

## Research Article

# Influence of Various Factors on Yield and Quality of Pumpkin Pectin Extracted with Water

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## Abstract

The study was conducted to extract pectin from pumpkin using water extraction method and evaluate its quality. Extraction conditions *i.e.* pulp-water ratio, extraction time and temperature, was found to have a significant impact on yield and quality of pectin. Out of various combinations of pulp-water ratio (1:2, 1:4 and 1:6), temperature (70, 80 90 and 100 °C) and time (60 and 120 min) used for water extraction method, the isolation with 1:4 pulp-water ratio at 90 °C for 120 min exhibited the maximum pectin yield (5.33 %) with better characteristics such as 8.21 per cent methoxyl, 74.84 per cent anhydrogalacturonic acid, 73.79 per cent degree of esterification and 753.00 equivalent weight. Henceforth, it is concluded that pumpkin is a source of good quality pectin and hence, can be successfully utilized for production of different value added products. It can be a substantial raw material for isolation of pectin due to higher availability and lower cost.

As the requirement of this food additive is steadily increasing in the country, this approach can consequently solve the problem of new sources required for extraction of pectin to meet the growing demand in food industry as well as other industries.

**Keywords:** Pumpkin, Pectin, water extraction, yield, quality

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## Introduction

Pumpkins are gourd squash of Cucurbitaceae family which includes around 119 genera and 825 species [1], derived from tropical and subtropical regions and out of which 26 species are cultivated as vegetables [2]. A large number of pumpkin varieties varying in shape, size and colour of flesh are available [3]. In India the varieties like CM-14, Pusa vishwas, Arka chandan, Arka suryamukhi, CM-350 and NDPK-24 are found [4]. The main growing season for cucurbits in India is the summer and rainy months. Pumpkin is a valuable source of functional components mainly carotenoids, lutein, zeaxanthin, vitamin E, ascorbic acid, phytosterols, selenium and linoleic acid, which act as antioxidants in human nutrition, hence, it has great economic potential for use both as food and industrial crop. Pumpkin is also a good source various polysaccharides such as cellulose, hemicellulose and pectin [5].

Pectin is a multifunctional polymer occurring naturally in cell wall of all non-woody plants [6]. It is found in higher concentration in middle lamella, with a gradual decrease as one passes through the primary wall towards the plasma membrane [7]. Pectic polysaccharides consist of linear polymer rich in galacturonic acid, containing significant amount of rhamnose, arabinose and galactose as well as other monosaccharides [8]. A number of methods are tried from time to time for extraction of pectin from various plant sources. Although pectin occurs commonly in most of the plant tissues but the number of sources that may be used for commercial manufacturing is limited. At present, commercial pectin is almost exclusively derived from citrus peel or apple pomace. The increasing world market demand for pectin has been in excess of 30,000 tonnes annually [9], with a growing trend of about 6 per cent per annum [6]. It is assumed that total production of pectin in India is about 70-75 tonnes per year while the production is little compared to the consumption, which is estimated to be around 180 tonnes per year [10]. Thus searching for new pectin containing raw material is an important task of science and industry.

Agriculture, food processing, pharmaceutical as well as feed industries are taking growing interest in pumpkin because of the nutritional and health productive value of the polysaccharides from the fruits as well as the protein and oil from the seeds [11]. Though worldwide, the attempts have been made to extract pectin from pumpkin but no information is available concerning its physiological properties and recovery when extracted with water. The most conventional and easiest means of extracting pectin is to use hot water. This method requires long time and high temperature to isolate all the available pectin. Hence, there is a great need to optimize this technique for development of sustainable methods for the extraction of pectin from pumpkin. Keeping in view the above facts, the present investigation was proposed to extract pectin from pumpkin using water.

