Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Production of Functional Edible Jelly from Extracted Pectin from Pumpkin Cap by Microwave Method

Sedigheh Yazdanpanah[1*],Zynab Shahamatpour [2]

1- Associate Professor, Department of Food Science and Technology, Kaz.C., Islamic Azad University, Kazerun, Iran

2- Graduated from Master's Degree in Food Industry Science and Engineering, Kaz.C., Islamic Azad University, Kazerun, Iran

ARTICLE INFO

Article History:

Received:2024/12/11

Accepted:2025/1/27

Keywords:

Pumpkin,
Pectin,
Microwave,
Degree of esterification.

DOI: 10.22034/FSCT.22.165.180.

*Corresponding Author E-

sedigheh_yazdanpanah@iau.ac.ir

ABSTRACT

Pectinic is a polysaccharide and is widely used in the food and pharmaceutical industries due to its unique technological and therapeutic properties. In this study, the production of edible jelly from pumpkin pectin was investigated. Pectin extraction from pumpkin head using microwave at two temperatures of 40 and 50 degrees Celsius, times of 10, 15, 20 and 30 minutes, pH 1.5 and 2 and sample to solvent ratio of 20 and 30% by weight/volume done Then, the next tests were performed on selected treatments (one treatment for 40°C temperature and one treatment for 50°C temperature) which had higher efficiency. Edible jelly based on apple juice was produced from selected treatments. The degree of esterification, stability and emulsion activity of the treatments were not statistically significant ($P<0.05$). According to the degree of esterification obtained for the samples, pumpkin pectin belongs to the low-ester category. Examination of FT-IR spectrum showed that the structure of extracted pectins at 40 and 50 °C did not differ significantly from each other ($P<0.05$). The highest stability of the emulsion related to extracted pectin was determined at 40°C. Examination of the FTIR spectrum showed that the extracted pectins confirm the presence of high concentration of galacturonic acid chains and carboxyl groups. The effect of different levels of pumpkin pectin (0, 10, 20 grams) on the chemical, textural and antioxidant properties of edible jelly based on apple juice was investigated. The chemical properties of treatments containing pumpkin pectin were determined more than the control treatment. The two characteristics of adhesion and elasticity were significantly reduced. The textural characteristics of firmness, cohesion, gummy and gummy state increased with increasing amount of pumpkin pectin. Pumpkin pectin has a high antioxidant effect compared to the control treatment. The anti-radical property was determined to be the highest amount and equal to BHT in the treatment containing 20 grams of pumpkin pectin. The results showed that pumpkin has a strong antioxidant property, usable and safer than synthetic antioxidants in the food and drug industry in order to maintain human health.

[DOI: 10.22034/FSCT.22.165.180]

1.Introduction

Pectin is a heteropolysaccharide derived from sugars and acids and an important component of plant cell walls. It is a chain of alpha-galacturonic acid, which is formed by a variety of methyl ester groups and sodium, ammonium and potassium salts. The gelatinous structures found in the cell walls and intracellular layers of fruits, the structural units of these walls, can be considered a symbol of the pectin arrangement in the walls of fruits and vegetables. Pectin is a preservative and is considered a common food [1]. Pectin can be used in the food industry to increase viscosity, stability and consistency, improve the suspension of substances in food systems, produce gels and other pplications including fat replacement, salad dressings, ice cream and emulsified meat products [1].Pectin is also used in the pharmaceutical industry to reduce blood cholesterol and relieve pain, prevent heart disease and gallstones [2]. The most basic raw materials used for pectin production include citrus peel and apple pulp. Pectin in citrus peel is the main component of the white, spongy part inside the fruit peel. Therefore, in the citrus industry, the process of recovering soluble solids after extracting the juice has become commercially popular [3]. Traditional methods, such as pectin extraction using hot acidified water, have led researchers to use new extraction methods due to reasons such as product quality degradation, damage to extraction equipment, and adverse effects on the environment. The use of microwaves is one of the new extraction methods that has recently attracted much attention from researchers. This method has many advantages over traditional methods, including shorter process time, less solvent consumption, higher production efficiency, higher quality product production, and lower cost [4, 5]. Today, researchers and producers are looking for new sources and have conducted many studies on pectin from other products, such as peach pulp, sunflower seeds, banana peels, and soybeans [6,7]. [The pumpkin, scientifically known as Moschata Cucurbita, is part of the Cucurbitaceae family [8]. It is a seasonal crop that is of great importance for human consumption. Pumpkin can be dried and used in powder form in various products in the food industry [8]. It is also of interest due to its mass production, good storage capacity, long availability, better transport quality, and therapeutic properties [9]. Similar studies have been conducted in the field of pectin extraction and edible jelly production, some of which are mentioned below. Maran et al. (2014) optimized the microwave extraction method of pectin from orange peel. In their study, these researchers investigated the effects of various process variables (microwave power, irradiation time, pH, and solid-liquid ratio) on the efficiency of pectin extraction. Stated that with increasing microwave power, the amount of extracted pectin increased, while with increasing time, pH, and solid-liquid ratio, its amount decreased [10]. Metacanon et al. (2014) extracted pectin from grapefruit peel and used response surface methodology for optimization and concluded that pH is the most important factor for pectin yield and the composition of side branches including arabinose and galactose [7]. In a study, Zuzan et al. (2016) investigated the extraction of pumpkin using microwave and examined various variables (microwave power, irradiation time, and solid-liquid ratio) and concluded that increasing the irradiation power and

[DOI: 10.22034/FSCT.22.165.180]

microwave time of pectin extraction increased the liquid-solid ratio and had the greatest effect on yield and molecular mass [11]. No study has been conducted so far on the production of functional jelly using waste. A large amount of pumpkin caps, which contain carotenoid compounds and various vitamins, are produced annually as waste in the agricultural sector. If such waste is used, a lot of added value can be added to the family basket and functional foods can be produced at low cost. In this study, pectin was first extracted from the skin and cap of pumpkin using microwaves, and then its efficiency and physicochemical properties were investigated and compared. Finally, edible jelly was produced using extracted pectin, and its textural and chemical properties and antioxidant capacity were investigated.

