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Evaluation of the Effect of Aqueous-Enzymatic Extraction Using Cellulase and Pepsin Enzymes on Phytochemical Compounds and Physicochemical Properties of Sesame Oil

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ABSTRACT

Sesame (*Sesamum indicum* L.) is highly valued both nutritionally and industrially due to its rich phytochemical composition, including lignans and unsaturated fatty acids. This study investigated the impact of aqueous-enzymatic extraction using cellulase and pepsin enzymes on sesame oil extraction yield and quality parameters. Results indicated that the application of cellulase and pepsin enzymes not only enhanced the extraction yield but also improved bioactive compounds such as total phenols, vitamin E, and antioxidant capacity. Quality indices, including acid value, saponification value, and unsaponifiable matter, showed no significant changes and remained within standard ranges. The iodine value also remained unchanged, reflecting the preservation of key fatty acids like oleic acid, linoleic acid, and palmitic acid. This stability was attributed to the gentle aqueous-enzymatic method, which prevents compound degradation. Furthermore, the peroxide value decreased to 8 milliequivalents per kilogram, indicating improved oxidative stability of the oil. Unsaponifiable compounds showed a significant increase, contributing to the overall enhancement of oil quality. Additionally, the anisidine value, a marker of secondary oil oxidation, was reduced, signaling diminished primary oxidation and improved oil stability. These findings confirm that the aqueous-enzymatic method can be a green, efficient, and environmentally friendly approach for sesame oil extraction.

1.Introduction

Sesame (*Sesamum indicum* L.) is an annual herbaceous plant belonging to the family Pedaliaceae, known as the "Queen of Oilseeds" due to its strong antioxidant properties [1]. The plant originated in India and later spread to Africa, Asia, and the southern regions of the Mediterranean basin. India, Sudan, China, and Burma are among the major producers of sesame, where the trade of this crop holds significant importance [2]. Sesame seeds contain various phytochemical compounds, including phenolic acids, flavonoids, phytosterols, tocopherols, phospholipids, and lignans such as sesamol, sesamin, and sesamolin [3]. Sesame oil is one of the main products derived from this plant and is traditionally used in cooking and as a flavor enhancer in both Asian and Western cuisines. Additionally, sesame oil is utilized in diets and for the treatment of certain health conditions. It is recognized as one of the finest vegetable oils and is an important source of energy [4]. In terms of nutritional value, sesame oil ranks second after olive oil [5]. It is primarily composed of oleic-linoleic fatty acids, with over 80% of its fatty acids consisting of linoleic acid (41.5% to 47.9%) and oleic acid (35.9% to 42.3%). Unlike many other oilseeds, the proportions of these two fatty acids in sesame oil are quite similar. Moreover, sesame oil contains less than 20% saturated fatty acids, mainly palmitic acid (7.9% to 12%) and stearic acid (4.8% to 6.1%) [6].

Thanks to the presence of active phytochemical compounds such as unsaturated fatty acids, phytosterols, tocopherols, and lignans like sesamin, sesamol, and sesaminol, sesame oil is capable of counteracting reactive oxygen species. These antioxidant compounds help prevent oxidation and contribute to maintaining the oil’s quality [7]. Various methods are used to extract oil from sesame seeds, including solvent extraction, cold pressing, and aqueous enzymatic extraction. In recent years, aqueous enzymatic extraction has gained attention due to its specific advantages, such as improved product quality and reduced use of chemical solvents. This method employs enzymes to break down the plant cell wall and simultaneously extract bioactive compounds such as oil and protein. By using selective enzymes, this process not only increases extraction yield but also enhances the flavor and aroma of the final products [8]. The plant cell wall is composed of cellulose, hemicellulose, pectin, and proteins. Enzymes can degrade the cell wall structure and release phenolic compounds, thereby boosting the yield of oil and other bioactive compounds [9]. The core principle of enzymatic extraction lies in the hydrolysis of the plant cell wall under optimal conditions. During this process, components of the cell wall bind to the active site of the enzyme, inducing a conformational change that facilitates stronger interaction between the enzyme’s active site and the cell wall components. As a result, cell bonds are broken and bioactive compounds are released more easily [10]. This method is recognized as a green extraction technique. Factors such as ground seed size, water to substrate ratio, enzyme concentration, stirring speed, and others influence the process. Additionally, optimal conditions, including pH and enzyme temperature, play a crucial role in the efficiency of the method [11]. The final quality of the extracted oil is affected by factors such as the chemical composition of the oilseeds and particle size. Due to safety concerns and environmental regulations, the use of aqueous enzymatic extraction is expanding. Compared to solvent extraction, this method is not only safer and more environmentally friendly, but also more economical. Furthermore, it requires less energy and has lower operational costs, making it a more efficient approach. Another advantage is the generation of valuable by products such as oilseed cake, fiber, and protein, owing to the mild operational conditions. Additionally, this method allows for the separation of phospholipids from the oil, eliminating the need for a degumming step and reducing overall processing costs [8].

1.1. Research Objective

This study was conducted to investigate the effect of using pepsin and cellulase enzymes in the aqueous enzymatic extraction process of sesame oil. While cellulase has previously been applied mainly in combination with other enzymes, in this research, its effect is examined independently. Moreover, for the first time, pepsin is used alone in the extraction of sesame oil. The main objectives of this research include evaluating the impact of these enzymes on oil extraction yield and analyzing the physicochemical properties of the extracted oil. The focus is placed on key indicators such as oxidative stability, bioactive compounds and the preservation of essential components such as essential fatty acids. This study aims to demonstrate that the application of this innovative method can lead to improved oil yield and overall quality, and potentially serve as a viable alternative to traditional and chemical extraction methods.