## Materials and Methods

### Preparation of pumpkin pulp for extraction

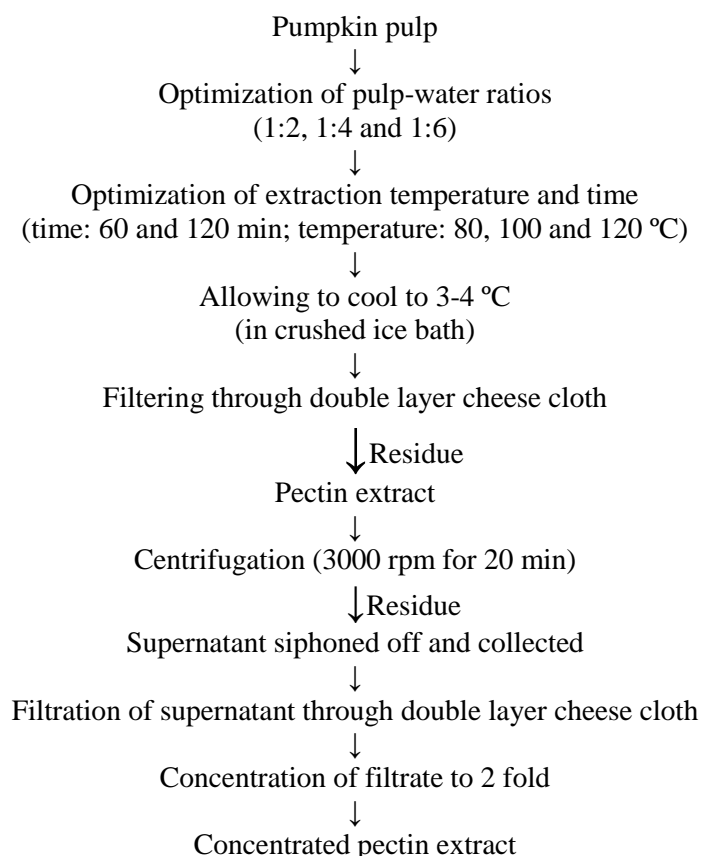
The pumpkin fruits have hard texture and generally require addition of water for converting pieces into pulp which affects the pulp-water ratio in subsequent studies along with sedimentation of solid particles with liquid during storage of pulp. Moreover, instead of small pieces, pumpkin has to be converted into shreds for pulp preparation due to its tough texture. The common process of grating pumpkin requires more labour and time. Hence, the method for preparation of pulp was modified for the isolation of pectin. The ripe pumpkins were washed and cut into halves. After removing the fibrous strands and seeds, the halves were cut into slices. The slices were peeled and made into small pieces by using stainless steel knife. The pumpkin pieces were crushed in a juicer which separated it into pomace and juice. The pomace and juice were then blended for 3-4 min in a mixer and grinder at room temperature to get a homogenous mixture. The pulp was packed in glass jars and stored in refrigerator for use in subsequent experimentation.

### Extraction of pectin

The pectin was isolated with water by using procedure as suggested by [12] for lemon pomace with modifications by varying the isolation parameters such as pulp-water ratio, time and temperature of extraction (**Table 1**).

**Table 1** Optimization of parameters for pumpkin pectin isolation with water extraction

Pulp-water ratio (w/v)	Extraction time (min)	Extraction Temperature (°C)
1:2, 1:4 and 1:6	60	70, 80, 90 and 100
1:2, 1:4 and 1:6	120	70, 80, 90 and 100