2- Materials and methods

2-1- Raw materials

In this study, both pumpkin and apple were purchased from the local market in Shiraz. Laboratory sucrose, hydrochloric acid, 96% ethanol, sodium hydroxide, phenolphthalein, hydrochloric acid, sodium azide, copper sulfate, sodium and potassium tartrate, methyl blue, zinc acetate, glacial acetic acid, Potassium ferrocyanide, sulfuric acid, boric acid, Folin's reagent, sodium carbonate, 2 and 2-diphenyl-1-picrylhydrazine solution, methanol, sodium phosphate, ammonium molybdate (Merck, Germany), diphenylpicrylhydrazine (Sigma Aldrich, Italy) were used.

2-2-Preparation of pumpkin cap and skin powder

The pumpkin cap was separated, then crushed and exposed to the sun for 4 days to dry against the air flow. Then the dried cap was ground (Kalorance, Germany) and a uniform powder was prepared from it. The obtained powder was stored in sealed containers, away from moisture.

2-3- Microwave extraction of pectin

Pectin was extracted from dried pumpkin powder with water in a microwave (Anton Paar SVM 3000, Austria) with a power program of 20 watts, time at four levels of 10, 15, 20 and 30 minutes and temperature of 40 and 50 ° C. The pH of the solution in all extraction treatments was adjusted to 1.5 and 2 using 1 N hydrochloric acid solution, and the ratio of sample to solvent was 20 and 30% w/v. After leaving the microwave, the solution was filtered with Whatman No. 1 filter paper and the residue remaining on the filter paper was washed twice with 96% alcohol. The filtered solution, which contained pectin, was adjusted to pH=3 with sodium hydroxide solution (weight concentration 1 to 5). Then, 96% ethanol was added to the resulting solution in a volume ratio of 1 to 1. The resulting solution was stored in a refrigerator at a temperature of about 5°C for one day. Then, the above solution was centrifuged (SIGMA 14-1, Germany) at 300 rpm for 30 minutes at laboratory temperature. After centrifugation, the precipitated pectin was separated. The precipitated pectin was again mixed with an equal volume of 96% ethanol to wash it better and centrifuged (10000g) for 10 minutes. Then the liquid phase was separated and the extracted pectin was dried in a dryer with an air flow of 60°C for 7 hours, then the extraction efficiency was calculated according to formula 1 [12].

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(1)

Yield(%) = mass of dried pectin (Ag) / mass of cap of pumpkin powder used for extraction (Bg) × 100

yield (%) is the percentage of extraction yield, Ag is the weight of pure pectin (grams), Bg is the weight of the initial dry powder (grams).

2-4-Measurement of the degree of esterification

The degree of esterification was measured according to the method of Bochek et al. (2001) [13]. First, 0.1 g of the dried pectin sample was transferred to a 250 ml rlenmeyer flask, moistened using 1 ml of ethanol, and 40 ml of distilled water was added to it. After about 10 hours of continuous stirring and complete dissolution, 2 drops of phenolphthalein (1% w/v) were added to the sample and titrated using 0.1 N sodium hydroxide until a pale pink color appeared. and was recorded as the initial volume (V1). This consumed volume of soda actually neutralized the free carboxylic acids. Then 10 ml of 0.5 normal soda was added to the samples and stirred for 15 minutes at 150 rpm to perform the saponification of pectin esters. Then 10 ml of 0.5 normal hydrochloric acid was added to the samples. And stirring was done to neutralize the used sodium hydroxide. Finally, the final solution was titrated with 0.1 normal sodium hydroxide until a pale pink color appeared and recorded as the secondary volume (V2). The degree of esterification was measured according to the formula (2) below.

(2)

DE (%)= (V2/(V2+V1)×100)

2-5-Measuring emulsion properties

For this purpose, first a 0.5% solution of selected pectin samples was prepared in distilled water. Then 5 ml of sunflower oil was mixed with 5 ml of pectin solution and 0.02% sodium azide was added to it to prevent the growth of microorganisms. In the next step, to prepare the emulsion, the samples were mixed with a homogenizer at 10,000 rpm for three minutes. Then, the resulting emulsion was transferred to a 15 ml centrifuge tube and centrifuged at 527 g for five minutes (formula 3).

(3)

$EA(\%)=(ELV / W_V)\times 100$

Emulsion activity, ELV is the volume of the emulsified layer and WV is the total volume of the system [14].

