

Research Article



A Comparative Study of Functional Properties of Pectin Isolated from Different Fruit and Vegetable Sources

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ABSTRACT

Pectin is a structural heteropolysaccharide contained in the primary cell walls of terrestrial plants. It is used in food as a gelling agent, particularly in jams and jellies. It is also used in fillings, medicines, sweets, as a stabilizer in fruit juices and milk drinks, and as a source of dietary fibre. Commercial pectin is mostly extracted from fruit sources like apple pomace and peels of citrus fruits. The current study was conducted to extract pectin from pomace of easily available vegetables like carrot (*Daucus carota* subsp. *sativus*), French bean (*Phaseolus vulgaris*) potato (*Solanum tuberosum* L.) and to compare the physiochemical characteristics of the extracted pectin with that extracted from pomace of apple (*Malus domestica*) and peels of citrus fruits like lemon (*Citrus limon*) and citron (*Citrus medica*). The extracted pectin was characterized extensively in terms of solubility, colour and quantitatively in terms of Yield, Equivalent Weight, Methoxyl Content, Anhydrouronic Acid content (AUA). The product was compared with pure pectin powder using technique of Thin Layer Chromatography (TLC). With the exception of potato all the other sources yielded appreciable amount of pectin. The highest yield (30.18%) was obtained from citron. The best quality gel was also formed from this pectin isolated from citron. All the extracted crude pectin showed significant Methoxyl content, Anhydrouronic content and degree of esterification. The TLC profile of the extracted pectin was found to be matching with pure pectin.

Keywords: Pectin, Methoxyl content, Anhydrouronic content, Esterification.

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INTRODUCTION

Pectin is a complex mixture of polysaccharides occurring in the primary cell wall of terrestrial plant and is a high value functional food ingredient.¹ Pectin forms gels when dissolved in water under suitable conditions. It is derived from the protopectin found in the middle lamellae of plant cells. Protopectin is insoluble but is converted to soluble pectin as fruit ripens or is heated in an acid medium.² Pectin is widely used in the food industry as a thickener, emulsifier, texturizer and stabilizer. Pectin is usually used in jams and jellies as a gelling agent and also used for fruit preparations, fruit drink concentrates, fruit juice, desserts and fermented dairy products.³

In addition, pectin has been shown to lower blood cholesterol levels especially low-density lipoprotein cholesterol fractions and reduces the risk for coronary heart diseases.⁴ The gelling properties of pectin have been known for centuries, but the isolation of commercial pectin only started at the beginning of the twentieth century.⁵ The common raw material for Commercial pectin is apple pomace or citrus peel.⁶ It is now required that alternative sources of Pectin must be investigated to meet the increasing demand. In recent

years the production of fruits and vegetables has been increased. Therefore, large amount of fruits and vegetables could be used value added products as well as good source of pectin.³

In the present study easily available vegetable sources like carrot (*Daucus carota* subsp. *sativus*), French bean (*Phaseolus vulgaris*) and potato (*Solanum tuberosum* L.) were explored for pectin extraction and functional properties of pectin extracted from these sources were compared with that isolated from pomace of apple (*Malus domestica*) and peels of citrus fruits like lemon (*Citrus limon*) and citron (*Citrus medica*).

MATERIALS AND METHODS

Preparation of Extracts

All sources were washed carefully with tap water to remove dirt soil from surface. The peels of lemon and citron were removed, then cut into smaller pieces, shade dried, and ground to a consistency intermediate to coarse and fine and stored at ambient temperature for further use. For apple, carrot and French bean entire materials were crushed in grinder and the juice was pressed out. The pulpy pomace is used for extraction of pectin.

The pectin extraction process was carried out at different pH values ranging from 1.2-4.2.¹

Extraction using citrus Peels.¹

A dry mass of 100g peel powder was subjected to extraction by adding 1600 mL of distilled water followed by 64g of citric acid. The mixture was then heated at



temperatures of 40 - 90°C and continuously stirred for 1 hour, filtered through a whatman No. 1 filter paper or muslin cloth, filtrate was coagulated using an equal volume of 95% ethanol and left for 2 h to allow the pectin to float on the surface. The gelatinous pectin flocculants were then skimmed off, filtered and washed 2 - 3 times with ethyl alcohol and finally, the precipitate was dried at 35 - 40°C in hot air oven and then grinded the extraction, the yield was in powder form. The yield was calculated.

