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Investigating the characteristics of pectin extracted from rapeseed meal in enzymatic hydrolysis method using Microwave-Assisted Extraction

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ABSTRACT

Pectin as a hydrocolloid located in the cell wall of plants can be a secondary product in food industry processing. Canola after de-oiling has high amounts of pectin. To extract pectin from rapeseed meal, the comparison method of enzymatic hydrolysis extraction was used with and without the presence of microwaves (with power of 600 W at four times zero, 1, 3, and 5 minutes). The effect of microwave irradiation time on extraction performance and physicochemical and mechanical characteristics of extracted pectin was investigated. Physical, mechanical, and chemical characteristics showed that the presence of the microwave process improved the functional properties of the extracted pectin and facilitated the extraction process ($p<0.5$). The highest yield of pectin extraction was 9.1% (W/W) in 5 minutes of radiation with 600 W power in the microwave-assisted process with 600 W power. This auxiliary process affected the degree of esterification and galacturonic acid content of pectin so that it can form a gel in the presence of low sugar amounts and is suitable in diet products. The content of galacturonic acid in all samples was higher than 60%, which indicates a high gel formation capacity. Pectin extracted during 5 minutes of microwave irradiation with 600 W power presented the best characteristics with the maximum content of galacturonic acid (76.51%), the highest emulsifying activity (58.01%), and emulsion stability (95.03%). The presence of the microwave-assisted process reduced the surface tension values of the pectin aqueous solution (43.11% in 5 minutes of irradiation) and affected and improved the foaming capacity of pectin (the highest value in 5 minutes of irradiation was 84.13%). Irradiation during microwave time caused significant changes in pectin properties, such as intrinsic viscosity, mean viscosity, and water holding capacity, to form a higher-quality gel.

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1-Introduction

Pectin is recognized as a polysaccharide that serves as one of the structural components in the primary cell walls and middle lamella of higher plants. This anionic polysaccharide complex consists mainly of (1-4) α-D-galacturonic acid and forms side chains with neutral sugars such as arabinose, galactose, and xylose. Pectins are primarily classified based on their degree of methylation into high-methoxyl (DM > 50%) and low-methoxyl (DM < 50%) categories [1].

Pectin is widely used in the food, biomedical, pharmaceutical, cosmetic, and personal care industries, not only because of its functional properties but also due to its proven health benefits as a soluble fiber [2]. Growing concerns for environmental preservation and social welfare have accelerated the development of alternative food sources. Consequently, various natural resources that are environmentally friendly, economically viable, and abundant have attracted attention from niche markets in the food industry [3]. On a commercial scale in the food and pharmaceutical industries, this acidic carbohydrate macromolecule is typically derived from citrus and apple waste due to the degree of methylation, molecular weight, and homogalacturonan content in the pectin structure of these sources [4]. Pectins are commonly used as gelling and emulsifying agents to stabilize jams, jellies, and acidic dairy beverages [5, 6]. Additionally, pectins can be utilized alone or in combination with other biopolymers to prepare micro- and nano-capsules containing bioactive compounds [7]. Moreover, the health-related roles of pectins, such as cholesterol reduction, lowering serum glucose levels, inhibiting cancer cell growth, and enhancing the immune system, have been reported [4, 8].

Rapeseed, as an oilseed crop, was cultivated in Europe during the early Middle Ages. Due to its ability to germinate and grow at low temperatures, rapeseed is one of the few oil crops that can be grown in temperate regions of the world. Both spring and winter forms of this plant exist, but the spring form is predominant in North American, European, and cold regions of Iran, such as Ardabil, East Azerbaijan, and Golestan. Canola oil is the primary product of this plant, constituting approximately 45% of the seed's weight. This oil is widely used in various industries, including cooking oil production, chocolate manufacturing, biscuit and confectionery industries, and more [9, 10]. Rapeseed meal is currently used as animal feed or in the cellulose industry [11]. Rapeseed meal contains high amounts of carbohydrates (over 30%), protein (over 30%), and fat (over 10%), making it a potential source for extracting the first two components [12]. The pectin content in rapeseed meal carbohydrates is relatively higher than other polysaccharides [13]. Therefore, exploring pectin extraction from rapeseed is expected to enhance the utilization efficiency of rapeseed meal carbohydrates and improve the economic aspects of rapeseed refineries. However, there are limited studies on pectin from this source, with most research focusing on cellulose and hemicellulose in rapeseed meal [11].

A microwave is a generator of electromagnetic waves with a corresponding frequency range of 0.3 to 300 GHz. In the entire electromagnetic spectrum, the microwave region lies between infrared and radio frequencies. As a type of electromagnetic wave, microwaves consist of electric and magnetic fields.

Based on the interaction between microwave radiation and exposed materials, substances can be classified into three types:

1. Absorptive materials, such as water and glycerol, which can absorb microwave

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waves and are also known as microwave dielectrics.

2. Conductors (mainly metals), which microwaves cannot penetrate, leading to most of the incident waves being reflected.
3. Insulators or transparent materials, such as quartz and Teflon, which allow microwaves to pass through without energy loss.

Microwave dielectrics are commonly used as heating mediums. The presence of microwaves offers numerous advantages in polysaccharide extraction, including the potential to reduce processing time and energy consumption, minimize wastewater production due to reduced use of organic solvents, increase the yield and purity of extracted polysaccharides, improve the heating rate during extraction, and provide better control over the system and extraction parameters throughout the process [14].

In this study, we investigated pectin extraction using a combined process under optimized conditions during enzymatic hydrolysis to achieve high pectin yield. Initially, rapeseed meal fat was removed through solvent extraction, followed by enzymatic hydrolysis at different hydrolysis times, enzyme-to-RSC ratios, and enzyme cocktail ratios (celluclast-to-alcalase ratio) under constant pH and temperature. Furthermore, the effect of enzymatic hydrolysis conditions on pectin extraction was examined to compare the degradation rate of rapeseed meal, the yield of released reducing sugars, and the extracted pectin yield.

