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# Study on the Physicochemical Characteristics and Extraction Kinetics of Pectin Derived from Indigenous Iranian Sugar Beet Pulp

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**ABSTRACT**

Pectin is a valuable hydrocolloid in the food industry, and its properties are largely influenced by its source. Understanding the characteristics, functional properties, and mathematical models of extracting this important hydrocolloid is essential. Therefore, this study focuses on identifying the intrinsic and functional properties of pectin extracted from Sugar beet pulp obtained from a sugar factory. In this research, pectin was extracted from sugar beet pulp at different extraction times. The pectin characteristics measured included yield, degree of esterification, galacturonic acid content, viscosity-average molecular weight, gel strength, color intensity, and water-holding capacity. In addition, the effective diffusion coefficient and kinetic models were fitted to describe the extraction behavior. Evaluation of the properties of pectin showed that increasing extraction time resulted in an increase in yield from 8.1±0.25% at 30 minutes to 13.05±0.20% after 120 minutes, while purity decreased. The extracted sample was classified as high-methoxyl pectin. The highest molecular weight (182.6 ± 1.1 kDa) was observed at 30 minutes, and the highest gel strength (31.63 ± 1.44 g-force) was detected at 60 minutes. The lowest color intensity corresponded to the 90-minute extraction time, while the highest color intensity was observed at 120 minutes. Water-holding capacity at 30 and 120 minutes was 8.2± 0.14 and 8.35±0.07 g/g, respectively. The second-order kinetic model provided an appropriate fit. Based on the characterization results, pectin extracted within the 30–60-minute time range exhibited the best qualitative properties.

## 1- INTRODUCTION

Sugar beet pulp is a valuable waste resource that, even after sugar extraction, still contains valuable compounds such as pectin, cellulose, lignin, certain amounts of protein, and phenolic compounds [1, 2]. Due to the operation of numerous sugar factories, the production volume of sugar beet pulp is significant, making it one of the main by-products of sugar refineries, and discarding it represents a waste of a valuable resource.

According to statistics provided by the Sugar Association of Iran, the amount of sugar beet consumed in the year (2024/2025) was 8,856,923 tons. On the other hand, 25 kg of pulp is obtained for every 100 kg of sugar beet [3]. Based on these two factors, it can be calculated that approximately 2,214,230 tons of sugar beet pulp were produced in the year 1403 SH, which accounts for a highly significant volume.

Pectin is an acidic colloidal heteropolysaccharide with high structural diversity. It mainly consists of homogalacturonan, xylogalacturonan, apiogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II subunits. This natural polymer is abundantly found in the primary cell wall of plants and leads to an increase in their mechanical strength. Commercial pectin is mainly extracted from fruit juice industry waste, including citrus peel (85%), apple pomace (14%), or sugar beet pulp (1%).

Pectin has the ability to entrap water to form gels at low concentrations. Therefore, it is widely used for diverse food applications such as a gelling, thickening, or stabilizing agent in jams, bakery products, confectionery, and dairy products. In addition, numerous bioactive and functional properties can be attributed to pectin. For instance, it is considered a dietary fiber, prebiotic, and fat replacer, making pectin a valuable ingredient in various industries, including the food industry.

It is worth noting that there are differences between pectin derived from sugar beet and that obtained from citrus peel or apple pomace. For example, its degree of esterification is somewhat lower than that of pectin derived from citrus peel or apple pomace, leading to different targeted applications. There are also differences in the sequence and distribution of non-esterified and esterified regions between them.

Pectin exerts physiological effects on the intestine by increasing transit time and reducing glucose absorption, thereby acting as a dietary fiber. On the other hand, pectin can be an important and effective precursor for pharmaceuticals; by modifying it to create modified pectin, extensive benefits are achieved, including immune system protection, inhibition of tumor development, cancer treatment, and wound healing [4-6].

Pectin is classified into two categories based on the degree of methylation (DM), which corresponds to the number of methylated carboxylic groups per 100 galacturonic acid units in the backbone chain: high-methoxyl pectin (HMP) and low-methoxyl pectin (LMP). In HM pectin, the degree of methylation is greater than 50%, and it predominantly occurs in nature as native pectin. In LM pectin, the degree of methylation is less than 50%, which is produced through the demethylation of HM pectin via alkaline or enzymatic treatments. High- and low-methoxyl pectins exhibit distinct physicochemical properties and are utilized for different applications. Therefore, the degree of methylation indicates the pectin's water dispersibility and gelling capability [7, 8].

Numerous methods exist for pectin extraction, such as hot water extraction, the use of chelating solutions like EDTA, and the application of dilute acids or sodium hydroxide solutions. A major drawback of using chelating

agents in extraction is the difficulty of separating them from the extracted pectin. On the other hand, utilizing alkaline solutions also leads to a reduction in the degree of methylation and the chain length of the resulting pectin. The most common method for pectin extraction from food waste is acid hydrolysis [9, 10].

In a study conducted by Kaya et al. (2021), the effect of high hydrostatic pressure-assisted extraction (HHPE) of pectin from sugar beet pulp was investigated in comparison with the conventional extraction method. Kaya et al. (2021) demonstrated that increasing the pressure in the hydrostatic process up to 450 MPa at 90 °C for 5 hours, combined with acid addition, resulted in the highest pectin extraction yield (12%), revealing a synergistic effect between high pressure and the acid.

Viscosity analysis revealed that the extracted pectins exhibited Newtonian behavior at a concentration of 2 g/L. Furthermore, unlike certain other methods, HHPE and acid addition did not exert a significant negative impact on viscosity compared to the conventional method, which represents a crucial advantage for industrial applications. Regarding pectin purity, although increasing the temperature, time, and HHPE led to a decrease in galacturonic acid (Gal-A) content due to the cleavage of glycosidic bonds and the β-elimination reaction, all HHPE-extracted samples maintained a Gal-A content higher than 65%. According to standard regulations, this qualifies the extracted pectin as having a suitable degree of purity for food applications [11].

In another study, Almohammed et al. (2017) investigated the feasibility of utilizing high-voltage electrical discharge (HVED) pretreatment for the extraction of pectin from sugar beet pulp. By optimizing parameters such as pulse amplitude (40 kV) and pulse number (100 pulses) with an energy consumption of 76.2 kJ/kg, these researchers successfully increased the pectin extraction yield by 23% in the HVED-treated sample (extraction conditions: temperature of 90 °C, pH of 2, and a duration of 1 hour). Results from FTIR spectroscopy and gas chromatography-mass spectrometry (GC–MS) indicated that the chemical composition and functional groups of the pectin extracted from the HVED-treated pulp were similar to both standard pectin and the pectin obtained from the untreated control sample [12].

