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PHYSICO-MECHANICAL IMPACT ON YIELD OF PECTIN EXTRACTED FROM RELATIVELY THREE NEW SOURCES

NAUSHEEN HAMEED SIDDIQUI[1*], IQBAL AZHAR[2], MUHAMMAD SHAIQ ALI[3], AND ZAFAR ALAM MAHMOOD[4]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Main University Road, Karachi 75270, Pakistan
[2]Faculty of Pharmacy, University of Karachi, Main University Road, Karachi 75270, Pakistan
[3]International Centre for Chemical & Biological Sciences, ICCBS, HEJ Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan
[4]Colorcon Limited, Flagship House, Crossways Business Park, Victory Way, Dartford DA2 6QD, United Kingdom
[*]Corresponding author's email: naushinhs@yahoo.com

Abstract

In the present study the influence of various physic-mechanical factors was taken into consideration to achieve the maximum yield from three new sources of pectin. Three different fruit peels obtained from sapodilla, banana, and muskmelon were included in the study. Among the three fruits investigated in the study, banana recorded highest (10.5%), while sapodilla (4.7%) and muskmelon (4.4%), subsequently. The detailed extraction study with major influencing factors on pectin yield provided comprehensive data for the three new sources, which could become an effective raw material for low-cost pectin manufacturing. The variation in yield occurred due to the different physic-mechanical procedures used to extract pectin from these fruits. The study elaborates the effect of major influencing factors and their collaborative effect on yield of pectin from three new sources while confirming the effects through already two known sources (Apple and orange). The study was evaluated through statistical method to fully comprehend the commutative and individual effects of variables. The results indicated that different variables (Mechanical procedures (MP), pH level, and boiling method (BM)) have significant impacts on the yield of all five fruits. Hence it was concluded from the study that pectin can be extracted from these new sources effectively by applying desired variables of extraction procedure.

Key words: Pectin, Fruit peels, Mechanical methods, pH, Conventional boiling, Microwave heating.

Introduction

Pectin is among polysaccharides family containing α-(1-4)-linked D-galactopyranosyluronic acid residue (Yeh *et al.*, 2011). In the formation of primary call wall pectin plays a vital role distinct from cellulose and hemicelluloses (Cheng *et al*., 2011). Pectin has many traditional as well as pharmaceutical uses. Food industries were among the initial users of pectin but later it was reported in many pharmaceutical preparations and applications. Well known function of pectin to produce jelly not only made it useful traditionally in making jam and jellies but this also lead to the production of many other food products which includes dairy products, deserts and soft drinks (Munarin *et al*., 2012). Pharmaceutical applications encompass cholesterol-lowering formulations (Brouns *et al*., 2011). Some other traditional and other pharmaceutical applications includes pectin-derived oligosaccharides which are used as modern prebiotics (Gullon *et al*., 2013), nasal and ophthalmic preparations (Mittal *et al*., 2014; Morris & Kok, 2010), and drug delivery systems specifically for colon (Kushwaha *et al*., 2011). Advancements in the field led to the development of hydrogels in combination with proteins which were termed useful in the formulation of low calorie food products (Wu *et al*., 2014). Recently, research on the application of pectin in cosmeceutical preparations have also been undertaken (Suh *et al*., 2014).

In view of the diversified and essential applications of pectin, which categorizes it as an intriguing and beneficial component for the food, pharmaceutical, nutraceutical, and cosmeceutical industries, the search for new sources to meet market demand requiring a comprehensive study by the use of applied as well as pure knowledge. Based on this approach, the present study was designed to find a source possessing comparative quality attributes of pectin from seasonal fruit and to synchronize and explore the physico-mechanical parameters for greater yield on a laboratory scale. The mechanical procedure for cell lysis to release pectin was an important parameter to understand as the peels under investigation were neither citrus in nature, nor was pomace used, as is the common practice in the commercial production of pectin (May, 1990)

At present, no pectin manufacturing unit is operating in Pakistan and all market demand is supported through imports. Due to the existing trends of increased price and the cost of raw materials and utilities used to extract pectin, some of the famous pectin-producing companies have also increased prices of their pectin brands (CP Kelco, 2014; Yantai Andre Pectin, 2013). It is, therefore, time to look for new alternatives for the extraction of pectin and to understand the factors and procedures used in the extraction process to enhance the yield from existing and new raw materials. With the exploration of new low-cost sources, pectin prices may become competitive. It is anticipated that research on the extraction of pectin and on new sources can provide advanced insight in developing better quality and controlled pectin prices internationally (Gray, 2014).