2- Materials and Methods

2.1. Sample preparation

Sesame seeds were from the Agricultural Jihad Center of Behbahan County. Pepsin enzyme was purchased from Merck, and cellulase enzyme was supplied by Bioluence Company. For sesame oil extraction, two aqueous enzymatic methods were employed using cellulase and pepsin enzymes independently. 10 g of ground sesame seeds were placed in a 250 mL Erlenmeyer flask with 20 mL of distilled water at a 1:2 ratio. The pH was adjusted to the optimum for each enzyme using 0.1 M sodium hydroxide and hydrochloric acid solutions (pH 7 for cellulase and pH 2 for pepsin). The enzymes were added at their optimal concentrations (2% w/w for pepsin and 3% w/w for cellulase). The mixture was incubated in a shaking incubator at 40°C and 150 rpm for 6 h. Afterwards, the mixture was transferred to Falcon tubes and centrifuged at 10,000 rpm. The upper phase containing the oil was separated using a micropipette and the enzymes were inactivated by heating at 90°C for 15 min in an oven. Samples were then stored at 4°C for subsequent analyses [12].

2.2. Extraction by maceration

To analyze the phenolic and antioxidant compounds soluble in alcohol, sesame oil extraction by maceration was performed using 70% ethanol. For this purpose, 2 g of sesame oil were mixed with 20 mL of 70% ethanol and incubated for 24 h at room temperature in a shaking incubator. The samples were then centrifuged at 8,000 rpm for 10 min, and the alcoholic extract was separated after centrifugation [13].

2.3. Oil extraction yield measurement

The oil extraction yield for all extraction methods was calculated according to the AOCS Af 3-54 standard using the following formula [14]:

$$\text{Oil extraction yield}(\%) = \frac{\text{Weight of extracted oil}(g)}{\text{Weight of initial sample }(g)} \times 100$$

2.4. Fatty acid analysis

The extracted oils were obtained using petroleum ether as the organic solvent. Fatty acid analysis was performed using a Gas Chromatography-Mass Spectrometry (GC-MS) system, model 6890 GC coupled with a 5973N MS detector (Agilent, USA). The instrument was equipped with an HP-5MS capillary column (5% methyl siloxane stationary phase), 30 meters in length and 0.25 mm internal diameter. The ionization energy of the mass spectrometer was set at 70 electron volts (eV). A volume of 1–2 μL of pure sample was injected using a 10 μL syringe. The oven temperature was initially set at 50°C and held for 3 min. Then, the temperature was increased at a rate of 10°C/min to 120°C, 150°C, and finally 190°C, with a 3-min hold at each temperature stage [15].

2.5. Antioxidant activity assessment by DPPH method

The antioxidant activity of the extracted oils was measured using the DPPH assay according to the Brand-Williams method. Initially, 0.1 mL of the extract was added to a test tube, followed by 3.9 mL of DPPH solution (0.002% concentration in 98% methanol). The samples were vortexed for a few seconds and then incubated in the dark for 30 min to allow the reaction to occur. During this process, the purple color of DPPH changed to yellow. The absorbance of the samples was measured at a wavelength of 517 nm using a spectrophotometer. The percentage of antioxidant activity was calculated using the following formula [16]:

Antioxidant activity % = (Control absorption - Sample absorption / Control absorption) *100

2.6. Total Phenolic Content Measurement

The total phenolic content was determined using a modified Folin Ciocalteu method. To 0.2 mL of the prepared extract, 1.5 mL of 10% Folin reagent was added. After resting for 6 min, 1.5 mL of 5% sodium carbonate solution was added, and the mixture was incubated in the dark for 60 min. The resulting dark blue color indicated the presence of phenolic compounds. The absorbance was then measured at 760 nm using a spectrophotometer. The total phenolic content was expressed as mg of gallic acid equivalents per 100 g of sample [17].

2.7. Vitamin E Content Measurement

The vitamin E content in sesame oil was measured using an HPLC system. The mobile phase consisted of 100% methanol with a flow rate of 1 mL/min. A reversed-phase column (250 mm length, 4.6 mm internal diameter, 5 µm particle size) and a UV detector set at 285 nm wavelength were used. The injection volume was 20 µL. For vitamin E extraction, 1 g of sesame oil was mixed with 2 mL of 12% potassium hydroxide and incubated in an oven at 82°C for 20 min. After cooling, 5 mL of hexane was added, and the mixture was centrifuged at 2000 rpm for 15 min. The upper hexane layer was separated, and the solvent was evaporated at 42°C using a rotary evaporator. The residue was dissolved in 420 µL of methanol and injected into the HPLC. Quantitative analysis was performed by comparing the chromatograms of each sample with corresponding standard chromatograms. Vitamin E concentration was expressed as mg per 100 g of oil [18].

2.8. Soap Number Determination

For the determination of the soap number, 0.5 g of each oil sample was mixed with 10 mL of KOH solution (2.8 g KOH dissolved in 100 mL of ethanol/water solution at a 1:1 ratio) and placed in a water bath at 80–85°C for 30 min. The progress of the reaction was indicated by the solution turning purple. After cooling to room temperature, 1–2 drops of 1% phenolphthalein indicator were added to each sample. Titration was then performed with 0.5 M hydrochloric acid (HCl) until the solution color changed from purple to colorless at the equivalence point. The same procedure was repeated for a blank solution containing only KOH solution [19]. The soap number was calculated using the following formula and expressed as milligrams of potassium hydroxide per 100 g of sesame oil:

$$\text{Saponification value}(\text{mgg}^{-1}) = \frac{28(5 \times (A - B) \times F}{S}$$

A = volume of HCl used for blank titration (mL)

B = volume of HCl used for sample titration (mL)

F = normality factor of 0.5 M HCl

S = sample weight (g)