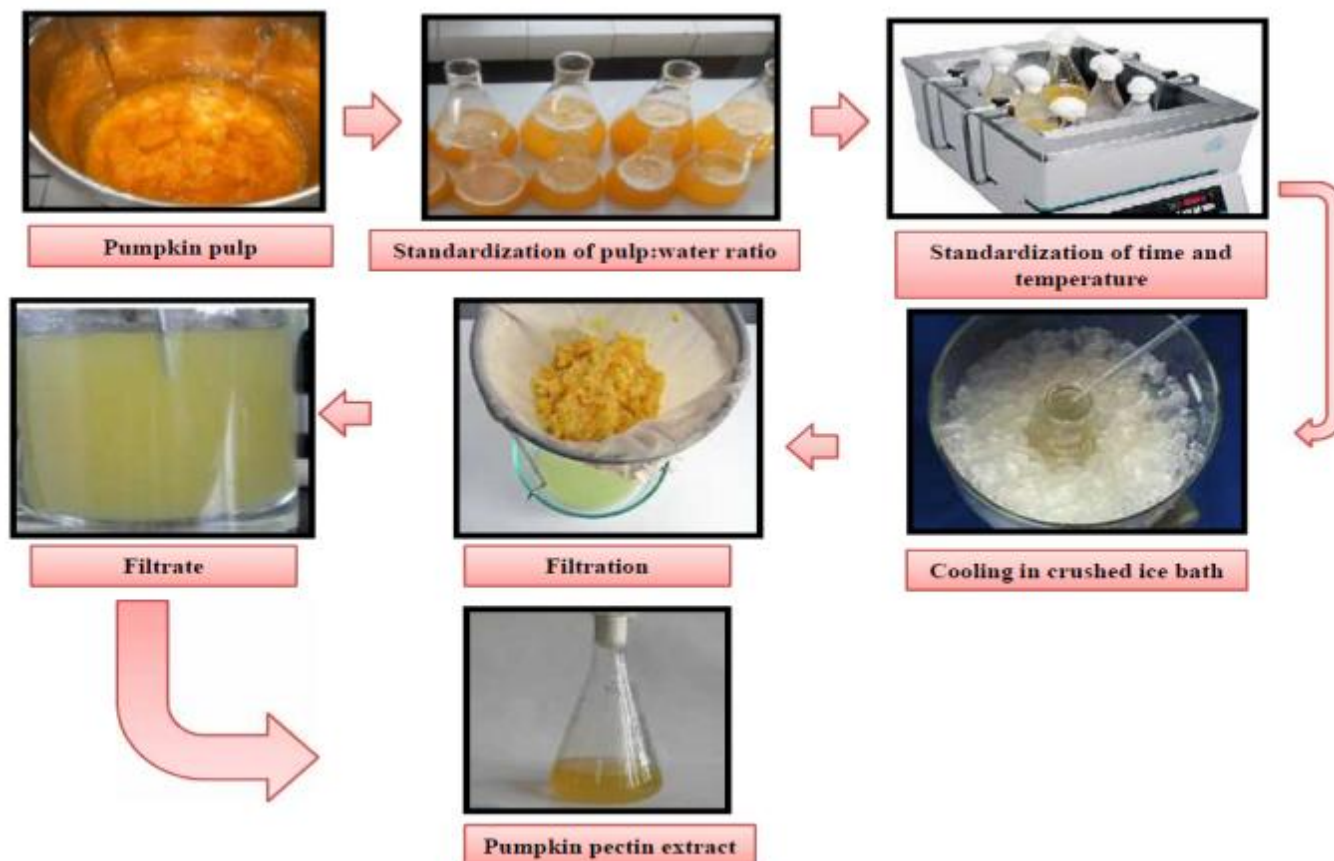


**Figure 1** Unit operations for preparation of pectin extract using water extraction

### Preparation of pectin extract

The pumpkin pulp was taken in 1000 mL conical flask and water was added. The temperature of water bath was adjusted (Table 1) and the flask was kept for definite time period. The mixture was agitated at an interval of 15 min until the extraction time elapsed. After completion of prescribed time, the content of flask was allowed to cool down rapidly (approximately 3-4°C) by immersing it in a bath of crushed ice. Then the mixture was filtered through two

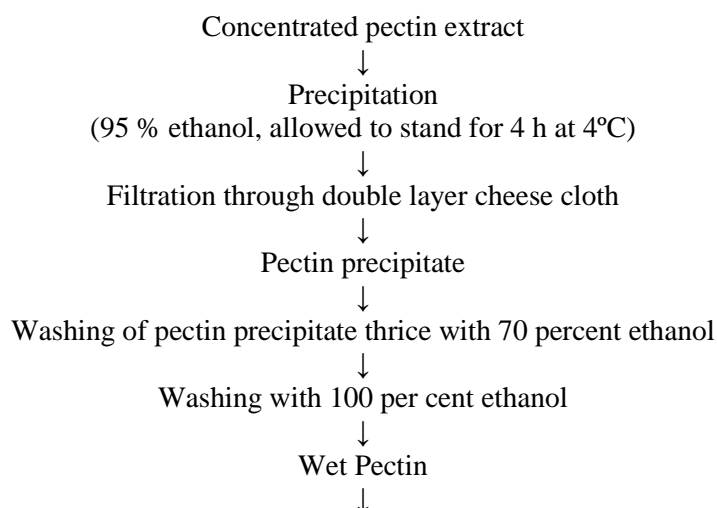
layers of cheese cloth in order to remove the suspended/insoluble particles. The extract was centrifuged at 3000 rpm for 20 min and supernatant was siphoned off and collected in a beaker (**Figures 1 and 2**).