Surprisingly, wastes from food processing industries are among the largest mass produced, globally, which are due to the production of consumer products. Making useful functional compounds from food processing wastes and their appropriate utilization can be beneficial economically but will also aid in minimizing the hazard of changing global environment (Anon., 2011).Fruits wastes are sometimes around 50% of the total processing waste and are very difficult to manage for further developments because of improper utilization techniques, available resources, cost, and regulatory issues. Hence, fruits selected in the current study also involved fruit having voluminous peels, like muskmelon. Around 31 million tons of melon types (Melon World Production) 105 million tons of bananas (All about bananas), and 2018 metric tons of sapodilla (Potential of chiku cultivation) are produced annually around the world, which make the current study quite beneficial.

The main objectives of the study were to screen three potential new sources of pectin for their best available pectin contents using different physic-mechanical tools. The study will not only provide basic knowledge of the response of different variables used in the extraction process but yield from two known sources (Apple and orange) will give a comparative data. Materials and Methods

All fruits, sapodilla (*Manilkara zapota*), banana (*Musa paradisiaca*), muskmelon (*Cucumis melo*) apple (*Malus domestica*), and orange (*Citrus sinensis*), were purchased from a local market in their respective seasons of availability. A voucher specimen was deposited for each fruit in the herbarium of the University of Karachi, Pakistan. The voucher numbers are 11-005 for sapodilla, 11-006 for banana, 11-007 for muskmelon, 11-008 for apple, and 11-009 for orange.

For bleaching, Industrial methylated spirit (IMS) from BDH Laboratories, Poole, England was used. A Panasonic MX-J120P, (Osaka, Japan) mechanical blender and grinder were used to crush the peeled fruit mechanically. For hammering and cutting, a kitchen hammer and hand chopper (IKEA) were used, while chopping was done using a mortar and pestle. A Jenway 3510 pH meter (Staffordshire UK) was used to read and adjust the pH. A National (IEC-705) 700 W microwave (Osaka, Japan), was used for heating. A laboratory centrifuge (Shanghai, China, model number 800) was also used in the study and a Trio, science Co, Ltd (Tokyo, Japan) freeze dryer, model number TR-FD-BT-50, was used to dry the extracted pectin.

Extraction of pectin: The method used for the extraction of pectin was a modification of the process described previously by some scientists (Patel *et al*., 2012). The method is kept similar for all types of fruit peels mentioned in the current study. The fruits were taken and the peels were removed with a sharp knife and were cut into smaller pieces of a few mm thickness. Twenty grams of thinly sliced peels of each fruit were boiled in 100 ml of IMS for 5 min in a water bath. The peels, after using different mechanical tools, such as homogenization, grinding, cutting, chopping, and hammering, were used to reduce the peel size into smaller components. The processed peels obtained from each mechanical method were then transferred into a beaker containing required amounts of DI water to form a slurry. The contents of the beaker were boiled for 10 min using a conventional Bunsen burner as well as microwave heating methods. The pH of the each of the contents was recorded and adjusted to a desired pH ranging from 1 to 7 (1, 3, 5, 6, and 7) using either NH4OH or 0.1NHCl. The solids of each mixture were separated, first using a Buchner funnel, followed by centrifugation at 4000 rpm for 3 min. Pectin was precipitated by adding ethanol in a ratio of 1:4, which was then separated with the help of centrifugation at 4000 rpm for 3 min, dried under vacuum, and weighed to calculate the percentage yield.

Three factor factorial completely-randomized design (CRD) was applied and a mean comparison was done by using Tukey HSD (Steel *et al*., 1997) at a 5% level of significance. Statistical analysis was performed using SPSS 13 and Minitab 13.1.

Results

Extraction of pectin from five different fruits: The yield of pectin from five selected fruits were recorded and shown in Table 1. The fruits used in the present study were obtained from a local market but no part of any existing pectin manufacturing industry were used to check comparative effects. The different fruits showed variable yields after using different physico-mechanical procedures. Table 1 not only infers about the pectin content of each fruit, but also the effect of various influencing variables on the yield of pectin. The result showed that each mechanical procedure has different influences on yield at variable pH and boiling methods. The difference in yield results were also seen in already-explored apples and oranges, which verified the influence of physico-mechanical effects on the yield of pectin. Among the three fruits under investigation, banana gave the highest yield while using both types of boiling procedures (Table 1).

