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Freeze assisted-aqueous extraction of rapeseed oil using Tween 20

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Abstract

The traditional oil extraction methods often rely on organic solvents, raising environmental and health concerns, while aqueous extraction offers a potentially greener alternative. Therefore, the present study attempted to investigate the feasibility of the aqueous extraction of oil from rapeseed (canola) using a food-grade surfactant (Tween 20). The physicochemical properties of the rapeseed (moisture, ash, protein, and oil content) were first determined. The effects of key parameters, including seed-to-water ratio, Tween 20 concentration, pH, and pre-treatment temperature and time, were evaluated using a one-factor-at-a-time approach. According to our findings, the optimal oil extraction conditions were as follows: seed-to-water ratio 1:10, Tween 20 concentration 1.4 wt%, pH 12.0, pre-treatment temperature/time combination 190°C/30 min. Under the optimal conditions, an oil extraction yield of 50.5% was achieved. The impact of the pre-treatment step (before or after grinding the seeds) also showed that thermal treatment (190°C/30 min) prior to grinding was much more efficient. Additionally, freezing and rapid defrosting treatments yielded comparable results to the optimized aqueous extraction. The results suggest that aqueous extraction with Tween 20, particularly with optimized pre-treatment, offers a viable alternative to solvent-based methods, although further optimization is needed to match the higher yield of the solvent extraction method.

Keywords: Aqueous extraction, Freeze-thaw, Microemulsion, Rapeseed (canola) oil, Surfactant, Tween 20.

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Contents

Contribution of this paper to the literature
The present study, for the first time, examined the capability of Tween 20 in recovering oil from crushed rapeseeds under optimized conditions. The findings proved the potential effectiveness of Tween 20 under alkaline pH and very low concentrations to recover up to 50% of rapeseed oil.

1. Introduction

Vegetable oils are used in numerous industries, including snacks, cakes, margarine, biscuits, cosmetics, detergents, plastics, etc. With a 42% share of the global market, vegetable oils are among the most important agricultural products (Hamm et al. [1]). Shahidi [2] noted that rapeseed normally contains 38-44% oil, which can be extracted by mechanical pressing (60% yield) and solvent extraction, primarily using hexane [2]. It is essential that an extraction process is used as part of food processing in order to obtain a particular compound from raw materials [3]. Generally speaking, traditional, industrial, and novel methods are all used to extract oil from oilseeds. As part of traditional methods, seeds are prepared and pressed by hand, which involves manual labor. Meanwhile, industrial methods utilize chemical and mechanical processes. By using cutting-edge technologies, the quantity and quality of oil extraction can potentially be improved. However, each of these approaches has advantages and disadvantages. Therefore, a number of factors are usually considered when choosing the most appropriate method of oil extraction, including the type of seeds, oil content, volume of production, quality of oil required, intended use of the oil, and the availability of appropriate technology [4]. The novel extraction methods, namely: Supercritical Fluid Extraction (SFE), Ionic Liquid Extraction (IL), Deep Eutectic Solvents (DES), Pressurized Liquid Extraction (PLE), Supercritical Liquid Extraction (SLE), Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), and Aqueous Extraction Processing (AEP) [5-7], can potentially offer less extraction time, use environmentally friendly solvents, reduce solvent consumption, provide full automation, and improve reliability [8, 9].

The AEP, as an innovative and sustainable method, is already used to extract edible oils from sesame seeds, babassu fruit, and other agricultural products. A water-based extraction process has a lower environmental impact and energy cost than chemical solvents, such as hexane. An aqueous extraction procedure consists of several steps, including pretreatment (milling), the formation of an emulsion, and the separation of the oil from the emulsion using a variety of methods [10]. Three common extraction methods are enzyme-assisted extraction (EAAE), ultrasound-assisted extraction (UAE), and surfactant-assisted extraction (SAAEP). In the latter, by using an appropriate surfactant at an optimized level, the oil extraction is conducted [11, 12]. A surfactant molecule has two distinct properties: a hydrophilic (water-loving) head and a hydrophobic (oil-loving) tail. This 'amphiphilic' structure allows it to position itself at the interface between oil and water, reducing the surface tension between the two and enabling them to mix together [13, 14]. The extraction process works by having surfactant molecules gather at the boundary between oil and water, which lowers the surface tension between them. The best results occur when the amount of surfactant reaches the ideal level to form micelles [15]. According to existing reports (mostly non-food), oil removal from various matrices can be achieved by three mechanisms, namely: roll-up or roll-back, snap-off (also known as necking and drawing/emulsification/solubilization), and diffusion or micellar solubilization [13, 14]. In roll-up or roll-back, when surfactant adsorbs at the oil–water interface, it increases the contact angle between the oil and the solid surface and, at the same time, decreases the interfacial tension (IFT) between oil and surfactant solution. Owing to the IFT reduction between the oil and surfactant solution, as well as the solid matrix and surfactant solution, the contact angle of the attached oil droplet increases. In ideal circumstances, the contact angle reaches ~180°, so that the oil droplet completely detaches from the solid matrix. In contrast to the roll-up mechanism, the snap-off mechanism is applicable when the contact angle is not high enough to detach an oil droplet from the solid surface. Therefore, an incomplete detachment of the elongated oil droplets occurs, and the detached oil droplets are then emulsified. Finally, in the diffusion or micellar solubilization mechanism, the surfactant micelles dissociate near the oil–water interface and then reform around the oil phase, essentially partitioning the oil into the aqueous phase [13].

