and effective treatment [18].

Conversely, larger particle size reduces contact between the material and the enzyme. Cells located deep within the seedmay not undergo the necessary changes under the influence of the enzyme, leading to less efficient oil extraction from these regions [19]. Considering the color and taste of the oil after pressing, it exhibits a light yellow color, a greasy taste, and the characteristic aroma of macadamia oil. Notably, the organoleptic quality of the oil obtained through treatment did not differ from that of the control. Therefore, based on these considerations, Viscozyme® L enzyme and a particle size smaller than 2 mm were selected for further experiments.

3.2. Effect of enzyme concentration

The experimental results show that the oil extraction efficiency significantly increased at all tested concentrations after 1 hour of enzyme treatment (Figure 4). With a gradual increase in enzyme concentration, the oil extractionefficiency likewise increased progressively. At concentrations of 0.5% and 0.6%, the highest oil recovery was achieved at 59.4 and 60.7%, respectively, with no statistical difference between these two concentrations (*p>0.05*). In contrast, the control formula without enzyme treatment resulted in an oil recovery efficiency of only 39.5%. The non-enzymatic procedure with added water achieved an oil extraction efficiency of 42.1%, with no significant difference compared to the control sample (Figure 4).

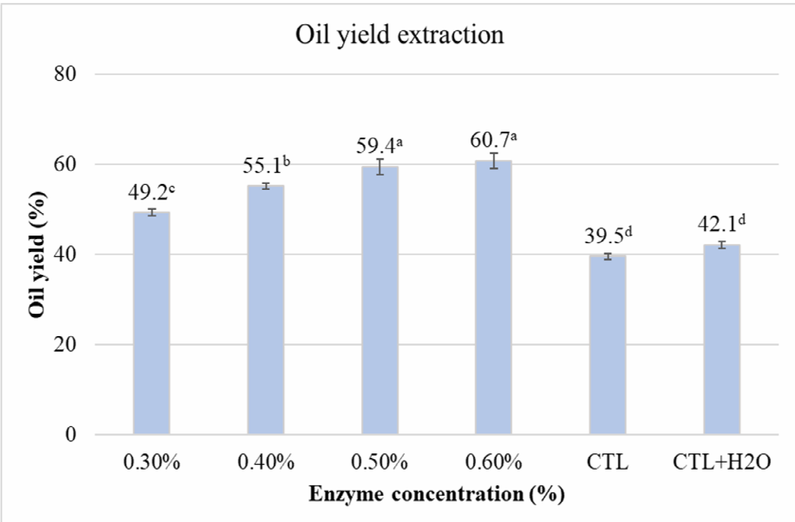

Figure 4. Oil extractionefficiency at different concentrations of Viscozyme® L enzyme (%)

a, b, c, d: Indicate values that have a statistically significant difference ($p<0.05$).

Several studies have mentioned the effect of enzyme addition on oil extraction efficiency and have similar results. For instance, Ezeh *et al.* (2016) concluded that, when comparing different seed pretreatment methods before pressing, the enzyme method resulted in the highest oil recovery yield, recovering approximately 90% of the oil contained in the oil in the seeds, while high-pressure treatment showed almost no increase in yield compared to the control [20]. Other studies have also confirmed that enzyme treatment enhances oil extraction efficiency from peanut seeds [21, 22]. Notably, among the enzymes used, Alcalase demonstrated the highest oil extraction efficiency, reaching around 73.45%, compared to the control treatment without enzymes, which yielded only 30.59%.

Furthermore, the acid and peroxide values were analyzed to provide an initial assessment of the quality of the obtained oil based on the preceding experimental results. These two parameters are crucial indicators of the oil quality and are susceptible to change depending on processing conditions, whereas the other values remain relatively stable.

In particular, the acid and peroxide values slightly decreased with increasing enzyme treatment concentration from 0.3-0.6%, although the difference was not statistically significant. However, a noticeable difference was observed between the enzyme-treated samples, the CTL and CTL+H_2O. The acid value and peroxide value in the CTL sample were higher than those in the enzyme-treated sample ($p<0.05$), while these values were highest in the CTL+H_2O sample. A study by Ma *et al.* (2022) also showed that the enzyme treatment positively affected the quality of macadamia oil when the acid value and peroxide value were lower than that of the solvent-based extraction method [23].