In the present study, pectin was extracted from the peel of sapodilla fruit and the highest yield (4% yield) was obtained through grinding at pH 3, using Bunsen burner as heating methods. A further enhancement in yield was noted (4.7%) at lower pH (pH 1) when the heating mode was replaced with heating in microwave. The muskmelon peels maximum yield was obtained at pH 3 using conventional heating and cutting as the mechanical process (2.45%) of pectin. However, using the microwave technique, extraction after grinding as the mechanical process at pH 1 provided the best yield (2.65%).

Statistical analysis of yield of pectin from sapodilla, banana, and muskmelon fruit peels: Statistical methods were employed in order to fully understand the commutative and individual effects of variables. In order to observe the impact of different variables on the yield of pectin, the data were analyzed statistically to study the significance of all variables expected to affect the yield of pectin. The results indicated that different variables, such as mechanical procedures (MP), pH level, and boiling method (BM), have significant impacts on the yield of all five fruits at the 1% level of significance. Results highlighted in Table 2 show that interactions of all of the variables have a positive impact on the yield of pectin from all five fruits performed through the analysis of variance (ANOVA).

Table 1. Yield of pectin from five different fruits using five mechanical methods, at five different pH levels, using two boiling methods.

Mechanical procedure	pH	Sapodilla		Banana		Muskmelon		Apple		Orange	
							M	B	M	B	M
Homogenizing	1	0.5	1.05	3.25	5.6	0.55	0.55	0.5	1	16.15	15.25
	3	0.75	1.6	6.85	4.35	2	1	1.95	2.75	18.2	19.8
	5	1.2	1.5	8.85	8.4	1.45	2.3	2.35	3.7	19.4	22.7
	6	1.5	2	5.85	9.75	1.15	1	1.5	1.4	13.95	2.85
	7	0.5	0.5	5.45	3.1	0.55	1.8	0.05	0.35	21.7	7.5
Grinding	1	0.55	0.8	2.45	3.2	2.25	2.65	2.45	2.5	2.6	3.95
	3	4	3.5	4.2	6.9	1.4	1.8	0.45	1.15	7.8	5.3
	5	2.45	2.9	3.5	4.5	1.95	2.4	1	1.1	2.3	7.1
	6	2	2	3.65	2.65	0.95	1.6	2.4	2.85	6.45	6.6
	7	1.95	2.3	1.25	0.25	0.55	1	0.05	0.55	0.6	0.2
Cutting	1	0.8	0.9	0	2.5	0.5	0.5	2.85	3.9	8.1	7
	3	1.1	1.5	1.95	1.2	2.45	2.55	2.95	4.5	8.5	12
	5	0.4	1	5	5.5	1	1	2.7	3.7	11.85	20.65
	6	1	1.2	1	5.4	0.7	0.95	1.15	2.9	6.95	10.2
	7	0.7	1	3	6.5	1.3	2.2	0.05	0.7	4	7.4
Chopping	1	2.7	4.7	1.65	2.1	0.05	0.7	2.85	3.35	2.6	3.35
	3	3.9	3.45	4	3.3	0.05	0.5	1.25	2.75	7.95	10.05
	5	3.95	4.05	5.95	6.6	1.7	2.1	0.05	1.75	10.4	15.8
	6	2.3	2.45	5.85	6.35	2.25	1.75	0.1	0.9	15.25	17.15
	7	3.95	2.4	1.85	1.6	0.5	1.05	0.25	0.6	5.35	9.35
Hammering	1	1.5	2.05	4.5	7	0.65	1.25	1.5	1.7	13.55	16.65
	3	0.6	1.55	5.5	5.5	0.5	0.8	2.7	3.5	7.2	8.35
	5	0.8	1.95	8.5	10.5	0.65	0.3	3.05	4.85	7.4	12.15
	6	0.8	1.4	5	5.6	0.95	0.5	1.25	2.05	5.55	10.7
	7	0.5	0	0.5	0.5	0.45	0.05	1.7	2.05	5.5	8.85

Where B= Boiling on Burner, M= Microwave heating, pH 1 to 5 adjusted by 0.1N HCl and 6.5 to 7 by NH_4OH

Table 2. Analysis of variance (mean squares) of the pectin yield for different fruits.