2-6-FTIR spectroscopy

FTIR spectroscopy was performed with an accuracy of 4/cm using a spectrophotometer (Perkin Elmer Co, MA, USA) using the potassium bromide tablet method in the range of 450 to 4000/cm. This test is performed to determine the presence of functional groups such as carboxyl and hydroxyl to confirm the quality of extracted pectin [15]. 2-7-Production of edible gel First, the gelling agents were mixed with sugar in the specified doses and other raw materials (powdered glucose) were added to it. On the other hand, the remaining sugar and apple juice were mixed in a cooking pot and heated to 40°C. Then the mixed raw materials were added to it and heated to 80°C with stirring. After the heat was turned off, the mixture was stirred for 20 minutes until the pectin in the medium completely absorbed water and was ready to form a gel. In the next step, the above-

[DOI: 10.22034/FSCT.22.165.180]

prepared solution was injected into the molds without wasting time and before it cooled. Finally, the molds were pasteurized in hot water baths at a temperature of 80-82 degrees Celsius for 10 minutes after being sealed. And then it formed a gel in cold water baths at a temperature of 20 to 30 degrees Celsius (Table 1) [14].

Table 1. Amounts of pectin used in apple jelly samples

Treatment			
Contains commercial pectin	20 g	10 g	0 g
Contains pumpkin pectin	20 g	10 g	0 g

2-7-1-Measuring the moisture content of the produced apple jelly

The moisture content in the tested sample was calculated using an oven (A121, Iran) at a temperature of 105°C using the method of Lee et al. (2006) [16].

2-7-2-Measurement of ash in produced apple jelly

The amount of ash in the tested sample was calculated using an electric furnace at 550°C (FTMF-701 Finetech, South Korea) using the method of Lee et al. (2006) [16].

2-7-3-Measurement of sugar in produced apple jelly

A standard glucose solution was prepared with different concentrations. The food sample was dissolved in distilled water and hydrolyzed with sulfuric acid. The lin-anion solution was added to the sample. Then, using an appropriate indicator, titration was performed to determine the end point. Using the glucose standard diagram and titer data, the sugar concentration in the food sample was calculated [16].

2-7-4-Measurement of protein in produced apple jelly

To measure protein by the Kjeldahl method in various powdered jelly dessert products, the method of Lee et al. (2006) was used [16].

2-7-5-Measurement of carbohydrates in produced apple jelly

The amount of carbohydrates was calculated using formula 4 [23].

(4)

$$\text{Carbohydrate} = (\text{Ash\%} + \text{Fat\%} + \text{Pro\%} + \text{MO\%} - 100)$$

2-7-6-Evaluation of the texture of the produced apple jelly

A texturometer (Brookfield, CT3, USA) was used to measure the textural characteristics of the produced samples. The loading of the device was set to 5 kg (50 N). Then, each of the samples was compressed in two reciprocating cycles by a cylindrical probe with a diameter of 35 mm and a moving speed of 60 mm/min to 70% of the initial height and then decompressed [17].

2-7-7-Evaluation of antioxidant activity of produced apple jelly

Investigation of antioxidant properties by DPPH method as a stable radical compound in this test is measured by the degree of

[DOI: 10.22034/FSCT.22.165.180]

decolorization of 2,2-diphenyl-1-picrylhydrazyl violet solution or (DPPH) in methanol. Thus, 50 microliters of different concentrations of the sample in methanol were added to 5 ml of 0.004% DPPH solution in methanol. After 30 minutes of incubation at room temperature, the optical absorbance of the samples was read at a wavelength of 517 nm. The percentage of DPPH free radical scavenging was calculated using the following formula 5 [18].

(5)

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Ablank is the optical absorption of the control, Asample is the optical absorption of different concentrations of the sample.

The concentration of the sample that had a radical inhibition percentage of 50%, EC50, was calculated from the graph. Obviously, the smaller this number, the greater the antioxidant power or free radical inhibition. In this test, a synthetic antioxidant (BHT butylated hydroxytoluene) was used as a positive control.

2-8-Statistical analysis

The test results were analyzed based on a completely randomized design at a probability level of 5%. To compare the mean of the tests related to pectin, the T-Test was used in three replicates. To compare the mean of the tests related to jelly, the Duncan test was used in three replicates. The data were statistically analyzed in a completely random model using analysis of variance (AVONA) and SPSS23 software. A confidence level of 5% was considered in all tests.

3-Discussion and Results

3-1-Investigation of pectin extraction from pumpkin caps

Investigation of microwave extraction The results of the efficiency of pectin extraction in pumpkin caps by the microwave method are shown in Table 2.

Table 2- Extraction efficiency of pectin from pumpkin head

Treatment	Temperature	Time	Weight/Volume	pH	Cap efficiency
1	40°C	10 min	20	2	11.36
2	40°C	15 min	20	2	12.29
3	40°C	20 min	20	2	15.41
4	40°C	30 min	20	2	17.68
5	50°C	10 min	30	1.5	17.14
6	50°C	15 min	30	1.5	18.87
7	50°C	20 min	30	1.5	19.65
8	50°C	30 min	30	1.5	20.27

Among the 8 cap treatments, 1 treatment at 40°C and 1 treatment at 50°C were selected as the highest efficiency. Treatments 4 and 8 were selected at 40 (17.68%) and 50 (20.27%) degrees Celsius for 30 minutes, respectively, at 20 and 30% w/v. One of the important factors in the extraction of various polysaccharide compounds such as pectin is to have the highest percentage of efficiency. Many researchers have reported temperature as one of the important factors in the extraction of various polysaccharide compounds such as pectin [19,20 and 21]. The yield of pectin increased with