2.9. Acid Value Determination

In this study, the acid value test according to the AOCS method was used to calculate the percentage of free fatty acids resulting from triglyceride hydrolysis. In a 250 mL volumetric flask, 1 gram of oil was dissolved in 10 mL of 96% ethanol. Then, 2 drops of 1% phenolphthalein indicator were added. The oil samples were titrated with 0.1 M potassium hydroxide (KOH) solution until a pale pink color appeared. The volume of titrant used was recorded, and the free fatty acid content was calculated using the following formula [14]:

$$\text{Acid Value} = \frac{(V - B) \times N \times 56.1}{W}$$

B = volume of KOH used for blank titration (mL)

V = volume of KOH used for sample titration (mL)

N = normality of KOH (0.1 N)

W = weight of the sample (g)

2.10. Iodine Value Determination

The iodine value was measured using the Hanus method according to AOAC standard 920-158. In this method, 0.25 g of oil was weighed into a 250 mL Erlenmeyer flask, followed by the addition of 10 mL of chloroform. The sample was dissolved by shaking the flask. Then, 2.5 mL of Hanus reagent was added, and the flask was kept in the dark for 30 min. After this period, 10 mL of 15% potassium iodide solution and then 10 mL of distilled water were added to the flask. Finally, the sample was titrated dropwise with 0.1 M sodium thiosulfate solution while continuously swirling the flask until the solution color changed to brick yellow. A few drops of starch indicator were then added, and titration continued until the blue color completely disappeared. The volume of sodium thiosulfate used was recorded. A blank test was conducted under the same conditions without adding the oil sample. The iodine value was calculated using the formula below [14]:

$$IV = \frac{(V_2 - V_1) \times N \times 12.69}{m}$$

IV = iodine value (g iodine absorbed per 100 g oil)

V_1 = volume of sodium thiosulfate solution used for oil sample titration (mL)

V_2 = volume of sodium thiosulfate solution used for blank titration (mL)

N = normality of sodium thiosulfate solution

m = mass of oil sample (g)

2.11. Determination of unsaponifiable matter in extracted oils

According to AOCS Official Method Ca 6a-40, 5 grams of oil were placed in a round-bottom flask, followed by the addition of 50 mL of 1 mol/L ethanolic potassium hydroxide (KOH) solution and a few boiling stones. The mixture was refluxed to complete the saponification process. After cooling, the contents of the flask were transferred to a 500 mL separatory funnel. The flask was rinsed three times with diethyl ether, and the rinses were added to the funnel. The funnel was allowed to stand until two distinct layers formed. The lower aqueous layer was discarded. The upper etheric layer, containing the unsaponifiable matter and ether, was transferred to a rotary evaporator. The solvent was evaporated at 75 °C. To achieve a constant weight, the residue was dried in an oven at 103 °C and then placed in a desiccator. A blank test (without oil) was performed under the same conditions. The percentage of unsaponifiable matter was calculated using the following equation [14]:

$$\text{Unsaponifiable matter (\%)} = \frac{m_1 - m_2 - m_3}{m} \times 100$$

2.12. Determination of peroxide value of extracted oils

The peroxide value (PV) was determined according to AOCS Official Method Cd 8-53. For this purpose, 0.5 g of the oil sample was mixed with 5 mL of an acetic acid chloroform solution in a 3:2 ratio. The container was shaken to dissolve the sample. Then, 0.5 mL of saturated potassium iodide solution was added, and the mixture was kept in a dark environment for exactly 1 min. After that, 30 mL of distilled water and a few drops of starch indicator were added. The mixture was then titrated with 0.01 N sodium thiosulfate solution until the blue color completely disappeared. The same procedure was performed for the blank sample (without oil) [14].

$$PV = \frac{(S - B) \times N \times 1000}{W}$$

PV = Peroxide value (milliequivalents of peroxide per kilogram of oil)

S = Volume of sodium thiosulfate used for the sample (mL)

B = Volume of sodium thiosulfate used for the blank (mL)

N = Normality of sodium thiosulfate solution

W = Weight of the oil sample (g)

2.13. Determination of Anisidine Value

The anisidine value was measured according to ISO 6885. For preparation of the test solution, 1 g of oil was weighed into a 25 mL volumetric flask and diluted to volume with iso octane. The absorbance of the sample solution was measured using a spectrophotometer via two procedures: First, 5 mL of the test solution was mixed with 1 mL of glacial acetic acid. After 8 min of incubation in the dark at 23 °C, the solution was transferred to a clean, dry cuvette within 2 min. After 10 min, the absorbance at 350 nm was measured and recorded as the absorbance of the non reacted test solution (A_0). Second, 5 mL of the test solution was mixed with 1 mL of p-anisidine reagent. After 8 min of incubation in the dark at 23 °C, the solution was transferred to the cuvette and its absorbance at 350 nm was recorded as the reacted test solution (A_1). To determine the absorbance of the blank, 5 mL of iso octane was mixed with 1 mL of p-anisidine reagent, and after 10 min of reaction, the absorbance at 350 nm was measured (A_2). The anisidine value (Q) was calculated using the following equation [20]:

$$Q = \frac{100 \times QV \times [1.2 \times (A_2 - A_1 - A_0)]}{m}$$

Q = Anisidine value (mg per g of sample)

V = Final volume of the sample solution (25 mL)

A_0 = Absorbance of the non-reacted sample

A_1 = Absorbance of the reacted sample

A_2 = Absorbance of the blank

m = Weight of the sample (g)

2.14. Statistical Analysis

Statistical analysis of the data was performed using SPSS software version 20. The mean values were calculated based on three independent replicates. Duncan's multiple range test was used to compare the means. Graphs of the means were plotted using Microsoft Excel.

