Optimization of the Conditions of Process of Production of Pectin Extracted from the Waste of Potato Peel

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ABSTRACT: *Potato peels contain valuable substances such as pectin extraction and the use of potato peels to produce pectin with appropriate properties can solve the biological problems resulting from these wastes in addition to value-added. The overall objective of this study is to investigate the effect of three variables including temperature (35, 65, and 95 °C), time (40, 120, and 200), and pH (1, 2, and 3) on the yield, galacturonic acid percentage and degree of pectin esterification extracted from the potato peels and optimization of the condition of extraction. The response surface method is used to optimize the conditions of extraction. The physicochemical properties of extracted pectin under optimum conditions were compared with commercial citrus and apple pectins by pectin flow behavior tests at different concentrations, FT-IR spectrum, and pectin molecular weight. The results of optimized single independent variables showed that the highest extraction yield of potato peel was 14.87% at 95 °C, 120 min time, and pH 1. The highest percentage of extracted galacturonic acid pectin in potato peel was 36.37% at 95 °C in 120 min and pH 1. The highest degree of esterification of extracted pectin from potato peel was 41.820% at 65 °C in 40 min time and pH 3.00. Simultaneous optimization of extracting pectin to achieve maximum yield was galacturonic acid with 100% desirability at 95 °C, 200 min time, and pH 1 in this condition, the yield was 15.23% and galacturonic acid was 38.0712%. The highest stability of extracted pectin emulsion from potato peel was observed at 4 °C on the first day. Also, the FT-IR results showed that the strong absorption between 3200-3500 cm^{-1} in the extracted pectin sample was related to the intracellular and extracellular vibration of the hydrogen bond in the galacturonic acid polymer. By increasing the concentration of pectin (0.1 to 2%) their viscosity was increased and the behavior of all samples was Newtonian and their flow index was about one. The molecular weight of extracted pectin from potato peel under optimum conditions was 53.46 kDa after 30 days of storage at 4 and 23 °C with emulsion stability 85.1 and 63.1, respectively. The results of this research showed that extracted pectin from potato peel can be introduced as a source of pectin to the market.*

KEYWORDS: *Potato peel; Response Surface Methodology (RSM); Pectin; Optimization.*

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INTRODUCTION

Pectin is a complex polysaccharide that is found in the wall of early plant tissues and in the intercellular layer. Pectin contains a group of rich polysaccharides of galacturonic acid units with lower amounts of different sugars [1]. Two commercial forms of pectin are available: high-methoxyl and low-methoxyl pectin (high-ester and low-ester pectin). High-ester pectin forms a gel in solutions containing highly soluble solids and acidic systems, whereas low-ester pectins form more gel at wider pH and range of solids content but they do require divalent cations to form the gel [2]. In the food industry, pectin is used as a jelly-making agent, especially in the production of jellies and jams. Pectin is also used in fillers, medicines, pastries, and bakery products and also as a stabilizer in juices and beverages, as well as in dietary fiber [3]. Pectin also has therapeutic benefits such as lowering blood cholesterol levels, removing heavy metal ions from the body, stabilizing blood pressure, and facilitating intestinal activity [4]. Pectin can be extracted from the cell wall material by hot or cold water, cool or hot solution of chelating agents, dilute hot acids, and cold diluted sodium hydroxide. The amount of water-extracted pectins is usually low. The disadvantage of extraction with chelating agents is that it is difficult to remove residues of chelating agents from extracted pectin. Alkaline extraction can reduce the degree of methylation, acetylation, and length of the main galacturonic acid chain. The most suitable method of industrial extraction of pectin is acid extraction. Temperature, pH, and acid extraction time are the most important factors affecting the extraction yield and quality of produced pectin [5]. Currently, almost all commercial pectins are produced from citrus or apple peels, both of which are juices' by-products [6]. However, much research has been done to find pectin-containing raw materials as well as to improve pectin extraction techniques. In this regard, *Nateghi et al.* [7] of eggplant wares, *Fathi et al.* [8] of nutty pumpkin lesions, *Hosseini et al.* [9] of orange peel, *Masibi et al.* [10] by waterlogging method from Blackberry Pulp, *Azadbakht et al.* [11] of Darabi fruit, *Keramat et al.* [12] of orange juice concentrate scraps extracted Pectin.

The potato plant, scientifically known as *Solanum tuberosum* L, is a one-year-old plant from the Solanaceae family and is one of the tuber products. The potato has compounded and cut leaves and white or purple flowers. Its fruit is small, spherical, and red. Potato stems and leaves contain a toxin called solanine that should not be used. But its root is edible and contains a lot of starch reserves [13]. The most important staple in potatoes is starch, which usually makes up 9 to 25 percent, so it is a good source of energy. In addition to various vitamins and minerals, potatoes contain important plant nutrients that have antioxidant activities [14]. Potato peel is high in fiber and other nutrients. Potato peel contains more iron, fiber, and folate than meat. In fact, potato peel has 2 to 3 times antioxidant more than its meat [1]. *Abang Zaidel et al.* [15] have reported the amount of pectin available in potato peel between 10% to 15%.

Pectin is a widely used polysaccharide in the food and pharmaceutical industries due to its unique technological and therapeutic properties. It has been used in many foods so far and it is possible to consider its use in other foods. Due to the existence of abundant and inexpensive resources to produce pectin in the country, producing this useful substance can prevent the import of this substance for industrial use. Due to the lack of research studies about the use of potato waste, this study attempted to use potato peel to extract pectin, a valuable ingredient in the food industry. Therefore, the main objective of this study was to optimize the conditions of extraction of pectin from potato peel by the response surface method and to compare the physicochemical properties of extracted pectin from potato peel under optimum conditions with commercial pectin of apple and citrus.

EXPERIMENTAL SECTION

Chemical and reagents

The Potato Granola variety was supplied by the local market in Ardebil. The chemicals used for the tests include tartaric acid, citric, hydrochloric, acetic, lactic, nitric, phosphoric, and sulfuric acid, 96% ethanol, methoxy hydroxyphenyl reagent, sodium tetra borate, sodium hydroxide, sodium phenolphthalein, 0. Sodium azide, sodium acetate, and sodium chloride supplied by Merck Company (Germany).

Extraction of pectin from potato peel

The potatoes were first washed with water and peeled. Next, were dried at 50 °C for 16 hours in the UF55 / UN oven made by Mommert Company (Germany) to reach a constant weight, then by using the mill under the brand of Pars Khazar Company (Iran) and model ML-320P formed flour granules granulated using the mill afterward sieved with the help of mesh 60. The produced powder was stored in polyethylene containers and in a dried place at 25 °C.

In the first stage, as an initial study and determination of the type of acid suitable for extraction of pectin from potato peel, the effect of tartaric acid, citric acid, lactic acid, nitric acid, phosphoric acid, and sulfuric acid on the yield of extracted pectin at 90 °C for 90 min in acidified water at pH 2.5 was tested. After determining the most suitable acid with the highest extraction rate, pectin was extracted by the following method. The method of *Raji et al.* [16] was used to extract pectin with slight modification as follows. First, 3g of dried powdered sample were poured into Erlenmeyer and mixed with 90 mL of acidified distilled water with the acid selected in the first step at specified pHs (1, 2, and 3) and mixed in the warm water bath at temperatures (35, 65, and 95 °C were stirred and heated by a magnetic stirrer (Genius, Germany) for a specified period (40, 120 and 200) minutes (the range of the test for each factor was selected based on the results of the initial tests). Response surface methodology (Box–Behnken) according to Table 1 was used to design the treatments. After passing the necessary time for extraction, the samples were removed from the hot water bath and the solution was filtered twice with filter paper, and the produced solution in order to separate the residual suspended particles was centrifuged at 4000 rpm for 20 minutes by centrifuge Model PIT320 R (Universal, Germany), and the supernatant phase was passed through filter paper 41 after centrifugation, and extract containing pectin was placed in the refrigerator. After the extraction reached 4 °C, ethanol 96% was added with a ratio of 1 to 2 (extraction to alcohol) to the solution in order to precipitate the pectin and kept overnight in the refrigerator for pectin complete precipitation and balance. The pectin precipitate was then separated by centrifugation at 4000 rpm for 15 min. The supernatant was removed and the remaining precipitate was washed to remove impurities with ethanol 70% and then with ethanol 96%. After each washing step with alcohol, the residual precipitate was removed from the liquid by centrifugation at 4000 rpm for 10 min. At the end of the extracted pectin samples by the frozen method were dried by freeze dryer model FD-5005-BT (Sana Pardaz Dena Co., Iran). The dried pectin after weighing to measure extraction yield was powdered and packed in polyethylene bags and stored in a refrigerator.