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increasing temperature from 40°C to 50°C. The increase in extraction yield with increasing temperature is probably due to the increase in the solubility of this polysaccharide in the solvent and the subsequent increase in its mass transfer from the solid compound particles into the solvent [21]. The extraction process time is another factor affecting the yield of extracted pectin. Many researchers have stated that extraction time has a direct relationship with extraction yield, such that the yield increases with increasing time [16]. In the present study, the extraction efficiency increased with increasing extraction time, so that the highest extraction efficiency occurred at 30 minutes. The present results are consistent with the results of the study by Zuzan et al. (2016) on pectin extraction by microwave method, which showed that the efficiency of pectin extraction increased with increasing microwave time and power [11]. The extraction efficiency for pH = 1.5 is higher than that for pH = 2. The high production efficiency at low pH is related to the effect of acid on the cell wall of the initial solid material and the release of pectin into the extraction solvent. Therefore, the lower the pH used for extraction, the greater the cell wall destruction and subsequent release and, consequently, the greater the production of pectin [22]. Maran et al. (2014) reported that at high concentrations of solid matter in the extraction solution, the mass transfer rate decreases due to the establishment of a dynamic equilibrium between the solid matter and the solution. [10] The results of the present study do not correspond to the results of Maran et al. (2014) because the extraction efficiency increased with an increase in the solid-to-liquid ratio from 20% to 30%.

3-2-Esterification degree study

The results of the esterification degree of pectin extracted from pumpkin caps in two selected treatments are given in Table 3. No significant difference was observed between the two treatments at the 5% level.

Table 3- The degree of esterification of pectin extracted from pumpkin caps

Temperature	Degree of esterification (% methoxy)
40°C	44.63 [a]
50°C	44.49 [a]

All numbers are the average (three repetitions), the same letters indicate the absence of significant differences (P<0.05).

One of the most important parameters determining the use of pectin is its degree of esterification, which is defined as the percentage of carboxyl groups esterified with methanol [23, 24]. The degree of esterification (methoxyl percentage) is one of the important qualitative characteristics of pectin in terms of its uses in the food industry and the conditions for gel production by pectin. Depending on the degree of esterification, pectins are classified into two groups: pectins with a high degree of esterification (with an esterification degree above 50%) and pectins with a low degree of esterification (with an esterification degree less than 50%) [20]. Pectin with a high degree of esterification forms gels in the presence of high sugar concentrations and at low pH. While gel formation in pectin with a low degree of esterification occurs over a wide range of pH, in the presence or absence of small amounts of sugar, the presence of

[DOI: 10.22034/FSCT.22.165.180]

divalent ions such as calcium is essential for gel formation of this type of pectin [6, 19]. According to the degree of esterification obtained for the samples, pumpkin pectin is of the low-ester category. The degree of esterification of pectin extracted under optimal conditions is shown in Table 3. According to the results obtained from Table 2, the highest degree of esterification is related to the sample extracted from pumpkin cap (44.63%) at a temperature of 40 °C. Using higher temperatures for extraction reduces the degree of esterification, which is due to the deesterification of pectin at high temperatures [15]. The lower the pH of the extraction solution, the lower the degree of esterification of the resulting pectin [22]. In general, pectins obtained from extractions using high temperature, long time, and low pH have a low degree of esterification, and this is due to the harsh conditions used for extraction, which cause the destruction and deesterification of pectin [15]. According to the results reported by Zarei (2008), the methoxyl content or degree of esterification in pectin extracted from pumpkin is 47.4%, which is consistent with the present study [25]. Pectin with different esterification degrees has different applications. Therefore, pectin obtained from this product is very suitable for the production of low-sugar products such as low-calorie jellies and jams due to its low esterification degree [15].

3-3-The results of the study of the emulsifier properties

Emulsifier activity and stability of pectin emulsions extracted from pumpkin caps during storage on the first and tenth day at 4 and 23 °C are shown in Tables 5 and 4.

Table 4- Emulsifying activity of 0.5% pectin solution

Treatment	Pumpkin cap
Pectin extracted at 40°C	14.48 [a]
Pectin extracted at 50°C	46.99 [a]

All numbers are the average (three repetitions), the same letters indicate the absence of significant differences (P<0.05).

Table 5-Emulsion stability of 0.5% pectin solution of pumpkin head

Treatment	First day	First day	10th day	10th day
	4 °C	4 °C	23 °C	23 °C
Pectin extracted at 40°C	91.56 [a]	90.20 [a]	88.80 [a]	86.56 [a]
Pectin extracted at 50°C	90.32 [a]	88.76 [a]	87.51 [a]	85.85 [a]

All numbers are the average (three repetitions), the same letters indicate the absence of significant differences in the column (P>0.05).

No significant difference was observed between treatments. The emulsifying properties of pectin depend on the presence of groups such as acetyl, ferulic acid and protein [15]. The highest emulsifying activity was observed at 40°C (48.14%). Many factors, including extraction parameters such as temperature and time, affect the emulsifying activity of pectin. The low molecular weight observed for the extracted pectin in this study is probably also a strong reason for the observed emulsifying activity [21]. According to Table 4, as can be seen, the highest emulsion stability is related to pectin extracted at 40°C, which is 91.56% and 88.80% at 4°C on the first and tenth day, respectively. Which indicates high stability

[DOI: 10.22034/FSCT.22.165.180]

of the emulsion, but the emulsion stability at 23°C on the first and tenth day was 90.20 and 86.56 percent, respectively, which indicates a decrease in emulsion stability at high temperatures. The stability of the pectin emulsion extracted at 50°C was reduced by 1.24 and 1.29 on the first and tenth days at 4°C, respectively, and by 1.44 and 0.71 on the first and tenth days at 23°C, respectively, compared to the pectin extracted at 40°C. The stability of the emulsion was satisfactory at both tested temperatures. It has been confirmed by many researchers that emulsions are more stable at low temperatures. High temperatures cause degradation and deesterification of pectin [21, 23]. According to the results of Biono (2009), citrus pectins with low molecular weight and high degree of methoxylation have high emulsifying properties and are able to stabilize oil-in-water emulsions [26].