**Figure 2** Preparation of pumpkin pectin extract with water extraction method

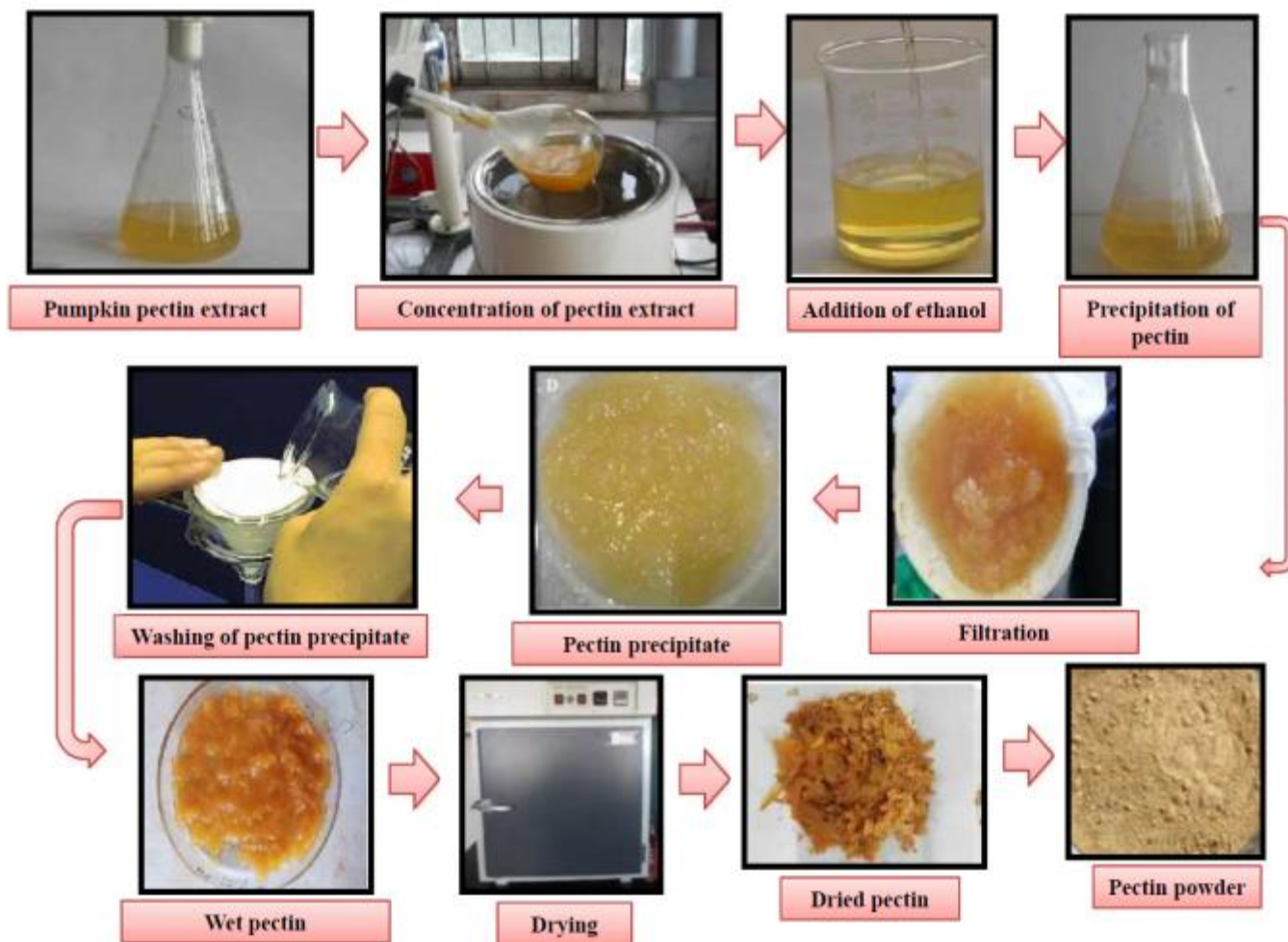
### *Precipitation of pectin*

The filtrate obtained above was concentrated two fold by using buch type vacuum evaporator (JSGW) and precipitated with 95 per cent ethanol twice the volume of filtrate. The solution was stirred continuously for 10 min and allowed to stand for 4 h at 4°C. The pectin was separated from the alcohol using a double layer cheese cloth. Samples were washed thrice with 70 per cent ethanol and once with undiluted alcohol to remove any impurities. The resulting pectin was transferred to petri-dish covered with aluminium foil and kept in hot air oven at a temperature of 50°C. The sample was dried up to the extent (approximately 2h) when it was easily removable from the foil. The samples were allowed to cool at room temperature, weighed and ground using a mortar and pestle till fine powder was obtained. The samples were stored in polyethylene pouches (**Figures 3 and 4**).



Drying (50°C)  
↓  
Allowing to cool at room temperature  
↓  
Dry pectin  
↓  
Grinding  
↓  
Packaging

**Figure 3** Unit operations for precipitation of pectin obtained by water extraction



**Figure 4** Preparation of water extracted pumpkin pectin extract from pumpkin

### ***Quality evaluation of extracted pectin from pumpkin***

#### ***Pectin yield***

The yield of pectin obtained was calculated by the formula given by [13] and is as under:

$$\text{Per cent pectin yield (\%)} = \frac{\text{Weight (g) of dried pectin}}{\text{Weight (g) of pumpkin pulp taken for estimation}} \times 100$$

#### ***Equivalent weight***

A known weight (0.5 g) of ammonia and ash free pectin was weighed into a 250 mL conical flask and was moistened with 5 mL ethanol. One gram sodium chloride was added to sharpen the end point. To the flask, 100 mL carbon dioxide free distilled water and 6 drops of Hinton's indicator was added. Precaution was taken to prevent lump

formation of pectic substances on the sides of flask so as to ensure complete dissolving. Sample was then titrated with 0.1 N NaOH until the colour of the indicator changed (pH 7.5) to magenta and persisted for at least 30 sec [14].