In addition, the quality of the oil was evaluated through sensory assessment. The results of the sensory quality assessment (Figure 5) showed no significant difference between the treatments in terms of color, flavor, taste, and overall evaluation. However, in the CTL+H_2O treatment, the oil's aroma received a lower assessment than the other samples. This finding is consistent with the earlier experimental results where the CTL+H_2O sample had the highest acid and peroxide value. Based on the analysis, it can be concluded that adding enzymes to the treatment of macadamia nuts before pressing does not significantly impact the physicochemical composition and overall quality of the macadamia oil product. As oil extraction efficiency tends to increase with the concentration of treated enzymes, it's worth noting that the color and flavor parameters maintain similar quality. However, when the enzyme concentration was increased to 0.6%, the results showed that, while the oil extraction efficiency increased (reaching 60.7%), this increase was not statistically significant compared to the 0.5% concentration (59.4 %).

A prior study by Picuric-Jovanovic *et al.* (1997), which involvedan aqueous-enzymatic extraction process of plum kernel oil, also showed that the maximum oil yield of approximately 70% was achieved at an enzyme concentration of 0.5% [24]. Therefore, considering the economic efficiency, the treatment of Viscozyme® L enzyme at a concentration of 0.5% was selected for further experiments.

Table 3. Quality parameters of macadamia oil

Targets _	Viscozyme® L. enzyme concentration (%)					
	0.3%	0.4%	0.5%	0.6%	CTL	CTL+H_2O
Acid number (mgKOH/g)	0.52 ^{AB}±0.01	0.51 ^{AB}±0.01	0.48 ^{A}±0.02	0.48 ^{A}±0.02	0.53 ^{B}±0.02	0.63 ^{C}±0.02
Peroxide Value (meq/kg)	1.34 ^{A}±0.03	1.33 ^{A}±0.03	1.29 ^{A}±0.03	1.28 ^{A}±0.03	1.57 ^{B}±0.04	2.57 ^{C}±0.04

A, B, C, D: Represent values with statistically significant difference (p<0.05) in the same row.

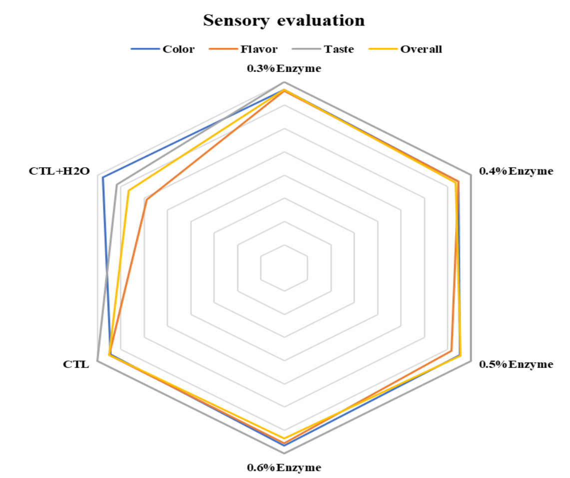

Figure 5. Sensory value of macadamia oil

3.3. Effect ofenzymetreatment time and added water ratio

In order to enhance the contact of the enzyme with the substrate, it is necessary to add water at the appropriate rate during the treatmentof the raw material, combined with the correct treatment duration. Macadamia kernel/nut material, after being ground to a size of <2mm, is soaked and treated with 0.5% Viscozyme® L enzyme for 3 different periods: 1 hour, 2 hours, or 3 hours, with water added at the rates of 20%, 30%, and 40% depending on the treatment (Table 4).

Table 4. Oil extraction efficiency (%) at different added water ratios and treatment time

Factor A (Processing Time)	Factor B (ratio of water/material)		
	B1 (20%)	B2 (30%)	B3 (40%)
A1 (1 hour)	$54.4^{Ab} \pm 0.85$	$58.3^{Ba} \pm 0.73$	$60.0^{Ba} \pm 0.77$
A2 (2 hours)	$55.3^{Ab} \pm 1.32$	$65.2^{Aa} \pm 0.78$	$64.7^{Aa} \pm 0.86$
A3 (3 hours)	$54.9^{Ab} \pm 1.40$	$66.0^{Aa} \pm 0.65$	$63.9^{Aa} \pm 0.84$

A, B, C, D: Represent values with statistically significant differences ($p<0.05$) in the same column; a, b, c, d: represent values with statistically significant differences ($p<0.05$) in the same row.