The yield of pectin production

The following equation was used to obtain pectin production yield [17]:

Table 1: Treatments studied with independent variables of time (X1), temperature (X2), pH (X3) by response surface methodology (Box–Behnken).

Run	pH	Time (Min)	Temperature (°C)
1	1	120	35
2	3	120	95
3	2	40	35
4	3	40	65
5	2	40	95
6	2	120	65
7	2	200	95
8	2	120	65
9	3	120	35
10	3	200	65
11	1	200	65
12	1	40	65
13	1	120	95
14	2	200	35
15	2	120	65

$$(\%)\text{Pectin produces yield} = \frac{Pectin\ Net\ Weight}{Raw\ material\ weight} \times 100$$

Measurement of galacturonic acid

The amount of galacturonic acid was measured according to *Mosayeni et al.* [18] method. The percentage of galacturonic extracted acid pectin from potato peel was measured by colorimetric method using meta-hydroxydiphenol reagent using an UltraViolet-Visible spectrophotometer (UK 2502 CE). 0.05 g of the extracted pectin sample was bulked in one Erlenmeyer 250 mL with deionized distilled water. The mixture was stirred with a magnetic stirrer until completely dissolved. 1 mL of this dilute solution containing pectin was transferred to three glass test tubes immersed in the ice-water mixture, one for measuring control sample absorption and two for measuring the absorption of sample pectin. Then, 6 mL of pre-prepared sodium tetra borate solution was added to each test tube and tubes were vortexed to mix their contents thoroughly. The contents of the tubes were heated in a boiling water bath 100 °C for 6 minutes and cooled

immediately to room temperature after the imitated time had elapsed. Two test tubes containing sample pectin 0.1 meta-hydroxydiphenol reagent and the 0.1 mL 0.5% sodium hydroxide solution to the test tube containing the control sample was added and the contents of each tube were completely mixed. The test tubes were left at room temperature for 15 min and then the absorbance of each tube was measured at a wavelength of 520 nm. The calibration curve was provided by measuring the absorbance of standard galacturonic acid solutions in the range of standard concentrations of 20, 40, 60, 80, and 100 µg/mL at 520 nm, and galacturonic acid concentration in each sample pectin was calculated by using the linear regression equation of the calibration curve and based on the dry weight of pectin [18].

Degree of esterification (DE)

The degree of pectin esterification was measured by titration method with slight changes. For this purpose, 0.1g of dried sample pectin with 3 mL of ethanol 98% was wetted and then 20 mL of deionized distilled water was added to the magnetic stirrer until completely dissolved at 40 °C. After the sample was completely dissolved, 5 drops of phenolphthalein were added. And it was titrated to pale pink with the sodium hydroxide 1% normal. The volume of consumed sodium hydroxide was recorded as V1. In the first step of titration 10 mL of 0.1 M NaOH was added to the neutralized solution and was stirred for 2 hours on a magnetic stirrer to make pectin in soap shape. 10 mL of hydrochloric acid 1% was added to it and stirred until the pink color disappeared. The additional acid was titrated with sodium hydroxide 1% normal to reach the same endpoint, and the volume of consumed sodium hydroxide was recorded as V2. The percent of esterification was calculated according to the following equation [19]:

$$DE\ (\%) = V2/(V1+V2) \times 100$$

Measurement of emulsion properties

Dalev et al. [20] method with slight changes was used to measure emulsifier activity and emulsion stability. For this purpose, 5 mL of sunflower oil was added to 5 mL volumetric 5% pectin solution (containing 0.02% sodium azide as antibacterial) for emulsification. Next, the samples were mixed with a homogenizer at 10,000rpm for 4 min. The emulsion was then centrifuged at 4000 rpm for 5 min. The emulsifier activity was calculated according to the following equation:

$$\text{Emulsifier activity}\ (\%) = \frac{\text{Emulsified layer volume}}{\text{Total sample volume}} \times 100$$

Also, to measure the emulsion stability, the sample was prepared as the above method and poured into 4 centrifuge tubes 10 mL. The samples were stored for 1 and 30 days at each 23 and 4 ◦C. Then this factor was calculated according to the following equation:

$$\text{Stability emulsion}(\%) = \frac{\text{Volume of remaining emulsion layer}}{\text{volume of initial emulsion layer}} \times 100$$

FT-IR spectrum

The FT-IR spectrum was drawn with the accuracy of 4on cm 4 cm by FT-IR spectrometer (Perkin Elmer Co., MA, USA) and using a potassium bromide plate method (KBr) in the range 4000 to 450 cm [21].

External Viscosity Measurement

The viscosity of different concentrations of pectin was measured by a programmable rotary viscometer model (LVDV-II Pro, Brookfield Engineering Inc., USA) with spindle LV at a constant temperature of 20 ° C. For each test, approximately 25 mL of each sample was transferred to the measuring cylinder, and the viscosity changes measured against the cut-rate increase ranged from 12.22 to 22.21 based on mPa/s [22]. The Herschel-Balckley model expressed by the power law equation was performed to study the flow behavior of samples:

$Z = k\ D^n$

Z: shear stress (N /m2)

K: Index of consistency or viscosity (Pa.sn)

D: Shear rate (S^{-1})

n: flow behavior index (without units)

Molecular Weight of Pectin

First, in order to measure the intrinsic viscosity of pectin ([η]), the concentrations of 0.1, 0.2, 0.3, and 0.4 of pectin in an aqueous solution containing 5 Molar sodium acetate and 0.1 Molar chloride sodium and 0.04, 0% sodium azide was prepared. The pectin solution was filtered through a 0.45-micron membrane filter and their flow time was measured by capillary tube viscometer No. 518.10. The solution temperature was kept constant by immersing the viscometer in a water bath at a controlled temperature

of 25 °C. The intrinsic viscosity of pectin was estimated by plotting the pectin concentration on the horizontal axis against the natural logarithm of relative viscosity divided by concentration (Keramer equation) and specific viscosity by concentration (Huggins equation) in the vertical axis [23].

(Keramer equation) $$\left(\ln \eta_{rel}\right)/C = [\eta] + K^{*}[\eta]^{2}$$

(Huggins equation) $$\left(\eta_{sp}\right)/C = [\eta] + K'[\eta]^{2}\, C$$

The intersection point means of these two graphs with a vertical axis was considered as the intrinsic viscosity of pectin according to DL/ G. Finally, the average molecular weight of pectin is from the Mark Houwink Sakurada equation, where *K* and *N* are constant numbers that depend both on the temperature and the soluble and solvent properties [24].

Equation Mark Houwink Sakurada $[\eta] = KM_{v}^{a}$

Statistical Analysis

In the current study, the effect of three independent variables including temperature (35, 65, and 95 °C), time (40, 120, and 200), and pH (1, 2, and 3) on the yield, galacturonic acid percentage and esterification degree of pectin extracted from the potato peels were examined. The response surface method is used to optimize the conditions of extraction. The designing of treatments was done by response surface methodology (Box- Behnken). Therefore 15 treatments were designed. Box Behnken method was used to analyze the yield data, degree of esterification, and galacturonic acid and analyze their optimum conditions. Analyzing the data of the emulsion stability section and determination of appropriate acid was performed using one-way ANOVA (Duncan) at 5% probability level using Minitab16 software.