Based on the origin of surfactants, they can be classified either as natural or synthetic, as well as being classified as food-grade or non-food-grade ones. Even though long-chain (or 'extended') non-food-grade surfactants are often very effective in forming emulsions and microemulsions, their toxicity and environmental concerns make them unsuitable for use in food applications [13, 16]. Tweens, which are also called polyoxyethylene sorbitan esters (polysorbates), are nonionic, hydrophilic food-grade emulsifiers. Sorbitan fatty acid esters are synthesized by adding ethylene oxide. These include Tween 20, 40, 60, 65, 80, and 83. Their solubility varies; Tween 20 is more hydrophilic than Tween 60 and Tween 80, while Tween 65 is the most lipophilic [13]. A yellow liquid made by the ethoxylation of sorbitan monolaurate is known as Tween 20 ($C_{58}H_{114}O_{26}$). It is soluble in both water and ethanol. Food manufacturers frequently use it in ice cream, baked goods, and salad dressings [17]. In addition, in order to reduce surface tension, Tween 20 can also be utilized to extract oil from seeds [14, 18].

By now, several reports have shown the effectiveness of Tween 20 on the extraction of oils. In the presence of ethanol, Tween 20 formed the largest microemulsion region and extracted 69% of the lipid components from bee propolis [19]. As a result of its use in combination with maceration techniques, Tween 20 increased the essential oil yield from rose by 94%, emphasizing its role in increasing cell wall permeability and softening tissues [20]. Using Tween 20 (0.5%) and heating (60°C for one hour), 80% oil (rich in unsaturated fatty acids and antioxidants) was extracted from oilseeds [21]. Similarly, Tween 20 (1.2% w/w) at pH 10 was able to extract 76.1% of peanut oil [22]. Using Tween 20 and Span 20 (1%), 80% of the oil was extracted while the bioactive compounds such as phenols and unsaturated fatty acids were preserved [15]. Furthermore, cyclic extraction of camellia seed oil was facilitated by Tween 20 at 70°C and pH 9.0, whilst interfacial tensions were reduced and overall yields increased [23]. Despite its higher hydrophobicity and ability to displace oleosin proteins, Tween 20 extracted 60% of walnut oil, whereas with Span 20, 91.2% of oil was extracted [24]. Lastly, Hasanah et al. [25] found that Tween 20 (0.5%) in combination with ultrasound treatment recovered 18.54% of rice bran oil [25]. Based on these studies, it is evident that Tween 20 can potentially improve oil extraction efficiency, quality, and sustainability.

Considering the potential capability of SAE and Tween 20, it is evident that Tween 20 can reduce interfacial tension and facilitate micelle formation, making it a promising candidate for an efficient and green oil extraction

method. This study aimed to evaluate and optimize the effects of different concentrations of Tween 20, along with the rapeseed-to-water ratio, temperature, pH, and freezing-defrosting treatments on the oil extraction yield from crushed rapeseeds.

2. Materials and Methods

2.1. Materials

The materials used in this study included Tween 20 (Thermo Fisher Scientific, USA), rapeseeds (obtained from Oksidaneh Oil Extraction Plant, Tehran, Iran), hexane (Dr. Mojallali Chemical Industries, Tehran, Iran), sodium hydroxide, and hydrochloric acid (Merck Chemical Industries, Germany). Double-distilled water was used for all sample preparations.

2.2. Methods

2.2.1. Determination of Selected Characteristics of Rapeseeds

The protein content of rapeseeds was determined via the Kjeldahl method using a Kjeltec 8400 (Foss, Sweden). A 0.3 g sample was mixed with 1 g of catalyst and 7 mL of concentrated sulfuric acid. Digestion was performed at 360°C for 3 hours. The resulting ammonia was captured in a titration solution and titrated against a standard acid to determine nitrogen content. Crude protein was calculated by multiplying the nitrogen content by 6.25 (the standard conversion factor for canola).

For ash content measurement, rapeseeds were cleaned, dried, and finely ground. A 2.0 g sample was weighed (Vibra AB 323, Japan) into a porcelain crucible. The sample was burned in a muffle furnace (Heareus, Germany) at 500-600°C for several hours. After cooling in a desiccator, the crucible and ash were reweighed. Ash content was calculated as the percentage difference between the initial and final weights, based on the dry sample weight [26].

In order to measure the moisture content, 5.0 g of the sample was accurately weighed and placed in the sample chamber of a moisture analyzer (Sartorius MA35, Germany). During the analysis, the instrument continuously monitored the sample weight until a constant weight was reached, indicating the complete removal of moisture. The instrument then automatically calculated and displayed the moisture content percentage of the sample.