2-Materials and Methods

2.1 Raw Materials

2.1.1 Preparation of Rapeseed Meal:

Rapeseed seeds (Nima cultivar, winter type, non-hybrid, with a seed purity of 99%) were obtained from the Agricultural Jihad Organization of Tehran Province. Initially, the seeds were completely ground using a laboratory mill (Iran Mill Co., Iran), and the seed husks and powder were separated using a 40-mesh sieve (Alak Zarrin Co., Iran).

The initial oil extraction process was performed using a cold press (Bekrdaneh Co., Iran) on the crushed seeds. In the second stage, the defatted seed powder was further subjected to solvent extraction using hexane at a 1:6 weight/volume ratio under magnetic stirring (with hexane replaced every 3 hours). Oil separation from the seed powder was completed by centrifugation at 10,000 rpm for 30 minutes at room temperature. Finally, the remaining seed flour was dried in a vacuum oven (Bahman Industrial Group, Iran) at 35°C under 2 bar pressure for 10 minutes to ensure complete hexane removal [13].

2.1.2 Pectin Extraction via Enzymatic Hydrolysis

After the fat removal process, two commercial enzymes from Novozymes Korea Ltd. (South Korea) were used for hydrolysis:

- Celluclast L 1.5 FGÒ (a cellulase produced from *Trichoderma reesei*), with a declared activity of 700 EGU/g.
- Alcalase L 2.5 EXÒ (a protease produced from *Bacillus licheniformis*), with a declared activity of 2.5 AU-A/g.

Five grams of defatted rapeseed meal powder was placed in a 250 mL Erlenmeyer flask, and 100 mL of 0.05 M sodium acetate buffer (pH 5.5) was added. Celluclast and Alcalase were applied at an enzyme-to-meal ratio of 1:50 (v/w). The enzymatic cocktail, consisting of a Celluclast-to-Alcalase ratio of 1:4 (v/v), was then added to the meal.

After incubation in a shaking incubator (Part Azma, Iran) at 50°C, 150 rpm for 90 minutes, the supernatant was filtered using Whatman No. 1 filter paper and centrifuged (19,620 ×g for 10 minutes) to collect the clarified

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supernatant. The pectic substances were then washed three times with 95% ethanol to remove hydrolyzed carbohydrates (such as mono- and disaccharides). Finally, the wet pectin was dried at 40°C for 10 hours in an oven and stored in Pyrex glass tubes [3].

2.1.3 Pectin Extraction Using Microwave-Assisted Method

The method described by Forouhar et al. (2023), with slight modifications, was used for pectin extraction from powdered rapeseed meal utilizing a microwave system equipped with a reactor chamber (MLS Ethos 1600 Microwave System, MLS, Leutkirch, Germany).

Based on preliminary evaluations for maximum extraction efficiency, the applied processing parameters included:

- 5 g of rapeseed meal powder in 100 mL of 0.05 M sodium acetate buffer (pH adjusted to 1.5 with citric acid),
- Microwave power: 600 W,
- Irradiation time: 1, 3, and 5 minutes.

After cooling, enzymes were added according to the method described in Section 2.1.2, and the process continued accordingly. The extracted solutions were filtered using Whatman No. 1 filter paper and centrifuged (19,620 ×g for 10 minutes) to collect the clarified supernatant. The pectic substances were then washed three times with 95% ethanol to remove hydrolyzed carbohydrates (such as mono- and disaccharides). Finally, the wet pectin was dried at 40°C for 10 hours in an oven and stored in Pyrex glass tubes [14].

2.1.4 Chemicals Materials

All chemicals used in this study were purchased from Sigma-Aldrich (USA).

2.2.1 Measurement of Extraction Yield

The pectin extraction yield (EY) obtained from both extraction methods was estimated using the following equation by dividing the weight (grams) of dried pectin (W_Dried-Pectin) by the weight (grams) of dried rapeseed meal powder (W_Dried-RSC) (Equation 1) [1]:

$$EY = \frac{W_{Dried-Pectin}}{W_{Dried-RSC}} \quad (1)$$

2.2.2 Measurement of Degree of Esterification

The classical titration method was used to determine the degree of esterification (DE). After adding 3 mL of 96% ethanol to 0.2 g of pectin, the mixture was dissolved in 20 mL of deionized water and stirred at 150 rpm on a magnetic stirrer (IKA, Germany). Two or three drops of phenolphthalein indicator were added to the solution, and it was titrated with 0.1 N NaOH.

Next, 10 mL of 0.1 N NaOH was added to the sample and stirred for 15 minutes under the same conditions to complete the hydrolysis reaction. Then, 10 mL of 0.1 N hydrochloric acid was added to the sample, and it was stirred until the pink color completely disappeared. Finally, the samples were titrated again with 0.1 N NaOH until the pink color reappeared.

The degree of esterification (DE) was estimated using Equation 2 [15]:

$$DE = \frac{v_2}{v_2 + v_1} \times 100 \quad (2)$$

2.2.3 Measurement of Total Galacturonic Acid Content

The colorimetric method based on 3,5-dimethylphenol reagent was used to determine the total galacturonic acid content of the extracted pectins from rapeseed meal using both extraction methods.

For this test:

- 1 mL of a pectin solution (200 µg/mL concentration) was placed in a test tube.
- 6 mL of sulfuric acid/sodium tetraborate solution was added.
- The mixture was immediately cooled in an ice-water bath.

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- The solution was then subjected to continuous processing, which included vortex mixing, heating in a water bath, and subsequent cooling.
- At this stage, the 3,5-dimethylphenol reagent was added, the mixture was shaken for 5 minutes, and the absorbance was measured at 520 nm using a UV-Vis spectrophotometer (SP-UV 500, China) [16].

2.2.4 Surface Tension, Foaming, and Water-Holding Capacity

The equilibrium surface tension was measured using the Du Noüy ring method with a Kruss K20 Easy Dyne tensiometer. For this test, sample solutions from both extraction methods (at a 1% w/v concentration) were prepared by dissolving 0.3 g of pectin in 30 mL of distilled water at 60°C. The sample solutions were stored at 4°C for 24 hours before measurement. Freshly quadruple-distilled water with a surface tension of 71 mN/m was used as the reference [14].