In another study, Rodsamran et al. (2019) utilized lime peel as the raw material for pectin extraction. They also employed hydrochloric acid; however, the process conditions were adjusted such that the extraction temperature was increased to 95 °C and the duration to 60 minutes (one hour). The results of this research demonstrated an extraction yield of 15.91%, a degree of methylation of 78.49%, and a galacturonic acid content of 89.8% [13].

On the other hand, due to the presence of feruloyl groups in its chain, sugar beet pectin is capable of cross-linking with additional free ferulic acid, thereby forming a thermostable gel characterized by covalent side-linkages. The formation of this pectin gel opens up further applications, such as the fabrication of superabsorbent hydrogels [14].

The objective of this study is to characterize and determine the functional properties of pectin obtained from native Iranian sugar beet, concurrently with evaluating mixing conditions and calculating the extraction coefficient. Although previous studies have addressed the multi-objective optimization of pectin extraction from sugar beet pulp, this research investigates the characteristics of pectin extracted from Iranian sugar beet pulp (cultivated in Shahrekord) and, by discretely tracking changes during the extraction period, delineates the evolutionary trajectory of each property over time. The more comprehensive set of optimization objectives in this study (yield, purity, gel strength, molecular weight, and water-holding capacity) complements the findings reported by other researchers regarding native sugar beet-derived pectin. Therefore, extraction is carried out under specific and mild

conditions to evaluate the functional properties of the obtained pectin. Thus, this study focuses on the simultaneous investigation of extraction kinetics alongside changes in molecular weight, gel strength, and other critical pectin properties across various extraction times, ultimately introducing an optimal time interval for scientific and industrial applications.

## 2- Materials and Methods

### 2-1- Materials

Sugar beet pulp from the Isfahan region was obtained from the Isfahan Sugar Factory. Hydrochloric acid and ethanol were purchased from Mojallali Company. Galacturonic acid standard was procured from Sigma Company (USA), while carbazole, sodium hydroxide, calcium chloride, and phenolphthalein were obtained from Merck Company (Germany).

### 2-2- Pectin Extraction from Sugar Beet Pulp

For the extraction of pectin from sugar beet waste, the method described by Faravash et al. (2008) was adopted with minor modifications. Initially, the obtained pulps were washed with water, and subsequently, an oven at 60 °C was utilized for 24 hours to dry the pulps until the waste reached a constant weight. The dried pulp was ground using a coffee grinder (Pars Khazar, Chili model, 200 W power) and passed through a 60-mesh sieve. Next, the dried pulp was mixed at a ratio of 1:20 with an acidic solution at pH 2 (prepared by adding hydrochloric acid to distilled water), and the extraction was performed for 30, 60, 90, and 120 minutes at a temperature of 90 °C. Filtration was then employed to separate the extract from the solid matter. After the extract had cooled down, 96% ethanol was added at a 1:1 ratio to precipitate the pectin. Finally, using a centrifuge at 5000 rpm for 15 minutes, the pectin precipitate was thoroughly separated from the supernatant. Ultimately, the resulting pectin was dried under vacuum at 45 °C for 24 hours [15].

### 2-3- Determination of Extraction Yield

To determine the extraction yield of pectin from sugar beet pulp, the final sample was weighed at the tested time intervals, and the extraction yield of each treatment was evaluated using Equation 1.

$$pectin\ extraction\ yield = \frac{weight\ of\ dried\ pectin}{weight\ of\ initial\ dried\ pulp} \times 100 \quad (1)$$

### 2-4- Determination of Galacturonic Acid Content

To determine the galacturonic acid content in the extracted pectins, the method described by Taylor (1993) was employed. The amount of galacturonic acid in the samples was measured, and the values obtained for pectins from different extraction times were compared with one another. Ten milligrams of pectin was added to 100 mL of water. Following dissolution, 200 µL of this solution along with 3 mL of concentrated sulfuric acid were added to a glass tube at a temperature of 4 °C, and then 200 µL of a 0.01% carbazole solution was added and mixed. The mixture was then left undisturbed at 60 °C for 2 hours to allow the formation of a pink color, and finally, the absorbance of the solution was read at 530 nm. The galacturonic acid content was calculated using a D-galacturonic acid standard solution (20 to 200 ppm) [16].

### 2-5- Determination of the Degree of Esterification

To measure the degree of esterification (DE), the method described by Wai et al. (2010) was employed, and the DE of different pectins was compared to investigate the effect of extraction time on this parameter. For this purpose, 2 mL of 96% ethanol was added to 0.1 g of the sample, followed by the addition of 20 mL of distilled water, and the mixture was stirred at 45 °C until the sample was completely dissolved. Subsequently, 2 drops of phenolphthalein were added, and the solution was titrated with 0.1 N sodium hydroxide (NaOH) until a pink color

appeared. The volume of NaOH consumed was recorded ($V_1$). To the previously neutralized solution, 10 mL of 0.5 M sodium hydroxide was added, mixed, and then left undisturbed for 20 minutes. Next, 10 mL of 0.5 M acid was added, and the mixture was titrated again with 0.1 N sodium hydroxide until the pink color reappeared ($V_2$). The calculation was performed based on Equation 2 [17].

$$DE = \frac{V_1}{V_1 + V_2} \times 100 \quad (2)$$

**2-6- Kinetic Modeling and Calculation of the Effective Diffusion Coefficient**

Kinetic modeling was performed to understand the mass transfer behavior and reactions during pectin extraction. The experimental data of pectin extraction were investigated using three mathematical models, including zero-order, first-order, and second-order kinetic models, using MATLAB software version 2016a. Based on the statistical indicators of the mentioned models, including the coefficient of determination ($R^2$) and root mean square error (RMSE), the most suitable model for describing the extraction process was determined. These models are 1, 2, and 3, respectively. In these models, the coefficient k represents the rate constant, Yeq indicates the equilibrium concentration or at infinite time, and t represents the time.

$$Y = K.t \quad (1)$$

$$Y = Y_{eq}(1 - e^{-k.t}) \quad (2)$$

$$Y = \frac{Y_{eq}^2 k.t}{1 + Y_{eq}.k.t} \quad (3)$$

Subsequently, to specify the extraction conditions, the Reynolds number and the effective diffusion coefficient were calculated. Mass transfer in solid particles obeys Fick's second law, assuming that the diffusion coefficient remains constant throughout the process. Assuming the particles are spherical, Crank's equation, derived from the analytical solution of mass transfer, describes the mass transfer radially. Based on Equation 4, the effective diffusion coefficient from the particle can be calculated. Additionally, the Reynolds number (Re) was calculated according to Equation 5 [18]. In this equation, N represents the rotational speed in revolutions per second, D is the impeller diameter, and μ denotes the fluid viscosity (acidic aqueous solution).

$$\frac{C_t}{C_\infty} = \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(-n^2 \frac{\pi^2 D_{ef}^t}{r^2}\right) \quad (4)$$

$$Re = \frac{\rho N D^2}{\mu} \quad (5)$$

**2-7- Water-Holding Capacity**

One gram of pectin was mixed with 10 mL of water and vigorously stirred for 1 minute. Subsequently, the mixture was centrifuged at 4000 g for 30 minutes. The supernatant was removed, and the remaining residue was weighed. The water-holding capacity was reported as the ratio of the weight of the retained water (the difference between the second weight and the first weight) per weight of the pectin (the first weight) [19].