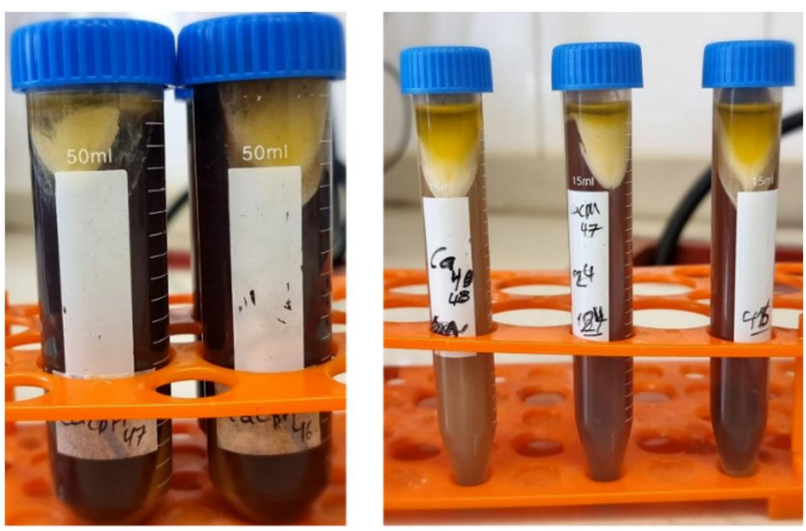
The oil of ground rapeseeds was extracted with hexane in a Soxhlet apparatus (GBG, Iran) at 60°C for 6 hours. The solvent was then removed using a rotary evaporator (IKA KS 4000i, Germany) under reduced pressure. The oil content was calculated using the equation [27].

2.2.2. Surfactant-Assisted Aqueous Extraction

An aqueous method assisted by Tween 20 was used to investigate the process of oil extraction from crushed rapeseeds. The seeds were obtained from an oil processing plant and stored at 5°C. Prior to the experiment, the seeds were cleaned and then subjected to a heat pre-treatment at temperatures ranging from 170 to 210°C. After cooling, the seeds were ground using a high-speed mill (Toos Shekan Khorasan, Iran) to reduce the particle size to < 400 µm. The concentration of Tween 20 solution (Thermo Fisher Scientific, USA) ranged from 0.8 to 1.6% (wt). The solution at various ratios (1:4 to 1:14 w/v) was added to the ground seeds. Mixtures were homogenized using a vortex mixer (Heidolph Reax Top, Germany), and the pH was adjusted (8.0 to 13.0) by adding KOH (2N) using a pH meter (Metrohm 827, Switzerland). The samples were then incubated for 40 min at 50°C while shaking at 165 rpm (IKA KS 4000i, Germany). They were then centrifuged (Sigma 3-30K, Germany) at 4000g and 25°C for 15 min to separate the oil, emulsion, and upper oil phases (Figure 1A). In order to improve the separation of the free oil, the samples were centrifuged (Figure 1B) again (5 min at 25°C, 4000g). Lastly, the separated oil was carefully weighed to determine its extraction yield (Figure 1). The following equation was used to calculate the free oil yield:

$$\text{Free oil content (\%)} = \frac{(A - B)}{(C \times D)} \times 100$$

Where: A: Weight of the tube with oil (g); B: Weight of the empty tube (g); C: Weight of the crushed rapeseed (g); D: Oil content of the crushed rapeseed (expressed as a decimal fraction).



(A) (B)

Figure 1. Demonstration of the separation of A) oil, emulsion, and solid phases and B) emulsion and free oil phases by centrifugation.

2.2.3. Extraction Yield Optimization (OFAT Approach)

To optimize the oil extraction yield, a one-factor-at-a-time (OFAT) approach was employed. The effects of pre-treatment temperature (170–210°C), Tween 20 concentration (0.8–1.6% wt), pH (8–13), crushed rapeseed: water ratio (1:4 to 1:14 w/v), and thermal pre-treatment time (20–40 min) were investigated. All other factors were held constant during each experiment. For each factor, several levels were tested. For example, when investigating the effect of the crushed rapeseed: water ratio, the following parameters were held constant: Tween 20 (1.2% wt), pH (10), pre-treatment temperature (190°C), and pre-treatment time (30 min). In addition, when investigating the effect of Tween 20 concentration, the following parameters were held constant: rapeseed: water ratio (1:10), pH (10), pre-treatment temperature (190°C), and pre-treatment time (30 min). A similar procedure was followed for the remaining factors, maintaining a similar level of control over the constant variables.

2.2.4. Freezing and Thawing

In order to investigate the effects of freezing and thawing on the yield of oil extraction, upon centrifugation of the samples from the previous step, they were frozen (-18°C for 24 h). Then, the effects of rapid (80°C, 10 min) and conventional (25°C, 4 h) thawing methods were tested. As soon as they were thawed, they underwent two additional centrifugations to ensure complete separation of the oil phase from the emulsion layer. The final extraction yield was calculated based on the weight of the extracted oil.

2.2.5. Statistical Analysis

Treatments were arranged in a complete factorial random design with three replicates. Data analysis was conducted using SAS JMP Statistical Discovery Pro 16.0 software, employing ANOVA to assess mean differences. EXCEL software was utilized to draw curves and distinguish their fittings with mathematical models if needed.

3. Results and Discussion

3.1. Chemical Composition of Rapeseeds

The average moisture, protein, oil, and ash contents of rapeseeds, measured in triplicate, were 4.88 ± 0.14%, 24.89 ± 0.02% (on a dry matter basis), 38.11 ± 0.77%, and 3.25 ± 0.01%, respectively. By subtracting the sum of these components from 100, the carbohydrate content was around 28.87 ± 0.06% (dry weight).

3.2. Comparing the Effects of Various Factors on Oil Extraction Yield

To evaluate the effects of various factors on oil extraction yield from rapeseeds, a one-factor-at-a-time (OFAT) approach was used. It was possible to determine the independent effects of each factor using this approach, including the ratio of crushed rapeseeds to water, surfactant concentration, pH, temperature, and pretreatment time. Utilizing this method, the optimal conditions for achieving maximum oil extraction yield were identified.