The foaming properties of the extracted pectin solutions were expressed as foam capacity (FC) and foam stability (FS) and were determined with slight modifications using the method described by Forouhar et al. (2023). Foam was prepared by homogenizing the pectin solution (1% w/v) at 11,000 rpm for 1 minute at 25°C using a homogenizer (Ultraturrax T25; IKA, Germany). The foam volume was recorded every 5 minutes up to 35 minutes using a digital camera and analyzed with ImageJ 1.46r software. FC and FS were determined by measuring the ratio of foam height to total height immediately after foam formation and after 35 minutes, respectively [14, 17].

Based on the kinetic modeling of foam stability, empirical models (Equation (3)) were used to correlate the experimental data of foam ratio, expressed as the foam height relative to the total height from t=0t = 0 to t=35t = 35 minutes. The half-time (t1/2t_{1/2}), which indicates foam stability, was calculated as shown in the following equation (Equation (4)). The kinetic parameters k1k_1 (foam volume per minute) and the constant number VrV_r were determined using regression analysis with the curve-fitting tool in MATLAB R2008a (The MathWorks, USA) [17].

$$V_r = V_{r0} - K_1 t^{0.01} \quad (3)$$

$$t_{1/2} = \left(\frac{V_{r0}}{2 \times K_s}\right)^{10} \quad (4)$$

The water-holding capacity (WHC) of the extracted pectin samples was evaluated at room temperature. A total of 0.3 g of each pectin sample was added to 10 mL of distilled water, vortexed for 1 minute, and incubated for 30 minutes before centrifugation at 7500 rpm for 10 minutes at 25°C. The remaining pectin, in the form of a pellet, was weighed after the removal of the supernatant. The WHC of the samples was expressed as the grams of water retained per gram of pectin [17].

2.2.5 Viscosity and Rheological Behavior

A programmable rotational viscometer (Brookfield Engineering Inc, DVBT, USA) equipped with an advanced UL adapter was used to determine the viscosity and flow behavior of pectin solutions (20 mL) at 24 ± 1°C, at concentrations of 0.1, 0.3, 0.5, 0.75, and 1% (w/v). A narrow-gap coaxial cylinder measurement system with an LV#16 spindle (probe) was utilized for this test. As recommended in the Brookfield manual, the spindle speed was adjusted to provide the highest percentage of torque within the range of 10–100%. The processing time for each measurement was approximately 5 minutes [1].

2.2.6 Emulsifying Activity and Emulsion Stability

Emulsions were prepared by mixing 5% corn oil and 5% pectin solutions (1:1 v/v) and subsequently homogenized using a rotor-stator homogenizer (Ultraturrax T25; IKA, Germany) at 7500 rpm for 5 minutes at 24°C. Coarse particle-containing emulsions were further ultrasonicated using a 24 kHz ultrasound

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system (UP400S, Hielscher Ultrasonics GmbH, Germany) with a maximum output power of 400 W to produce finely dispersed emulsions.

Immediately after preparation, 10 g of the emulsion was transferred into a test tube and stored at 4°C and 24°C as storage temperatures for 28 days. The emulsifying activity (EA (%), Equation 5) and emulsion stability (ES (%), Equation 6) of pectins extracted using both methods were calculated.

$$EA = \frac{V_{el}}{V_t} \quad (5)$$

$$ES = \frac{EA_{f-d28}}{EA_{i-0d}} \quad (6)$$

where Vel and Vt represent the volume of the emulsified layer and the total emulsion volume, respectively. EAi-0d and EAf-28d denote the emulsifying activity at the initial time (day 0) and the final time (day 28) of the storage period, respectively [1, 18].

2.2.7 Antioxidant Activity

2.2.7.1 DPPH Radical Scavenging Ability

A modified method by Yamaguchi et al. (1998) was used to evaluate the free radical scavenging activity of pectins extracted by both methods. Briefly, 1 mL of pectic extract at various concentrations (3–15 mg/mL) was mixed with 2 mL of a 0.2 mM DPPH solution and incubated at 24°C for 30 minutes. The absorbance (Abs) was recorded against methanol using a UV/Vis spectrophotometer (SP-UV 500, China) at a wavelength of 517 nm. BHA and vitamin C were used as positive controls, and the scavenging activity was estimated using the following equation (Equation 7) [19].

$$SA_{DPPH} = \left(1 - \frac{Abs_{Sample}}{Abs_{Control}}\right) \times 100 \quad (7)$$

2.2.7.2 ABTS Radical Scavenging Capacity

The ABTS radical scavenging activity (SA_ABTS) of pectin solutions was measured following the method described by Thambiraj et al. (2015) with slight modifications. Briefly, 0.35 mL of 7.4 mM ABTS diammonium salt was mixed with 0.35 mL of 2.6 mM $K_2S_2O_8$ and incubated in a dark environment at 24 ± 1°C for 15 hours to generate the ABTS radical. The ABTS solution was then diluted with 96% ethanol to reach an absorbance of 0.70 ± 0.02 at 734 nm.

Subsequently, 2 mL of the ABTS solution was mixed with 0.2 mL of pectin solution at varying concentrations (3–15 mg/mL), and the absorbance was measured at 734 nm after 20 minutes of incubation using a UV/Vis spectrophotometer (SP-UV 500, China). The absorbance was recorded against 96% ethanol as the negative control. BHA and vitamin C were used as positive controls. The SA_ABTS percentage was calculated using the following equation (Equation 8) [20].

$$SA_{ABTS} = \left(1 - \frac{Abs_{Sample}}{Abs_{Control}}\right) \times 100 \quad (8)$$

2.3 Statistical Analysis

The obtained data were analyzed using IBM SPSS Statistics (version 25.0.2.2, USA). Significant differences between measured groups were determined using one-way analysis of variance (ANOVA) at a 95% confidence level. After verifying normality and homogeneity of variances, the Tukey HSD test was applied. Linear Discriminant Analysis (LDA) was conducted to assess the discriminability of each type of extracted pectin. In cases of significant differences, the Tukey test was performed at a 5% significance level. All experiments were conducted in triplicate.