**2-8- colorimetry**

The color parameters (L*, a*, b*) of the extracted pectin were measured using a colorimeter (Model ZE-6000, Nippon, Japan) based on the CIE LAB color system, with a D65 light source and a 10° observer angle. Chroma (color intensity) was calculated using Equation 6 based on the parameters a* (positive a* values indicate redness and negative a* values indicate greenness) and b* (negative b* values indicate blueness and positive b* values indicate yellowness) [20]:

$$\text{Chroma} = \sqrt{(a^*)^2 + (b^*)^2} \quad (6)$$

### 2-9- Investigation of Viscosity-Average Molecular Weight

The intrinsic viscosity of dilute pectin solutions (5 mg/mL) was measured using a capillary viscometer (Ostwald model, Hayan Company, Shiraz, Iran) fixed in a water bath at a constant temperature of 25 °C. The pectin samples were dissolved in a 0.2 M sodium chloride solution. The intrinsic viscosity was calculated using Equation 7. Here, $t_0$ and ts were recorded as the efflux times of the solvent and the sample solution from the capillary tube, respectively, and $[\eta]$ represents the intrinsic viscosity [21].

$$[\eta] = \frac{1}{c}\sqrt{2\left(\frac{(t_s - t_0)}{t_0} - \ln\left(\frac{t_s}{t_0}\right)\right)} \quad (7)$$

Subsequently, the Mark-Houwink equation was utilized to determine the relationship between the molecular weight and intrinsic viscosity.

### 2-10- Preparation of Pectin Gel

To formulate the pectin gel, 45% sucrose, 2% pectin, and 80 mg of calcium chloride per gram of pectin were utilized for gel formation. To prepare the gel, pectin was initially added to water and heated under agitation to achieve complete dissolution, after which calcium chloride was introduced. Finally, the fluid was allowed to cool, and the gel strength was evaluated after 24 hours [22].

### 2-11- Pectin Gel Stiffness Investigation

A Centam texturometer (STM1, made in Iran) was used to measure the stiffness of gels obtained from extracted pectins. The device was equipped with a load cell with a capacity of 20 kg and a cylindrical probe with a diameter of 5 mm. The gel samples were placed in a container with a diameter of 40 mm and a height of 40 mm, and the probe penetration process was carried out at a constant speed of 60 mm/min. In this test, gel stiffness was considered as the force required to reach a penetration depth of 12 mm, and the data obtained from the force-displacement curve were stored and analyzed.

### 2-12- Optimization

In order to determine the optimal extraction time that leads to the achievement of the best properties of the extracted pectin, a multivariate optimization method based on numerical interpolation was used with the MATLAB 2016 software. All index data (including yield, degree of esterification, degree of galacturonic acid, molecular weight, gel strength and water holding capacity) were normalized using a linear scaling method in the range [0-1]. The PCHIP method was used to model the changes in variables in time intervals between experimental data. This method was chosen because it maintained the monotonicity of the data and prevented unrealistic fluctuations. In this study, equal weights were considered for all variables and the goal was to maximize all responses. The optimal time was determined by numerical search in the range of 30 to 120 minutes and with time steps of 1 minute, at the point that had the highest total score [23].

### 2-13- Statistical analysis

Laboratory data were analyzed using SPSS version 17 statistical software. Experiments were performed in three replications in a completely randomized design, and significant differences between treatments were determined using analysis of variance and Tukey-Kramer test at a confidence level of 95% ($p < 0.05$).

## 3- Results and discussion

### 3-1- Pectin extraction yield

Pectin extraction from plant sources such as sugar beet is affected by several factors, the extraction time being one of the most important.

The experimental data (Figure 1) shows that the pectin extraction efficiency increases almost linearly with increasing time and then enters a region with a reduced growth rate. In the range of 0 to 90 minutes, the efficiency reaches 12 ± 0.15, but then, up to 120 minutes, another increase of about 1% is observed (13.05 ± 0.20). This trend indicates that the extraction process has reached a plateau and saturation. However, the difference at this time is still significant ($p<0.05$). From a kinetic perspective, the extraction process of pectin from sugar beet can be divided into three stages: In the first stage (0–30 minutes), surface and accessible compounds are extracted rapidly. In the second stage (30–90 min), the solvent penetrates deeper areas and dissolves the more difficult-to-access pectic compounds (linked to other components of the cell wall). In the third stage (90–120 min), the process approaches equilibrium and the extraction rate decreases. This pattern can be explained by solid-liquid mass transfer and Fickian diffusion models [24]. Energy is one of the key parameters in industrial and economic processes. The balance between quantity, quality and economy determines the optimum point in extraction. From an economic perspective, a time of 90 min is suggested as the optimum point. At this time, 92% of the maximum yield is achieved, while a 25% reduction in process time compared to 120 min results in significant savings in energy consumption and operating costs. Therefore, choosing the right extraction time not only ensures the desired yield but also improves the efficiency of the process. In the process of extracting pectin from sugar beet, the extraction time plays a decisive role in the quality and quantity of the final product. Studies have shown that a time interval of 30 to 90 minutes provides the best conditions for effective pectin extraction [25].