3.3. Effect of Seed: Water Ratio (Tween 1.2%, pH: 10, Pretreatment Temp 190°C, 30 Min)

Statistical analysis of the data (Figures 1, 2) showed that different seed-to-water ratios had a significant effect on oil recovery ($p < 0.05$). Furthermore, based on the comparison of means, the highest oil recovery (50.96%) was observed at a ratio of 1:10, while the lowest oil recovery was observed at a ratio of 1:4. Additionally, there was no significant difference in oil recovery between the 1:4 and 1:6 ratios. It appears that as the oil content of the seeds decreases, the seed-to-water ratio required for optimal oil extraction increases. Zhang and Wang [22] also reported a direct relationship between oil content and the required seed-to-water ratio, where the total oil content of peanuts was 55%, and the optimal ratio was 1:4. However, in the case of rice bran oil extraction, as its total oil content was low (15%), the optimal seed-to-water ratio was 1.5:10 [22, 28].

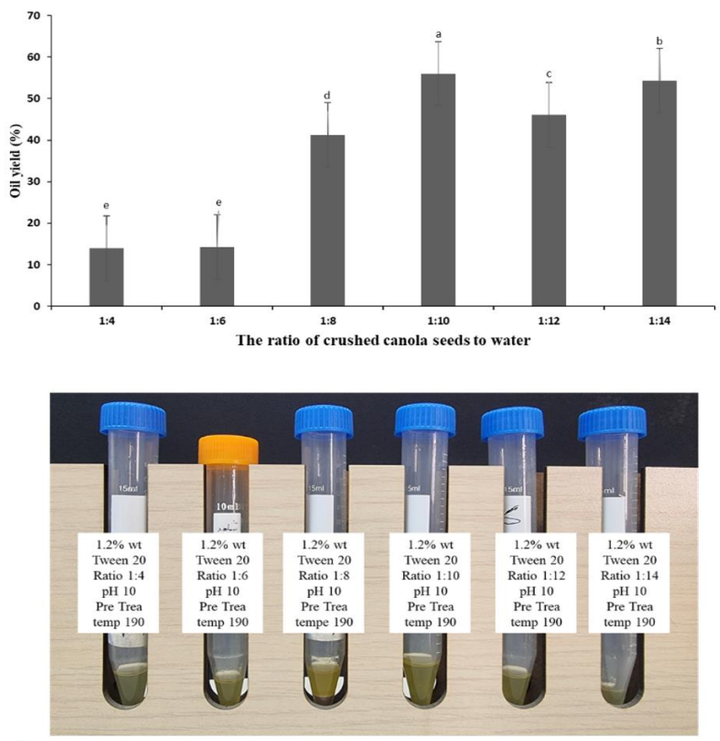


Figure 2. Comparison of the effect of rapeseed: water ratio on oil recovery under constant Tween 20 concentration (1.2 wt%) and pH 10.

3.4. Effect of Tween 20 Conc (Seed: Water Ratio 1:10, pH 10, Pretreatment Temp 190°C, 30 Min)

As can be seen (Figure 3), Tween 20 concentration had a significant effect on oil extraction yield ($p<0.05$). The highest oil extraction yield (52.23%) was obtained at 1.4% wt of Tween 20, while the lowest yield (20%) was attained at 0.8% wt of Tween 20.

As a result, no significant differences were observed between 1.2% and 1.6% wt concentrations of Tween 20. Several studies have shown the effects of Tween 20 concentration (1– 2% wt) on the enhancement of the oil extraction efficiency through aqueous extraction methods. Zhang and Wang [22] achieved a 76% oil extraction yield from peanuts using 1.2% of Tween 20. Surlehan et al. [15] extracted 80% of oil from passion fruit seeds using 1% of Tween 20.

In the same context, Geng et al. [24] reported a 58% oil extraction yield from walnut seeds using 1.7% wt Tween 20. Surfactants aid in oil removal by disrupting the protein matrix that surrounds oil bodies within oilseeds [29]. Additionally, they form channels filled with water between the oil phase and the solid surface [30]. A gel layer occurs at the interface between water and solid, as a result, water molecules penetrate the oil-water interface. Surfactant-stabilized oil microdroplets undergo extraction by mass transfer of extractant into the droplets, followed by upward movement within the aqueous phase [31].