3-4-Far Infrared Spectroscopy (FTIR)

Examination of the far infrared spectrum of the extracted pectin sample (pectins extracted at 40 and 50°C) by microwave method from pumpkin caps to identify functional groups in the pectin structure is shown in Table 6.

Table 6- FTIR spectroscopy of pectin sample extracted by microwave method from pumpkin caps to detect functional groups

functional groups	Frequency range (cm^{-1})	functional groups	Frequency range(cm^{-1})
C-H bending	500-600	C=O stretching	1700-1750
N-H bending			
O-H bending			
C-H bending	600-700	C=O stretching	1750-1800
C-C bending			
N-H bending			
C-H bending	800-900	C≡C stretching	2000-2100
C-O stretching	900-1000	C≡N stretching	2100-2200
C-O stretching	1000-1100	C-H stretching	2600-2800
C-C stretching	1100-1200	C-H stretching	2900-3000
C-H bending	1200-1300	C-H stretching	3000-3100
C-H bending	1300-1400	N-H stretching	3100-3200
O-H bending	1400-1500	O-H stretching	3200-3400
N-H bending	1500-1600	O-H bending	3400-3600
C=C stretching	1600-1700	N-H bending	3600-3700

The use of FTIR spectrum is a new method for studying the structure of pectin [21]. Table 6 shows that the peaks formed by extracted pectins (at 40 and 50 °C) from pumpkin caps are not different from each other, indicating the absence of significant differences in the structure of their pectins. Strong peaks are usually seen in the region of 3300-3200 cm, peaks related to hydroxyl groups (–OH), functional groups of carbohydrates, and peaks related to C–O and C–C vibrations are observed in the region of 1000-1200 cm. Carbonyl peaks in the region of 1700-1600 cm-1, peaks related to carbonyl groups (C=O) can be present. A temperature of 40°C may indicate pectin with special characteristics, such as a higher concentration of hydroxyl groups that contribute to greater hydration.

Temperatures above 50°C may cause changes in the structure of pectin, especially in the peaks related to functional groups, which can lead to decomposition or

[DOI: 10.22034/FSCT.22.165.180]

changes in the physical and chemical properties of pectin. Hydroxyl groups (–OH) indicate hydrogen bonding and gel formation. Carbonyl groups (C=O) can contribute to the gelling reactions and viscosity properties of pectin. C=O stretching indicates the presence of carbonyl groups in various compounds, which can be related to esters, carboxylic acids, and other chemical compounds. C-O stretching These fluctuations are commonly seen in alcohols, ethers, and carbohydrate compounds and indicate the presence of oxygen functional groups. According to the different peaks and regions of the FTIR spectrum, what changes in functional groups and properties does the extracted pectin have under different temperature conditions? In general, the strong absorption region between 3300-3500 cm-1 is related to the OH groups present in different parts of the galacturonic acid polymer structure. The sum of the peaks in the region between 800 and 1200 is called the fingerprint region, which is a unique region and is difficult to interpret [15, 27]. The results obtained from this spectrum confirm the presence of a high concentration of galacturonic acid chains in the extracted pectins.

3-5-Chemical properties of jelly

The chemical results including ash, moisture, carbohydrate, protein, reducing sugar are shown in Table 7.

Table 7- Checking the chemical properties of apple jelly production From pumpkin pectin

Treatment	Ash	Moisture	Carbohydrate	Protein	Sugar
Control	0.269 [b]	18.884 [b]	8.343 [b]	1.086 [b]	51.833 [b]
Contain pumpkin pectin (10g)	0.325 [b]	20.233 [a]	14.656 [a]	1.188 [a]	54.000 [b]
Contain pumpkin pectin (20g)	0.437 [a]	21.155 [a]	15.493 [a]	1.192 [a]	60.366 [a]

All numbers are the average (three repetitions), the same letters indicate the absence of significant differences in the column (P>0.05).

In the ash study, no significant difference was observed between the treatment containing 10 grams of pectin and the control, but the treatment containing 20 grams of pectin with the highest ash content (0.437%) had a significant difference with the control. Pumpkin is rich in carotene, fiber, vitamins C, B, B_6, K, and minerals such as potassium, phosphorus, magnesium, iron, and selenium, protein, and fat [8]. The reason for the increase in ash content in treatments containing pumpkin pectin compared to the control is the high mineral content in its structure. A significant difference was observed in the moisture content between the treatments and the control. The highest moisture content was observed in the treatment containing 20 grams of pectin (21.155%). The moisture content of the 20 g pectin and 10 g pectin treatments was significantly higher than the control treatment. The results of Khalilian et al. (2010) showed that with the increase of xanthan gum in the formulation of cantaloupe pastille, the moisture content of the samples increased [28]. Pectin is a functional food of a hydrocolloid molecule, and its hydrophilic ability allows it to absorb a large amount of water and is widely used as a gelling agent and stabilizer. [3] Pectin has a high water retention capacity due to its hydrophilic structure, which has led to an increase in the