The neutralised solution was further used for determination of methoxyl content.

$$\text{Equivalent weight} = \frac{\text{Weight of sample} \times 1000}{\text{mL of alkali} \times \text{Normality of alkali}}$$

#### *Methoxyl content*

To the neutral solution titrated for equivalent weight, containing 0.5 g pectin, 25 mL of 0.25 N sodium hydroxide was added, shaken thoroughly, and was allowed to stand for 30 min at room temperature in a stoppered flask. To this 25 mL 0.25 N HCl was added and titrated with 0.1 N NaOH to the end point as mentioned above in equivalent weight [14].

$$\text{Per cent methoxyl content (\%)} = \frac{\text{mL of alkali} \times \text{Normality of alkali} \times 3.1}{\text{Weight of sample (g)}} \times 100$$

#### *Anhydrogalacturonic acid (AGA) content*

Making use of equivalent weight and methoxyl content, anhydrogalacturonic acid content was calculated from the expression given below [15]:

$$\text{Per cent AGA content} = \frac{176 \times 0.1z \times 100}{w \times 100} + \frac{176 \times 0.1y \times 100}{w \times 100}$$

When molecular weight of AGA (1 unit) = 176 g

Where, z = mL (titre) of NaOH from equivalent weight determination, y = mL (titre) of NaOH from methoxyl content determination, w = weight of sample.

#### *Degree of esterification*

Degree of esterification of pectin was measured as the ratio of methoxyl content and anhydrogalacturonic acid content [14] and was calculated by following formula:

$$\text{Per cent degree of esterification (\%)} = \frac{176 \times \text{methoxyl content (\%)}}{31 \times \text{anhydruronic acid content (\%)}} \times 100$$

#### *Statistical Analysis*

The data on pectin isolated was analysed by using Completely Randomized Design (CRD) as given by Cochran and Cox [16].

## **Results and Discussion**

The results pertaining to the effect of extraction conditions *i.e.* pulp-water ratio, time and temperature on the yield and chemical characteristic of pumpkin pectin extracted with water are presented and discussed below.

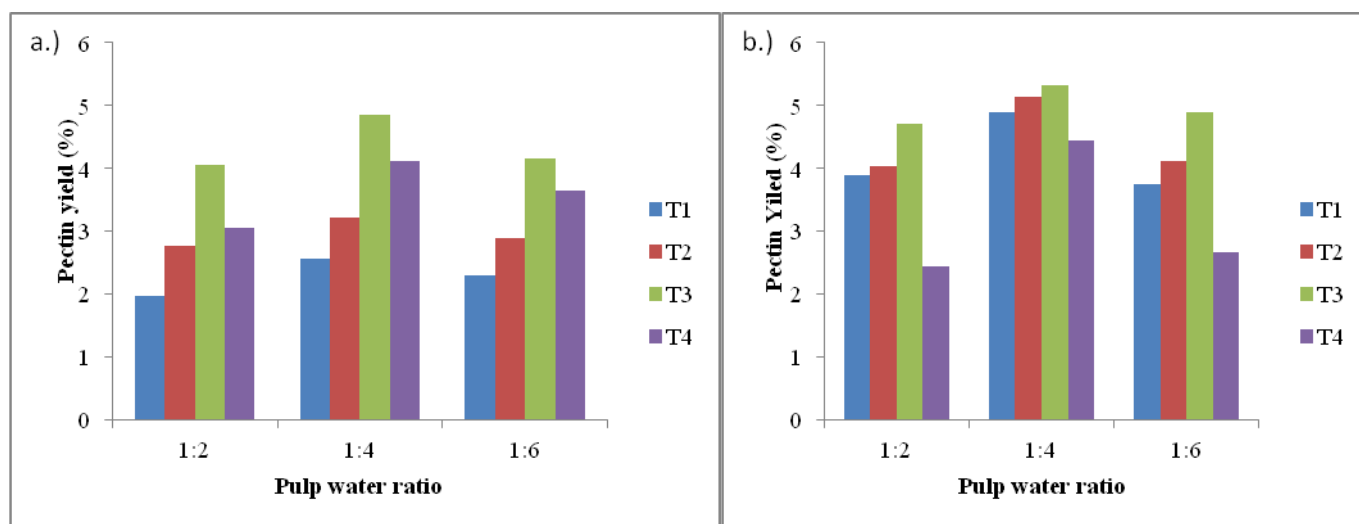
#### *Effect of extraction parameters on yield of pumpkin pectin*