RESULTS AND DISCUSSION

Determination of the suitable acid for extracting pectin from potato peel

The type of acid is an important factor in the pectin extraction process. This not only affects the performance of the extracted pectin but also determines its chemical and functional properties such as DE, molecular weight, emulsion activity, and gel strength [16]. In this study, the evaluation of pectin yield was evaluated by three types of organic tartaric acid, citric acid, lactic acid, and 3 types of mineral nitric acid, phosphoric acid, and sulfuric acid under constant conditions (90 °C, 90 min extraction time at pH 2.5). The results of determining the appropriate acid for extraction of pectin from potato peel are reported in Table 2. As can be seen, the acid type had a significant effect ($P \leq 0.05$) on the amount of extracted pectin. The highest amount of extracted pectin (8.63%) was citric acid and the lowest amount of pectin (2.18%) was related to sulfuric acid. The high rate of pectin extraction with citric acid due to its chelating properties and the presence of adsorbents in it made it more effective in extracting pectin than other acids [16].

Jamsazzadeh Kermani et al. [25] reported in their studies that citric acid was able to extract higher levels of pectin than hydrochloric acid and sulfuric acid. They also attributed it to the chelating property of citric acid. Citric acid can bind and extract more amount of solution pectin. Therefore, the yield of extraction of pectin with citric acid is higher than that of the other two acids which don't have chelating nature. *Azadbakht et al.* [11] reported that the use of hydrochloric acid and ethanol separation had the best yield of pectin extraction from *citrus decumana murry*.

Lin et al. [26] reported that adding small amounts of hydrochloric acid to the precipitation of apple pectin causes better pectin purification with ethanol. These researchers also used different solvents to extract pectin at temperatures of 36 and 60 °C and reported that the highest extraction yield was achieved respectively by hydrochloric acid, sulfuric acid, oxalate-ammonium, and sodium hexametaphosphate at temperatures of 60 and 36 °C. *Arsalan* & *Togrul* [27] could extract acid pectin by hydrochloric with 21.8% yield of *grapefruit peel*. After extraction with acid the precipitation of pectin and its purification by using ethanol. On the other hand, *Keramat et al.* [12] extracted the available pectin in the pulp of citrus with three different acids including nitric acid, sulfuric acid, and hydrochloric acid, and reported that the highest extraction yield was related to sulfuric acid.

The yield of Pectin Extraction from Potato Peel

The yield of extraction pectin from potato peel and the predicted yield of extraction pectin from potato peel are reported in Table 3. As can be seen, the different extraction conditions had a significant effect on the pectin yield ($P \leq 0.05$). The results showed that increasing temperature (from 35 to 95 °C), extraction time (from 40 to 120 min), and decreasing pH (from 3 to 1) had a significant effect on increasing pectin yield ($P \leq 0.05$). The highest yield of

Table 2: Evaluation of pectin efficiencies extracted from potato peel by different acids

Acid type	Pectin Extraction Rate (%)
tartaric acid	7.35±0.32[a]
Citric acid	8.63±0.26[a]
lactic acid	4.19±0.24[b]
nitric acid	3.82±0.48[b]
phosphoric acid	3.46±0.39[bc]
sulphuric acid	2.18±0.19[c]

Results are presented as mean ± SD. The lowercase letters indicate a significant difference (p≥0.05) in each column.

extraction of pectin from potato peel 14.87% was obtained at 95 °C, 120 min time, and pH 1, and the lowest yield 7.15% was achieved at 65 °C, 40 min time, and pH = 3.

The time of the extraction process is one of the factors influencing the yield of the extracted pectin. Extraction time has a direct relationship with the extraction yield so the yield increases as time increases [28]. The reason for the increase in yield with an increase in extraction time can be due to the increase in solubility and complete release of insoluble polysaccharides and pectin into the acidic solution [24]. Temperature and pH variables have the greatest effect on pectin yield. Many researchers have reported temperature as one of the important factors in the extraction of various polysaccharide compounds such as pectin [19]. Many researchers have reported that high temperatures increase extraction yield [29]. The reason for the increase in yield with an increase in the temperature of extraction can also be related to the greater solubility of polysaccharides and pectin in the extraction solvent and increased mass transfer from the solid to the solution [30]. Studies showed that as the temperature increases, the solvent penetration coefficient increases in the solid. Therefore, the rate of release of pectin into the solvent is enhanced [31]. The reason for the increase in pectin extraction yield with the decrease in pH may be due to the effect of acid on the cell wall of dried powder of potato peel and hydrolysis of insoluble pectin into soluble pectin, which increases pectin extraction yield. The stronger the acid, the more it breaks down the cell wall, thus releasing pectin into the acid causing increasing pectin [32]. To confirm the results of the present study, the solvent must first penetrate into the dry pulp tissue, dissolve the pectin and subsequently seep it out of the pulp tissue. *Nateghi et al.* [33] investigated the efficacy and physicochemical properties of pectin extracted from eggplant peel lesions. The researchers stated that in order to investigate the physicochemical properties of the produced pectin, the emulsion stability test at different days and temperatures was performed to study the flow behavior at different concentrations. According to the results, the highest extraction yield of eggplant peel was observed at 30.28% under severe extraction conditions at 90 °C, 150 min, and pH 1.5. Analysis of variance showed that extraction temperature was the most effective factor in the yield and degree of esterification of the extracted pectin.

Chaharbaghi et al. [34] investigated the extraction conditions of available pectin in pistachio green husk and the results showed that the highest pectin extraction yield was at 90°C, pH 0.5, and 30 min. In the study of *Raji et al.* (2017), acid extraction (citric acid) was used to extract pectin from watermelon shell and the effect of temperature (95-35 °C), time (200-200 μg), pH (3-1) was investigated. The highest extraction yield (29.48 ± 1.7) was obtained under optimum conditions (pH 1, temperature 95 °C, and after 200 min). *Abid et al.* [35] investigated the properties of pectin extracted from pomegranate peel by citric acid. According to the results, the highest extraction yield of pomegranate peel was observed between 6.8 and 10.1% at conditions of 86 °C, 80 min. and pH 1.5.

Analysis of variance was performed on the quadratic polynomial model whose results are shown in Table 4. As can be seen in Table 4, the coefficient of explanation of this model (R^2) was 97.36% and its modified coefficient of explanation (R^2-adj) was 92.62%, indicating a good fit to the experimental data. According to Table 5, the linear effects of all three variables such as time, temperature, and pH of extraction on pectin yield were significant (P≤0.05). The extraction rate increased significantly with increasing temperature and time and decreasing pH. Also, the quadratic effects of temperature, pH, time, and the interaction between temperature × time and time × pH and temperature × pH on pectin yield were not significant (P> 0.05).

Influence of extraction conditions on pectin yield

The results of surface and interaction effects on pectin extraction yield from potato peel are shown in Fig. 1. As can be seen, Fig.1 (a) shows the yield of pectin extraction from potato peel at constant pH 2 and temperature × time variations. As is known, the rate of yield operation has

Table 3: Comparison between yield, galacturonic acid and degree of esterification of extracted pectin from potato peel tested with predicted conditions.

Treatment	Pectin yield (%)		Galacturonic Acid (%)		Degree of esterification (%)	
	Extraction yield	Anticipated yield	Extracted Pectin	Predicted extract pectin	Extracted Pectin	Predicted extract pectin
1	12.23	11.842	29.14	28.570	17.45	18.599
2	8.32	8.726	17.73	18.300	35.78	34.631
3	8.54	8.299	18.34	18.045	31.45	30.480
4	7.15	7.210	14.45	13.550	41.82	41.471
5	10.84	10.374	21.19	21.520	27.45	28.948
6	10.23	10.320	23.78	23.650	24.45	24.373
7	11.44	11.681	28.06	28.355	20.87	21.840
8	10.34	10.320	23.63	23.650	24.36	24.373
9	7.35	7.531	15.67	16.865	38.23	39.549
10	8.54	7.892	19.57	18.705	36.17	36.349
11	13.75	13.69	33.23	34.130	16.45	16.799
12	11.02	11.667	25.84	26.705	19.65	19.471
13	14.87	14.689	36.37	35.175	15.35	14.031
14	9.23	9.696	24.12	23.790	31.29	29.793
15	10.39	10.320	23.54	23.650	24.31	24.373

Table 4: Response surface model of pectin extraction from potato peel.