The results presented in table 4 initially show that the enzyme treatment duration of 2 hours and 3 hours resulted in higher oil extraction efficiency compared to a 1-hr treatment. In the formulation with an additional 20% water content, longer treatment times they led to increased oil recovery efficiency. In particular, the 30% and 40% water additions during 2 hours or 3 hours of enzyme treatment yielded the highest oil recovery. The fact can explain this that adding sufficient water provides the enzyme with a better opportunity to contact the cells within the material, thereby supporting the hydrolysis reaction of substances surrounding the oil, ultimately facilitating the release of oil. Additionally, adequate water addition alters the physical properties of the nuts, making them more porous and better suited to support the oil pressing process. However, excessive water does not further enhance the oil extraction process; on the contrary, it increases the cost of the drying process and can lead to higher viscosity in the raw material fed into the press, consequently increasing the production cost of the machine and final product.

Table 5. Quality parameters of macadamia oil

physicochemical properties	Factor A (Time)	Factor B (Rate of added H_2O)			Mean
		B1 (20%)	B2 (30%)	B3 (40%)	
Acid value (mgKOH/g)	A1 (1 hour)	0.54 ± 0.04	0.52 ± 0.02	0.51 ± 0.02	$0.52^{B} \pm 0.03$
	A2 (2 hours)	0.53 ± 0.01	0.47 ± 0.03	0.48 ± 0.02	$0.49^{A} \pm 0.03$

	A3 (3 hours)	0.53 ± 0.03	0.48 ± 0.02	0.49 ± 0.03	0.50 ^{AB} ± 0.03
	Mean	0.53 ^{B} ± 0.02	0.49 ^{A} ± 0.03	0.50 ^{A} ± 0.02	
Peroxide value (meq/kg)	A1 (1 hour)	1.46 ±0.01	1.33 ±0.03	1.34 ±0.02	1.38 ^{B} ±0.06
	A2 (2 hours)	1.41 ±0.02	1.29 ±0.03	1.29 ±0.03	1.33 ^{A} ± 0.06
	A3 (3 hours)	1.42 ±0.02	1.30 ±0.01	1.32 ±0.01	1.34 ^{A} ±0.05
	Mean	1.43 ^{B}±0.02	1.31 ^{A}±0.03	1.32 ^{A}±0.03	

A, B, C, D: Represent values with statistically significant differences (p<0.05) in the same column or in the same row.

Similar to the previous findings, in order to evaluate how the quality of the obtained macadamia oil is influenced by the enzymatic treatment time and the percentage of added water, the acid value and the peroxide value were analyzed. As shown in table 5, the processing conditions, specifically the enzyme treatment time and the percentage of added water, had a slight impact on the quality of the macadamia oil, as reflected in the acid and peroxide values. When evaluating the effect of processing time in the enzyme treatment, oil obtained after pressing for 2-3 hours tended to have lower acid and peroxide values than oil treated for 1 hour. Furthermore, the use of 30% and 40% water in the enzyme treatment demonstrated a positive effect on the quality of macadamia oil after pressing, with lower acid value and peroxide value (*p<0.05*) compared to the formula with 20% water. An earlier study by Concha *et al.* (2004) had similar results, claiming that a water ratio of 30% gave a higher probability of oil extraction yield than treatment with a water ratio of 20% or 40% [25]. Based on these initial results, the combination of the addition of 30% water and treating macadamia with enzymes within 2 hours was chosen to complete the oil extraction process in the next stage.

3.4. Effect of pressing temperature

Macadamia nuts are highly sensitive to heat due to their rich content of unsaturated fatty acids, vitamins, and other biologically active compounds. In the oilpressing process, high temperatures can lead to oil denaturation, and other components such as glucid can easily be caramelized, causing the oil to darken. Additionally, oxidation may occur, adversely affecting the overall quality of the oil. In conventional hydraulic pressing method, the pressing temperature typically ranges from 30°C to 50°C. It's noting that at temperatures below 35°C, most of the characteristics related to the quality of macadamia oil are preserved. To evaluate the impact of temperature, the experimental process was conducted within the range of 25 to 40°C, meaning that all the quality characteristics of macadamia oil were maintained and better oil press efficiency.