3.1. Changes in oil yield

The results of the comparison of mean oil yield from sesame extracted by different methods showed that the oil yield increased in sesame oil extracted using cellulase and pepsin enzymes compared to the control sample. The highest oil yield was observed in the sample extracted with cellulase enzyme ($p < 0.05$) (Figure 1). Aqueous-enzymatic extraction facilitates oil release through the breakdown of the plant cell wall. The yield of oil extraction depends on various parameters such as particle size, enzyme type and concentration, water-to-seed ratio, extraction time, temperature, and pH [21]. Extraction of oil using cellulase and pepsin enzymes from sesame seeds shows that cellulase enzymes degrade the cell wall, releasing the oil trapped within protein and polysaccharide layers [22]. Additionally, pepsin hydrolyzes the peptide bonds in polypeptide chains where oil droplets are accumulated, breaking them into shorter peptide fragments. This process contributes to the increased oil yield in enzyme-assisted extraction [23].

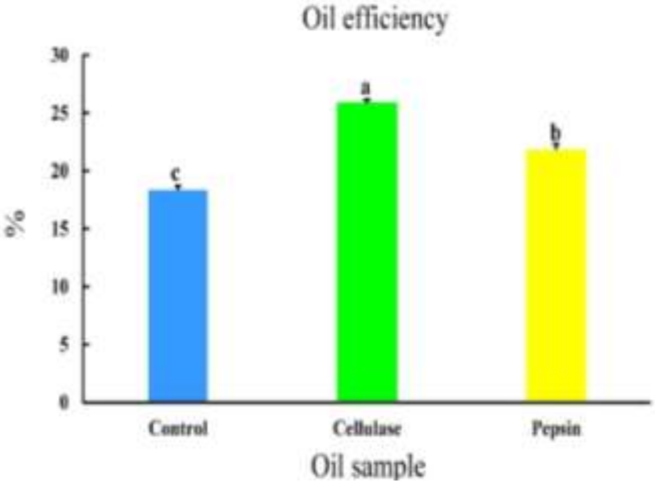

Figure 1. Yield of extracted sesame oil. The mentioned values are the average of 3 repetitions ± standard error. Similar letters indicate no significant difference between the means.

3.2. Changes in fatty acid composition

Based on the GC-MS data of sesame seed oil extracted using different methods, four main fatty acids were identified: palmitic acid, linoleic acid, oleic acid, and stearic acid. In the control condition, an appropriate amount of each of these fatty acids was present, and no significant difference was found compared to the oils extracted using cellulase and pepsin enzymes (Figure 2). Fatty acids are important indicators for evaluating the quality and stability of oils. In sesame oil, oleic acid and linoleic acid make up more than 80% of the total fatty acids and are considered to have high nutritional value

[24]. Polyunsaturated fatty acids, such as linoleic acid, help reduce cholesterol and prevent atherosclerosis [25,26]. Enzymatic extraction does not affect the fatty acid composition, whereas factors such as heat and oxidation can alter their structure. Low extraction temperatures and short processing times prevent the oxidation and degradation of unsaturated fatty acids and help preserve sensitive compounds [27]. The cellulase enzyme increases oil yield by breaking down the seed cell wall without damaging the fatty acid composition. Similarly, pepsin does not directly interact with lipids or fatty acids and does not affect the chemical composition of the oil [28].

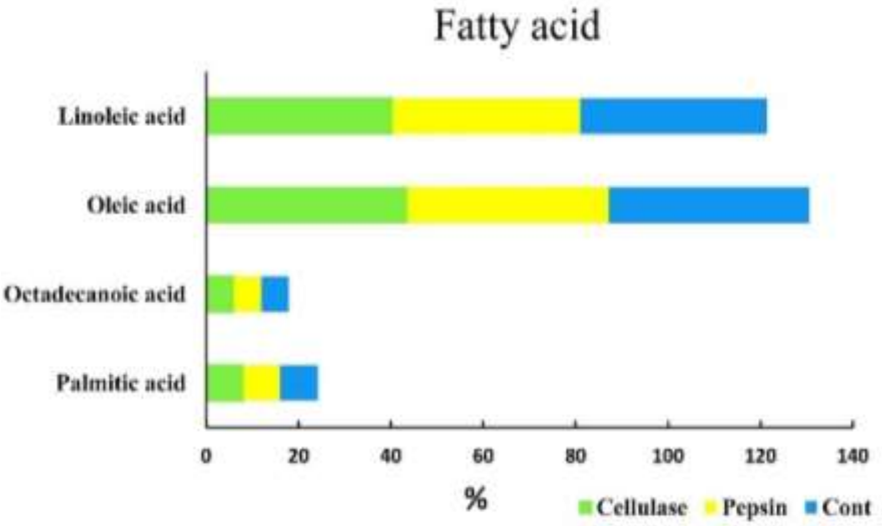

Figure 2. Content of main fatty acids in extracted sesame oil.

3.3. Changes in antioxidant capacity (DPPH method)

The results of the mean comparison of antioxidant capacity in sesame oil extracted using different methods showed that the antioxidant capacity increased in the oils extracted with pepsin and cellulase enzymes compared to the control sample. The highest antioxidant capacity was observed in the sesame oil extracted using pepsin enzyme ($p < 0.05$) (Figure 3). DPPH is a stable free radical with maximum absorption at 520 nm. When DPPH encounters a proton donor, the free radical is neutralized, resulting in a decrease in absorbance. Therefore, the antioxidant activity of a substance is related to its ability to scavenge DPPH radicals [29]. Sesame oil extracted by the aqueous-enzymatic method exhibits higher antioxidant capacity compared to conventional methods. This method enhances the release of more bioactive compounds such as phenols, tocopherols, volatile substances, carotenes, xanthophylls, and chlorophylls into the oil phase, improving flavor quality and product shelf life. Additionally, the reduced complexation of polyphenols with seed polysaccharides further increases the antioxidant capacity of the oil [30].