3- Results and Discussion

3.1 Pectin Extraction Yield

The extraction yields of pectin obtained using EH and MAE methods are presented in Figure 1. As observed, the highest pectin yield was obtained in the MAE5 sample (600 W power and 5 minutes duration) with a value of 9.1%, while the lowest yield was recorded in the EH sample (extraction without microwave-assisted processing) with a value of 7.6%. The presence

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of an auxiliary process enhanced the pectin extraction yield from rapeseed meal powder. Microwave irradiation time is one of the key factors influencing extraction efficiency [17]. An increase in yield from 8.4% to 9.1% was observed as the irradiation duration increased from 1 to 5 minutes. During the extraction process, microwave heating generates significant pressure inside the powdered particles, altering the physical properties of the raw material, such as porosity. Many researchers have confirmed that microwave waves can enhance solvent penetration into the tissue, energy transfer to the mixture, and release of target compounds [21]. Increasing the process duration can facilitate greater pectin release into the acidic solution. Previously, Jeong et al. (2013) extracted pectin from rapeseed using an enzymatic hydrolysis method, achieving an extraction yield of 6.23%. The differences in extraction yields (7.6% in this study) may be attributed to variations in plant species and varieties [3]. However, microwave-assisted pre-treatment for pectin extraction from oilseeds has not been reported in previous studies. Among other plants, Jiang et al. (2012) demonstrated that pectin extraction yield from watermelon rind using MAE reached 19.6% under 500 W power, pH 1.5, and a 7-minute extraction time [22].

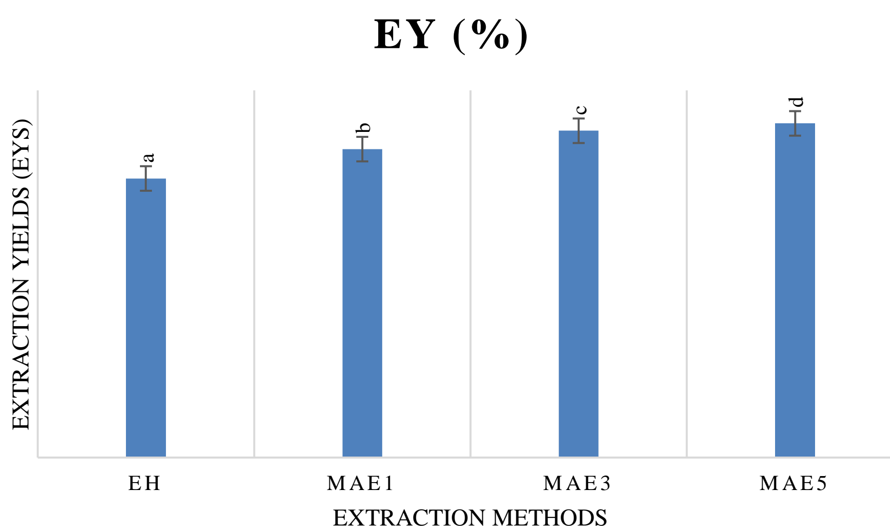

Fig 1. The effect of Enzymatic and microwave-assisted treatment on pectin extraction efficiency of defatted-cake rapeseed [a] [‡]

[a] Operating conditions in an acidic medium (pH 1.5): EH (Enzymatic Hydrolysis), MAE (Microwave-Assisted) (600 W, 1,3 and 5 min)
[‡] Means within each column with the same letters are not significantly different ($P < 0.05$).

3.2 Determination of the Degree of Esterification

The application of pectin largely depends on its degree of esterification (DE). According to Figure 2, all extracted pectins belonged to the low-methoxy (LM) pectin category, as their DE values were below 50%. The results indicate that the microwave irradiation duration influences DE values. As shown in Figure 2, the DE of pectin extracted using enzymatic hydrolysis reached 45.4%. A significant difference was observed in DE values depending on the extraction duration.

Microwave-assisted extraction (1, 3, and 5 minutes) resulted in DE values ranging from 42.81% to 45.17%. The extraction process, under thermal conditions and microwave exposure, created harsh conditions that increased de-esterification of polygalacturonic acid chains, leading to lower DE values [23]. Previous studies have confirmed that three factors—irradiation time, applied power, and

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process temperature—can influence DE reduction [17]. However, in this study, only the process duration was variable, while power remained constant, and temperature likely increased with prolonged irradiation.

Low-methoxy pectins (DE < 50%) form gels regardless of sugar content, even in its absence or at very low levels. They are also more chemically stable against moisture and heat than high-methoxy pectins (DE > 50%) [24]. These pectins are commonly used in the formulation of low-sugar or low-calorie jams, jellies, and dairy-based desserts [1]. Additionally, LM pectins are more pH-resistant than high-methoxy pectins, enabling gel formation over a wider pH range [25].

Pectins with DE < 50% can gel in the presence of divalent cations, typically calcium ions (Ca^{2+}). This gelation process can be easily reversed by adding monovalent cations such as sodium (Na^{+}) and potassium (K^{+}) [26]. Jeong et al. (2014) reported a DE of 47% for ultrasound-assisted extraction of pectin from rapeseed meal, which was approximately 7% lower than commercially available pectins [12]. In another study, Iglesias et al. (2004) found that pectin extracted from sunflower meal had a DE of about 11%, which was significantly lower than that of rapeseed pectin [27].

