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EFFECT OF Ph AND HEATING TECHNIQUES ON EXTRACTION OF PECTIN FROM DIFFERENT SOURCES AVAILABLE IN PAKISTAN

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ABSTRACT

Pectin is complex heteropolysaccharide primarily present in the cell wall of terrestrial plants cell wall. Although it is obtained from citrus peels and apple pomace, but new sources have been investigating to fulfill the increased demand of pectin. The current study aimed to evaluate the effect of three parameters (pH level and heating methods) on the yield of pectin from peels of five different sources. Pectin was extracted from peels of sapodilla, banana, muskmelon, orange and apple at different pH levels (pH 1-pH 7) and with two heating methods include heating on Bunsen burner and microwave heating and after keeping the extracting mixture for 24hrs at room temperature before final precipitation of pectin. Although the results of the current study showed highest pectin yield from orange peels but among the three new sources (sapodilla, banana and muskmelon), banana peels pectin was found to show highest yield at pH 3. While the lowest yield was resulted from muskmelon peels among the five fruits peels. Pectin yield was found to be significantly influenced by pH level and heating method after 24hrs curing. Microwave heating showed significantly increased yield of pectin from all the investigated fruits peels. Thus concluded that these new sources of pectin can play promising role in order to fulfill the global requirement of pectin production.

1. Introduction

Pectin is complex heteropolysaccharide that is primarily present in the cell wall of dicotyledonous plant (Hamidon 2017). The higher concentration of pectin is present in middle lamella of cell wall of plants. It has wide range of applications in food, pharmaceutical (Sandarani 2017), cosmetic products (Marić et al., 2018), personal care and nutraceutical products (Ciriminna, 2016). In addition it has versatile gelling property because of source dependent verity of molecular size, degrees of methylation and acetylation and amount of galacturonic acid and neutral sugar moieties (Gawkowska, 2018). Demand of pectin is increasing due to wide range of applications. On commercial scale apple pomace and citrus peels are used for production of pectin (Hamidon, 2017).

Different pectin polysaccharides are present in plant cell wall which are covalently linked domains that may be distinguished as homogalacturonan(HG), hamnogalacturonan I (RGI), rhamnogalacturonan II (RGII), xylogalacturonan (XGA), apiogalacturonan (AGA), arabinan, galactan, arabinogalactan I (AGI) and arabinogalactan II (AGII) whereas 65% of HG (homogalacturonan) is present

which is most abundant, 20-35% of RG-I (rhamnogalacturonan-I) and less than 10% covers RG-II (rhamnogalacturonan-II) and XGA (xylogalacturonan) is present (Harholt, 2010). Pectin can be divided into two types according to degree of methylation (Vanitha, 2019). The pectin with more than 50% of degree of methylation referred to as high methoxy pectin. It can form gels in acidic medium in pH from 2 to 3.5, in presence of sucrose with more than 55% by weight. Pectin referred to as low methoxy pectin when it has degree of methylation less than 50%. It also forms gels in higher range of pH from 2 to 6 in presence of divalent cations such as calcium. Pectin is also treated with ammonia which results amidated pectin. Pectin contains 65% of galacturonic acid units in food industries (Ciriminna, 2016). Commercially, pectin produced from citrus sources contributes 85% of pectin production where as 14% of pectin is obtained from apple pomace and little amount is obtained from sugar beet in addition commercially pectin extraction involved acid extraction, filtration and precipitation with ethanol (Gawkowska, 2018). Different types of pectin extraction techniques are well described by Sandarani (2017). Acid extraction of Pectin, Microwave assisted extraction of Pectin, Enzymatic extraction of Pectin. In Acid extraction of pectin the method involved utilization of chemical agents which include water, buffer, acid, base and calcium ion chelating agents. Among these, acid is found to be most effective extracting agent for pectin while commonly used acids are hydrochloric acid, acetic acid, citric acid and tartaric acid. By increase in strength of acid it causes increase in galacturonic acid content. The vield, physicochemical and functional properties of pectin also depend on the type and concentration of acid used for extraction of pectin. Nitric acid is also use in common practice for acidifying hot water for pectin extraction.