Source	Model	R^2	R^2-adj
Pectin yield (%)	10.32 - 2.5638 A +0.6762 B + 1.0150 C + 0.2375 A^2 -0.4425 B^2+0.1350 C^2 - 0.3350 AB - 0.4175 AC - 0.0225BC	97.36	92.62
Galacturonic Acid (%)	23.65 - 7.145 A + 3.145 B + 2.01C+0.7112 A^2 -1.0887B^2 +0.3663C^2 - 0.5675 AB -1.2925AC +0.2725 BC	98.70	96.36
Degree of esterification (%)	24.3733 +10.3875 A -1.9487 B - 2.3712 C + 1.5433 A^2 + 2.6058 B^2 + 0.7858 C^2 - 0.6125 AB -0.0875 AC-1.6050 BC	96.40	98.71

A: Temperature, B: Temperature, C: Sample weight

increased with increasing time and temperature. The yield was observed 11.5% and higher than it at 90 °C and above it between 130 and 200 minutes. Fig. 1 (b) shows the yield of extraction of pectin from potato peel at a constant temperature of 65 ° C, and variation of pH × time. As is known, by increasing the time and decreasing the pH the rate of yield is increased. The yield was observed 13% and above it at 110 min and above it at pH 1. Fig. 1 (c) shows the yield of extraction of pectin from potato peel under constant time conditions of 120 minutes with changes in pH and temperature. As is known, the yield was increased by increasing the operating temperature up to 90 °C and decreasing pH. A yield of 14 and above was observed at temperatures above 83 °C and pH 1.

Optimal conditions of pectin extraction yield from potato peel

According to the results, the maximum yield or extracted pectin amount of potato peel was predicted to be 15.25% with a 100% desirability related to 95 °C temperature, 200 min time, and pH 1. The predicted results were confirmed by two replicate tests and the predicted optimum conditions yield to extract pectin from potato peel were practically applied in the laboratory and its yield

Table 5: Results of analysis of variance response model of pectin extraction from potato peel.

Source of Changes	Pectin yield (%)		Galacturonic Acid (%)		Degree of esterification (%)	
	F-value	P-value	F-value	P-value	F-value	P-value
regression	20.52	0.002*	42.23	0.00*	42.66	0.000*
Linear effects	59.51	0.000*	123.01	0.000*	122.19	0.000*
Temperature (A)	145.57	0.000*	22.94	0.05*	17.57	0.009*
Time (B)	10.13	0.024*	56.17	0.001*	11.87	0.018*
pH (C)	22.82	0.005*	289.92	0.000*	337.15	0.000*
Quadratic effects	1.00	0.466	1.73	0.276	4.25	0.077
Temperature×Temperature (A2)	0.58	0.482	0.35	0.579	0.89	0.389
Time × Time (B2)	2.00	0.216	3.11	0.138	9.79	0.026*
pH (C2) ×pH	0.19	0.684	1.33	0.302	3.44	0.123
interaction	1.06	0.444	1.96	0.239	1.54	0.313
Temperature × Time (A × B(	0.01	0.943	0.21	0.665	4.02	0.101
Temperature × pH (A × C)	1.93	0.223	4.74	0.081	0.01	0.917
PH × time (A × C)	1.24	0.316	0.91	0.383	0.59	0.478
Lack of fit	89.19	0.11	195.05	0.096	847.11	0.071

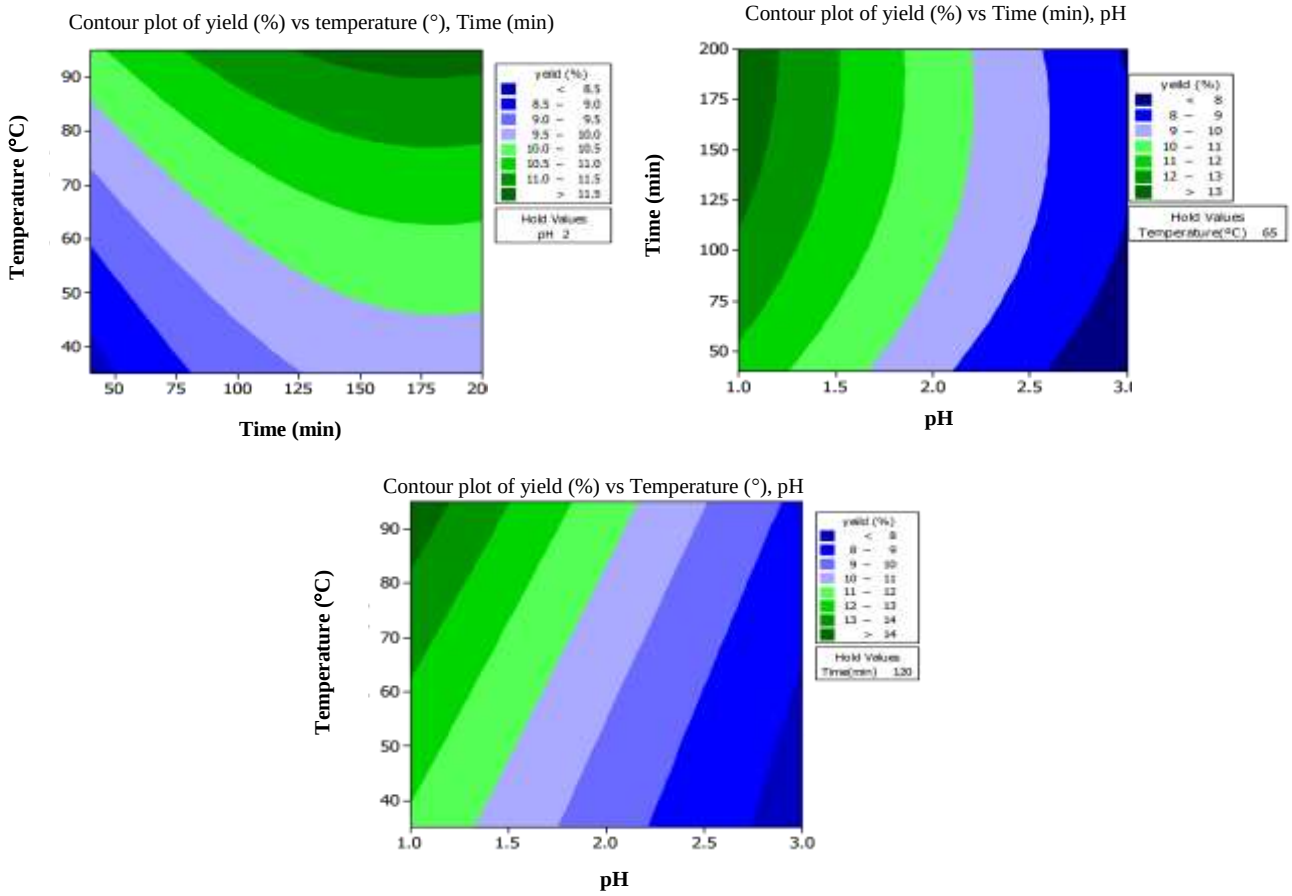

Fig. 1: Interactions of independent variables on pectin extraction yield from potato peel a) Temperature × extraction time, b) pH × time, c) Temperature × pH.

was 15.28% which was not significantly different from the predicted yield of 15.23%. *Jafari et al.* [36] reported that under optimum conditions the extraction of pectin from carrot pulp (pH 1.3, 90 °C, 79.8 min, liquid to solid ratio 23.3 v/w), the yield was 15.6% that was close to the predicted level (16%).