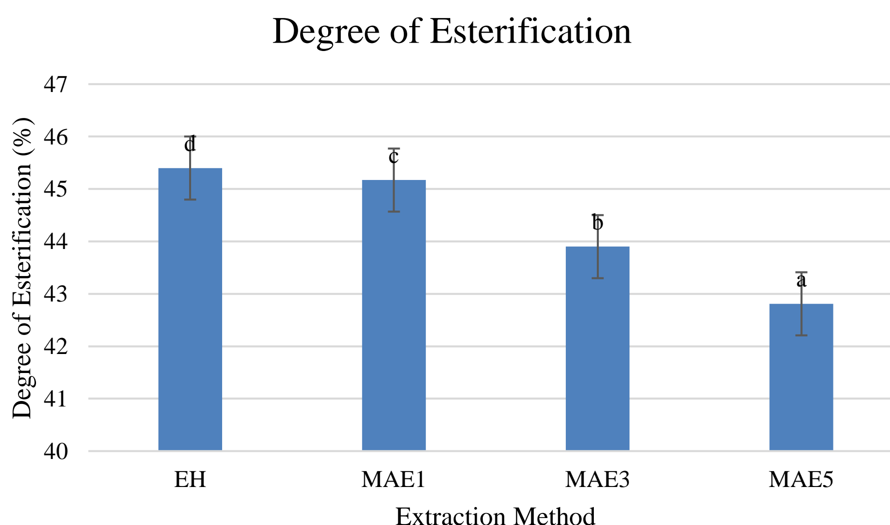

Fig 2. The effect of Enzymatic and microwave-assisted treatment on pectin Degree of Esterification (DE) of defatted-cake rapeseed [a ‡]

[a] Operating conditions in an acidic medium (pH 1.5): EH (Enzymatic Hydrolysis), MAE (Microwave-Assisted) (600 W, 1,3 and 5 min)
[‡] Means within each column with the same letters are not significantly different ($P < 0.05$).

3.3 Measurement of Total Galacturonic Acid Content

The quality of the extracted pectin can be evaluated based on the percentage of DE and TGA, as these chemical parameters significantly affect gelation properties and texture formation [23]. The TGA of pectin extracted by enzymatic hydrolysis (65.21%) was significantly lower than that of commercially available pectins ($p < 0.05$). The application of the microwave process significantly increased the TGA in pectin. The maximum TGA content was 76.51% after 5 minutes of microwave processing. According to FAO and EU regulations, the TGA content in industrial pectins must be at least 65% [28]. Therefore, pectin extracted using the microwave process is suitable for forming strong gels in the food industry. The high gel-forming capacity of pectin obtained through MAE may be attributed to the dominant effect of microwave radiation in creating a medium

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for complete and rapid extraction compared to other conditions [29]. For this reason, the combination of this type of pectin with acid at high temperatures has a high ability to fully release pectic compounds with higher galacturonic acid content from the deeper parts of the plant structure [30].

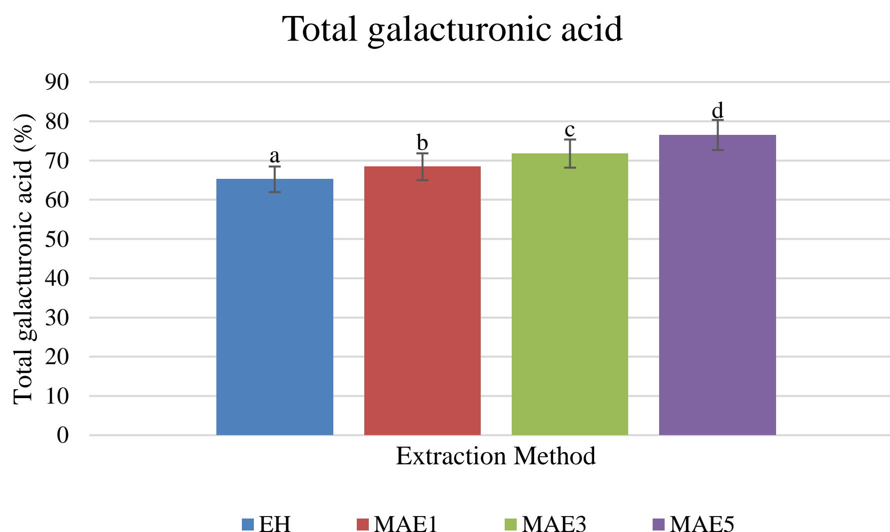

Fig 3. The effect of Enzymatic and microwave-assisted treatment on total galacturonic acid content of defatted-cake rapeseed [a ‡]

[a] Operating conditions in an acidic medium (pH 1.5): EH (Enzymatic Hydrolysis), MAE (Microwave-Assisted) (600 W, 1,3 and 5 min)
‡ Means within each column with the same letters are not significantly different ($P < 0.05$).

3.4 Surface Tension, Foaming, and Water-Holding Capacity

The surface tension of deionized water, used as a reference, was measured at 71 mN/m. As shown in Table 1, the surface tension values of the 1% (w/v) pectin solution extracted from rapeseed meal using EH (enzymatic hydrolysis) were lower than 71 mN/m (45.45 mN/m), indicating that the extracted pectin can reduce the surface tension of the solution. This parameter is one of the most important physical properties of biopolymers for foam formation, as lower surface tension increases the foaming ability [31]. The surface tension values of pectin extracted using MAE were 44.38 mN/m at 1 minute, 43.69 mN/m at 3 minutes, and 43.11 mN/m at 5 minutes. However, compared to the commercially available sample, the surface tension values of rapeseed meal pectin solutions extracted using both methods (1% w/v) were nearly equal (45.8 mN/m). Schmidt et al. (2014) also obtained a similar value for a commercially available citrus pectin solution (1% w/v) in the Iranian market [32].

The FC and FS values of rapeseed meal pectin aqueous solutions are presented in Table 1. Statistical analysis showed that the extraction pre-treatment duration could significantly affect FC ($P < 0.05$). The pectin obtained using EH (enzymatic hydrolysis) exhibited a significant difference in FC and FS compared to the MAE sample. These FC results for extracted pectin solutions corresponded with surface tension measurements and protein data. The FS values of the samples ranged from 30.48% to 31.87%.