In microwave assisted extraction of pectin dielectric heating of plant molecules through exposure of microwave is carried out. Microwave energy absorption is taken place causing dipolar rotation of water which leads to

heat generation inside the plant tissue. Studies showed improved pectin yield by microwave assisted pectin extraction, the inactivation of pectin esterase enzyme due to microwave radiation causes better pectin extraction. Further due top disintegration of parenchyma cells causing increase in specific surface area which improve the water absorption capacity of plant cell which leads to decease extraction time and energy. Yield of pectin is directly related to the power of microwave. The increase in microwave irradiation improves penetration of solvent into plant matrix and when molecular irradiation interacts with electromagnetic field results rapid transfer of energy to the solvent and matrix that facilitates dissolution of components for extraction. As water is a polar solvent, it effectively absorbs microwave energy and thus promotes effective heating the cells rupture increases due to sudden increase of temperature and pressure rise inside the plant cells which further potentiates the exudation of pectin within plant cells into the surrounding solvents. Enzymatic extraction of pectin is not only safe environmentally but also important for good pectin yield. In this type of extraction, cell wall degrading enzymes which have minimum pectinolytic activity are used to hydrolyse non pectic components present in plant cell wall where different enzymes are used in this pectin polygalacturonase, extraction such as hemicellulose, protease and microbial mixed enzymes, alpha amylase, cellulose, alpha amylase and neutrase, xylase, b-glucosidase, pectinesterase celluclast, alcalase, and endopolygalacturonase. These enzymes are responsible for degradation of pectin and modification of its physicochemical properties.

Objectives of Study Considering the ecofriendly environmental and economical value of pectin sources and it has wide range of applications

2. Materials and Methods

2.1. Sources of Samples

Sapodilla, Orange, Banana, Muskmelon and Apple.

2.2. Extraction of Pectin

Pectin extraction is carried out by following the method, with slight modification, adopted by Siddiqui (2018). The fruits were taken from local market which were identified by experts and their herbarium numbers were deposited in department of pharmacognosy. the The following steps were taken place to extract orange, pectin from sapodilla, banana. muskmelon and apple. Fruits were washed and peeled off with sharp knife. Peels were sliced of a few mm in thickness. 40 g of pieces of each fruit peels was taken in beaker and 200 ml of IMS was added to each. Now allow these to boil for 5 minutes in a water bath. IMS was carefully decanted and 120 ml DI water was added and these were blended for 30 seconds in a mechanical blender to form slurry. The slurry of each sample was then boiled for 10 minutes using Bunsen burner and microwave heating. The contents were then allowed to cool and pH from 1 to 7 was for each sample by either 0.1 NH4OH or 0.1N solution of HCl. The contents were kept overnight for 24hrs at room temperature. The solids of each mixture were removed by filtration with the help of muslin cloth. The pectin for each sample was then precipitated by adding ethanol in a ratio of 1:4. The precipitated pectin for each sample was separated by using Buchner funnel and weighed. Wet pectin was freeze dried by and then weighed. Percentage yield of pectin from each source was calculated by using following formula,%Yield = Weight of dried pectin/ weight of peels \times 100. The dried pectin from all the three sources were then sieved with mesh number 60 and then desiccated.

2.3. Identification Tests for Pectin

A few tests like stiff gel test, test with ethanol, iodine and potassium hydroxide were performed to identify the extracted pectin according to the method followed by (Qadir, 2019)

2.4. Statistical Analysis

Statistical analysis was performed using SPSS 13 and Minitab 13.1. The mean comparison was done by using Tukey HSD (Steel, 1997) at a 5% level of significance.

3. Results and discussion

The non-edible parts of plants are the most focusing area for the researchers for the isolation and evaluation biological activities of bioactive molecules/compounds. Pectin is one of them which is also obtained from the waste of various fruits. It is reported that global market has increased upto 60,000 tonnes per year. The reported growth rate is 6% with price esteemed at \$12.90/kg for LM pectin whereas for HM pectin, it is esteemed at \$11.00/kg (Ciriminna et al., 2016).

Different extraction factors affecting the yield of pectin are playing effective roles for the optimization of extraction of pectin and can improve the yield of pectin from such economical source of pectin. Although apple pomace and citrus fruits peels are the commercial sources of pectin but researchers are now searching for new sources of pectin.

Current study designed to put great attention toward the economical source of pectin and optimization of pectin extraction to improve the yield of pectin. Considering the economical and eco-friendly environmental value of pectin source, the study involved the extraction of pectin from peels of five fruits. For factors affecting the yield of pectin, pH level heating techniques and curing time were used to optimize the extraction process for evaluating the positive effect on yield of pectin. The current study involved extraction of pectin from peels of different fruits (sapodilla, banana. five muskmelon, apple and orange) as shown in table 2. After extraction of pectin, few identification tests were performed for qualitative assessment which were stiff gel test, test with ethanol, iodine test and test with potassium hydroxide.