The initial results in Table 6 showed that the oil extraction efficiency is higher when the oil is pressed at higher temperatures. At a temperature of 40°C, the highest oil extraction efficiency reached 70.7%, and this difference was statistically significant compared to the other conditions. At 30°C and 35°C, the oil extraction efficiencies were 66.8% and 68.1%, respectively, with no statistically significant difference (*p>0.05*). The lowest oil extraction efficiency was observed at 25°C. Therefore, in terms of oil recovery efficiency, a temperature of 40°C appears to be suitable for use in the cold pressing process. However, since temperature often impacts the quality of oil, it is essential to analyze the quality parameters and storage time when using a temperature of 40°C. To evaluate the quality of macadamia oil obtained and understand how is it affected by pressing temperature, we analyzed two important quality parameters of oils: acid value and peroxide value. The analysis results for the acid value and peroxide value are presented in table 7, showing that pressing temperature indeed affects both acid value and peroxide value. Specifically, as the temperature gradually increases, the acid value and peroxide value tend to increase, with the highest values observed in the pressed sample pressed at 40°C. Previous studies have also mentioned the changes and influence of pressing temperature on oil quality.

Table 6. Oil extraction efficiency (%) at different pressing temperatures

	Oil pressing temperature (°C)			
Oil extraction efficiency (OY, %)	25	30	35	40
	64.6 ^{C} ± 0.84	66.8 ^{B} ± 0.57	68.1 ^{B} ± 0.75	70.7 ^{A} ± 0.74

A, B, C, D: Represent values with statistically significant difference (p<0.05) in the same row.

Enzymes typically function within a specific temperature range. According to Rui *et al.* (2009), the optimum temperature range for enzymatic hydrolysis falls between 40 - 55°C. As a result, most studies are often performed within this temperature range [26]. In practical application, it is generally preferred to use the lowest possible temperature while still achieving adequate enzyme activity [27]. For instance, when dealing with olives, temperatures lower than 30°C are considered particularly favorable for oil preserving oil quality [28, 29, 30, 31]. Gros *et al.* (2003) also used a temperature of 34°C for similar reasons in the extraction of linseed oil [32]. Temperature plays a significant role in influencing oil yield, as demonstrated by Sharma *et al.* (2002), where the efficiency of peanut oil extraction was highest at 40°C and significantly decreased when the temperature was lowered to 37°C [33]. According to Zuniga *et al.* (2003), temperatures exceeding 45°C led to a decreasein the enzyme hydrolysis due to enzyme inactivation, resulting in lower oil yield [34].

In addition, the presence of soluble sugar in the mixture can limit oil release from the cell and cause caramelization during drying. As a result, oil output generally increases up to a certain temperature, after which it stabilizes or gradually decreases. This trend is observed in the majority of studies. Therefore, when selecting an enzyme treatment temperature, both oil yield and oil quality characteristics must be taken into account.

Table 7. Macadamia oil quality parameters

Targets	Oil pressing temperature (°C)			
	25	30	35	40
Acid value (mgKOH/g)	0.51 ^{A} ± 0.02	0.53 ^{A} ± 0.04	0.76 ^{B} ± 0.05	1.34 ^{C} ± 0.08
Peroxide value (meq/kg)	1.29 ^{A} ± 0.05	1.32 ^{A} ± 0.03	1.98 ^{B} ± 0.07	3.71 ^{C} ± 0.06

A, B, C, D: Represent values with statistically significant difference (p<0.05) in the same row.

It has been well known that fatty acids are recognized as the primary components of macadamia oil, and they play an important role in determining the quality of macadamia oil. In order to have a better understanding of how diferent treatment methods influence the quality of macadamia oil, we analyzed the fatty acid composition of macadamia oil. The analytical results presented in table 8 showed that the fatty acid composition in macadamia oil primarily consists of unsaturated fatty acids, accounting for > 80% of the total fatty acids. Specifically, the fatty acids found in large amounts in macadamia oil include oleic acid (52~55%), palmitoleic acid (20.5~24%), palmitic acid (10.3~11.8%), stearic acid (2.54~5.53%), linoleic acid (1.92~3.13%).