WHC can influence physical properties such as viscosity, thickening, texture formation, and volume production in food products formulated with pectin [38]. As shown in Table 1, the WHC of rapeseed meal powder obtained via MAE ranged from 11.71 to 11.75 g water/g pectin, demonstrating suitable WHC values compared to commercially available citrus pectin (10.2 g

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water/g pectin). Based on experimental results (Table 1), the WHC of pectin obtained through MAE was higher than that obtained via EP, with a significant difference observed only between the MAE and EH samples when pre-treatment was applied for 5 minutes at 600 W. The physical structure and chemical composition of pectin can influence WHC. Therefore, microwave pre-treatment may alter the physical structure or chemical composition of pectin, affecting this parameter and reducing the hydrophilicity of the samples [39]. The WHC value of rapeseed meal pectin, with or without microwave pre-treatment, was higher than that of guar gum (4.8 g water/g pectin) and carboxymethyl cellulose (10 g water/g pectin), which are used in cosmetic and personal care products as thickeners and to reduce moisture loss from the skin [33]. Since pectin is classified as a thickening agent and possesses significant WHC, rapeseed meal pectin can be proposed for use in cosmetic and personal care products.

Table 1. Effect of extraction method (1% (w/v) pectin solutions at 25 °C) on Surface Tension, Water holding capacity, foam properties, Viscosity, emulsifying activity, emulsion stability, SA_{DPPH}, and SA_{ABTS} [‡*]

Characters[a]	**Pectin types obtained with different extraction methods** [b]			
	EH	MAE1	MAE3	MAE5
SF (%)	45.45±0.1[d]	44.38±0.24[c]	43.69±0.17[b]	43.11±0.15[a]
WHC (%)	11.67±0.11[a]	11.72±0.1[b]	11.71±0.18[b]	11.75±0.39[c]
FS (%)	30.48±0.12[a]	31.21±0.23[b]	31.8±0.05[c]	31.87±0.41[d]
FC (%)	81.1±0.09[a]	82.45±0.21[b]	84.12±0.09[c]	84.13±0.14[c]
Viscosity (Pa.s)	8.54±0.09[a]	9.34±0.31[b]	11.57±0.2[c]	12.39±0.33[d]
EA (%, 4°C)	50.6±0.13[a]	52.33±0.15[b]	55.87±0.19[c]	58.01±0.39[d]
EA (%, 24°C)	49.87±0.11[c]	50.04±0.14[c]	51.08±0.27[f]	52.1±0.11[a]
ES[a] **(%, 4°C)**	81.7±0.12[a]	85.54±0.2[b]	89.88±0.31[c]	93.05±0.21[d]
ES[a] **(%, 24°C)**	79.09±0.1[a]	83.68±0.09[b]	88.16±0.33[c]	92.04±0.24[d]
SA_{DPPH}[a]	67.3±0.2[b]	69.9±0.19[b]	70.5±0.14[c]	71.2±0.26[d]
SA_{ABTS}[a]	70.5±0.13[b]	72.4±0.16[b]	75.1±0.15[c]	78.8±0.47[d]

[a] SF (Surface Tension), WHC (Water Holding Capacity), FS (Foam Stability), FC (Foam Capacity), EA (emulsifying activity), ES (emulsion stability), SA_{DPPH} (DPPH scavenging activity), SA_{ABTS} (ABTS scavenging activity)
[b] Operating conditions in an acidic medium (pH 1.5): EH (Enzymatic Hydrolysis), MAE (Microwave-Assisted) (600 W, 1,3 and 5 min)
[‡] Means within each row followed with the same letters are not significantly different ($P < 0.05$).
[*] Data are means ± SD.

3.5 Rheological Behavior

The viscosity of 1% (w/v) pectin solutions indicated that pectin solutions extracted via MAE had higher viscosity than those obtained through EH ($p < 0.05$, Figure 4). This table illustrates the flow behavior of pectins obtained through MAE (at three durations: 1, 3, and 5 minutes at 600 W) and EH at a 1% (w/v) concentration. A significant increase in viscosity was observed in all pectin solutions extracted using microwaves ($p < 0.05$). Newtonian flow behavior was observed in the EH sample, whereas shear-thinning (pseudoplastic) behavior was predominant in the pectin samples extracted using microwave-assisted extraction (Figure 4).

The transition from Newtonian to pseudoplastic behavior at longer extraction times can be attributed to the disruption of the biopolymer network under shear stress and the partial alignment of pectin chains in the direction of shear flow due to the weakening of some weak physical interactions [1, 34]. Jun et al. (2006) demonstrated in their study on pumpkin pectin that viscosity gradually decreases with increasing shear rate, eventually reaching an almost constant value due to structural anisotropy caused by shear deformation [35]. A similar result was previously reported by Chen et al. (2014) regarding the rheological behavior of pectin extracted from okra [36]. Additionally, Gharibzahedi et al. (2019) found that different

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pectin extraction methods from dried figs showed that microwave-assisted pre-treatment could yield similar results in increasing pectin viscosity (Figure 4) [1].

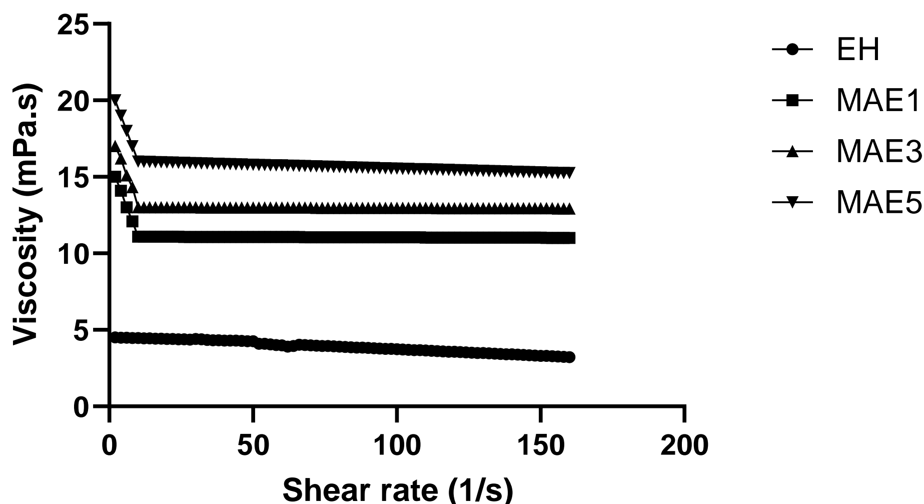

Fig. 4. The flow behavior of pectic solutions containing RSC pectins extracted by Enzymatic Hydrolysis (EH) and Microwave-Assisted Extraction (MAE) methods at different times