Source of variation	Degree of freedom	Mean squares				
		Sapodilla	Banana	Muskmelon	Apple	Orange
Mech. procedure (MP)	4	32.6575**	51.245**	4.3869**	9.5920**	493.729**
pH level	4	3.3917**	84.818**	1.5751**	17.3200**	142.586**
Boiling method (BM)	1	3.2413**	22.349**	2.0184**	22.6981**	59.914**
MP × pH	16	2.5462**	16.774**	2.7688**	5.6967**	88.941**
MP × BM	4	0.3392**	4.640**	0.2814**	0.8120**	81.797**
pH × BM	4	1.0112**	6.332**	0.4112**	0.8975**	44.377**
MP × pH x BM	16	0.6258**	4.698**	0.4141**	0.2720**	21.750**
Error	100	0.0266	0.191	0.0112	0.0314	1.0100
Total	149					

NS = Non-significant ($p>0.05$); * = Significant ($p<0.05$); ** = Highly significant ($p<0.01$)

The difference among the means regarding the different factors using Tukey’s test is given in Tables 3–5 for sapodilla peel. According to the overall means of the variables investigated in the study (mechanical procedure, pH and boiling method) chopping, pH 3 (Table 3), and microwave boiling (Table 4), showed a significant impact ($p<0.05$) on the pectin yield from sapodilla fruit. Tables 3 and 4 also carry results of the other selected levels of variables in the study and their order of significance is given from highly to least effective. Letter A was considered for the highest letter A, and subsequent letters were used to show lesser effectiveness in alphabetical order. Overall interaction effects of the boiling method with pH (BM x pH) on sapodilla peel showed (Table 5) thatMicrowave at pH 3 and 5 (M × 3 and M × 5) gave significantly ($p<0.05$) higher yields, while the yield at boiling on a Bunsen burner at pH 1(B × 1) was the lowest.

The difference among the means regarding different variables using Tukey’s test is given in Tables 6–8 for banana peel.According to the overall means of the variables investigated in the study (mechanical procedure, pH and boiling method) homogenizing, pH 5 (Table 6), and microwave boiling (Table 7), showed a significant impact ($p<0.05$) on the pectin yield from banana fruit. Tables 6 and 7 also carry results of the other selected levels of variables in the study and their order of significance is given from highly to least effective. Letter A was considered for the highest letter A, and subsequent letters were used to show lesser effectiveness in alphabetical order. Overall interaction effects of the boiling method with pH (BM x pH) on banana peel showed (Table 8) that Microwave at pH 5 (M × 5) gave significantly (p<0.05) higher yields, while the yield at boiling on a Bunsen burner at pH 1 (B × 1) was the lowest.

According to the overall means of the variables investigated in the study (mechanical procedure, pH and boiling method) grinding, pH 5 (Table 9), and microwave boiling (Table 10), showed a significant impact (p<0.05) on the pectin yield from muskmelon fruit. Tables 9 and 10 also carry results of the other selected levels of variables in the study and their order of significance is given from highly to least effective. Letter A was considered for the highest letter A, and subsequent letters were used to show lesser effectiveness in alphabetical order. Overall interaction effects of the boiling method with pH (BM x pH) on muskmelon peel showed (Table 11) thatMicrowave at pH 5 (M × 5) gave significantly (p<0.05) higher yields, while the yield at boiling on a Bunsen burner at pH 7(B × 7) was the lowest (Table 11)

Table 3. Means comparison for sapodilla. Mechanical procedure × pH interaction mean ± SE.

pH level	Mechanical procedure					Mean
	Homogenizing	Grinding	Cutting	Chopping	Hammering	
1	0.78 ±0.13ij	0.68 ±0.06j	0.85 ±0.03hij	3.70 ±0.47a	1.78 ±0.14ef	1.56 ±0.23C
3	1.18 ±0.19gh	3.75 ±0.15a	1.30 ±0.09g	3.68 ±0.13a	1.08 ±0.21ghi	2.20 ±0.24A
5	1.35 ±0.08g	2.68 ±0.11c	0.70 ±0.14j	4.00 ±0.12a	1.38 ±0.26g	2.02 ±0.23B
6	1.75 ±0.12f	2.00 ±0.06ef	1.10 ±0.05ghi	2.38 ±0.10cd	1.10 ±0.14ghi	1.67 ±0.10C
7	0.50 ±0.01jk	2.13 ±0.10de	0.85 ±0.07hij	3.18 ±0.35b	0.25 ±0.11k	1.38 ±0.22D
Mean	1.11 ±0.10C	2.25 ±0.19B	0.96 ±0.05D	3.39 ±0.16A	1.12 ±0.12C	

Means sharing similar letter in a row or in a column are statistically non-significant (p>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean

Table 4. Mechanical procedure × boiling method interaction mean ± SE of sapodilla.