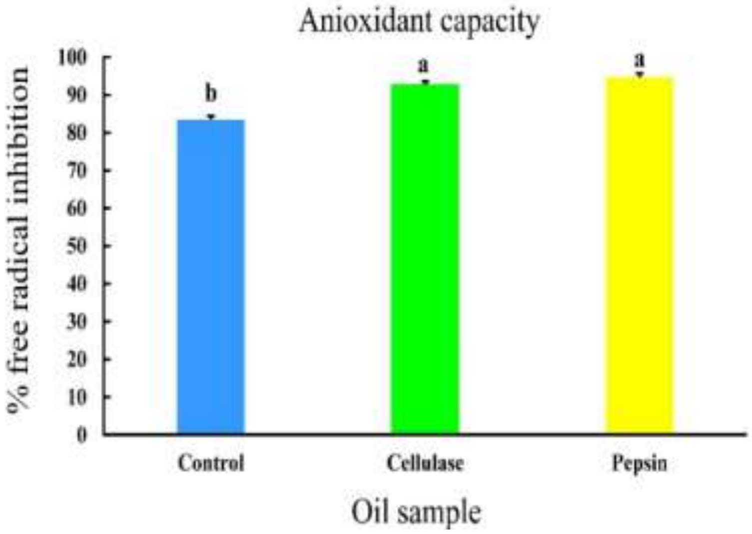

Figure 3. Content of antioxidant capacity in extracted sesame oil. The mentioned values are the average of 3 repetitions ± standard error. Similar letters indicate no significant difference between the means.

3.4. Changes in total phenolic content

The results of the mean comparison of total phenolic content in sesame oil extracted by different methods showed that the phenolic content increased in the oils extracted using cellulase and pepsin enzymes compared to the control sample. The highest phenolic content was observed in the oil extracted with pepsin enzyme ($p < 0.05$) (Figure 4).

Phenolic compounds are recognized as natural antioxidants due to their ability to neutralize free radicals and inhibit their formation. Additionally, their capacity to chelate metal ions contributes to the inhibition of lipid peroxidation [31]. Enzymatic pretreatment in oil extraction promotes the hydrolysis of cell walls, releasing phenolic compounds and enhancing the bioactive components of the oil. This process, by reducing the complexation of phenolic compounds with seed polysaccharides, facilitates their transfer into the oil phase [32].

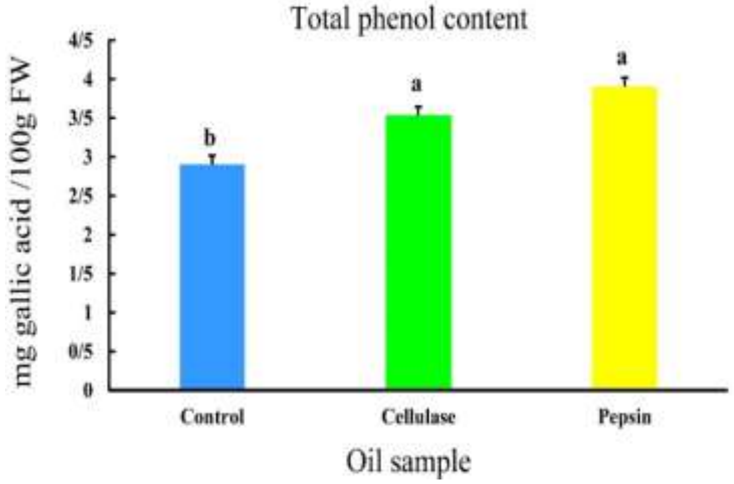

Figure 4. Total phenolic content of extracted sesame oil. The mentioned values are the average of 3 repetitions ± standard error. Similar letters indicate no significant difference between the means.

3.5. Changes in vitamin E content

The results of the comparison of the mean Vitamin E content in sesame oil extracted by different methods showed that the Vitamin E levels in oils extracted using cellulase and pepsin enzymes increased compared to the control sample ($p < 0.05$) (Figure 5). Vitamin E, which includes tocopherols and tocotrienols, prevents oxidative degradation through its antioxidant properties and stabilizes oils and fats [33]. During enzymatic extraction, the hydrolysis of the seed cell walls leads to the increased release of tocopherols and phenolic compounds, thereby enhancing the bioactive components of the oil. This increase is likely due to the reduction of complexes formed between bioactive compounds and seed polysaccharides, facilitating their transfer into the oil phase [30]. Additionally, pepsin enzyme increases the Vitamin E content by breaking the bonds between Vitamin E and proteins [34].

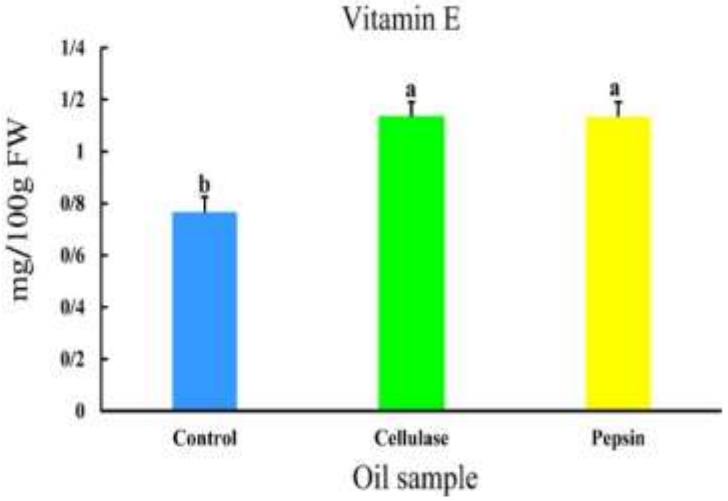

Figure 5. Vitamin E content of extracted sesame oil. The mentioned values are the average of 3 repetitions ± standard error. Similar letters indicate no significant difference between the means.