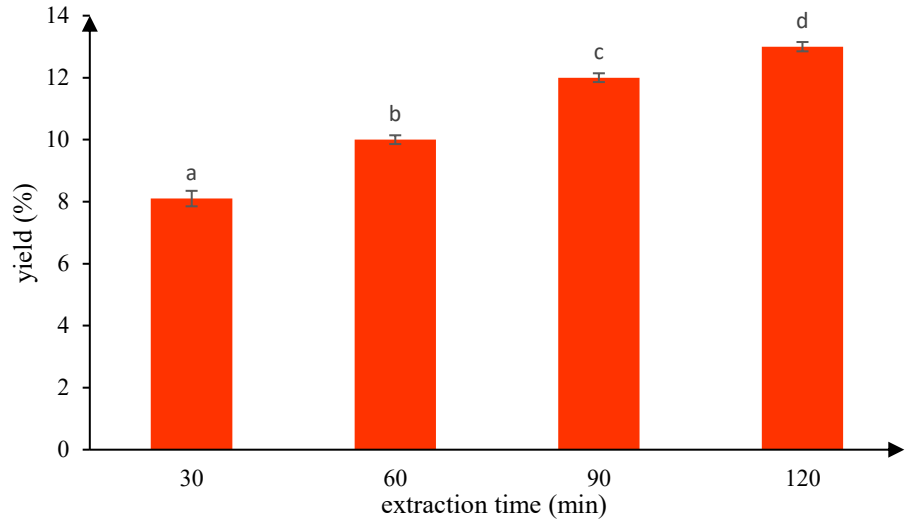


Figure 1- The effect of time on pectin extraction yield
Different letters in each column indicate a significant difference between the data ($p<0.05$)

### 3-2- Effect of time on the amount of galacturonic acid and the purity of extracted pectin

The amount of galacturonic acid indicates the degree of purity of pectin. The results of the effect of extraction time on the amount of galacturonic acid in the resulting pectin are shown in Figure 2. Based on these results, the purity of extracted pectin decreases after 60 minutes. In addition to affecting the ability of pectin to form gel, the decrease in pectin purity also affects the rate of pectin dissolution. Statistical analysis of the data clearly showed that the amount of galacturonic acid in the samples extracted from 90 and 120 minutes of extraction did not differ statistically

significantly ($p<0.05$); also, the amount of galacturonic acid in the samples obtained from 30 and 60 minutes of extraction did not differ statistically significantly ($p<0.05$). However, the difference between these two subgroups is statistically significant, such that the highest amount of galacturonic acid was obtained at 30 and 60 minutes. Maric et al. (2018) also reported the pectin purity in the same optimal time interval [25]. The significant decrease in galacturonic acid percentage at 90 and 120 min can be attributed to three main factors: first, thermal degradation, which breaks polygalacturonic chains and converts them to simpler sugars due to prolonged exposure to high temperature; second, the beta-elimination mechanism, which destroys the pectin structure under acidic conditions and high temperature; and third, the introduction of impurities into the final product, which can reduce pectin purity [26, 27]. Overall, extraction time is known to be a key variable in determining the percentage of extracted galacturonic acid. The data show that 30 and 60 min times are significantly ($p<0.05$) better than 90 and 120 min times. Among these times, 60 minutes with an average of 75.52±2.0% was determined as the appropriate time to achieve maximum purity of the extracted sample.

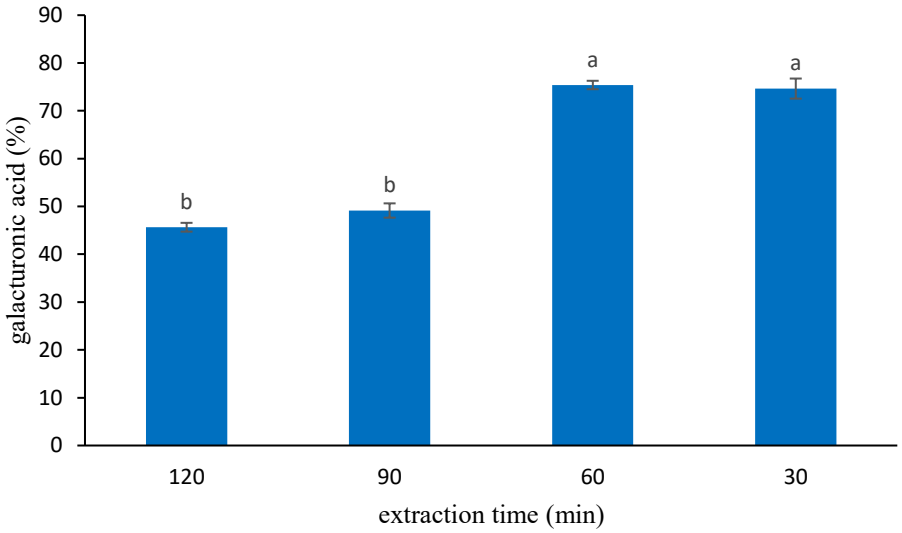


Figure 2- The effect of time on the amount of galacturonic acid present in the extracted pectin
Different letters in each column indicate a significant difference between the data ($p<0.05$)

### 3-3- Effect of Time on the Degree of Esterification of Extracted Pectin

In the pectin extraction process, the degree of esterification (DE) is an important product characteristic, as DE directly affects the functional properties of pectin such as gelation, viscosity. The results of one-way ANOVA test showed that extraction time has a statistically significant effect on DE (Sig. = 0.006, $p < 0.01$). This finding indicates that time changes in the extraction process affect not only the quantity but also the structural quality of pectin.

The results of the effect of extraction time on the degree of esterification of sugar beet pectin are shown in Figure 3. Based on these results, pectin extracted from sugar beet mainly falls into the category of pectins with high methoxyl degrees. However, at longer extraction times (120 minutes), a decrease in the degree of esterification below 50% was observed, indicating the breakage of the polymer chain due to prolonged heat treatment. The study of the changes showed that in a short time interval (30 to 60 minutes), the degree of esterification did not differ significantly and the pectin structure was maintained; however, with

increasing time from 60 to 90 minutes and then to 120 minutes, a significant decrease in the degree of esterification occurred ($P < 0.01$). This pattern suggests that longer extraction times lead to structural degradation of pectin and, as a result, the degree of esterification decreases significantly. These findings are also consistent with the results obtained from measuring the galacturonic acid content. Specifically, with increasing extraction time from 30 to 120 minutes, the DE value decreased from 66.5±2.1% to 47.5±2.9%. This significant decrease can be attributed to the process of breaking ester bonds that occurs under high temperature and acidic environment. Under these conditions, the methoxy groups ($-OCH_3$), which are responsible for the esterification of the polygalacturonic chain, gradually dissociate, causing a decrease in DE. This phenomenon is not only explainable from a chemical point of view, but also important in terms of industrial application, since pectin with a lower DE will behave differently in the food, pharmaceutical and cosmetic industries. Previous studies have also confirmed this trend. Kravchenko et al. (1999) in a comparative study of industrial pectins showed that prolonged exposure to high temperatures leads to the destruction of methoxy groups and a decrease in DE. Also, in acidic environments, the beta-elimination mechanism is activated and causes the breakage of pectin chains. These mechanisms not only reduce DE, but may also lead to the introduction of impurities into the final product, which affect the purity and performance of pectin [27].