The data related to effect of pulp-water ratio, time and temperature on yield of pectin from pumpkin by using water extraction technique are highlighted in **Table 2 (Figure 5)**. Among different treatments, the isolation pectin up to 90 °C (T<sub>3</sub>) showed an increase in yield (from 3.04 to 4.29 %) and thereafter it was found to decrease (3.77 %) irrespective of time of extraction and pulp-water ratio. The pulp-water ratio of 1:4 indicated the highest yield (4.32 %) among all the treatment and time of extraction as compared to 1:2 and 1:6. The effect of extraction time on yield of pectin reflected a value of 3.30 and 4.19 per cent at 60 and 120 min, respectively. A critical look at the combined effect of extraction time (E), pulp: water ratio (S) and temperature (T) on recovery of pectin indicated that higher yield (5.33 %) was achieved with 1:4 pulp-water ratio at 90 °C for 120 min. The increase in pectin yield with increase

in temperature might be due to heated solvent that helped to solubilise the pectin held in cell wall (protopectin) which was not possible at low temperature. The decreased yield after a certain temperature (90°C) may be attributed to breakdown of pectin molecule at higher temperature. Similar trend of pectin yield with respect to extraction time and temperature was reported by [17-23]. The lowest yield in 1:2 pulp-water ratio might be due to the insufficient quantity of extracting medium required to solubilise pectic substances and hold the extracted pectin while the decreased yield at higher pulp-water ratio (1:6) may be due to excessive dilution of the solution that results in hydrolysis of extracted pectin. A similar trend with increase in pectin yield was noticed by [19, 22] in pectin recovered from mango and passion fruit peel, respectively, but no decreasing trend in yield was reported by them with increase in pulp-water ratio.

**Table 2** Effect of extraction parameters on yield (%) of pumpkin pectin isolated with water

E	Extraction Time (E)										
	60 minutes				120 minutes						
	S	Pulp-water ratio (S)			Mean	Pulp-water ratio (S)			Mean		
1:2		1:4	1:6	1:2		1:4	1:6				
T <sub>1</sub>	1.97	2.57	2.31	2.28	3.76	4.89	3.82	4.18			
T <sub>2</sub>	2.78	3.21	2.90	2.96	4.04	5.13	4.12	4.43			
T <sub>3</sub>	4.05	4.85	4.15	4.35	4.71	5.33	4.90	4.98			
T <sub>4</sub>	3.06	4.11	3.64	3.60	2.45	4.45	2.66	3.19			
Mean	2.97	3.67	3.25		3.77	4.95	3.86				
Mean (E)	3.30				4.19						
T x S interaction Table					CD <sub>(0.05)</sub>						
T	Pulp-water ratio (S)				Extraction Time (E)	Pulp-water ratio (S)	Treatments (T)	E x S	E x T	S x T	E x S x T
	1:2	1:4	1:6	Mean (T)							
T <sub>1</sub>	2.93	3.73	3.04	3.23							
T <sub>2</sub>	3.41	4.17	3.51	3.70							
T <sub>3</sub>	3.89	4.72	4.27	4.29							
T <sub>4</sub>	3.25	4.65	3.41	3.77							
Mean (S)	3.37	4.32	3.56								
T <sub>1</sub> : 70 °C; T <sub>2</sub> :80°C; T <sub>3</sub> :90°C;T <sub>4</sub> :100 °C											



**Figure 5** Effect of temperature and pulp-water ratio on yield (%) of pumpkin pectin at 60 min (a) and 120 min (b) during water extraction Effect of extraction parameters on different quality characteristics of pumpkin pectin

#### *Effect of extraction parameters on quality characteristics of pumpkin pectin*

**Table 3** reflects the effect of extraction time (60 and 120 min), temperature (70, 80, 90 and 100 °C) and pulp-water ratio (1:2, 1:4 and 1:6) on equivalent weight of pectin. The mean maximum equivalent weight (840.00) was recorded at 60 min extraction time while at 120 min the recovered pectin had the mean minimum value (753.92). Increase in extraction temperature (70 to 100 °C) significantly decreased the mean equivalent weight (from 799.83 to 794.00). The increase pulp-water ratio (from 1:2 to 1:6) also had significant effect on the equivalent weight

as the values decreased from 798.00 to 796.00. The decrease in equivalent weight with increase in extraction time and temperature may be attributed to depolymerisation and de-esterification of pectin leading to formation of pectic acid. The results in the present study are in agreement with [17, 22, 24, 25] but contradictory to [20, 26, 27] who have reported an increase in equivalent weight with increase in temperature of isolation.

The data pertaining to the effect of extraction time in combination with different extraction temperature and pulp-water ratio on methoxyl content of pectin are shown in **Table 4**. The results elucidate that the 60 min extraction time recorded a value of 9.34 per cent for methoxyl content while at 120 min a lower value of 8.83 per cent was observed. Further, the methoxyl content was found to decrease from 9.73 to 8.92 per cent at 60 min and 9.16 to 8.50 per cent at 120 min of extraction with increase in temperature from 70 to 100 °C. An interaction between extraction temperature and pulp-water ratio, reveals that mean minimum (8.77 %) methoxyl content was recorded in 1:2 pulp-water ratio while maximum (9.38 %) was in 1:6. The decrease in methoxyl content with increase in extraction time and temperature might be due to the partial degradation of pectin. Almost similar observation was reported by [17, 22, 24, 25, 27] in pectin isolated from mango peel, passion fruit peel, cocoa husk, grapefruit peel and apple pomace, respectively. However, a vice versa results were recorded by [26] in Assam peel pectin and [20] in citrus peel pectin.