3. Changes in Soap Number

The comparison of the soap number of sesame oil extracted by different methods showed no significant difference in the soap number of sesame oils extracted with cellulase and pepsin enzymes compared to the control sample ($p > 0.05$) (Figure 6). The soap number is a key indicator used to identify oils and fats, estimating the average chain length of fatty acids [35]. This index has an inverse relationship with the fatty acid chain length; a decrease in the soap number indicates an increase in the chain length of fatty acids. Long-chain fatty acids, due to their higher molecular weight, have fewer molecules per gram of oil and therefore require less alkali for saponification [36]. Different extraction methods did not significantly affect the soap number because the fatty acid composition and chain length in oils with oleic-linoleic acid profiles are almost identical [12]. The aqueous-enzymatic extraction method, by hydrolyzing the cell wall and oleosome membrane, releases the oil but does not change the fatty acid composition or soap number [37].

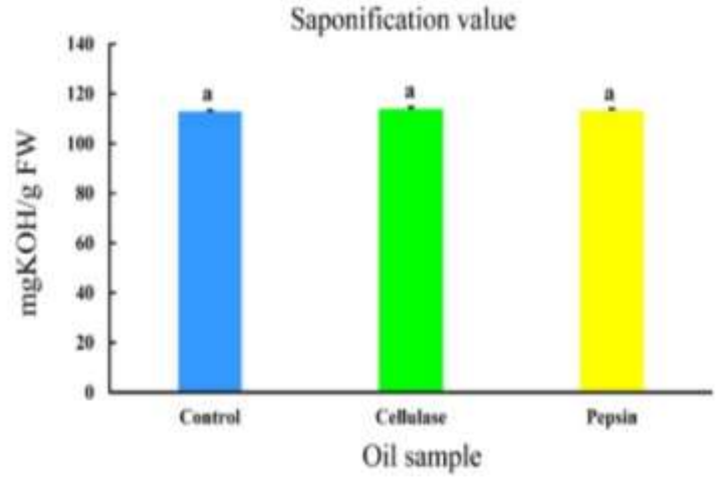

Figure 6. Saponification value of extracted sesame oil. The mentioned values are the average of 3 repetitions ± standard error. Similar letters indicate no significant difference between the means.

3.4. Changes in Acid Value

The comparison of acid values of sesame oil extracted by different methods showed no significant difference in the acid values of sesame oils extracted with cellulase and pepsin enzymes compared to the control sample ($p > 0.05$) (Figure 7). Free fatty acids are secondary compounds in edible oils, and their increase negatively affects oil quality, including aroma, appearance, and stability. Therefore, their levels must remain within standard limits. Low free fatty acid levels indicate the absence of hydrolytic reactions that could cause rancidity and triglyceride breakdown. The enzymatically extracted oil, due to its low free fatty acid content and an average of 92.11% acylglycerols, confirms the high efficiency of the aqueous-enzymatic extraction method in preserving and enhancing oil quality [38]. In this method, the short extraction time and low temperature are insufficient for hydrolyzing triglycerides into smaller components, thus the acid value remains unchanged [39].

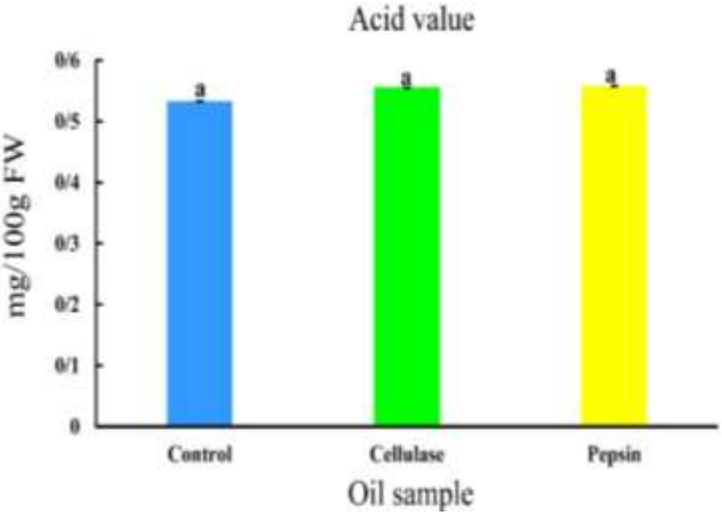

Figure 7. Acid value of extracted sesame oil. The mentioned values are the average of 3 repetitions ± standard error. Similar letters indicate no significant difference between the means.

3.5. Changes in iodine value

The comparison of iodine values of sesame oil extracted by different methods showed no significant difference in the iodine values of sesame oils extracted with cellulase and pepsin enzymes compared to the control sample ($p > 0.05$) (Figure 8). The iodine value is an index used to measure the degree of unsaturation and the number of double bonds in fats and oils. A decrease in iodine value indicates a reduction in double bonds and increased oil oxidation. This value provides information about the structure, nutritional value, and oxidative stability of oils. Higher iodine values (greater unsaturation) indicate increased susceptibility to oxidation [40]. Iodine value is typically used to evaluate oil quality and is not affected by extraction methods [12]. It depends only on the seed type [41].

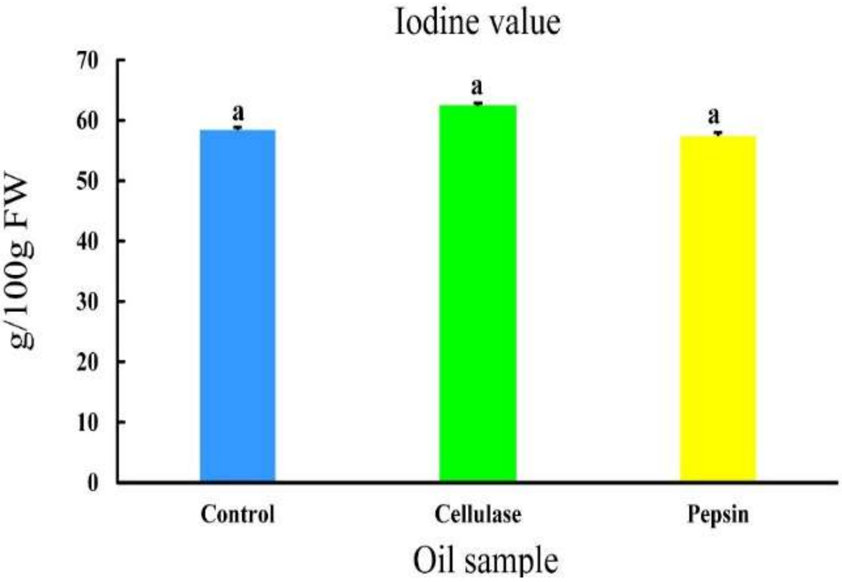

Figure 8. The Iodine value of extracted sesame oil. The mentioned values are the average of 3 repetitions ± standard error. Similar letters indicate no significant difference between the means.