From a practical perspective, these findings suggest that the extraction time should be adjusted to maintain a balance between yield and structural quality. Shorter times, such as 30–60 min, not only provide acceptable yields but also preserve the pectin structure. In contrast, longer times, although they may slightly increase the yield, may compromise the functional quality of the pectin by reducing the DE. Therefore, the selection of the optimal extraction time should be based on the ultimate goal of the pectin application; for example, in gelling applications, maintaining a high DE is a priority, while in some thickening applications, a lower DE may be more desirable.

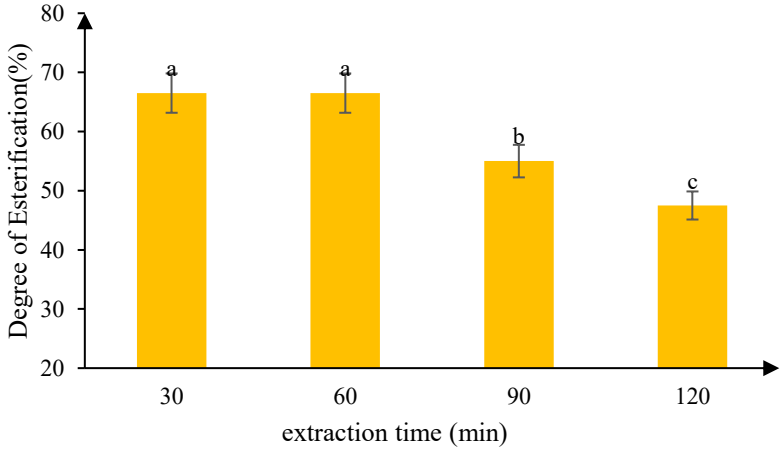


Figure 3- The effect of extraction time on the degree of esterification of pectin
Different letters in each column indicate a significant difference between the data ($p<0.05$)

### 3-4- Kinetic Modeling Results and Effective Extraction Coefficient

In the process of pectin extraction from sugar beet, kinetic analysis plays an important role in understanding the mass transfer behavior and chemical reactions. In this study, experimental data of pectin extraction were examined with three different mathematical models: zero-order, first-order and second-order kinetic models (Figures 5, 4 and 6). Based on statistical

indices including coefficient of determination ($R^2$) and root mean square error (RMSE), the second-order kinetic model was identified as the most appropriate model to describe the extraction process. This model showed a very good fit to the data with $R^2$ of 0.998 and RMSE of 0.441, and its parameters included final equilibrium concentration (c∞) of 16.41% and extraction rate constant (k1) of 0.0018/min. In comparison, the first-order model also performed reasonably well with an $R^2$ of 0.990 and an RMSE of 0.581, but the second-order model showed better fit coefficients in accurately predicting the extraction behavior. This model estimated the equilibrium concentration to be 13.09% and the rate constant to be 0.029/min. In contrast, the zero-order model with an $R^2$ of 0.726 and an RMSE of 2.421 failed to provide a good fit to the data and was not considered suitable for describing the extraction process.

The results of this section based on the quadratic model show that the extraction of pectin from sugar beet is a complex mechanism and not only a linear relationship of time and concentration but also several mechanisms simultaneously with chemical reactions affect mass transfer. The findings of this study are consistent with previous research in the field of pectin extraction from other plant sources and emphasize that nonlinear models such as the quadratic model are a suitable tool for kinetic analysis of such processes [28, 29].

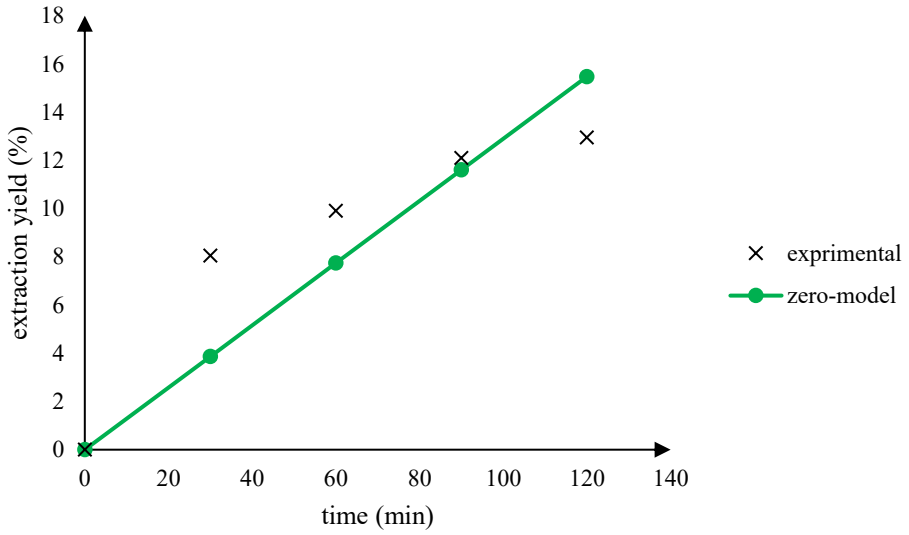


Figure 4- Zero-order kinetic model, crosses represent the experimental data, and the circles and line indicate the model data.

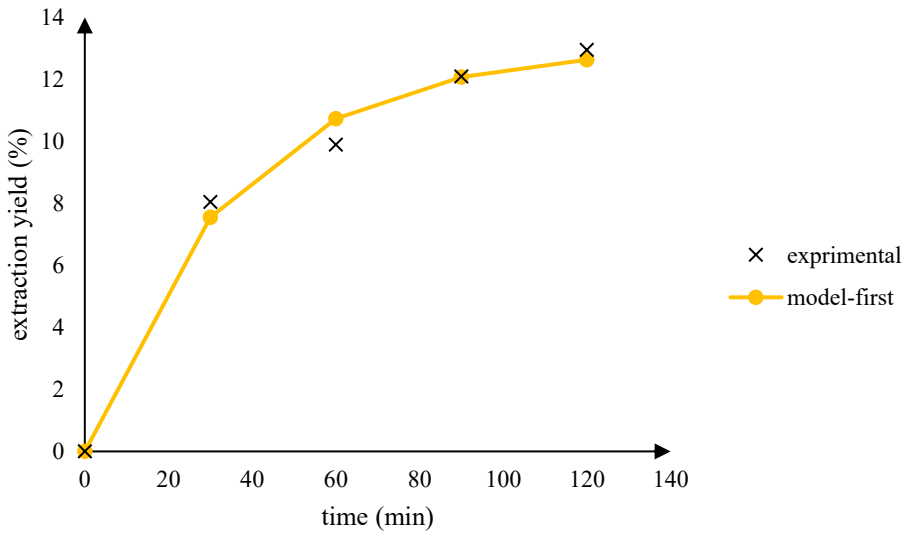


Figure 5- First-order kinetic model, crosses represent the experimental data, and the circles and line indicate the model data