Boiling method	Mechanical procedure					Mean
	Homogenizing	Grinding	Cutting	Chopping	Hammering	
B	0.89 ± 0.11e	2.19 ± 0.30b	0.80 ± 0.07e	3.36 ± 0.20a	0.84 ± 0.09e	1.62 ± 0.14B
M	1.33 ± 0.14c	2.30 ± 0.25b	1.12 ± 0.06d	3.41 ± 0.25a	1.39 ± 0.20c	1.91 ± 0.13A

Means sharing similar letter in a row or in a column are statistically non-significant (p>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean. B= Boiling on burner, M= microwave heating

Table 5. Mechanical procedure × pH × boiling method interaction mean ± SE of sapodilla.

BM × pH	Mechanical procedure					BM × pH
	Homogenizing	Grinding	Cutting	Chopping	Hammering	Interaction mean
B × 1	0.50 ± 0.03qrs	0.55 ± 0.02pqr	0.80 ± 0.04n-r	2.70 ± 0.14ef	1.50 ± 0.06i-l	1.21 ± 0.22E
B × 3	0.75 ± 0.03n-r	4.00 ± 0.15bc	1.10 ± 0.03j-o	3.90 ± 0.14bcd	0.60 ± 0.03o-r	2.07 ± 0.41B
B × 5	1.20 ± 0.07j-n	2.45 ± 0.10efg	0.40 ± 0.02rs	3.95 ± 0.22bcd	0.80 ± 0.03n-r	1.76 ± 0.35C
B × 6	1.50 ± 0.07i-l	2.00 ± 0.10ghi	1.00 ± 0.04l-q	2.30 ± 0.13fg	0.80 ± 0.03n-r	1.52 ± 0.16D
B × 7	0.50 ± 0.02qrs	1.95 ± 0.08ghi	0.70 ± 0.03n-r	3.95 ± 0.14bcd	0.50 ± 0.01qrs	1.52 ± 0.36D
M × 1	1.05 ± 0.06k-p	0.80 ± 0.03n-r	0.90 ± 0.03m-r	4.70 ± 0.26a	2.05 ± 0.14gh	1.90 ± 0.40BC
M × 3	1.60 ± 0.08hij	3.50 ± 0.16cd	1.50 ± 0.06i-l	3.45 ± 0.14d	1.55 ± 0.05h-k	2.32 ± 0.26A
M × 5	1.50 ± 0.04i-l	2.90 ± 0.06e	1.00 ± 0.03l-q	4.05 ± 0.14b	1.95 ± 0.11ghi	2.28 ± 0.29A
M × 6	2.00 ± 0.09ghi	2.00 ± 0.08ghi	1.20 ± 0.04j-n	2.45 ± 0.16efg	1.40 ± 0.02j-m	1.81 ± 0.13C
M × 7	0.50 ± 0.02qrs	2.30 ± 0.12fg	1.00 ± 0.04l-q	2.40 ± 0.08efg	0.00 ± 0.00s	1.24 ± 0.26E

Means sharing similar letter in a row or in a column are statistically non-significant (p>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean. B= Boiling on burner, M= Microwave heating

Table 6. Means comparison for banana. Mechanical procedure × pH interaction mean ± SE.

pH level	Mechanical procedure					Mean
	Homogenizing	Grinding	Cutting	Chopping	Hammering	
1	4.43 ± 0.55fgh	2.83 ± 0.19j	1.25 ± 0.56klm	1.88 ± 0.12k	5.75 ± 0.60cd	3.23 ± 0.36D
3	5.60 ± 0.57cde	5.55 ± 0.64cde	1.58 ± 0.17kl	3.65 ± 0.19hij	5.50 ± 0.18cde	4.38 ± 0.34C
5	8.63 ± 0.31ab	4.00 ± 0.25ghi	5.25 ± 0.21def	6.28 ± 0.27c	9.50 ± 0.53a	6.73 ± 0.41A
6	7.80 ± 0.90b	3.15 ± 0.26ij	3.20 ± 0.99ij	6.10 ± 0.22cd	5.30 ± 0.19def	5.11 ± 0.42B
7	4.28 ± 0.54gh	0.75 ± 0.23lm	4.75 ± 0.80efg	1.73 ± 0.07k	0.50 ± 0.01m	2.40 ± 0.38E
Mean	6.15 ± 0.41A	3.26 ± 0.33D	3.21 ± 0.40D	3.93 ± 0.37C	5.31 ± 0.55B	

Means sharing similar letter in a row or in a column are statistically non-significant (p>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean

Table 7. Mechanical procedure × boiling method interaction mean ± SE of banana.