3.6. Changes in unsaponifiable matter

The comparison of unsaponifiable matter in sesame oil extracted by different methods showed no significant difference in unsaponifiable content between sesame oils extracted with cellulase and pepsin enzymes and the control sample ($p > 0.05$) (Figure 9). In enzymatic extraction, reactions such as hydrolysis of proteins and carbohydrates in seeds occur, which can increase unsaponifiable matter in the oil [42]. Compounds such as phenolics, lignans, sterols, tocopherols, and hydrocarbons are considered unsaponifiable and have significant importance in oils [43]. In this study, no significant difference was observed in the amount of unsaponifiable matter extracted by different methods, indicating these compounds were not affected by the extraction technique. Moreover, the enzyme composition and process conditions in aqueous-enzymatic extraction significantly influence the amount of unsaponifiable matter extracted [12].

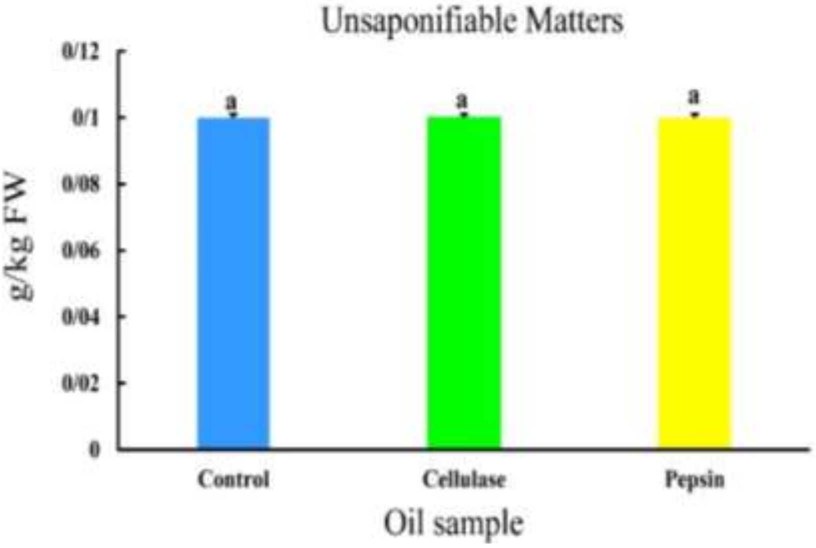

Figure 9. Unsaponifiable matter of extracted sesame oil. The mentioned values are the average of 3 repetitions ± standard error. Similar letters indicate no significant difference between the means.

3.7. Changes in peroxide value

The comparison of peroxide values of sesame oil extracted by different methods showed a decrease in peroxide value in sesame oils extracted with cellulase and pepsin enzymes compared to the control sample ($p > 0.05$) (Figure 10). Peroxide value indicates the presence of hydroperoxide compounds formed by oxidation of edible lipids and serves as a primary quality assessment measure of oils. Sesame oil naturally has a low peroxide value and high oxidative stability, due to the presence of natural antioxidants such as sesamol and sesamolin and its high antioxidant activity [22]. The enzymatic extraction method, by increasing bioactive compounds in the oil, enhances oxidative stability and leads to a reduction in peroxide value. The mild temperature (40°C) used during extraction also helps maintain oil stability against oxidation [44]. Protease enzymes, by hydrolyzing peptide bonds in proteins, increase the oil's antioxidant capacity, thereby reducing peroxide value [45]. Additionally, enzymatic extraction with cellulase breaks down the cell wall, releasing more bioactive compounds (such as phenolics, tocopherols, carotenoids, xanthophylls, and chlorophylls) into the oil phase, which contribute to lowering peroxide value due to their antioxidant properties [30].

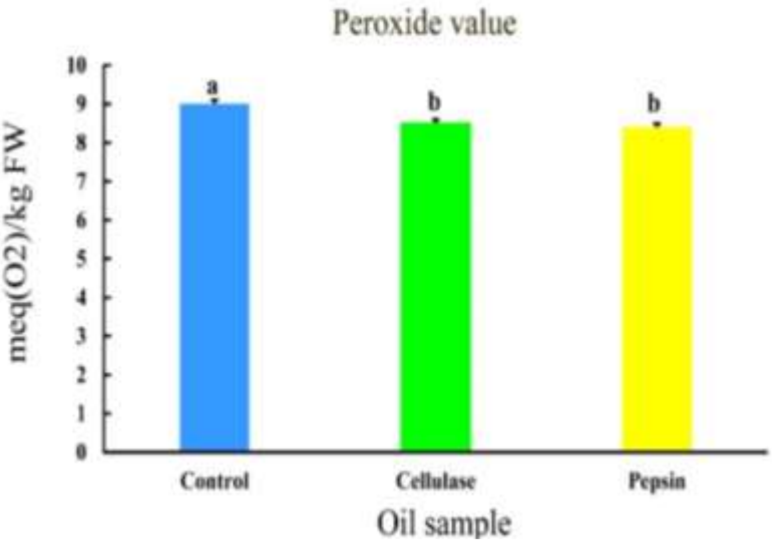

Figure 10. Peroxide value content of extracted sesame oil. The mentioned values are the average of 3 repetitions ± standard error. Similar letters indicate no significant difference between the means.