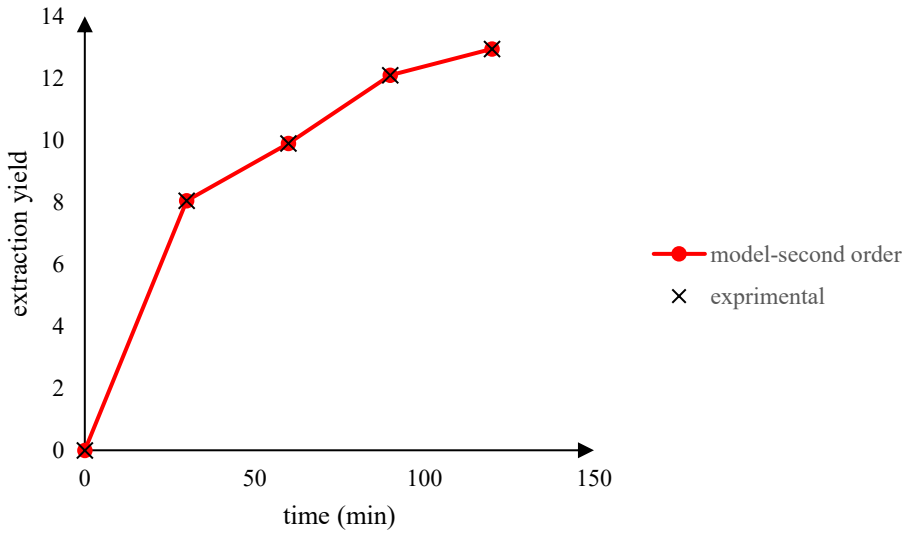


Figure 6- Second-order kinetic model, crosses represent the experimental data, and the circles and line indicate the model data

Since extraction conditions can affect some of the properties of pectin, such as yield or esterification degree, in this part of the research, the conditions were calculated numerically based on the characteristics of the fluid flow and the coefficients of the transfer phenomena in the mass transfer process.

The results of Reynolds number calculations and effective extraction coefficient calculations are necessary to express the real conditions governing extraction, along with temperature, pH, and time. Therefore, to investigate the mixing conditions and fluid flow inside the beaker, the Reynolds number of the magnetic stirrer was calculated by considering the effective length of the magnet (3 cm) as the diameter of the stirrer. The Reynolds number was calculated to be 6875. This value largely indicates turbulent vortex flow in stirring

systems inside the beaker. Such conditions indicate effective and uniform mixing of the pulp particles in the solvent and minimize the resistance of the boundary layer around the particles. Also, in order to determine the speed of movement of pectin inside the solid matrix of pulp particles, the effective diffusion coefficient (Deff) was calculated using the Crank model for spherical particles and using experimental extraction data at different times. Initially, the maximum extractable concentration or concentration at infinite time was selected as 16.41% based on the quadratic kinetic model (optimal mathematical model) and based on solving equation 4, the effective extraction coefficient was estimated to be $3.16\times10^{-13}$. AlMohammed et al. (2017) reported the effective diffusion coefficient of pectin extraction from sugar beet by acid extraction with electrical discharge pretreatment in the range of $7\times10^{-13}$ to $9\times10^{-13}$ $m^2/s$ assuming particle sphericity. These researchers also reported the use of pretreatment in significantly increasing mass transfer coefficients. This coefficient is a function of the solid-to-solvent ratio and particle size [12]. According to these results, while the calculated Reynolds number indicates excellent mixing in the liquid phase, the very small value of the effective diffusion coefficient (on the order of $10^{-13}$) indicates that the intraparticle diffusion step acts as the main bottleneck of the extraction process. In other words, despite the turbulent and suitable external environmental conditions, pectin molecules face significant resistance to exiting the compact structure of sugar beet pulp. For comparison, AlMohammed et al. (2017) reported a diffusion coefficient in a higher range than the present study using electrical discharge pretreatment, indicating the positive effect of pretreatment in reducing the internal resistance of the solid material [12].

**3-5- Water holding capacity**

Statistical analysis of the results of the investigation of the water holding capacity of sugar beet pectin ranges from 6.85±0.07 to 8.30±0.07 g/g of sample, which is given in Table 1. Water holding capacity is one of the important properties that refers to the ability of 1 g of wet material to hold water. The retained water is the sum of bound water, hydrodynamic water, and physically trapped water. In fact, pectin with higher WHC can be used to retain water and improve sensory properties in food systems. Based on the results of this study, the water holding capacity of Iranian sugar beet pectin is higher than that of eggplant pectin reported by Kazemi et al. (6.2 g/g of sample) [30]. Also, the WHC of sugar beet pectin is higher than that of tomato WHC reported by Namir et al. [31].

It was also found that extraction time has a significant effect on the water holding capacity of pectins extracted from sugar beet pulp ($p < 0.05$). The findings of this study showed that the water holding capacity of pectin extracted from sugar beet pulp at 30 and 120 minutes was 8.2±0.14 and 8.35±0.07 (g/g), respectively, significantly higher than those at 60 and 90 minutes of extraction and were not statistically significantly different from each other (30 and 120 minutes). The significant decrease in WHC at 90 minutes (6.8±0.7 g/g) compared to shorter times (30 and 60 minutes) could be due to structural degradation of pectin due to prolonged contact with acidic solvent and high temperature. This degradation may lead to a decrease in the ability of pectin chains to retain water molecules through hydrogen bonds. However, it was found that the WHC of the sample extracted at 120 min not only did not decrease, but increased. This re-increase can be attributed to several factors. First, with more time, more pectin and associated compounds (such as hemicelluloses) are extracted, which may themselves have a high water-holding capacity. Also, the controlled degradation of the pectin structure over a long time can lead to the creation of shorter chains with a higher number of free hydroxyl groups that have a higher affinity for water.