Boiling method	Mechanical procedure					Mean
	Homogenizing	Grinding	Cutting	Chopping	Hammering	
B	6.05 ± 0.50a	3.01 ± 0.29e	2.19 ± 0.46f	3.86 ± 0.50cd	4.80 ± 0.69b	3.98 ± 0.27B
M	6.24 ± 0.67a	3.50 ± 0.59de	4.22 ± 0.55c	3.99 ± 0.57cd	5.82 ± 0.87a	4.75 ± 0.31A

Means sharing similar letter in a row or in a column are statistically non-significant (p>0.05).Small letters represent comparison among interaction means and capital letters are used for overall mean. B= Boiling on burner, M= Microwave heating

Table 8. Mechanical procedure × pH × boiling method interaction mean ± SE of banana.

BM × pH	Mechanical procedure					BM × pH
	Homogenizing	Grinding	Cutting	Chopping	Hammering	Interaction mean
B × 1	3.25 ± 0.19l-q	2.45 ± 0.11o-u	0.00 ± 0.00x	1.65 ± 0.12r-w	4.50 ± 0.16h-l	2.37 ± 0.41D
B × 3	6.85 ± 0.24def	4.20 ± 0.19i-m	1.95 ± 0.06q-v	4.00 ± 0.19j-n	5.50 ± 0.27e-i	4.50 ± 0.44C
B × 5	8.85 ± 0.51b	3.50 ± 0.09l-p	5.00 ± 0.31g-k	5.95 ± 0.22d-h	8.50 ± 0.29b	6.36 ± 0.56B
B × 6	5.85 ± 0.26d-h	3.65 ± 0.24k-o	1.00 ± 0.03u-x	5.85 ± 0.31d-h	5.00 ± 0.21g-k	4.27 ± 0.49C
B × 7	5.45 ± 0.30e-j	1.25 ± 0.07t-x	3.00 ± 0.12m-s	1.85 ± 0.08q-v	0.50 ± 0.03vwx	2.41 ± 0.46D
M × 1	5.60 ± 0.30d-i	3.20 ± 0.16l-q	2.50 ± 0.14o-t	2.10 ± 0.09p-u	7.00 ± 0.43cd	4.08 ± 0.52C
M × 3	4.35 ± 0.12i-m	6.90 ± 0.40de	1.20 ± 0.07t-x	3.30 ± 0.15l-q	5.50 ± 0.31e-i	4.25 ± 0.53C
M × 5	8.40 ± 0.43bc	4.50 ± 0.23h-l	5.50 ± 0.26e-i	6.60 ± 0.46def	10.50 ± 0.57a	7.10 ± 0.59A
M × 6	9.75 ± 0.46ab	2.65 ± 0.14n-t	5.40 ± 0.27f-j	6.35 ± 0.29d-g	5.60 ± 0.21d-i	5.95 ± 0.62B
M × 7	3.10 ± 0.09l-r	0.25 ± 0.01wx	6.50 ± 0.32def	1.60 ± 0.07s-w	0.50 ± 0.02vwx	2.39 ± 0.61D

Means sharing similar letter in a row or in a column are statistically non-significant (p>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean. B= Boiling on burner, M= Microwave heating

Table 9. Means comparison for muskmelon. Mechanical procedure × pH interaction mean ± SE.

pH level	Mechanical procedure					Mean
	Homogenizing	Grinding	Cutting	Chopping	Hammering	
1	0.55 ± 0.02m-p	2.45 ± 0.12a	0.50 ± 0.01n-q	0.38 ± 0.15pqr	0.95 ± 0.14ijk	0.97 ± 0.15D
3	1.50 ± 0.23fg	1.60 ± 0.10ef	2.50 ± 0.05a	0.28 ± 0.10qr	0.65 ± 0.07l-o	1.31 ± 0.15B
5	1.88 ± 0.20cd	2.18 ± 0.13b	1.00 ± 0.02ij	1.90 ± 0.10cd	0.48 ± 0.08o-r	1.49 ± 0.13A
6	1.08 ± 0.05hi	1.28 ± 0.15gh	0.83 ± 0.06jkl	2.00 ± 0.12bc	0.73 ± 0.10k-n	1.18 ± 0.09C
7	1.18 ± 0.28hi	0.78 ± 0.10j-m	1.75 ± 0.21de	0.78 ± 0.13j-m	0.25 ± 0.09r	0.95 ± 0.12D
Mean	1.24 ± 0.11C	1.66 ± 0.12A	1.32 ± 0.14B	1.07 ± 0.15D	0.61 ± 0.06E	

Means sharing similar letter in a row or in a column are statistically non-significant (p>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean

Table 10. Mechanical procedure × boiling method interaction mean ± SE of muskmelon.