[DOI: 10.22034/FSCT.22.165.180]

moisture content of the samples. In the study of carbohydrates, a significant difference was observed between the treatments and the control, and the highest amount was determined in the 20 g pectin treatment (15.493%). Pumpkin is rich in minerals and carbohydrates, and in addition, due to its high beta-carotene content, it is also rich in vitamin A [29]. Proteins are organic compounds with high molecular weight. Like carbohydrates and lipids, they contain carbon, oxygen, and nitrogen, and all of them also contain nitrogen and generally sulfur. Protein is one of the most important macronutrients needed by the body and should be consumed in sufficient amounts throughout the day [30]. In the protein analysis, the treatments had a significant difference with the control and the highest amount was observed in the 20 g pectin treatment (1.192%). According to recent research, some protein compounds such as alpha and beta mucin are present in pumpkin, which can show antimicrobial activity due to their inhibitory activity (a process in protein production) [31]. It seems that the reason for the increase in protein content in treatments containing pumpkin pectin is the presence of protein compounds alpha and beta mucin. In the sugar analysis, a significant difference was observed between the treatments, but only the treatment containing 20 grams of pectin had a significant difference with the control. The highest sugar content was observed in the treatment containing 20 grams of pectin (60.366%). Pectin is a heteropolysaccharide derived from sugar and acid, which is obtained from the gelatinous structure found in fruits and vegetables. It is found in the cell walls of plants and in a layer between cells called the middle lamella. It plays an important role as a cementing material, and in general, among the polysaccharides extracted from plant materials, pectin has the highest amount [32]. In some pectins, amide groups may also be present. In addition to D-galacturonic acid, rhamnose, arabinan, galactan or arabinogalactan, and some other sugars are also present in the pectin molecule. The amount of these sugars in commercial pectin is about 10 to 15 percent and are called composite substances or neutral sugars [33].

3-6-Examination of textural properties

Examination of textural results including firmness, adhesion, cohesion, elasticity, gummy state, and gummy state are shown in Table 8.

Table 8- Examining the textural characteristics of different concentrations of jelly

Treatment	Hardness (N)	Adhesiveness (Ns)	Cohesiveness	Elasticity (mm)	Gumminess (N)	Chewiness (Nmm)
Control	9.696 [b]	-0.74 [a]	0.433 [b]	3.453 [a]	2.260 [c]	18.563 [c]
Contain pumpkin pectin (10g)	10.497 [b]	-1.503 [b]	0.504 [b]	3.030 [b]	4.775 [b]	23.143 [b]
Contain pumpkin pectin (20g)	13.214 [a]	-2.473 [c]	0.791 [a]	2.930 [b]	6.889 [a]	25.373 [a]

All numbers are the average (three repetitions), the same letters indicate the absence of significant differences in the column (P>0.05).

Both descriptive sensory analysis and instrumental measurements are useful methods for evaluating the textural properties of foods. Mimicking tests, such as texture profile analysis, attempt to simulate the mechanical movements of biting or chewing. This method has gained wide popularity due to its ability to measure multiple textural parameters that are highly correlated with sensory data [5]. The

[DOI: 10.22034/FSCT.22.165.180]

firmness of a food is the resistance of the food to the application of pressure force. [34] Pectin from pumpkin caps increased the firmness of the jelly. A significant difference was observed in the firmness between the treatment containing 20 grams of pectin and the control and the treatment containing 10 grams of pectin, but there was no significant difference between the treatment containing 10 grams of pectin and the control.

The highest firmness was determined in the treatment containing 20 g of pectin (13.214 Newtons). Bland et al. (2004) in a study on gelatin, pectin and starch gels stated that flavor release was significantly related to gel texture [35]. Gelatin gels, due to their firmer texture, release less flavor. Taken together, these events reduced the overall acceptance score of gelatin-containing samples. Flavor perception in gel systems depends on the firmness of the texture and the type of gelling agent [36]. The time to peak flavor release varies with different degrees of firmness. The firmer the tissue, the slower the time to peak flavor release [37]. Therefore, treatments containing pumpkin pectin require more time to release flavor than the control treatment due to their high hardness. Adhesion is the work required to overcome the attractive forces between the food surface and other surfaces such as the tongue, teeth, and palate, or in other words, the work required to pull the food from the surfaces. In examining the adhesion between the treatments and the control treatment, a significant difference was observed. The highest adhesion value was observed in the control treatment (-0.74 Newton seconds) and the lowest value was observed in the treatment containing 20 grams of pectin (-2.473 Newton seconds). The results of Khazai et al. (2013) showed that increasing the amount of agar in the fruit gel formulation reduces the level of adhesion. The results of the present study are consistent with the results of Khazai [38]. By increasing pectin in edible jelly, the amount of stickiness was reduced. Cohesion is the strength of the internal bonds that form the body of the product, and the higher this value, the greater the product's cohesion. Cohesion is the ratio of the work done to compress the food in two consecutive cycles by the device [7, 9, 39]. In examining the correlation between the treatment containing 20 grams of pectin and the control treatment and the treatment containing 10 grams of pectin, a significant difference was observed, but there was no significant difference between the treatment containing 10 grams of pectin and the control. The highest correlation value was observed in the treatment containing 20 grams of pectin (0.791) and the lowest value was observed in the control treatment (0.443).