**Table 3** Effect of extraction parameters on equivalent weight of pumpkin pectin isolated with water

E	Extraction Time (E)							
	60 minutes				120 minutes			
	S	Pulp-water ratio (S)			Mean	Pulp-water ratio (S)		
1:2		1:4	1:6	1:2		1:4	1:6	
T <sub>1</sub>	844.00	843.00	842.00	843.00	758.00	756.00	755.00	756.67
T <sub>2</sub>	842.00	841.00	840.00	841.00	756.00	755.00	754.00	755.00
T <sub>3</sub>	840.00	839.00	838.00	839.00	754.00	753.00	752.00	753.00
T <sub>4</sub>	838.00	837.00	836.00	837.00	752.00	751.00	750.00	751.00
Mean	841.00	840.00	839.00		755.00	753.75	753.00	
Mean (E)	840.00				753.92			
T x S interaction Table					CD <sub>(0.05)</sub>			
T	Pulp-water ratio (S)			Mean (T)				
	1:2	1:4	1:6		Extraction Temperature (E)	0.47		
T <sub>1</sub>	801.00	799.50	799.00	799.83	Pulp-water ratio (S)	0.58		
T <sub>2</sub>	799.00	798.00	797.00	798.00	Treatments (T)	0.67		
T <sub>3</sub>	797.00	796.00	795.00	796.00	E x S	NS		
T <sub>4</sub>	795.00	794.00	793.00	794.00	E x T	NS		
Mean (S)	798.00	796.88	796.00		S x T	NS		
T <sub>1</sub> : 70°C; T <sub>2</sub> : 80°C; T <sub>3</sub> : 90°C; T <sub>4</sub> : 100°C					E x S x T	NS		

**Table 4** Effect of extraction parameters on methoxyl content (%) of pumpkin pectin isolated with water

E	Extraction Time (E)							
	60 minutes				120 minutes			
	S	Pulp-water ratio (S)			Mean	Pulp-water ratio (S)		
1:2		1:4	1:6	1:2		1:4	1:6	
T <sub>1</sub>	9.56	9.66	9.96	9.73	8.78	9.17	9.53	9.16
T <sub>2</sub>	9.34	9.42	9.67	9.48	8.52	8.95	9.39	8.95
T <sub>3</sub>	9.04	9.28	9.35	9.22	8.21	8.73	9.11	8.68
T <sub>4</sub>	8.71	8.93	9.13	8.92	8.01	8.59	8.91	8.50
Mean	9.16	9.32	9.53		8.38	8.86	9.24	
Mean (E)	9.34				8.83			
T x S interaction Table					CD <sub>(0.05)</sub>			
T	Pulp-water ratio (S)			Mean (T)				
	1:2	1:4	1:6		Extraction Temperature (E)	0.01		
T <sub>1</sub>	9.17	9.41	9.75	9.44	Pulp-water ratio (S)	0.01		
T <sub>2</sub>	8.93	9.19	9.53	9.22	Treatments (T)	0.01		
T <sub>3</sub>	8.63	9.01	9.23	8.95	E x S	0.02		
T <sub>4</sub>	8.36	8.76	9.02	8.71	E x T	0.02		
Mean (S)	8.77	9.09	9.38		S x T	0.03		
T <sub>1</sub> : 70°C; T <sub>2</sub> : 80°C; T <sub>3</sub> : 90°C; T <sub>4</sub> : 100°C					E x S x T	0.04		

**Table 5** highlights the data for anhydrogalacturonic acid (AGA) content of recovered pectin as affected by pulp-water ratio, time and extraction temperature. It can be clearly seen that the AGA content increased with increase in extraction time. The value was 75.52 per cent at 120 min while at 60 min of extraction the mean AGA was 73.44 per cent. At various extraction temperatures, maximum AGA content was seen in pectin isolated at 70 °C while minimum at 100°C thus indicating a decrease with increase in temperature. An increase in AGA content with increase in extraction time can be attributed to hydrolysis of pectin into D-anhydrogalacturonic acid. The decrease reported in AGA content with increase in temperature may be due to the accelerated degradation of pectin sugar side chain. The results obtained in present investigation are in conformity with [28] in apple pomace pectin, [23, 24] in cocoa husk pectin.