Extraction using pomace.⁷

The pre-treatment such as blanching (boiling at 95°C for 5 minutes followed by cooling) and drying in mechanical by oven at $40 \pm 2^\circ\text{C}$ was used for extraction of pectin. Method for extraction of pectin consisted of boiling the pomace in distilled water, 0.05N HCL and 0.75% ammonium oxalate-oxalic acid (1:1) for 1 hour at 95°C. Ratio of 1:2 of apple pomace to extraction media was used for extraction. After extraction, the extract was separated from the solid residue by filtration through a muslin cloth prior to its use for pectin precipitation. Liquid pectin extraction was used for the precipitation either with 95% ethanol or by using Aluminum chloride. The precipitate were then separated, washed successively with 70 and 95% ethanol, dried in oven at 50°C, grinded to powder form of Pectin. The yield of dried pectin was calculated and the pectin is stored in ambient temperature for further use.

Identification test of Pectin by Thin Layer Chromatography (TLC).⁸

10 ul of sample was loaded on pre-coated silica gel 60 F 254 aluminium plate and developed in the using suitable solvent system under appropriate condition. TLC Separation was performed for the pectin extracted from apples, carrots, French beans, lemon peels and citron peels. Standard Pectin (procured from Hi-media) was taken as reference. The Solvent system used were Butanol: Formic acid: water (2:3:1, 3:2:1 v/v), Butanol: Water: Acetic acid (2:3:1, 3:2:1 V/V). Iodine chamber was used for detection of spot. R_f values were determined after the appearance of spots.

Qualitative Characterization of Pectin⁹

Stiff gel Test

1g of pectin was heated with 9ml of water on a water bath till a solution was formed, on cooling stiff gel was formed.

Test with 95% Ethanol

On adding an equal volume of Ethanol (95%) to 1% w/v solution of pectin sample, a translucent, gelatinous precipitate was produced.

Test with Potassium Hydroxide (KOH)

To 5ml of a 1% w/v solution of pectin sample, 1ml of a 2% w/v solution of KOH was added and set aside for 15 minutes. A transparent semigel is produced when the above gel was acidified with dilute HCl and shaken well, a voluminous, colourless gelatinous precipitate forms which upon boiling became white and flocculent.

Iodine test

To 5ml of recently boiled and cooled 2% w/v solution of sample, 0.15 ml of Iodine solution was added. Absence of blue colour gives positive test.

The Quantitative Characterization of Pectin¹

Pectin Yield

The precipitate was dried at 40°C in hot air oven and percentage yield was calculated.

$$Y_{pec} (\%) = P / B_i \times 100$$

Where, Y_{pec} is the yield of pectin in (%), P is the amount of extracted pectin in g and B_i is the initial amount of pomace or peel powder.

Equivalent Weight¹⁰

Pectin sample (0.5g) was weighed into 250 ml conical flask and moistened with 5ml ethanol. A 1.0g NaCl was added to the mixture followed by 100ml distilled water and 6 drops of phenol red indicator.

Titrated with 0.1 M NaOH to an end point where pink colour is obtained. This neutralized solution was stored for determination of methoxyl content.

$$\text{Equivalent weight} = \frac{\text{Weight of sample (g)} \times 1000}{\text{Volume of alkali} \times \text{Normality of alkali}}$$

Methoxyl Content (MeO)¹⁰

This was done using the neutralized solution from equivalent weight determination, by the saponification of pectin followed by titration of the liberated acid. 25ml of 0.25M NaOH was added to neutralize solution and the mixture was stirred thoroughly and allowed to stand for 30 min at room temperature.