Hernandez et al. (1999) noted that changes in the texture of gels made from strawberry pulp and gelatin depend on the concentration of hydrocolloid used and the fruit pulp. In their study, increasing the fruit pulp or decreasing the hydrocolloid concentration had a negative effect on the final texture of the gel [39]. In confectionery jellies, interactions occur between gelatin and other compounds, and sucrose helps stabilize this structure. One of the characteristics of pectin gels is the creation of a continuous and dense network structure [15]. A similar result was obtained in the present study. The reason for the formation of a continuous and dense phase of pectin gels is their strong tendency to form numerous interactions with water molecules [40]. The results of increasing pectin concentration are increased texture firmness and texture cohesion of the

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product, and ultimately the formulation components come into contact with each other with greater strength [40,41]. Elasticity from a sensory perspective is the degree or intensity with which a sample returns to its original shape and size after slight pressure between the tongue and the roof of the mouth. In examining elasticity, there is no significant difference between the treatments, but there is a significant difference between the treatment containing 10 and 20 grams of pectin and the control treatment. The highest elasticity was observed in the control treatment (3.453 mm) and the lowest in the treatment containing 20 g of pectin (2.93 mm). In the application of hydrocolloid mixtures, non-gelling and viscosity-imparting agents are commonly used to increase viscosity or to provide better properties in gels such as elasticity [40]. Yarmand et al. (2008) in a study combining xanthan, locust bean gum and guar gum with gellan (as a gel-giver) observed that a sharp decrease in the hardness of the gel was created, resulting in the gel becoming brittle and its hardness decreasing, and with the increase of guar gum, xanthan gum and locust bean gum, elasticity increased. The results of the present study are consistent with the results of Yarmand et al. [41]. In edible jellies, hardness and elasticity are inversely related to each other. It was observed that jelly containing 20 grams of pectin, despite being the hardest, had the least elasticity compared to other treatments. Gummy state is the energy required to crush a semi-solid food until it is ready to be swallowed [42]. There was also a significant difference in gummy state between treatments and between treatments and the control. The highest gummy state was observed in the treatment containing 20 grams of pectin (6.889 Newtons) and the lowest value was observed in the control treatment (2.26 Newtons). The results of Khazai et al. (2013) showed that increasing agar and guar gum in kiwi pastille formulation increases the gummy texture parameter, which they attributed to the synergistic effect between these two hydrocolloids [38]. In the present study, increasing the amount of pectin increased the amount of gummy state, which is consistent with the results of Khazai's study. Gummy state is the energy required to chew a solid food until it is ready to swallow [42]. There was also a significant difference in gummy state between treatments and between treatments and the control. The highest gummy state was observed in the treatment containing 20 grams of pectin (25.373 Newton mm) and the lowest value was observed in the control treatment (18.563 Newton mm). Hernandez et al. (1999) also pointed out the same issue and evaluated the effect of gelatin content on the chewability of the direct and significant tissue [39]. The effect of gelatin on tissue gummification can be explained by the relationship between tissue stiffness and chewability, as noted by Bland et al. (2004). They stated that the time required to chew the gel before swallowing increased significantly with gel stiffness, and harder gels were chewed for longer periods of time [35]. In the present study, the time required to chew the gel before swallowing it significantly increased with gel hardness.

3-7-Investigation of inhibitory effects

The results of the percentage of inhibition of different concentrations of jelly and BHT are shown in Table 9.

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Table 9 - Comparison of the average DPPH radical inhibition percentage of different concentrations of jelly and BHT

Treatment Concentration(µg/ml)	Control	10 grams of pectin	20 grams of pectin	BHT
12.50	8.71 ^{Cf}	9.93 ^{Cf}	13.88 ^{Be}	18.89 ^{Af}
25	22.67 ^{Be}	38.66 ^{Ae}	39.92 ^{Ad}	23.01 ^{Be}
50	40.41 ^{Dd}	50.89 ^{Cd}	76.89 ^{Ac}	62.11 ^{Bd}
100	52.69 ^{Dc}	68.41 ^{Cc}	82.76 ^{Bb}	90.65 ^{Ac}
200	86.93 ^{Bb}	87.58 ^{Bb}	94.12 ^{Aa}	94.97 ^{Ab}
500	95.05 ^{Aa}	92.11 ^{Ba}	95.03 ^{Aa}	96.82 ^{Aa}

All numbers are the average (three replicates), the same letters indicate the absence of significant differences (uppercase letters in rows and lowercase letters in columns) (P>0.05).

One of the methods for evaluating the antioxidant effects of plants is the use of free radicals, and by eliminating these radicals, antioxidant capacity can be easily, quickly, and accurately assessed [43]. In this experiment, BHT was considered as a positive control. Antiradical activity was concentration-dependent in all treatments. Among the treatments, the 20 g pectin treatment had the highest antiradical activity, which even had higher inhibitory activity than BHT at concentrations of 25 and 50 µg/mL, and there was no significant difference with BHT at concentrations of 200 and 500 µg/mL. At all concentrations, the free radical scavenging activity of 0 and 10 g pectin treatments was less than or equal to BHT and 20 g pectin treatment.

3-8-Examination of the average EC50 on different treatments and BHT in the DPPH free radical scavenging method

The average EC50 value (micrograms per milliliter) on different treatments and BHT in the DPPH free radical scavenging method is shown in Table 10.

Table 10- The average value of EC50 (µg/ml) on different treatments of jelly and BHT in DPPH free radical inhibition method

Treatment	EC50
Control	81.63^{a}
10 grams of pectin	47.91^{b}
20 grams of pectin	32.25^{d}
BHT	34.77^{c}

All numbers are the average (three replicates), same letters indicate no significant difference (P>0.05).