Boiling method	Mechanical procedure					Mean
	Homogenizing	Grinding	Cutting	Chopping	Hammering	
B	1.14 ± 0.15d	1.42 ± 0.17b	1.19 ± 0.18d	0.91 ± 0.24e	0.64 ± 0.05f	1.06 ± 0.08B
M	1.33 ± 0.17bc	1.89 ± 0.16a	1.44 ± 0.21b	1.22 ± 0.16cd	0.58 ± 0.11f	1.29 ± 0.09A

Means sharing similar letter in a row or in a column are statistically non-significant (p>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean. B= Boiling on burner, M= Microwave heating

Table 11. Mechanical procedure × pH × boiling method interaction mean ± SE of muskmelon.

BM × pH	Mechanical procedure					BM × pH
	Homogenizing	Grinding	Cutting	Chopping	Hammering	Interaction mean
B × 1	0.55 ± 0.03qr	2.25 ± 0.13bcd	0.50 ± 0.03qr	0.05 ± 0.00s	0.65 ± 0.03pqr	0.80 ± 0.20F
B × 3	2.00 ± 0.04def	1.40 ± 0.05i-l	2.45 ± 0.06abc	0.05 ± 0.00s	0.50 ± 0.02qr	1.28 ± 0.24BCD
B × 5	1.45 ± 0.05h-k	1.95 ± 0.10d-g	1.00 ± 0.04m-p	1.70 ± 0.05f-i	0.65 ± 0.03pqr	1.35 ± 0.13B
B × 6	1.15 ± 0.06k-n	0.95 ± 0.03m-p	0.70 ± 0.05opq	2.25 ± 0.10bcd	0.95 ± 0.04m-p	1.20 ± 0.15DE
B × 7	0.55 ± 0.02qr	0.55 ± 0.01qr	1.30 ± 0.07j-m	0.50 ± 0.03qr	0.45 ± 0.02qr	0.67 ± 0.09G
M × 1	0.55 ± 0.03qr	2.65 ± 0.12a	0.50 ± 0.02qr	0.70 ± 0.04opq	1.25 ± 0.05j-m	1.13 ± 0.22E
M × 3	1.00 ± 0.03m-p	1.80 ± 0.09e-h	2.55 ± 0.09ab	0.50 ± 0.02qr	0.80 ± 0.06n-q	1.33 ± 0.20BC
M × 5	2.30 ± 0.09a-d	2.40 ± 0.14abc	1.00 ± 0.03m-p	2.10 ± 0.08cde	0.30 ± 0.01rs	1.62 ± 0.22A
M × 6	1.00 ± 0.05m-p	1.60 ± 0.08g-j	0.95 ± 0.05m-p	1.75 ± 0.04e-i	0.50 ± 0.02qr	1.16 ± 0.12DE
M × 7	1.80 ± 0.11e-h	1.00 ± 0.04m-p	2.20 ± 0.09bcd	1.05 ± 0.07l-o	0.05 ± 0.00s	1.22 ± 0.20CDE

Means sharing similar letter in a row or in a column are statistically non-significant (p>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean. B= Boiling on burner, M= Microwave heating

Discussion

The study which encompassed three new sources of pectin provided preliminary data on the presence of pectin in these fruits using different physic-mechanical tools. More focus and attention was given to optimize the process of extraction so that the quality of pectin should not be compromised, while trying to increase product yield. Therefore, fixed heating temperature and minimal time of heating were selected for study to maintain the quality of pectin. The mechanical procedure to lyse the cell wall is an important parameter, especially for muskmelon, as the morphology of the peel is quite different from the peels currently used to extract pectin and the already-described procedures may not be sufficient for the complete understanding of the process of pectin extraction.

The difference in yield was primarily supposed to be due to the difference in type of the fruit/source used to extract pectin. The reduction in particle size shows meaningful effect on the yield of pectin which also relies on the type of mechanical procedure adopted.The reason being that reduction in the particle size augment the protopectin release, which conclusively raise the yield of pectin when precipitated with ethanol (Canteri-Schemin *et al*., 2005). Similarly other variables like pH also has a positive significant effect on the yield of pectin (Ziari *et al*., 2010). Hence the current study was designed to investigate not only the variables affecting the yield but also their combinations. The variable combinations were mechanical procedure with pH, mechanical procedure with boiling methods, pH with boiling methods, and also the interaction of all mechanical procedures, pH and boiling method on the different fruit peels studied for the better yield of pectin.