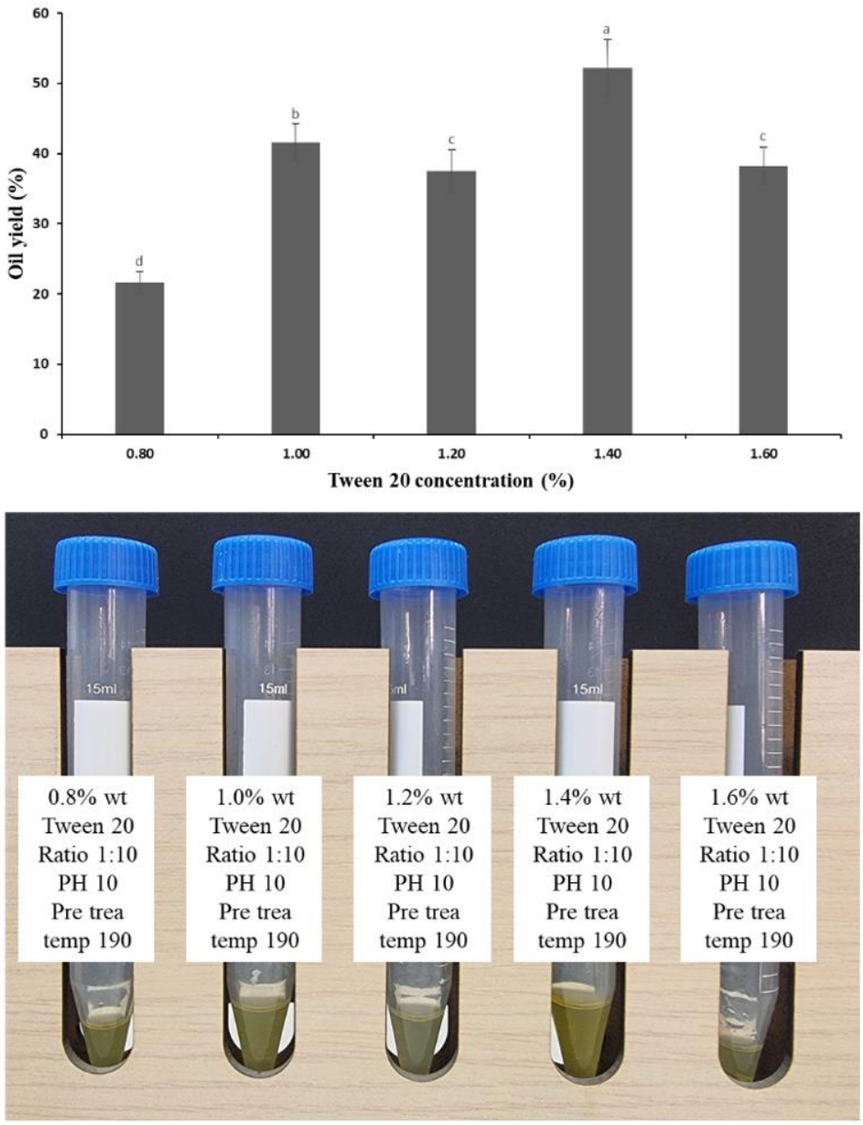


Figure 3. Comparison of the effect of Tween 20 concentration on the amount of oil extracted from rapeseed (Rapeseed: Water 1:10 and pH 10).

3.5. Effect of pH (Seed: Water Ratio 1:10, Tween 1.4%, Pretreatment Temp 190°C, 30 Min)

The results of the statistical analysis showed that pH had a significant impact on oil extraction yields ($p<0.05$). According to Figure 4, the maximum yield (54.76%) was obtained at pH 12.0, while the minimum (36.4%) was obtained at pH 11.0. Oil extraction yields were not significantly different between pH values of 9.0, 11.0, and 13.0. Furthermore, other studies have demonstrated that by using an alkaline pH, the oil extraction yield can be increased. Hanmoungjai et al. [28], for example, successfully extracted 80% of the oil from rice bran using an aqueous extraction method at a pH of 12.0 [28]. Canola oil extraction with Tween 20 is likely to be influenced by pH by reducing protein adsorption at the interface, which destabilizes emulsions and increases oil yield at higher pH levels. With alkaline pH, Tween 20 has an advantage over proteins in competition for the interfacial layer, which allows it to adsorb and dominate the interface more rapidly [22].

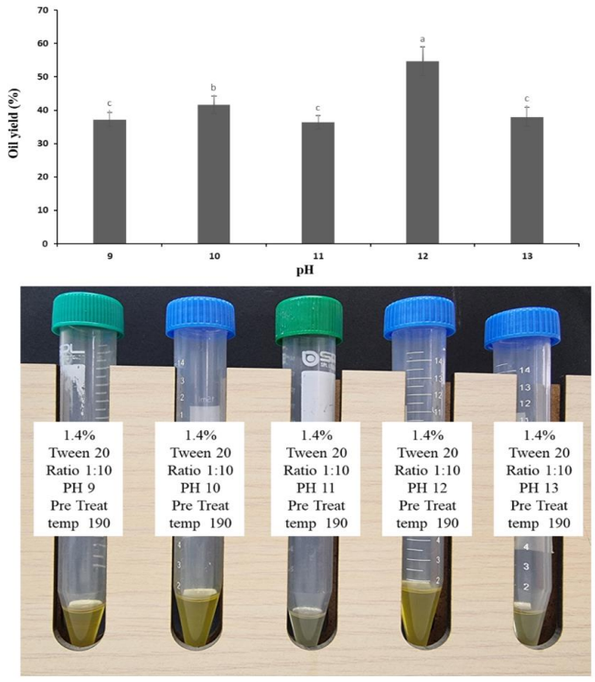


Figure 4. Comparison of the effect of aqueous pH on oil recovery from rapeseed (rapeseed: water ratio 1:10, and Tween 20 concentration 1.4% wt).