3.8. Changes in anisidine value

The comparison of anisidine values of sesame oil extracted by different methods showed a decrease in anisidine value in sesame oils extracted with cellulase and pepsin enzymes compared to the control sample (*p* > 0.05) (Figure 11). Anisidine value is an important index for controlling and assessing the quality of edible oils and indicates secondary oxidation or oil rancidity. It reflects the odor and stage of oil oxidation. A decrease in anisidine value indicates lower oxidative spoilage and absence of unpleasant rancid compounds [6]. Enzymatic extraction methods increase phenolic compounds by breaking down the seed cell wall structure. Phenolic compounds in enzymatically extracted oil act as antioxidants and reduce anisidine value. This method increases oil resistance to oxidation due to higher extraction of phenolic and tocopherol compounds [12].

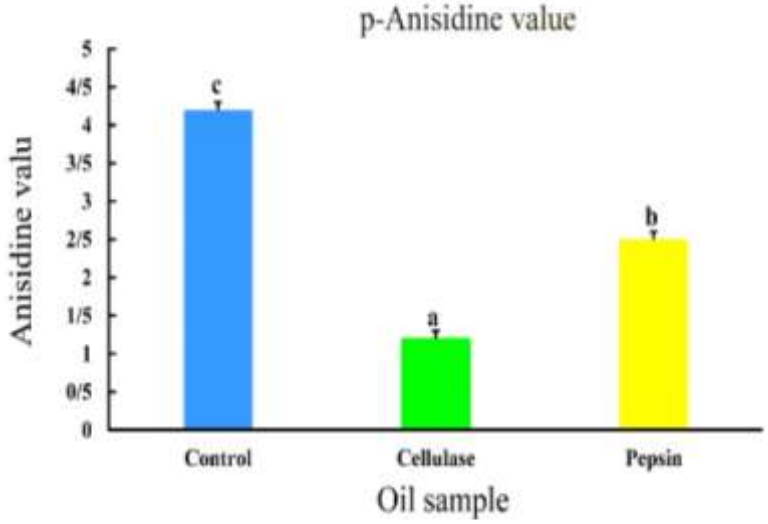

Figure 11. p-Anisidine value content of extracted sesame oil. The mentioned values are the average of 3 repetitions ± standard error. Similar letters indicate no significant difference between the means.

4-Conclusion

The results of this study demonstrate that the use of the aqueous-enzymatic extraction method employing cellulase and pepsin enzymes has a significant impact on improving the extraction yield of sesame oil and enhancing its quality parameters. This method not only increases the oil extraction efficiency but also preserves the main compounds present in the oil, such as essential fatty acids, positively affecting the final product quality. Additionally, the oxidative stability of the oil is noticeably improved, and the content of bioactive compounds, such as total phenolics, is increased, which contributes to strengthening the antioxidant properties and nutritional value of the oil. The reduction in anisidine value further indicates improved oxidative characteristics and a decrease in lipid degradation during the extraction process. These findings highlight that the aqueous-enzymatic method, especially with the enzymes used, can serve as an innovative, efficient, and environmentally friendly technology for extracting high-quality sesame oil. This method reduces the reliance on chemical solvents and minimizes environmental waste, making it not only economically viable but also environmentally commendable.

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مقاله علمی_پژوهشی

بررسی اثر استخراج آبی-آنزیمی با استفاده از آنزیم های سلولاز و پپسین بر ترکیبات فیتوشیمیایی و خواص فیزیکو شیمیایی روغن کنجد

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چکیده

کنجد (*Sesamum indicum* L.) به دلیل دارا بودن ترکیبات فیتوشیمیایی ارزشمند مانند لیگنان‌ها و اسیدهای چرب غیراشباع، از اهمیت تغذیه‌ای و صنعتی بالایی برخوردار است. در این پژوهش، تأثیر روش آبی-آنزیمی با آنزیم سلولاز و پپسین بر استخراج روغن کنجد و شاخص‌های کیفی آن بررسی شد. نتایج نشان داد که استفاده از آنزیم‌های سلولاز و پپسین نه تنها بازده استخراج را افزایش می‌دهد، بلکه ترکیبات زیست‌فعال نظیر فنول کل، ویتامین E و ظرفیت آنتی اکسیدانی را نیز بهبود می‌بخشد. شاخص‌های کیفی شامل عدد اسیدی و عدد صابونی و مواد غیر قابل صابونی تغییر محسوسی نشان ندادند و در محدوده استاندارد باقی ماندند. عدد یدی نیز بدون تغییر ماند که نشان‌دهنده حفظ ترکیب اسیدهای چرب اصلی مانند اولئیک اسید، لینولئیک اسید و پالمیتیک اسید است. این پایداری به دلیل روش ملایم آبی-آنزیمی است که از تخریب ترکیبات جلوگیری می‌کند. از سوی دیگر، عدد پراکسید کاهش یافت و به ۸ میلی‌اکی‌والان بر کیلوگرم رسید که نشان‌دهنده بهبود پایداری اکسیداتیو روغن است. میزان ترکیبات غیر صابونی نیز افزایش معناداری نشان داد که به ارتقای کیفیت کلی روغن کمک می‌کند. علاوه بر این، عدد آنیزیدین، که نشان‌دهنده اکسیداسیون ثانویه روغن است، کاهش یافت، که کاهش این عدد نیز حکایت از کاهش اکسیداسیون اولیه و بهبود پایداری روغن دارد. این نتایج تأیید می‌کند که روش آبی-آنزیمی می‌تواند به‌عنوان یک رویکرد سبز، کارآمد و با اثرات زیست‌محیطی کمتر برای استخراج روغن کنجد استفاده شود.