A significant difference was observed between the treatments and the control sample. The higher inhibitory activity can be attributed to the phenolic content [44]. The 20 g pectin treatment had the highest amount of phenolic compounds. A factor called EC50 is used to compare the anti-radical activity of the treatments. By definition, EC50 is the concentration of the treatment at which 50% of DPPH radicals are inhibited. According to the values presented in Table 10, BHT with an EC50 of 34.77 µg/mL had a higher inhibitory activity than the 0 and 10 g pectin treatments. Among the treatments, the 20 g pectin treatment had the lowest EC50 (25.32 µg/mL) and consequently the highest antiradical activity, and the 0 g pectin treatment had the lowest antiradical activity (63.81 µg/mL). This difference in EC50 values is due to the difference in phenolic content of the treatments. Samples

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with higher polyphenol content showed lower EC50. The results of the present study are consistent with those of Young-Kil et al. (2009) [45].

4-Conclusion

Pectin is a widely used polysaccharide in the food and pharmaceutical industries due to its unique therapeutic technology properties. Given the abundant and cheap resources for pectin production in the country, its importation for industrial use can be prevented. Natural products are healthier and safer than synthetic antioxidants. Pumpkin has strong antioxidant properties and can be used in the food and pharmaceutical industries to maintain human health. Considering the results obtained from pumpkin caps and the jelly produced, it seems that it can be used as a source of pectin to meet the needs of the food industry.

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[DOI: 10.22034/FSCT.22.165.180]

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تولید ژله خوراکی فراسودمند از پکتین کلاهک کدو حلوایی استخراج شده به روش ماکروویو

صدیقه یزدان پناه[1*]، زینب شهامت پور[2]

۱-دانشیارگروه علوم و صنایع غذایی، واحد کازرون، دانشگاه آزاد اسلامی، کازرون، ایران

۲-دانش آموخته کارشناسی ارشد علوم و مهندسی صنایع غذایی، واحد کازرون، دانشگاه آزاد اسلامی، کازرون، ایران

اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۳/۹/۲۱

تاریخ پذیرش: ۱۴۰۳/۱۱/۸

کلمات کلیدی:

کدو حلوایی،

پکتین،

ماکروویو،

درجه استریفیکاسیون

DOI: 10.22034/FSCT.22.165.180.

* مسئول مکاتبات:

sedigheh_yazdanpanah@iau.ac.ir

چکیده

پکتینیک پلی‌ساکارید است و به دلیل ویژگی‌های تکنولوژیکی و درمانی منحصر به فرد این ماده در صنایع غذایی و دارویی بسیار پر کاربرد می‌باشد. در این مطالعه تولید ژله خوراکی از پکتین کلاهک کدو حلوایی بررسی شد. استخراج پکتین از کلاهک کدو حلوایی با استفاده از ماکروویو در دو دمای ۴۰ و ۵۰ درجه سانتی‌گراد، زمان های ۱۰، ۱۵، ۲۰ و ۳۰ دقیقه، pH ۱/۵ و ۲ و نسبت نمونه به حلال ۲۰ و ۳۰ درصد وزنی/حجمی انجام شد. سپس آزمایشات بعدی بر روی تیمارهای منتخب (یک تیمار برای دمای ۴۰ درجه سانتی‌گراد و یک تیمار برای دمای ۵۰ درجه سانتی‌گراد) که راندمان بالاتری داشتند، انجام شد. ژله خوراکی بر پایه آب سیب از تیمارهای منتخب تولید شد. درجه استریفیکاسیون، پایداری و فعالیّت امولسیونی تیمارها از لحاظ آماری تفاوت معنی داری نداشتند($P<0/05$). طبق درجه استریفیکاسیون بدست‌آمده برای نمونه‌ها، پکتین کدو حلوایی از دسته کم‌استر می‌باشد. بررسی طیف FT-IR نشان داد ساختار پکتین های استخراجی در دمای ۴۰ و ۵۰ درجه سانتی‌گراد تفاوت معنی‌داری با یکدیگر ندارند ($P<0/05$). بالاترین پایداری امولسیون مربوط به پکتین استخراجی در دمای ۴۰ درجه سانتی‌گراد تعیین گردید. بررسی طیف FTIR نشان داد، پکتین‌های استخراجی حضور غلظت بالای زنجیره‌های گالاکتورونیک‌اسید و گروه‌های کربوکسیل را تأیید می‌کند. اثر سطوح مختلف پکتین کدو حلوایی (۰ ، ۱۰ ، ۲۰ گرم)، بر ویژگی‌های شیمیایی، بافتی و آنتی‌اکسیدانی ژله خوراکی بر پایه آب سیب مورد بررسی قرار گرفت. خصوصیات شیمیایی تیمارهای حاوی پکتین کدو حلوایی بیشتر از تیمار شاهد تعیین گردید. دو ویژگی چسبندگی و الاستیسیته به طور معنی‌داری کاهش یافت. ویژگی‌های بافتی سفتی، پیوستگی، حالت صمغی و آدامسی با افزایش مقدار پکتین کدو حلوایی افزایش داشت. پکتین کدو حلوایی از اثر آنتی‌اکسیدانی بالایی نسبت به تیمار شاهد برخوردار است.خصوصیت آنتی‌رادیکالی در تیمار حاوی ۲۰ گرم پکتین کدو حلوایی بالاترین مقدار و معادل BHT تعیین گردید. نتایج نشان داد که کدو حلوایی دارای خاصیّت آنتی‌اکسیدانی قوی، قابل استفاده و ایمن‌تر از آنتی‌اکسیدان‌های سنتزی در صنعت غذا و دارو در جهت حفظ سلامت انسان می‌باشد.

[DOI: 10.22034/FSCT.22.165.180]