A 25 ml of 0.25 N HCL was added and titrated with 0.1 N NaOH. The same end point observed as earlier.

$$\text{Methoxyl Content \%} = \frac{\text{Volume of alkali} \times \text{Normality of Alkali} \times 3L \times 100}{\text{Weight of sample pectin}}$$

Anhydrouronic Acid content (AUA)¹⁰

The alkali milli-equivalents from equivalent weight, methoxyl content, and alkalinity of ash were taken to calculate anhydrouronic acid (AUA) content.



$$\text{Anhydrouronic Acid content} = \frac{176 (\text{m.e. for free acid} + \text{m.e. for saponification} + \text{m.e. for titrable ash}) \times 100}{\text{Weight of the sample (mg)}}$$

m.e. =milli-equivalents of alkali

Degree of Esterification (DE) ⁷

The DE of extracted pectin calculated from methoxyl and anhydrouronic acid content using the following expression.

$$\text{DE\%} = \frac{176 \times \text{MeO(\%)} \times 100}{31 \times \text{AUA(\%)}}$$

Moisture Content and Ash Content was estimated as per guidelines of Association of Analytical Chemists (AOAC 1995)¹¹ and (AOAC 1975).¹²

RESULTS AND DISCUSSION

All the sources except for potato pomace were found to be excellent source of pectin (Fig.1). The pH determination for maximum pectin yield were found to be for apple 3.6, lemon 4.1, carrot 3.8, citron 4.3 and French bean 4.0 which is in agreement with previous report of instability of pectin under alkaline condition.¹³

Maximum yield under optimum pH condition as mentioned in Table 1 was obtained from citron (30.18%) followed by lemon (18.75 %), apple (16.12 %), carrot (14.61%) and French bean (12.15%) whereas, in a previous work, amount of pectin obtained from citron, lemon, apple and carrot under optimum condition was 65%,¹⁴ 17.36%,¹⁵ 20.60%⁸ and 15.6%¹⁶ respectively.

There is no previous data of pectin extraction from French bean. Preliminary qualitative identification of the isolated Pectin was performed via TLC by co-chromatography technique using Standard Pectin as reference. Of all the solvent system used the system comprising of Butanol: Water: Acetic acid (3:2:1) was found to be the best. The R_f value of all pectin was in range of 0.61-0.62 (Fig. 2; Table 2) and was found to be matching with that of Standard pectin (R_f 0.63) The result is in agreement with previous work carried out on pectin extracted from Apple and Citrus Pomace.⁸

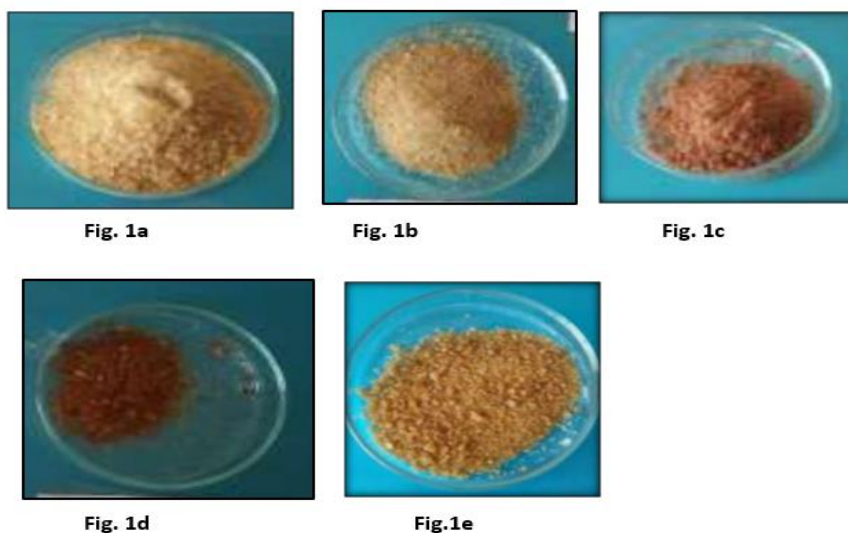


Figure 1: Yield of pectin in powder form;

Figure 1a: citron, Figure 1b: lemon, Figure 1c: carrot, Figure 1d: apple, Figure 1e: French bean

Table 1: Percentage (%) Pectin Yield

Sr.no.	Pectin Source	Pectin Yield (%)
1	Apple	16.12 ± 0.15
2	Lemon	18.75 ± 0.22
3	Carrot	14.61 ± 0.34
4	Citron	30.18 ± 0.45
5	French Bean	12.15 ± 0.70