3.6. Effect of Pretreatment Temperature (Seed: Water Ratio 1:10, Tween 1.4%, pH 12, 30 Min)

Regarding the effect of pretreatment temperature on rapeseed oil recovery, the present study found a significant correlation between temperature and extraction yield ($p<0.05$). According to Figure 5, 190°C yielded the highest yield (50.5%), while 120°C yielded the lowest (14.6%). These findings indicate that temperature plays an important role in determining the efficiency of oil extraction. Studies on oil extraction from sesame seeds have also demonstrated that higher temperatures resulted in higher yields of oil [32]. The extraction of oil can be significantly enhanced by pretreatment at 180°C. According to Jia et al. [33], germinated corn meal was four times more likely to yield oil after pretreatment at this temperature. The same was observed by Wang et al. [34] who observed a 40% increase in oil extraction from peanuts after a 180°C pretreatment. Oil extraction yields are increased when thermal treatment is conducted at temperatures exceeding 180°C. Chemical reaction kinetics is accelerated at higher temperatures, resulting in improved oil absorption. During this process, proteins are hydrated and swollen, which aids in the collection of soluble solids, reduces the formation of emulsions, and increases the amount of free oil recovered. During roasting, proteins become more hydrophilic, resulting in the aggregation and coalescence of oil bodies, as well as cell disruption, which facilitates the oil release [34, 35].

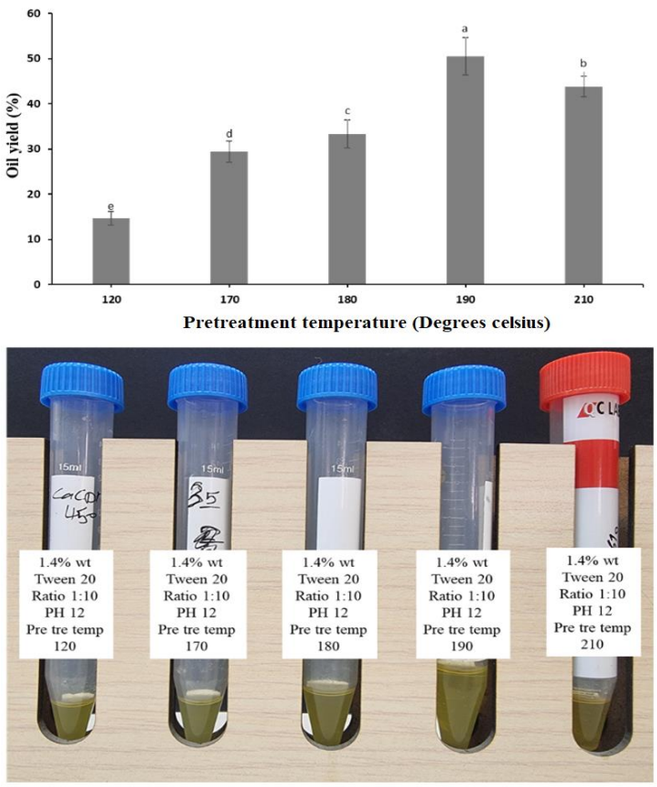


Figure 5. Comparison of the effect of pretreatment temperature on the oil recovery rate from rapeseed (rapeseed: water ratio 1:10, Tween 20 concentration 1.4% wt, pH 12.0, and thermal pretreatment duration 30 min).

3.7. Effect of Pretreatment Time (Seed: Water Ratio1:10, Tween1.4%, pH 12.0, Temp 190°C)

The present study demonstrated a significant influence ($p < 0.05$) of pretreatment time on oil extraction yield (Figure 6). A maximum yield of 50.4% was obtained after 30 minutes of pretreatment, compared to a minimum of 30.7% at 20 minutes. As a result, thermal pretreatment time was identified as an important factor in optimizing aqueous oil extraction, primarily through its effects on the structure of the cell wall and the subsequent release of oil from the cell. The oil extracted from peanuts and canola seeds was 93% and 95%, respectively, after the seeds were pretreated at 104°C for 35 minutes using extended surfactants [11].