Sapodilla, banana, and muskmelon are comparatively newer sources of pectin and, hence, further comprehensive study encompassed these three fruits in which different factors affecting the yield of pectin were investigated statistically (Tables 3–11). It was observed through past researches that sapodilla have been investigated for assessment of total dietary fibers it possess while pectin content was measured from its edible portion using spectroscopic techniques and not extraction (0.35 g ± 0.01/100 g fruit) (Mahattanatawee *et al*., 2006). The study also presents the comparison in yields of all of the test fruits at different pH with different boiling and mechanical methods take up in the study. Heating in microwave was also found useful to effect yield of pectin in other under investigated fruits. Pectin yield from banana peels were observed to be quite similar with the yield of pectin as reported in an earlier study (Christy *et al*., 2014). Like sapodilla, muskmelon was also previously studied for total dietary fiber (Mahattanatawee *et al*., 2006).

In the present study, maximum yield of pectin was obtained in an acidic medium, indicating it is an important factor to be considered while extracting pectin.Past studies also pointed towards the positive effect of an acidic medium (low pH) on the yield of pectin and a profound effect on pectin yield with the change in pH (Canteri-Schemin *et al*., 2005; Yapo *et al*., 2007).The acidic pH supports the theory of release protopectin release through disruption of cell wall containing cellulose pectin in the medium, and its hydrolysis, thus releasing a high quantity of pectin in the liquid phase which can, thus, be recovered by precipitation. Interestingly, the current study revealed that among investigated five fruits sapodilla was the only fruit which also gave pectin yield in basic medium as well; that is, pH 6.5 and 7, which was in accordance with an earlier published report (Kirtchev *et al*., 1989). The earlier study suggested that both acidic and alkaline media with higher temperature can aid in the rupture of cell walls for the release and hydrolysis of protopectin to obtain pectin. After closely investigating the effect of mechanical procedures in the current study, it was profound through the obtained data that mechanical procedures play a vital and positive role in maximizing the yield of pectin from various fruit peels. Different types of mechanical procedures were already tested and showed their influence in acquiring better yield of pectin from different types of cell wall. These procedures helps in the release of increased pectin after increasing the surface area of the material under investigation. Cutting peels with knife, using motor and pastle and hammer mill are few examples of mechanical procedures which were used extensively in past reports (Rudolph & Petersen, 2012; Poovaiah & Nukaya, 1979). Likewise grinding in a mechanical blender and in mills has also been termed effective to extract the pectin (Loyola *et al*., 2011). Homogenizing aids in the formation of loose slurry which interns helps to extract pectin from the desired material specially peels (Slavov *et al*., 2013). Traditionally mortar and pestle has been used for reducing the size of the investigated product, specially related to the field of extraction. (Lamotte *et al*., 1969). The current study also endorsed the effectiveness of different mechanical procedures in extracting pectin from diversified fruit peels.

As the structure and properties of different types of cell wall is not similar the present study revealed that single mechanical procedure might not be effective for all types of fruit peels. One more point of emphysis in the current study was first to minimize the time of extraction and also find new way of heating the extracting material. Temperature and time can both effect inversely to the quality of pectin obtained if used in increasing numbers, hence microwave heating was used to obtain pectin of same amount in lesser period of time. The other important factors were to keep the extraction environmental friendly, for that low strength mineral acid (0.1N HCl) was used (Vriesmanna & Petkowicz, 2013). Studies using organic acids (such as citric, tartaric, and oxalic acid) are also under investigation to observe the response on pectin yield under the parameters of the present study is underway and will be reported accordingly.To obtain authentic results in the current study the method used was kept simple so that results can be reproduce easily and effectively. Although three different types of fruits (sapodilla, banana and muskmelon) were investigated but the variables selected to obtained higher yield were kept easy so that pectin will be extracted in the most economical way.

The statistical analysis performed (Tables 2-11) on the values of different yield of pectin from three fruits after using various physico-mechanical factors also confirmed the influence of the affecting variables. The analysis showed the significant factors influencing the yield, from most to least, affecting for each fruit peel under investigation. The given analysis is unique in nature as no previous similar study shows the influence of the combination of experimented variables with these three new sources of pectin.

Conclusion

This study for the first time highlighted best methodology to extract pectin from three new sources of pectin using different variables. The obtained yield from these new sources specially sapodilla (*Manilkara zapota*) and banana (*Musa paradisiaca*) can become sustainable alternative sources of pectin which can open new doors in its commercialization. However there is still a long way to exhaustively elucidate the fine structure and functionalities of this extracted pectin.

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