The values represent mean of three replicates ± Standard deviation

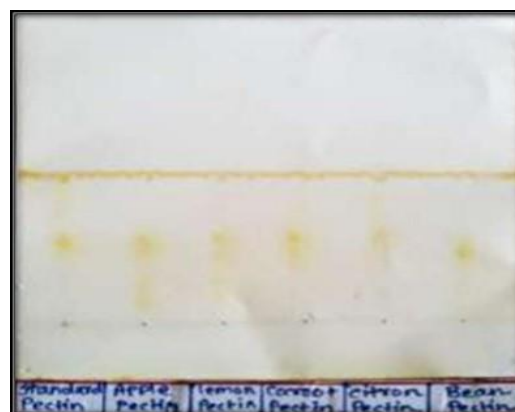


Figure 2: TLC profile of extracted pectin with reference to that of standard pectin

Table 2: R_f values of pectin extracted from different sources

Sr.no.	Pectin Source	R _f Value
1	Standard Pectin	0.63
2	Apple	0.62
3	lemon	0.62
4	Carrot	0.62
5	Citron	0.62
6	French Bean	0.61

Characteristic Variation was observed among the pectin gel obtained from the different sources. Apple, lemon, citron and French bean form stiff gel, whereas carrot formed gelatinous liquid. Ethanol, KOH and Iodine test showed positive result with all the pectin samples (Table 3).

The equivalent weight was found to be highest for pectin obtained from citron and least for the pectin obtained from carrot (Table 4). Equivalent weight of pectin is the total content of free galacturonic acid (not esterified) in the molecular chains of pectin.¹⁰

Table 3: Qualitative tests of Pectin

Sr. No.	Methods	Source				
		Apple	Lemon	Carrot	Citron	French Bean
1	Stiff gel test	gelatinous gel	Sticky gel	gelatinous gel	Sticky stiffgel	Sticky gel
2	Ethanol (95%) test	+	+	+	+	+
3	KOH test	+	+	+	+	+
4	Iodine test	+	+	+	+	+

+ Positive response

Methoxyl content is an important factor in controlling the setting time of pectins and the ability of the pectin to form gels.¹⁷ In the current study, the methoxyl content varied from 2.3% to 6.8% (Table 4).

The methoxyl content of pectin usually varies from 0.2 – 12% depending on the source and mode of extraction. The values exhibited that the isolated pectin are within the preferred range as also reported in a previous work.¹

The Degree of Esterification (DE) is the ratio of the esterified galacturonic acid groups to the total galacturonic acid groups present. It is an important property which determines the gelling nature of pectin.¹⁸

In the present investigation the highest DE value (Table 4) was observed in citron (74.64%) followed by apple (68.74%). The pectin obtained from these two sources are categorized as High methoxyl pectin (HMP) as their DE value is higher than 50%. Pectin obtained from lemon,

carrot and French bean fall under the category of low methoxyl (LM) pectins as their DE value is less than 50%.¹⁹ However in another report it has been stated that DE value of LM-pectins range from 20 to 40%.²⁰ So accordingly lemon and French bean with DE value of 42.80% and 41.72% can also be considered as High methoxyl pectin.

Anhydrouronic acid content of pectin is an important parameter which indicates the suitability of pectin for its use in jams, jellies etc.²¹ The content of anhydrouronic acid (AUA) indicates the purity of the extracted pectin with a recommended value of not less than 65%.²² Results of this study (Table 4) showed that the pectin obtained from all the sources can be considered to be falling within the purity range in terms of Anhydrouronic acid content with pectin extracted from French bean showing the maximum value of 80.95%. The percentage moisture content obtained from the five sources was 58.72, 75.80, 82.70, 66.60 and 80.95% respectively (Table 4).

Table 4: Physico-Chemical Characteristics of extracted Pectin

Parameters	Pectin Source				
	Apple	Lemon	Carrot	Citron	French beans
Equivalent Weight (mg/mL)	594.86	386.45	253.70	694.44	293.60
Methoxyl Content (%)	6.840	4.460	2.348	4.460	3.900
Anhydrouronic Acid Content (%)	79.72	75.80	72.70	76.60	80.95
Degree of Esterification (%)	68.74	42.80	39.48	74.64	41.72

CONCLUSION

In the present study, pectin extracted from the various sources was found to possess all physical and chemical characteristics ideal to be used for commercial purpose. It was also observed that pectin extracted from sources which are commonly used for commercial purpose was

superior as compared to the unconventional sources like carrot and french bean. But in view of easy availability and relative low-cost, these sources can be considered as cheap alternative source of pectin particularly when food additives from natural sources are preferred to avoid health hazards associated with synthetic additives.



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