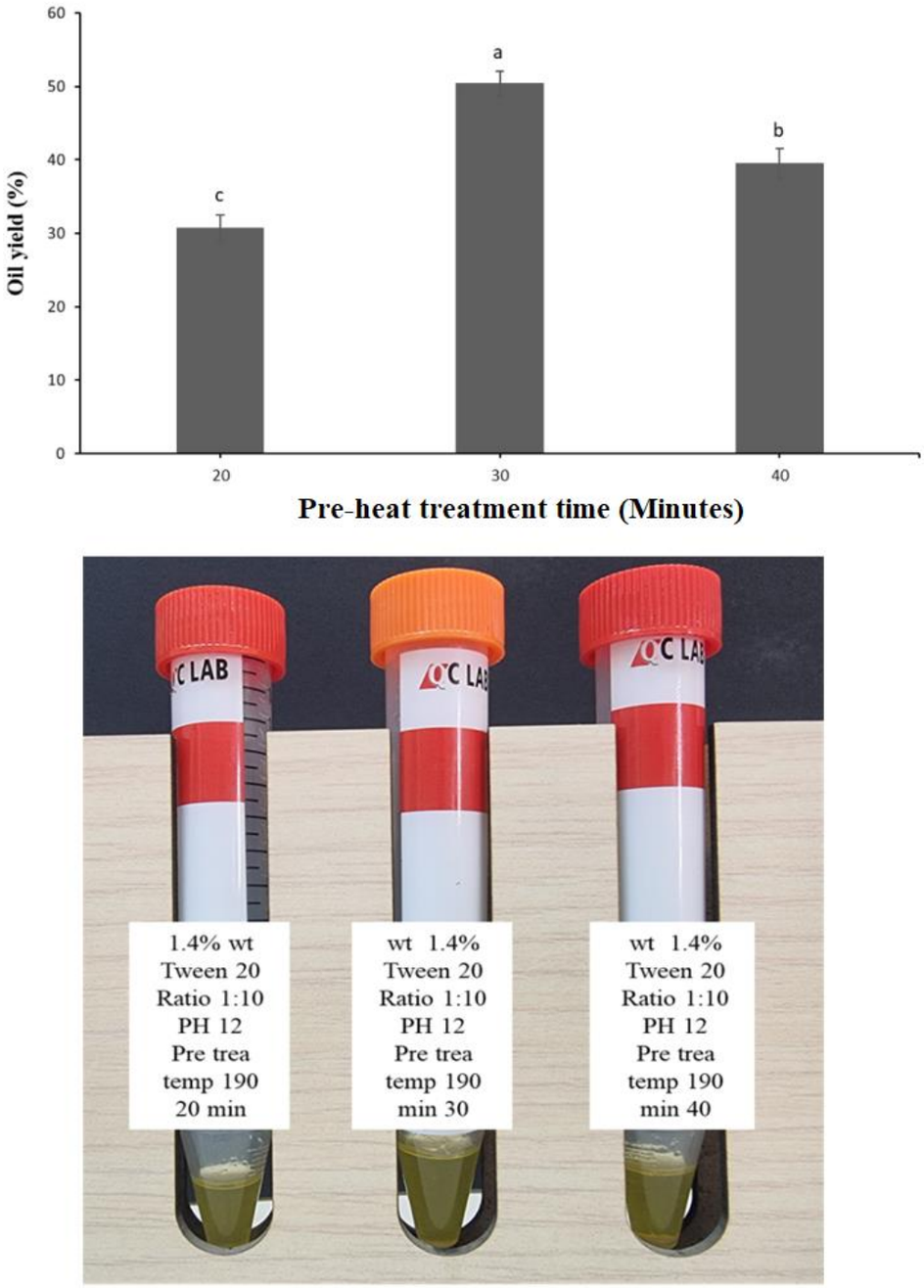


Figure 6. Comparison of the effect of the duration of thermal pretreatment of rapeseed on the oil extraction yield (rapeseed: water ratio 1:10, Tween 20 concentration 1.4%wt, pH 12.0, and temperature of 190°C).

3.8. Effect of Preheat Stage (Before or After Grinding the Seeds) and Temperature (170-210°C for 30 Min) Under Constant Condition (Seed: Water Ratio 1:10, Tween 1.4%, pH 12.0)

It can be seen (Figure 7) that both the stage of thermal pretreatment (before or after grinding) and the pretreatment temperature independently had a significant impact on the extraction of rapeseed oil yield. In addition, a significant effect ($p<0.05$) was observed between the pretreatment stage and temperature, indicating that they interact to influence each other. The highest oil extraction yield was obtained at 190°C when heat pretreatment was applied before grinding. This is likely due to the fact that, not only does reducing particle size increase the surface area for oil-solvent interaction, but also applying heat treatment before grinding leads to more extensive cell disruption, thereby facilitating oil release. This finding is in agreement with that reported by Sagili et al. [36], where the highest crude oil yield (28% from hemp seeds) was obtained with the smallest particles (0.25-0.5 mm). Shejawale et al. [37] also observed an increase in soybean oil extraction when the particle size was reduced, although only up to a certain level (0.239-0.353 mm). In addition, further reductions in particle size (to 0.129 and 0.122 mm) resulted in a reduction in yields as a result of reduced porosity and bed compaction. It is believed that the superior effectiveness of pre-grinding heat treatment is due to the fact that grinding can increase surface area but also cause physical damage to the seed matrix, which entraps oil, preventing its release. Alternatively, heat treatment before grinding increases oil accessibility and enhances extraction yields by disrupting the cellular structure more effectively.