The amount of galacturonic acid pectin extracted from potato peel

The percentage of galacturonic acid indicates the purity of the pectin and by determining its amount the purity of precipitated pectin can be estimated with alcohol. The higher this parameter, the purer the resulting pectin is [37]. According to the results of Table 3, at pH and different temperatures of extraction by increasing temperature from 35 to 95 °C, increasing time from 40 to 200 min, and decreasing pH from 3 to 1% galacturonic acid the acid significantly increased. The highest percentage of galacturonic acid for potato peel at 95 °C, 120 minutes, and pH 1 was 36.37%. While the lowest percentage of galacturonic acid for extracted pectin was 14.45% at 65 °C, 40 minutes, and pH 3.

The reason for the increase in purity due to the decrease in pH and increase in temperature may be related to the basic hydrolysis of neutral pectin sugars at pH below 2 and high temperature [37]. As the temperature and time increase, a number of non-pectic materials in the cell wall such as cellulose, hemicellulose, galactan, and Arabian are hydrolyzed and combined with pectin during extraction, thus this factor causes the increase in the amount of galacturonic acid at elevated temperatures [38]. In fact, the reason for the increased galacturonic acid content of the extracted pectin is due to the separation of non-pectin compounds because other cell walls of polysaccharides such as cellulose, hemicellulose, Araban, and Galatians are also extracted. Another reason for the increase in the content of galacturonic acid of extracted pectin by passing time is the increased hydrolysis of neutral sugars side chains belonging to the pectin structure itself. These side chains, which are part of the pectin structure itself and are covalently linked to the linear chain segment, have formed mainly of arabinogalactan 1 in section rhamnogalacturonan and substituted with section rhamnogalacturonan 2 and also 4 side chains of 11 different sugars including apiose. As the extraction time increases, these lateral chains become further separated and bound to pectin [18].

Nateghi et al. [33] investigated the efficacy and physicochemical properties of extracted pectin from eggplant peel lesions. The highest percentage of galacturonic acid extracted from pectin was 50.76% at 75 ° C, 100 min., and pH 0.25. *Raji et al.* [16] studied extracted pectin with acid from watermelon shells. The results showed that the galacturonic acid pectin amount was 48% in optimum conditions (pH 1, temperature 95°C, and 10 w/v ratio after 200 minutes). *Keramat et al.* [12] extracted the available pectin in orange pulp and reported that the highest level of galacturonic acid was at 90°C, pH 1.8, and 50 minutes. *Yapo et al.* [37] extracted pectin from sugar beet under different conditions and found that pH was the most effective factor in the percentage of galacturonic acid and the extracted pectin at pH 1.5 was purer than the extracted pectin at pH 2. Temperature also has a moderate effect on the percentage of galacturonic acid.

Analysis of variance was performed on this quadratic polynomial model and the results are shown in Table 4. As can be seen in this table, the coefficient of determination of this model (R^2) was 98.70% and its modified coefficient of determination (R^2-adj) was 96.36%, indicating a good fit of the model to the experimental data. According to Table 5, the linear effects of all three variables of time, temperature, and pH extraction on galacturonic amount were significant ($P \leq 0.05$). The extraction rate increased significantly with increasing temperature and time and decreasing pH. Also, the quadratic effects of temperature, pH, time, and the interaction between temperature × time and pH × time and temperature × pH on pectin yield were not significant ($P > 0.05$).

The effect of extraction conditions on the amount of galacturonic acid pectin extracted from potato peel

The results of surface and interaction effects on galacturonic acid extraction of pectin from potato peel are shown in Fig. 2. According to Fig. 2 (a), the interaction of temperature × time on the amount of galacturonic acid pectin extracted from potato peel was not significant ($p > 0.05$). Increasing temperature and extraction time increased the percentage of galacturonic acid so that galacturonic acid was observed at amounts higher than 28 at times more than 180 minutes and temperatures above 90 °C at constant point pH = 2.

As shown in Fig. 2 (b), the interaction effects of pH × time on the amount of galacturonic acid pectin extracted from

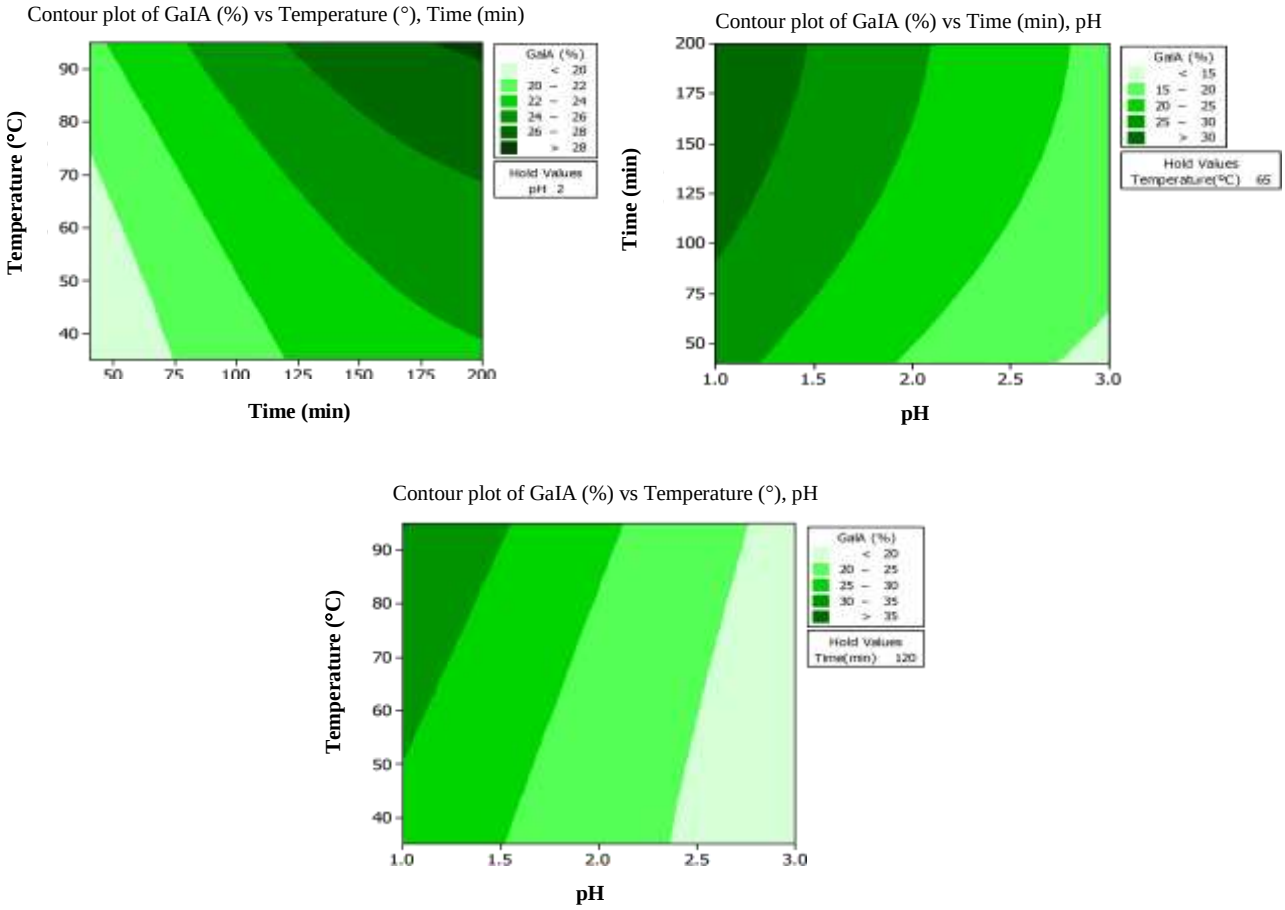

Fig. 2: Interaction effects of independent variables on the percentage of galacturonic acid pectin extracted from potato peel a) Temperature × time of extraction, b) Time × pH, c) Temperature × pH.

potato peel were not significant (p> 0.05). As the extraction time increased and pH decreased the amount of galacturonic acid increased as if the highest amount of galacturonic acid i.e. 30% and higher was observed at times above 90 minutes and pH ranging from 1 to 1.5 at a constant temperature of 65 °C.