3.6 Emulsifying Activity and Emulsion Stability

The emulsifying activity (EA) of all three pectin samples extracted via MAE at 1, 3, and 5 minutes was influenced by storage temperature (ST) ($p < 0.05$). However, the EA of all pectin types decreased as ST increased from 4°C to 24°C (Table 1). A decrease in emulsion stability (ES) was also observed with an increase in ST from 4°C to 24°C. Although ST significantly affected this parameter in MAE-extracted pectins ($p < 0.05$), no significant change in ES was observed in EH-extracted pectins with increasing ST. The pectin extracted via MAE exhibited the highest and lowest EA and ES values, respectively (Table 1). Therefore, rapeseed meal pectin extracted via MAE can be considered a powerful emulsifier and stabilizer for incorporation into various food and bioproduct formulations.

Pectins appear to stabilize emulsion systems by reducing the surface tension of oil droplets through the formation of electrostatic repulsions on their surfaces [37]. Additionally, pectin can enhance emulsifying properties by forming strong three-dimensional networks and improving the rheological characteristics of the continuous aqueous phase [38].

3.7 Antioxidant Activity

Table 1 and Figure 5 present the DPPH (Figure 5a) and ABTS (Figure 5b) radical scavenging activities of pectins extracted from rapeseed meal using EH and MAE, along with the positive control compounds BHA and vitamin C.

DPPH is a nitrogen-centered free radical with an unpaired electron, making it susceptible to reduction upon exposure to proton radicals. Its solution appears dark purple due to the presence of an unpaired electron, leading to strong absorption at 517 nm. Therefore, the color change from purple to yellow after DPPH removal by antioxidants serves as a quantitative measure of antioxidant activity. Similarly, ABTS is a well-known nitrogen-centered synthetic radical that can be produced by the

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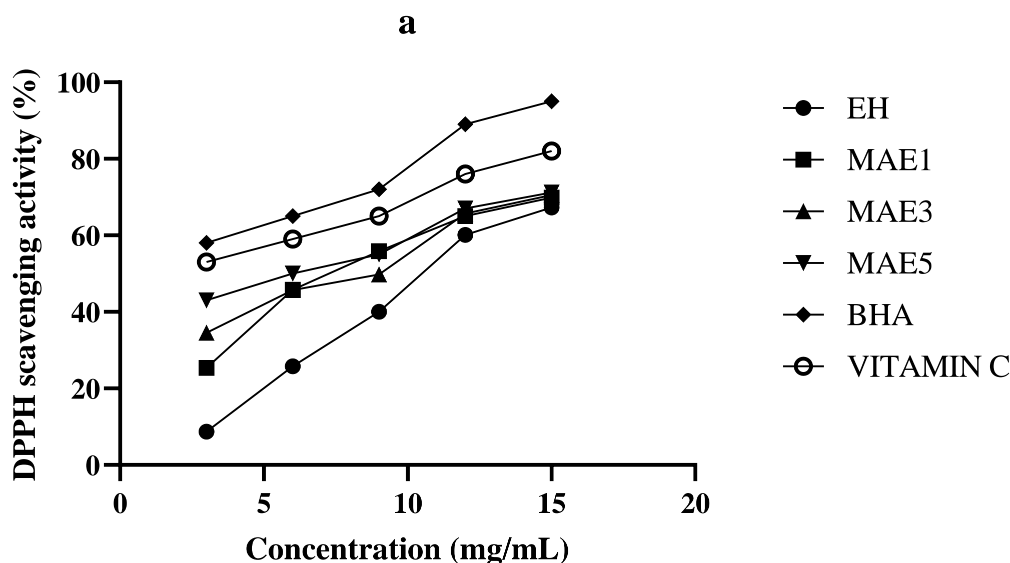
oxidation of ABTS with potassium persulfate. Upon reaction with an antioxidant, ABTS donates an electron and converts into its non-radical form [25].

The antioxidant activity of extracted pectins increased in a dose-dependent manner from 3 to 15 mg/mL ($p < 0.05$). According to Table 1, no significant difference was observed in the DPPH radical scavenging activity (SADPPH) at 15 mg/mL between EH- and MAE-extracted pectins ($p > 0.05$). Overall, the radical scavenging activity of pectins in both groups was lower than that of BHA and vitamin C. SADPPH values ranged from 67.3% to 71.2% (Table 1 and Figure 5a).

However, at 15 mg/mL pectin extract, the highest and lowest SAABTS values were observed in pectins extracted via MAE5 (5 minutes at 600 W) (78.8%) and EH (70.5%), respectively ($p < 0.05$) (Table 1 and Figure 5b).

These results indicate that the ability to scavenge DPPH and ABTS radicals increased with prolonged microwave irradiation up to 5 minutes, enhancing the antioxidant properties of MAE5-treated pectin compared to other treatments.

The presence of multiple hydroxyl and carboxyl groups in extracted pectins likely plays a key role in donating electrons and transferring them to free radicals under investigation. Additionally, pectin molecules can terminate radical chain reactions by interacting with radical ions essential for oxidation processes. These findings are generally consistent with the results reported by Gharibzahedi et al. (2019), Marzouki et al. (2018), who investigated the DPPH and ABTS radical scavenging properties of pectin extracted from *Suaeda fruticosa* leaves, and Jeong et al. (2013) [1, 12, 39].