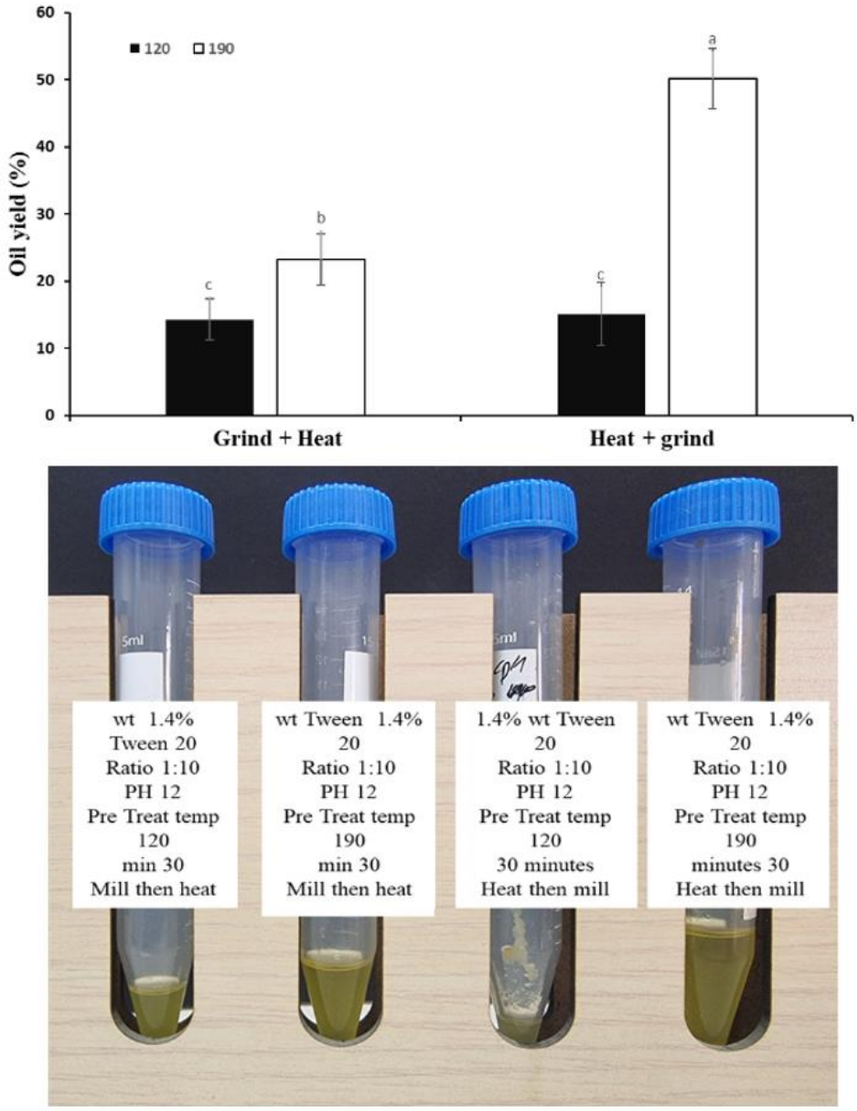


Figure 7. Comparison of the effect of the thermal pretreatment step (190 and 120°C) before grinding rapeseed and the temperature of thermal pretreatment on the oil extraction rate (Rapeseed: Water ratio 1:10, tween 20 concentration 1.4% wt, pH 12).

3.9. Effect of Freezing and Thawing (Seed: Water Ratio 1:10, Tween 1.4%, pH 12, Pretreatment Temp 190°C, 30 Min)

The freeze-thaw treatment yielded the second highest oil extraction rate (45.2%). Li et al. [38], by optimizing freeze-thaw conditions, recovered 82.28% free oil from soybean during the enzyme-assisted aqueous extraction process [38]. It was reported that freezing destabilized soybean oil emulsions and increased the free oil recovery from 3% to 22% by reducing oil droplet size [39]. Zhang et al. [40] also found that freeze-thaw cycles improved the yield and quality of tiger nut oil, with 6-8 cycles being considered optimal. According to Ghosh et al. [41], ice crystals formed during freezing disrupt the emulsion structure and encourage oil droplet coalescence. In addition, freezing reduces the emulsifying capacity by affecting the protein's secondary structure, resulting in the release of oil [38]. Furthermore, thawing can further disrupt the weakened emulsion network and aggregate the oil droplets, causing the trapped phases to be released. During freeze-thaw cycles, some surfactants also lose their efficacy [42]. In Table 1, it can be seen that Tween 20 effectively extracts oil from rapeseeds under all tested conditions. In the final separation process of oil and emulsion, pH primarily showed a significant influence. By changing the pH of the emulsion, the emulsion destabilized, thus improving oil recovery. While pH may influence the competition between Tween 20 and proteins during oil extraction, its primary function is to break the final emulsion and release the oil from the emulsion. According to Hao et al. [43], high pH reduces the stability of oil-in-water emulsions by altering surface tension and surfactant behavior. By altering surface properties and reducing repulsive forces between droplets, cations (Na^+, Ca^{2+}, Mg^{2+}) intensify this effect [43].

Table 1. Comparison of the effects of thawing conditions, pH, presence/absence of Tween 20 (0, 1.4% wt), and thermal pretreatment (25 or 190°C, 30 min) under constant seed: water ratio (1:10) on rapeseed oil extraction rate.

Treatment variables	**Thawing conditions**													
	Ambient temp (25°C, 4 h)							**High temp (80°C, 10 min)**						
Tween 20 (%)	0	0	1.4	1.4	1.4	1.4	0	0	0	1.4	1.4	1.4	1.4	0
pH	6.8	6.8	6.8	6.8	12.0	12.0	12.0	6.8	6.8	6.8	6.8	12.0	12.0	12.0
Pretreatment temp (°C)	25	190	190	25	25	190	190	25	190	190	25	25	190	190
Free oil recovered (%)	3.8e	3.1e	43.3b	26.7d	38.8c	45.2a	3.2e	2.9e	3.4e	44.5b	22.3d	27.5c	49.2a	3.2e

4. Conclusions

In this study, Tween 20-assisted aqueous extraction was examined as an alternative to traditional solvent-based extraction methods for extracting oil from crushed rapeseeds (canola seeds). Based on the results, this method can achieve a notable oil recovery of 50.4% under optimized conditions: a rapeseed-to-water ratio of 1:10, Tween 20 concentration of 1.4 wt%, pH 12.0, and a 30-minute thermal pretreatment at 190°C. Alkaline pH significantly increased oil recovery as it destabilizes emulsions and allows Tween 20 to dominate the oil and water interface. Likely, Tween 20, by reducing the interfacial tension and disrupting the protein matrix, facilitated the oil release. Furthermore, freeze-thaw treatment (freezing at -18°C for 24 hours, followed by rapid thawing at 80°C for 10 minutes) led to a 49% oil recovery rate, offering an alternative approach to thermal pretreatment. In addition, the study emphasizes the importance of thermal pretreatment before grinding in order to achieve better cell disruption and greater oil release compared with post-grinding treatment. Although it seems that wet-heat treatment should be much more effective than dry heat, which was tested in this study.

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