As shown in Fig. 2 (c), the surface and interaction effects of temperature × pH on galacturonic acid amount extracted from potato peel was not significant (p>0.05). As the extraction temperature increased and pH decreased, the amount of extracted pectin increased as if the highest extraction rate i.e. 35% and above was observed at temperatures above 65 °C and pH ranging from 1 to 1.4 at a constant time i.e. 120 minutes.

Optimal conditions of Galacturonic acid pectin extracted from Potato Peel

The optimum condition of galacturonic acid percentage in extracted pectin from potatoes was predicted. The maximum galacturonic acid in the amount of extracted pectin from potato peel was 38.07% with 100% utility related to the temperature at 95°C, time 200 minutes, and pH 1. Predicted results were performed by confirmatory tests with two replications and no significant difference was observed between predicted and actual amounts. *Hosseini et al.* [9] reported 71% of galacturonic acid in extracted pectin from orange peel under optimum conditions using microwave waves.

Degree of esterification of potato peel

The reaction of a carboxylic acid (COOH conjugate group) with alcohol (OH conjugate group) in which an ester and water are obtained is called an esterification reaction. In this reaction, based on its mechanism OH is extracted from acid, and H from alcohol and water is produced. The esterification reaction is an equilibrium reaction that is carried out in the presence of a suitable

catalyst (such as $4SO_2H$). The degree of esterification of pectin is one of the most important parameters for identifying its application, which by definition indicates the number of moles of methanol per 100 moles of galacturonic acid. The importance of Methoxyl groups derives from their close association with other pectin properties such as solubility, gel-forming ability, type and properties of the gel produced, salt sensitivity, and so on. The amount of Methoxyl determines the type of pectin and its consumption [39]. According to the results of Table 3, the degree of esterification of pectin extracted from potato peel significantly increased with decreasing temperature, time, and increased pH. Among the factors studied, the effect of pH on changes in the degree of pectin esterification was more significant than the other factors. The highest amount of esterification for extracted pectin from potato peel was 41.820% at 65 °C, 40 minutes, and pH 3.00. The lowest degree of esterification was observed for pectin 15.35% at 95 °C, 120 minutes, and pH 1. As the degree of esterification of extracted pectin from potato peel was less than 50% therefore it was classified as low-esterified pectin.

Extracted pectin at high pH, low temperature, and short time has the ability to produce more favorable gels, lower yield, and lower galacturonic acid amount. Because the pectin chain is not extracted under extreme conditions and is less hydrolyzed, so the pectin chain length becomes longer. Since the yield of pectin extraction is one of the main goals, it is not possible to extract pectin in conditions where the yield is low, even if the ability to produce the gel is favorable under these conditions.

In fact, by decreasing pH, increasing temperature, and extraction time despite increasing yield, the degree of esterification of pectin is significantly reduced, which may be due to the breakdown of pectin ester bonds under harsh acidic hydrolysis conditions [19]. High esterification rates are thought to indicate less damaged pectin because ester bonds have less resistance to acid hydrolysis than Glycosidic alpha 1 and 4 bonds between galacturonic acids [40].

Zhemerichkin and *Ptitchkin* [41] compared the acidic and enzymatic methods of pumpkin pulp and reported the degree of esterification of extracted pectin by the acidic method at 66% and by the enzymatic method at 68%. *Nateghi et al*. [33] reported that the percentage of pectin esterification extracted from eggplant peel lesions at optimum conditions (60 °C, 50 min time, and pH 3) was 83.22%. *Raji et al*. [16] used citric acid to extract pectin from a watermelon shell and the effect of temperature (35-95 °C), time (200-200 µg), pH (3-1), and solvent to sample ratio (v/w 50-10) was applied to the degree of stabilization. The results showed that the degree of sterilization was from 1.33 to 29.33%. *Keramat et al.* [12] extracted pectin from orange juice concentrate of factory waste and reported that the degree of esterification decreased with increasing temperature and time. The highest degree of esterification was 80.8% at 90 °C, pH 1.8, and 50 min.

Analysis of variance was performed on this quadratic polynomial model which results are shown in Table 4. The coefficient of determination of this model (R^2) was 98.71% and its modified coefficient of determination (R^2-adj) was 96.40%, indicating a good fit of the model to the experimental data. According to Table 5, the linear effects of temperature (A), time (B), and pH (C) and quadratic effects of time were significant expressions ($p\leq0.05$) in this model. Quadratic effects of temperature and pH and the interaction effects of temperature × time, temperature × pH × time were not significant ($p> 0.05$).

The effect of extraction conditions on the degree of esterification of pectin extracted from potato peel

The results of surface and interaction effects on the degree of esterification of pectin extraction from potato peel are shown in Fig. 3. According to Fig. 3 (a), the interaction and surface temperature effects × time on the degree of pectin esterification extracted from potato peel was not significant ($p>0.05$). The degree of esterification by increasing the pH and decreasing time and temperature. degree of pectin esterification extracted from potato peel was 30% and higher in the scope of time from 45 to 48 minutes and at a temperature ranging from 35 to 42 °C at constant pH 2.

According to Fig. 3 (b), the interaction and surface effects of pH × time on the degree of esterification of pectin extracted from potato peel was not significant ($p\leq0.05$). By increasing pH and decreasing the time, the degree of esterification of the extracted pectin increased. The degree of esterification of pectin extracted from potato peel was observed at amounts of 40% and higher in the scope of time from 45 to 50 min. at pH 2.8 to 3 at a constant temperature of 65 °C. According to