Among the unsaturated fatty acids, oleic was the most abundant, while among the saturated fatty acids, palmitic acid predominant. These results in our study are similar with those of previous studies when analyzing the fatty acid composition in macadamia oil. For example, Aquino-Bolaños *et al.* (2017) analyzed 20 fatty acids and found that the most common ones in macadamia nut oils were oleic acid (40 - 51%), palmitoleic acid (24 - 36%)) and palmitic acid (8.4 - 13.1%) [35]. Akhtar *et al.* (2006) also reported that unsaturated fatty acids in macadamia oil accounted for 80 - 84%, with the 03 acids present in the highest content content being oleic acid (54 - 68%), palmitoleic acid (16 - 23%) and palmitic acid (7 - 10%) [36]. Recently, Shuai *et al.* (2021) analyzed the acid composition in 15 macadamia varieties harvested in China and obtained similar results. Specifically, the unsaturated fatty acid composition in macadamia oil ranged from 82.8 - 85.9%, while saturated fatty acids accounted for 14.1 - 17.2%. Furthermore, there was no significant difference in fatty acid composition among these varieties [37]. In another study conducted by Shuai *et al.* (2022), it was similarly shown that the fatty acid composition of macadamia oil did not significantly vary based on different extraction methods [2]. The results of the present analysis in this experiment also showed that there was no major difference in the fatty acid composition of the samples treated under various conditions. It is evidence that the enzyme method offers advantages such as higher oil recovery efficiency and consistent oil quality. The results of this experiment further reinforce the fact that macadamia oil contains a high content of unsaturated fatty acids (constituting more than 80% of the total fatty acid content in the oil), while the percentage of saturated fatty acids is low (<15%). This information reaffirms the quality and health benefits of macadamia nut oil.

Table 8. Fatty acid composition and some quality values in macadamia nut oil

Fatty acid composition (g/100 g)	Enzyme	CTL+H_2O	CTL
Butyric acid	0.068	0.039	0.054
Caproic acid	0.075	0.061	0.098
Caprylic acid	0.077	0.085	0.091
Capric acid	0.057	0.174	0.059
Undecylic acid	0.025	0.038	0.057
Lauric acid	0.081	0.091	0.084
Myristic acid	0.815	0.83	0.726
Methyl Cis-10-Pentadecanoate	0.046	0.086	0.048
Palmitic acid	10.8	11.0	10.3
Palmitoleic acid	24.0	20.5	21.1
Stearic acid	2.54	3.25	5.53
Oleic acid	55.0	53.1	54.4
Linoleic acid	2.13	2.07	1.92
α-Linolenic acid	0.11	0.11	0.12
Arachidic acid	Nd	4.63	0.23
cis-11-Eicosenoic acid	1.44	1.44	1.35
Behenic acid	2.52	1.29	2.56
Erucic acid	0.079	1.15	1.26

Nd: Not detected (LOD=0.008)

4. CONCLUSION

In this study, we evaluated the impact of using enzymes in the pretreatment stage on the pressing performance and some quality parameters of the resulting macadamia nut oil. Out of the three enzymes investigated, Viscozyme® L was selected as the most effective. When combined with Viscozyme® L and a crushed particle size less than 2 mm, with the 0.5% enzyme concentration, the highest oil recovery efficiency reached 59.4%, which is approximately 20% higher than the control samples without enzymes. When using a 0.5% enzyme concentration in combination with 30% or 40% added water during a 2 hours or 3 hours treatment, the highest oil extraction efficiency was achieved. The optimal temperature achieving good oil pressing results was found to be 30°C, where the oil extraction efficiency reached 66.8%. Quality parameter of the oil, including the acid value, peroxide value, fatty acid composition, and others, were analyzed, demonstrating that the oil met the quality standards expected of vegetable oils. Our current research has demonstrated that enzyme treatment has a positive impact on increasing macadamia oil yield while maintaining its quality characteristics. However, before considering large-scale implementing to replace traditional method with enzymes treatment, it is imperative to conduct a thorough economic assessment.

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