### 3-6- Colorimetry

Color is an important indicator for pectin because it plays a significant role in its acceptance before use as a food additive. During the extraction process, the degradation of the cell wall makes water-soluble pigments available for dissolution in the solvent. These pigments are trapped in the pectin during the precipitation step and affect the color of the pectin [32]. Visual results showed that the samples had a whitish-yellow color. The color parameters are shown in Table 1. Based on these results, it can be said that the color of sugar beet pectin samples is lighter and yellower than the color of pectin extracted from tomato peels extracted by ultrasonication, which was reported by Sengar et al. in the range (a=8-11 and b=21-24) [32]. The results of one-way ANOVA test with Tukey test showed that the extraction time has a significant effect on the chroma index of the extracted pectin samples ($p < 0.05$). The values show that with the increase in extraction time from 30 to 90 minutes, the color intensity tends to decrease, which could be due to thermal degradation of pigments due to longer contact with acidic solvent. However, at the extraction time of 120 minutes, the color intensity reaches its highest value. Considering that no statistically significant difference was observed between 120 minutes and 30 minutes, it cannot be concluded that the increase in color intensity at 120 minutes is definite. Therefore, it can be said that the overall trend of color intensity in the time range from 30 to 120 minutes lacks a uniform increasing or decreasing pattern and the extraction time has a complex and nonlinear effect on this index. In short, although the extraction time is statistically effective on chroma, this effect is not simply incremental and is probably the result of the simultaneous interaction of two opposing phenomena: the release of pigments from the matrix and their thermal degradation.

table 1- Colorimetric Parameters and Water Holding Capacity of Sugar Beet Pectin

| Extraction time (min) | $b^*$ | $a^*$ | Chroma | WHC |
|---|---|---|---|---|
| 30 | 19.7±0.5$^a$ | 4.5±0.4$^a$ | 20.2±0.7$^a$ | 8.2±0.14$^a$ |
| 60 | 18.5±1.5$^a$ | 3.8±1.0$^a$ | 18.8±2.0$^{ab}$ | 7.7±0.14$^b$ |
| 90 | 16.1±1.1$^b$ | 3.9±0.7$^a$ | 16.6±0.7$^b$ | 6.9±0.07$^b$ |
| 120 | 20.6±0.2$^a$ | 4.2±0.9$^a$ | 20.9±0.2$^a$ | 8.3±0.07$^a$ |

*Different letters the indicator Significant difference It is between samples ($p<0.05$)

### 3-7- Rheological and molecular properties of extracted pectin

The functional properties of pectin are influenced by molecular properties such as molecular weight and intrinsic viscosity. In this study, the effect of extraction time on these two key parameters was also investigated and the results showed that increasing the time from 90 to 120 minutes resulted in a 34.5% decrease in molecular weight and intrinsic viscosity. The decrease in intrinsic viscosity and molecular weight indicates the occurrence of degradation of polygalacturonic chains at longer extraction times. This phenomenon arises from two main mechanisms including hydrolytic degradation and beta-elimination. In hydrolytic degradation under high temperature and acidic conditions, glycosidic bonds between galacturonic monomers are broken and long chains are converted into shorter oligomers. This mechanism has been introduced as the main

factor of molecular weight reduction in industrial pectins. The beta-elimination degradation mechanism also occurs at high temperatures and low pH, resulting in the random breakage of pectin chains. Unlike hydrolytic degradation, which is relatively controllable, beta-elimination can irreversibly alter the structure of pectin and impair its functional properties. [33].

The relationship between molecular weight and intrinsic viscosity is expressed by the Mark-Hovink equation. This equation includes [η], intrinsic viscosity, molecular weight (M) and is expressed as $[\eta] = K \times M^{\alpha}$. K and α are constants depending on the type of polymer and solvent. Harding et al. (2017) noted that this relationship shows that intrinsic viscosity can be used as a rapid and non-destructive indicator to estimate molecular weight and evaluate the structural quality of pectin. This feature is very valuable for quality control in industrial production lines [34]. Studies by Panouillé et al. (2014) and Maric et al. (2018) have also confirmed the decrease in molecular weight and intrinsic viscosity of pectin at longer extraction times. These studies showed that over-extraction not only reduces the gelling performance of pectin, but may also result in a product with lower purity and poorer rheological properties [35, 36].

Based on the results in Table 2, it is clear that the extraction time had a significant effect on the molecular weight of pectin. With the increase in the extraction time from 30 minutes to 120 minutes, both the intrinsic viscosity and molecular weight indices showed a significant decreasing trend. The intrinsic viscosity decreased by approximately 43% and the molecular weight decreased from 182.6±1.1 to 119.2±1.2 kDa (approximately 35% decrease). These results indicate that with the extension of the extraction time, the phenomenon of thermal degradation occurred in the pectin chains. The glycosidic bonds in the main body of pectin were broken by heat and the extraction medium (acidic) and the long molecules were converted into shorter fragments.

Table 2- Molecular characteristics of the extracted pectin

| Extraction Time (min) | Viscosity-Average Molecular Weight (K Da) | Intrinsic Viscosity (mL $g^{-1}$) |
|---|---|---|
| 30 | 182.6±1.1[a] | 15.71±0.10[a] |
| 60 | 179.6±1.9[a] | 15.21±0.21[a] |
| 90 | 162.7±0.7[b] | 13.55±0.09[b] |
| 120 | 119.2±1.2[c] | 8.88±0.11[c] |

*Different letters the indicator Significant difference It is between samples (Duncan's test, $p<0.05$)

From an industrial perspective, choosing the right extraction time can purposefully adjust the functional properties of pectin. For example, high molecular weight pectin is suitable for applications such as strong gelling in jams, jellies and dairy products, while low molecular weight pectin is used for emulsification, beverage stabilization and pharmaceutical applications. The use of intrinsic viscosity as a quality control indicator allows for rapid and accurate monitoring of the extraction process and prevents the production of undesirable products [37].

**3-8- Gel Stiffness of Pectin**

Analysis of the effect of extraction time on the gel stiffness of sugar beet pectin not only indicates the functional changes of this biopolymer under different processing conditions, but also reflects its structural and molecular changes. The data from the histometric test clearly show that the extraction time plays a decisive role in the gel forming ability of pectin (Figure 7). In the range of 30 to 60 minutes, the gel stiffness is 30.10 ± 0.72 and 31.63 ± 1.44 g-force, respectively, which is not statistically significant. By increasing the

extraction time to 90 and then 120 minutes, the gel stiffness decreased significantly and reached 15.56 and 12.5 g-force, respectively. This functional loss can be attributed to the thermal degradation of polysaccharide chains, the decrease in molecular weight and the decrease in the degree of esterification. Under these conditions, the long chains of pectin are converted into shorter oligomers and the methoxyl groups are broken down by diesterification. These structural changes lead to a decrease in the ability of pectin to form a coherent gel network, as the intermolecular interactions are weakened and the three-dimensional structure of the gel is partially collapsed [38]. Therefore, the samples extracted at short extraction times (30–60 min) had better gelation properties than those obtained at longer extraction times. This improvement can be attributed to the increased extraction of high molecular weight pectins, maintaining the structure and degree of esterification suitable for gel formation. At this stage, the polygalacturonic structure is still preserved and the methoxy groups (-$OCH_3$) remain in sufficient quantities in the chain, which is essential for the formation of a strong gel network [39]. This pattern indicates the existence of an optimum point in the extraction process; The point at which both the extraction yield and the functional quality of pectin are in the desired state. Previously, Faravash and Ashtiani (2008) also showed in a study that controlled extraction conditions can lead to the production of pectin with desirable functional properties [40].