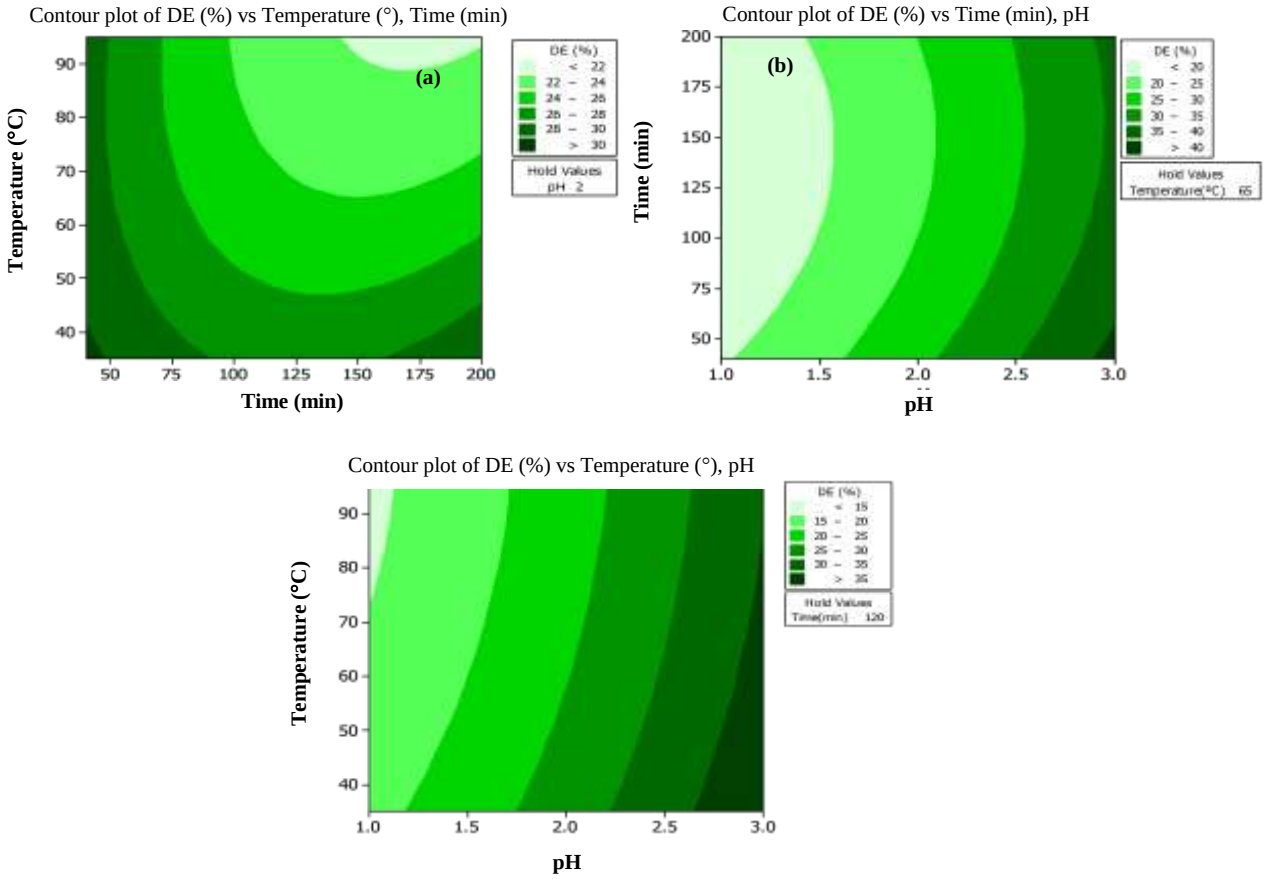

Fig. 3: Interactions of independent variables on the percentage of galacturonic acid pectin extracted from potato peel a) Temperature × time of extraction, b) Time × pH, c) Temperature × pH.

Fig. 3 (c), the interaction and surface effects of temperature × pH on the degree of pectin esterification extracted from potato peel were not significant ($p > 0.05$). It must be mentioned that decreasing the temperature and increasing pH the degree of esterification was increased. The degree of pectin esterification extracted from potato peel was 52% and higher at temperatures below 60 °C and pH ranging from 2.6 to 3 with a constant time of 120 minutes.

Optimal conditions of the degree of esterification of pectin extract from potato peel

The optimum condition of the degree of esterification of pectin extracted from potato peel was predicted. The maximum degree of esterification of potato peel was 43.11% with 100% utility at 35 °C, 40 minutes, and pH 3. Predicted results were performed by confirmatory tests with two replications and no significant difference was observed between predicted and actual amounts. *Hosseini et al.* [9] investigated the optimum conditions for pectin extraction from orange peel and reported that the degree of esterification of extracted pectin ranging from 17% to 30.5% and the mentioned pectin were classified in the low esterification pectin group.

Multiple optimizations of pectin extraction conditions on yield percentage, galacturonic acid percentage of pectin extraction from potato peel

Fig. 4 shows the optimum yield and the percentage of galacturonic acid pectin extracted from potato peel. As can be seen, the optimum extraction conditions to achieve maximum yield and galacturonic acid were obtained at a temperature 95 °C, time 200 min, and pH 1. In the mentioned conditions predicted the yield and galacturonic acid of pectin extracted from potato peel were 15.23%, 38.0712% respectively with 100% desirability.

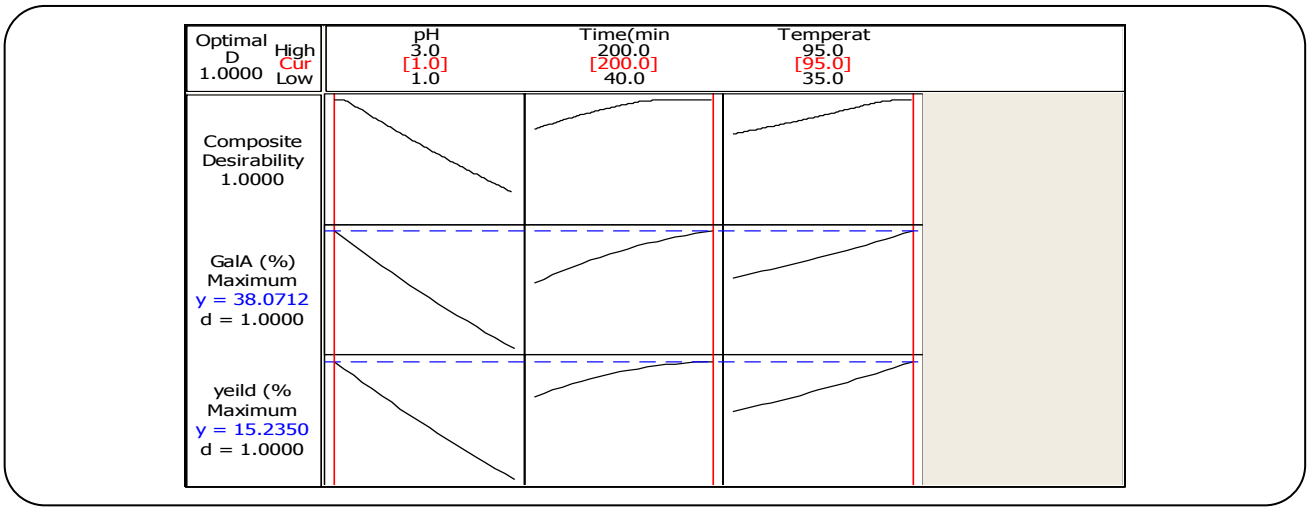

Fig. 4: Multiple optimization conditions of the percentage of yield and galacturonic acid extracted from potato peel

The predicted results were performed by confirmatory tests with two replications and no significant difference was observed between the predicted and actual amounts. *Raji et al.* [16] also evaluated the optimum conditions of temperature, time, pH, and solvent/sample ratio to extract pectin from melon peel through the acid method and reported that the highest amount of pectin extraction yield from melon peel was 29.48% that was obtained at 95 ° C, 200 min extraction time, pH 1 and solvent to sample ratio of 10 W/V.

Measurement of emulsion stability

The emulsifier resistance and emulsion stability of extracted pectin from potato peel under optimum conditions were studied by using a solution 0.5% W/W pectin in water (Table 6). After centrifugation of the emulsions, three detectable phases were observed in the environment. The oil phase with low density was at the top, the emulsion phase was due to the medium density was in the middle, and the Aqueous phase with higher density was at the bottom. As can be seen, the emulsifier resistance of pectin extracted from potato peel under optimum conditions after 30 days of keeping at 4˚C and 23˚C respectively was 85.1% and 63% and also, emulsion stability after 1 day of keeping at 4˚C and 23˚C was respectively 87% and 73%. The results showed that the temperature of keeping had a significant effect ($p \leq 0.05$) on the resistance changes of the emulsions extracted from pectin. The results showed that the resistance of pectin emulsion extracted from potato peel at 4 °C was significantly higher than 23 ° C. *Ma et al.* [42] examined the emulsifying properties of sugar beet pectin. These researchers stated that the resistance scope of emulsion extracted from different acids at 4 °C after one day was between 79.4 % and 74.3% and between 69.3% and 62.1% at 23 °C after one day. Also, this factor after 30 days at 4 °C was between 74.2% and 79.3% and between 62.1% and 69.2% at 23 °C. As a result, it can be stated that the emulsion resistance varies at different temperatures and is much higher at 4 °C than 23 °C. *Raji et al.* [16] also reported that extracted pectin from melon peel through the acid method at 1 w/v concentration produced weak gel and the emulsion resistance at 4 °C was above 23 ° C. *Jafari et al.* [36] extracted pectin from carrot pulp and reported that pectin extracted from carrot pellet was classified in the pectin group with low esterification degree that showed high resistance at keeping temperatures of 4 and 23 ° C.