**Table 5** Effect of extraction parameters on anhydrogalacturonic acid content (%) of pumpkin pectin isolated with water

E	Extraction Time (E)							
	60 minutes				120 minutes			
	S	Pulp-water ratio (S)			Mean	Pulp-water ratio (S)		
T	1:2	1:4	1:6		1:2	1:4	1:6	
T <sub>1</sub>	82.76	77.79	73.11	77.88	85.54	80.57	75.89	80.66
T <sub>2</sub>	79.34	75.17	70.32	74.94	83.12	77.95	72.10	77.72
T <sub>3</sub>	74.29	72.06	66.89	71.08	79.07	74.84	69.11	74.34
T <sub>4</sub>	71.89	69.09	63.17	68.05	75.67	71.47	66.39	71.41
Mean	77.07	73.53	68.37		80.85	76.21	70.88	
Mean (E)	73.44				75.52			
<b>T x S interaction Table</b>					CD <sub>(0.05)</sub>			
<b>Pulp-water ratio (S)</b>								
T	1:2	1:4	1:6	Mean (T)	Extraction Temperature (E)	0.01		
T <sub>1</sub>	84.15	79.18	74.50	79.27	Pulp-water ratio (S)	0.01		
T <sub>2</sub>	81.23	76.56	71.21	76.33	Treatments (T)	0.02		
T <sub>3</sub>	76.68	73.45	68.00	72.71	E x S	0.02		
T <sub>4</sub>	73.73	70.28	64.78	69.61	E x T	0.03		
Mean (S)	78.96	74.86	69.62		S x T	0.03		
T <sub>1</sub> : 70°C; T <sub>2</sub> :80°C; T <sub>3</sub> :90°C; T <sub>4</sub> :100°C					E x S x T	0.05		

**Table 6** Effect of extraction parameters on degree of esterification (%) of pumpkin pectin isolated with water

E	Extraction Time (E)							
	60 minutes				120 minutes			
	S	Pulp-water ratio (S)			Mean	Pulp-water ratio (S)		
T	1:2	1:4	1:6		1:2	1:4	1:6	
T <sub>1</sub>	68.33	74.24	70.50	71.02	63.25	65.68	64.62	64.52
T <sub>2</sub>	69.20	75.41	71.15	71.92	64.14	67.09	65.19	65.47
T <sub>3</sub>	73.84	77.31	76.26	75.80	68.35	73.79	69.35	70.50
T <sub>4</sub>	69.77	71.55	70.36	70.56	63.98	67.99	65.16	65.71
Mean	70.28	74.63	72.07		64.93	68.64	66.08	
Mean (E)	72.33				66.55			
<b>T x S interaction Table</b>					CD <sub>(0.05)</sub>			
<b>Pulp-water ratio (S)</b>								
T	1:2	1:4	1:6	Mean (T)	Extraction Temperature (E)	0.01		
T <sub>1</sub>	65.79	69.96	67.56	67.77	Pulp-water ratio (S)	0.01		
T <sub>2</sub>	66.67	71.25	68.17	68.70	Treatments (T)	0.01		
T <sub>3</sub>	71.09	75.55	72.80	73.15	E x S	0.02		
T <sub>4</sub>	66.87	69.77	67.76	68.13	E x T	0.02		
Mean (S)	67.61	71.63	69.07		S x T	0.03		
T <sub>1</sub> : 70°C; T <sub>2</sub> :80°C; T <sub>3</sub> :90°C; T <sub>4</sub> :100°C					E x S x T	0.04		

**Table 6** indicates that the degree of esterification (DE) of isolated pectin was significantly affected by extraction time in combination with pulp-water ratio and extraction temperature. The mean value at extraction time of 60 min was 72.33 per cent while a value of 66.55 per cent was recorded at 120 min. Among different extraction



temperatures and pulp-water ratios, 90 °C and 1:4 gave pectin with maximum degree of esterification irrespective of extraction time. The combined interaction of extraction temperature and pulp-water ratio reflected that the mean maximum (73.15 %) degree of esterification was recorded in T<sub>3</sub> (90 °C) while mean minimum (67.77 %) in T<sub>1</sub> (70 °C). An interaction among extraction time, pulp-water ratio and extraction temperature revealed that extraction condition of 90 °C for 120 min with 1:4 pulp-water ratio produced pectin with maximum (77.31 %) degree of esterification while the minimum (63.25 %) value was at 70 °C for 60 min with 1:2 pulp-water ratio. A decrease in DE with increase in extraction time might be due to degradation of methyl ester group of pectin into carboxyl group as suggested by [24]. The findings are in conformity with [22, 24, 25, 29].

## Conclusion

The best optimized condition for extracting pumpkin pectin with water is concluded to be pulp-water ratio 1:4, temperature 90 °C and time 120 min based upon higher yield (5.33 %) and better quality characteristics as per the results discussed. The pectin extracted with this treatment combination possessed 753.00, 8.73 per cent, 74.84 per cent and 73.79 per cent of equivalent weight, methoxyl, anhydrogalacturonic acid and degree of esterification, respectively. The presence of 8 per cent methoxyl content and 50 per cent degree of esterification is the indication of high methoxyl pectin, therefore, the pectin obtained with water extraction falls under the category of high methoxyl pectin which can easily form jelly in presence of sugar and acid.

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