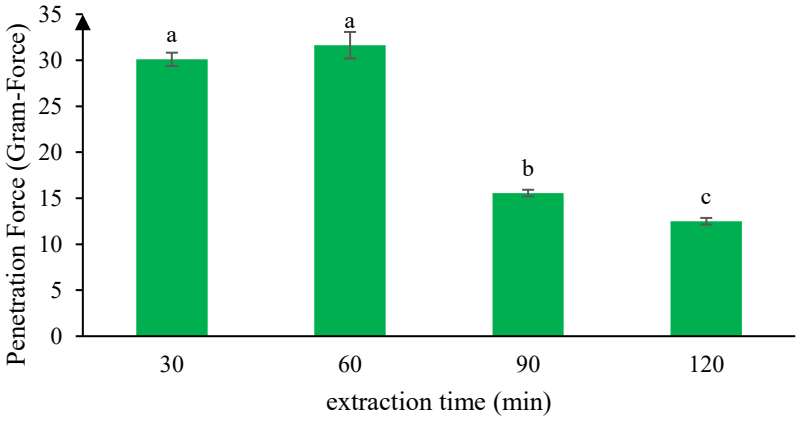


**Figure 7- Effect of Extraction Time on Pectin Gel Strength**

Different letters in each column indicate a significant difference between the data ($p<0.05$)

### 3-9- Optimization

The results of the optimization algorithm were based on the highest extraction efficiency and at the same time the highest purity of the extracted product based on the galacturonic acid percentage index, the highest preservation of the initial structure based on the maximum esterification degree index and viscosity-dependent molecular weight, the highest gelation quality based on the maximum gel stiffness index and the highest water retention capacity. Accordingly, the extraction time of 60 minutes was calculated to have the highest quality and highest efficiency of extracting pectin from sugar beet. In this period of time, a balance of product quality and extraction efficiency is achieved.

## 4- Conclusion

Based on the findings of this study, pectin extraction from sugar beet pulp is a complex process with a nonlinear pattern in terms of yield, quality characteristics, and performance. Time, as one of the key factors, has a direct

impact on the extraction yield, purity, and chemical structure of pectin. The second-order kinetic model was able to express the pectin extraction efficiency mathematically with sufficient accuracy. The results showed that with increasing extraction time, the yield increases significantly, but at long extraction times, the product quality decreases due to thermal degradation of polysaccharide chains and the introduction of impurities into the final product. This decrease includes a decrease in purity, molecular weight, and gel strength of pectin. Finally, considering the balance between yield and quality, an extraction time of 60 minutes was identified as the most optimal condition to achieve the highest extraction yield while maintaining the most appropriate quality characteristics of pectin.

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### Data Availability

Research data are included in the text and will not be shared outside of the rules of this journal.

### Conflict of Interest

The authors declare that there is no financial or personal conflict of interest in relation to this research.

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### Author Contributions

It is hereby confirmed that all authors have actively participated in the design, analysis, writing and approval of the final version of this article.

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# بررسی ویژگی های فیزیکی-شیمیایی و سینتیک استخراج پکتین از تفاله چغندر قند بومی ایران

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**چکیده**

**پکتین یک هیدروکلوئید ارزشمند در صنعت غذا می‌باشد که ویژگی‌های آن تا حد زیادی به منبع آن وابسته است. به منظور تعیین کاربرد، شناخت ویژگی‌ها، خواص عملکردی و مدل‌های ریاضی استحصال پکتین، ضروری می‌باشد. بنابراین، این پژوهش بر شناسایی ویژگی‌های ذاتی و عملکردی پکتین استخراج شده از تفاله چغندر قند حاصل از کارخانه قند تمرکز دارد. در این پژوهش پکتین از تفاله چغندر قند بومی ایران در زمان‌های مختلف با حلال اسیدی استخراج گردید. ویژگی‌های پکتین شامل بازده، درجه استریفیکاسیون، درصد اسید گالاکتورونیک، وزن مولکولی وابسته به ویسکوزیته، سفتی ژل، شدت رنگ و ظرفیت نگهداری آب اندازه‌گیری شد. همچنین ضریب انتشار موثر و مدل‌های سینتیکی برای توصیف رفتار استخراج برازش گردید. نتایج نشان داد با افزایش زمان استخراج، بازده از ۰/۲۵ ± ۸/۱۰ درصد در ۳۰ دقیقه به ۱۳/۰±۰۵/۲۰ درصد بعد از ۱۲۰ دقیقه افزایش می‌یابد، اما خلوص از ۱/۵۰±۷۴/۶۵ به ۴۵/۰±۶۵/۲۰ درصد کاهش می‌یابد. نمونه حاصل در دسته پکتین‌های با متوکسیل‌بالا قرار گرفت. بیشترین وزن مولکولی (۱/۱±۱۸۲/۶ کیلودالتون) در ۳۰ دقیقه و بیشترین سفتی ژل (۱/۴۴ ± ۳۱/۶۳ گرم-نیرو) در ۶۰ دقیقه مشاهده شد، بدون تفاوت معنادار بین این دو زمان ($p<0.05$). بیشترین شدت رنگ مربوط به ۱۲۰ دقیقه بود. ظرفیت نگهداری آب در زمان‌های ۳۰ و ۱۲۰ دقیقه به ترتیب ۸/۲±۰/۱۴ و۸/۳±۰/۰۷ (گرم/گرم) بالاتر از سایر زمان‌ها بود و مدل سینتیکی مرتبه دوم برازش مناسبی نشان داد. بر اساس تعیین ویژگی‌ها، پکتین استخراج شده در بازه زمانی۳۰-۶۰ دقیقه دارای بهترین ویژگی‌های کیفی شامل خلوص بالا، وزن مولکولی مناسب، قدرت ژل مطلوب، شدت رنگ قابل قبول و ظرفیت نگهداری آب مناسب می‌باشد.**