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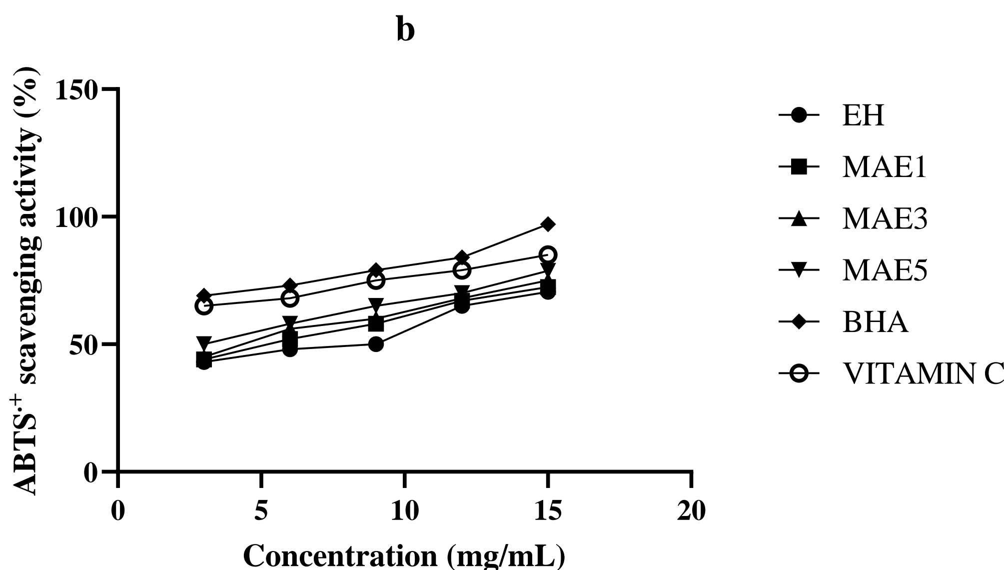

Fig. 5. DPPH• (a) and ABTS• (b) scavenging activities of the RSC pectins extracted by different methods at various concentrations. Vitamin C and BHA were used as positive controls

4- Conclusion

In this study, enzymatic hydrolysis (EH) and microwave-assisted extraction (MAE) (600 W for 1, 3, and 5 minutes) were used to extract pectin from rapeseed meal. The 5-minute irradiation time at 600 W was a successful strategy for extracting high-molecular-weight pectin with the highest yield. Based on the findings, the highest extraction efficiency of pectin from rapeseed meal powder was 9.1%, which was higher than that obtained through enzymatic extraction. This increase in yield, as a quantitative factor, along with improvements in qualitative parameters, indicated enhanced pectin extraction from the selected source.

The performance of this pectin in terms of DE and TGA showed that with increased microwave irradiation time, the degree of esterification decreased while the total galacturonic acid content increased. Overall, all pectin extracted in this study had a DE of less than 50. Therefore, they can be classified as low-methoxy pectin (LMP) and used in stable formulations for many low-sugar dietary foods.

These changes also influenced the viscosity of the pectin solution, where exposure to microwave irradiation and increased irradiation time led to a transition from Newtonian to pseudoplastic behavior, making it more suitable for food products that require such rheological properties. Additionally, the emulsifying activity (EA) and emulsion stability (ES) at different temperatures and times confirmed the potential use of this pectin in food emulsions, as O/W emulsions yielded promising results.

This research highlights the potential of rapeseed meal pectin extracted via MAE as a valuable functional ingredient in various food and bioproduct formulations.

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مقاله علمی_پژوهشی

بررسی ویژگی‌های پکتین استخراج شده از کنجاله کلزا در روش هیدرولیز آنزیمی با استفاده از استخراج به کمک مایکروویو

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چکیده

پکتین به عنوان هیدروکلوئید که در دیواره سلولی گیاهان قرار دارد می‌تواند یک محصول ثانویه در فراوری صنایع غذایی باشد. کلزا پس از روغنکشی دارای مقادیر بالایی از پکتین است. برای استخراج پکتین از کنجاله کلزا از روش مقایسه استخراج هیدرولیز آنزیمی با حضور و عدم حضور به کمک مایکروویو (با قدرت ٦۰۰ وات در چهار زمان صفر، ۱، ۳ و ۵ دقیقه) مورد استفاده قرار گرفت. بررسی تأثیر زمان تابش مایکروویو بر عملکرد استخراج و ویژگی‌های فیزیکوشیمیایی و رئولوژیکی پکتین استخراج‌شده انجام شد. ویژگی‌های فیزیکی، رئولوژیکی و شیمیایی نشان دادند که حضور فرآیند مایکروویو منجر شد تا خواص عملکردی پکتین استخراج شده بهبود یابد و فرآیند استخراج را تسهیل کرد ($p < ۰/۵۰$). بالاترین بازده استخراج پکتین ۹/۱ ٪ (وزنی/وزنی) در ۵ دقیقه تابش پرتو با قدرت ٦۰۰ وات در فرایند کمکی مایکروویو با قدرت ٦۰۰ وات بود. این فرآیند کمکی بر درجه استری شدن و محتوای اسید گالاکترونیک پکتین تأثیر گذاشت تا توانایی تشکیل ژل در حضور مقادیر قند کم را داشته باشد و در محصولات رژیمی مناسب باشد. محتوای اسید گالاکترونیک در تمام نمونه ها بالاتر از ٦۰٪ بود که نشان از ظرفیت تشکیل ژل بالا است. پکتین استخراجی در زمان ۵ دقیقه تابش مایکروویو با قدرت ٦۰۰ وات بهترین ویژگی‌ها را با حداکثر محتوای اسید گالاکترونیک (۷٦/۵۱ ٪)، بالاترین فعالیت امولسیون کنندگی (۵۸/۰۱٪) و پایداری امولسیون (۹۵/۰۳٪) را ارائه داد. حضور فرآیند کمکی مایکروویو مقادیر کشش سطحی محلول آبی پکتین را کاهش داد (٤۳/۱۱٪ در ۵ دقیقه تابش) و ظرفیت ایجاد کف پکتین (بیشترین مقدار در ۵ دقیقه تابش ۸٤/۱۳٪) آن را تحت تأثیر قرار داده، بهبود بخشید. تابش پرتو در طول زمان مایکروویو باعث تغییرات قابل‌توجهی در ویژگی‌های پکتین، مانند ویسکوزیته ذاتی، میانگین ویسکوزیته، ظرفیت نگهداری آب شد تا ژلی با کیفیت بالاتر را تشکیل دهد.

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