FT-IR spectrum of pectin extracted from potato peel under optimum conditions

One way to identify the pectin structure is to use the FT-IR spectrum. Fig. 5 shows the FT-IR spectrum of pectin extracted from potato peel under optimum conditions (Fig. 5). According to the results of all three samples of commercial pectin of citrus, extracted pectin from potato peel and commercial pectin from apple had strong adsorption in the range of 3500-2900 cm^{-1} indicating OH stretching adsorption and related to vibration of intra- and extra-molecular hydrogen bands. OH stretching bands occur at a range of frequencies and represent various components, including stretching bands derived from free carboxyl groups in the vapor phase

Table 6: Emulsifier stability (%) of pectin extracted from potato peel in optimal conditions.

	Emulsion stability (%)			
Pectin type	One day		Days 30	
	4 ˚C	23 ˚C	4 ˚C	23 ˚C
Potato peel	87±35.90aA	73±16.69aB	85.1±15.04aA	63.0±14.39aC

- The lowercase letters indicate a significant difference ($p \leq 0.05$) in each column.

- The different uppercase letters indicate a significant difference ($p \leq 0.05$) in each row.

as well as available bending bands in carboxylic acid groups. The mentioned region in the pectin sample extracted from potato peel was also related to intracellular and extracellular vibration of hydrogen bonds in the galacturonic acid polymer. The available peak in the range of 2800 to 3000 cm^{-1} for the studied samples was related to CH vibration modes including stretching and bending vibrations of CH_3, CH_2, and CH [39]. Peaks between 1411and 1750 cm^{-1} were attributed to free carboxyl groups and carboxylic methyl esters. Previous studies have shown that the relative intensity of these peaks can be related to the degree of methoxyl pectin [16]. Therefore, the degree of esterification increases by increasing the relative intensity of ester bands and decreasing the relative intensity of carboxylic groups. Peaks between 800-1200 cm^{-1} are a unique fingerprint of available compounds in extracted pectin that are difficult to interpret.

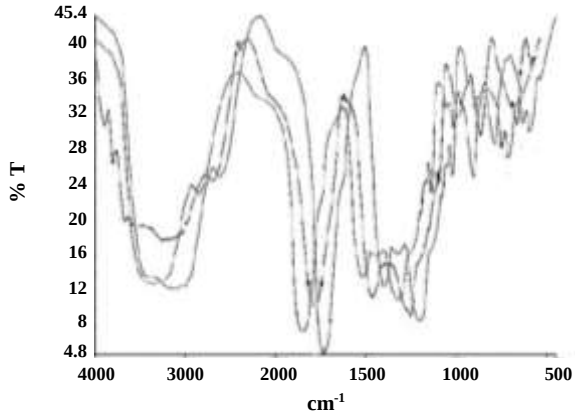

Fig. 5: FT-IR spectrum 1) Commercial pectin of citrus 2) extracted pectin from potato and 3) Commercial pectin of apple

Viscosity

In Table 7, the values of rheological indices including flow index (n) and coefficient of consistency (k), and viscosity of different concentrations of pectin extracted from potato peel in optimal conditions were obtained by fitting the data to Newtonian and power models. The results of data fitting show that the behavior of all samples was Newtonian and their flow index was about one. As expected, as the concentration of pectin samples increased, their consistency coefficient increased. In fact, as the concentration of pectin increases, the viscosity also increases [43]. Research done by *Chen et al.* [32] about the rheological properties of extracted pectin from Okra, *Jafari et al.* [36] about extracted pectin from carrot pulp, and also *Hosseini et al.* (2016) about extracted pectin from orange peel showed that extracted pectin at low concentrations, i.e. less than 1% V/ W had Newtonian behavior and by increasing concentration the behavior changed from Newtonian to diluting or cutting profits and plastic. The viscosity of different concentrations of extracted pectin from potato peel at optimum conditions at a constant cut rate (50 rmp) are also shown in table 7. The results of the viscosity measurements showed that the viscosity produced by the different samples are similar at low concentrations and were not significantly different. Common and current behavior for pectin solution was pseudoplastic behavior. *Panouille et al.* [44] attributed the pseudoplastic behavior of pectin to the orientation of its polymer chains in the related stream direction therefore this material is less resistant to flow. On the other hand, it is stated in various studies that the viscosity of pectin is also dependent on the concentration of its galacturonic acid, and as its concentration increases, the viscosity created by pectin increases [44].

Molecular Weight of Pectin

The molecular weight of extracted pectin from potato peel under optimum conditions was 53.46 KDa. Given the molecular weight of commercial pectin ranging from 50 to 150 KDa, the pectin extracted in this study was within the range of commercial pectin. The molecular weight of pectin varies greatly depending on the raw material and the extraction method. The higher the molecular weight of the extracted pectin, the stronger and more coherent the gel [45]. The longer the extraction temperature and time under acidic conditions, the longer the hydrolysis in the pectin chain,

Table 7: Indices of fitting viscosity measurement data on different rheological models for pectin extracted from potato peel under optimal conditions

Pectin type	Density (W/V%)	Viscosity	Model	n	K (Pa S^n)	R^2 (%)
	0.1	1.63	Newtonian	1	0.014	98.2
			Power law	0.97	0.016	99.2
	0.2	1.92	Newtonian	1	0.015	98.6
Pectin extracted from potato peel			Power law	0.96	0.018	97.4
	1.0	2.89	Newtonian	1	0.025	97.8
			Power law	0.99	0.036	99.3
	2.0	5.35	Newtonian	1	0.049	98.9
			Power law	0.96	0.058	99.7

the shorter the length of the pectin chain due to hydrolysis, and the lower the molecular weight of the chain. As one of the major factors in the gel strength is the molecular weight of pectin, as the molecular weight of pectin decreases, the polygalacturonic chains of junctions decrease, and the formed gel thereby becomes weak. *Mosayebi et al.* [18] studied the optimized conditions of the pectin extraction using ultrasonic waves from the Blackberry pulp. The results of their study showed that the molecular weight of the extracted pectin was 50.03 KDa. *Bagherian et al.* [45] reported the molecular weight of pectin extracted from grapefruit ranged from 56.4 to 84.4 KDa. On the other hand, *Chen et al.* [32] reported the molecular weight of extracted pectin from sugar beet to be 89.4 KDa. *Emaga et al.* [5] investigated the effect of extraction conditions on the molecular weight of extracted pectin from the banana peel and reported that the molecular weight of pectin varied from 87 to 248 KDa under different extraction conditions.

CONCLUSIONS

In this study, pectin was extracted from potato peel by the acidic or citric acid method under temperature, time, and pH conditions. Results showed that pectin yield from potato peel were different from 7.15 to 14.87%, galacturonic acid percentage from 14.45 to 36.37%, and degree of esterification from 15.35 to 41.82% in 15 extraction treatments. The highest yield was 14.87% from potato peel in the most severe extraction conditions at 95 °C, 120 min time, and pH 1, which galacturonic acid pectin in potato peel was equal to 36.37% and the esterification rate for potato peel under this condition was equal to 15.35%. According to the results of the analysis of variance, temperature, time, and pH conditions were the most important factor for pectin extraction from potato peel. The results showed that the stability of pectin emulsion extracted from potato peel at 4 °C and the first day was more than 23 °C and 30th day. The FT-IR results showed that all three samples of citrus commercial pectin, potato-extracted pectin, and apple commercial pectin had strong adsorption in the range of 3500-2900 cm^{-1}, indicating that intracellular and extracellular vibration of hydrogen bond in the galacturonic acid polymer was related to the pectin sample. As the concentration of the pectin samples increased, the coefficient of consistency increased, the behavior of all samples was Newtonian and their flow index was close to one. The molecular weight of pectin extracted from potato peel in optimum condition was 53.46 Kilo Dalton. Therefore, the pectin produced in this study could be introduced as a marketable source. Wastes can also be used optimally. Finally, citric acid was selected as the most suitable acid for the extraction of pectin from potato peel. In addition, it can be stated that potato peel can produce pectin with Newtonian behavior and optimal gel grade.

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