These tests for qualitative analysis exhibited positive results for pectin from each source (table 1). The gel forming property of pectin was confirmed for each pectin by stiff gel test. The formation of translucent and gelatinous precipitate with 95% ethanol was resulted for each pectin which also confirmed the presence of pectin. Test of each pectin showed no blue coloration which differentiated pectin from starch. Each pectin was also differentiated from most gum by their test with potassium hydroxide due to their positive response. Yield of pectin was primarily supposed to be affected by its source (Siddiqui, 2018). Aina (2012) also found influence of source on yield among three citrus fruits peels pectin (orange, lemon and grape fruit).Five sources are used in this study, although the results of the current study showed highest pectin yield from orange peels but among the three new sources (sapodilla, banana and muskmelon), banana peel pectin exhibited the highest yield while the lowest yield was resulted from muskmelon peels (table 2).

| Test | Sapodilla | Banana | Muskmelon | Apple | Orange |
|----------------------------------|-----------|--------|-----------|-------|--------|
| Stiff gel test | + | + | + | + | + |
| Test with ethanol | + | + | + | + | + |
| lodine test | + | + | + | + | + |
| Test with potassium hydroxide | + | + | + | + | + |

Table1. Results of tests of pectin identification

| | | | | | | pectin from different fruits | | | | | |
|----|-------|------|-----|-----|-------|------------------------------|------|-------|------|--------|--|
| ъЦ | Sapod | illa | Ban | ana | Muskm | elon | Ар | Apple | | Orange | |
| рН | В | М | В | М | В | М | В | М | В | М | |
| 1 | 1.45 | 2 | 5.5 | 5.8 | 0.75 | 0.8 | 1.55 | 4.5 | 3.45 | 4.25 | |
| 3 | 3.5 | 2.5 | 8.5 | 10 | 0.75 | 0.4 | 4.25 | 4.45 | 8.45 | 9.35 | |
| 5 | 2.5 | 3 | 4.5 | 7.5 | 0.4 | 0.9 | 2.05 | 3.55 | 6.35 | 7.5 | |
| 6 | 1.5 | 2 | 4 | 3.5 | 0.9 | 2.1 | 1.35 | 2.2 | 5.7 | 7.25 | |
| 7 | 0.5 | 0.5 | 6 | 7 | 0.35 | 0.7 | 4.65 | 4.9 | 5.05 | 6.05 | |

Table 2. Yield of pectin from different fruits

The current results also favor the results of previous investigation on pectin yield from same fruits peels which also showed the highest pectin yield from banana peels and lowest from muskmelon peels (Siddiqui , 2018). As the experiment involved two heating methods for each pectin source, it was noted that the resulted high yield of banana obtained when the heating mode was microwave. Similarly the lowest yield of muskmelon was resulted when heating was carried out by the use of Bunsen burner. The experimental results favor the use of microwave heating for improved quantity of pectin as compared to the heating technique of utilizing Bunsen burner.

Previously sapodilla was assisted for total dietary fibers (Mahattanatawee et al., 2006) but

recently pectin extraction from sapodilla was also investigated from its peels (Siddiqui, 2018). The yield of pectin from sapodilla peels by using heating technique of Bunsen burner was in the range of 0.5% to 3.5% while the yield of pectin from the same source by the use of microwave heating was found to be in the range of 0. 5% to 3% keeping all the parameters same for both heating techniques (Fig.1). These ranges are almost comparable to each other.

In case of banana pectin yield after the use of Bunsen burner was found to be from 4% to 8.5% but yield range was widen by the use of microwave heating as 3.5% to 10% (Fig.2) with same experimental conditions. Similarly microwave heating resulted broader range of pectin yield from muskmelon peels with same experimental conditions, ranged from 0.4% to 2.1%, as compared to the pectin yield obtained by the use of heating on Bunsen burner which was ranged from 0.35% to 0.9% (Fig.3). muskmelon Although was previously investigated for total dietary fibers (Mahattanatawee, 2006) but recently it was also investigated for pectin extraction from its peels (Siddiqui , 2018). Earlier recorded yield of pectin from muskmelon peels (2.1 to 3.8) was relatively higher extracted at pH, temperature and duration ranges from 1 to 1.5, 70 to 90°C 30 to 60 minutes. So variation could be due the differences in the experimental conditions (Muthukumaran, 2017). The lowest yield could also be due to the variation in the cultivar and maturity state which affect the polysaccharide composition in the cell wall (Simandjuntak, 1996), so ultimate effect produces on the pectin yield (Bhardwaj et al., 2012). The experimental

results favor the use of microwave heating for improved quantity of pectin as compared to the heating technique of utilizing Bunsen burner.

The two other sources, apple and orange, which are commonly used for commercial pectin (Ciriminna,2016; Srivastava 2011) were used investigated for evaluation of pectin vield from their peels with same experimental conditions. Apple peels contain 16.95% of pectin (Virk, 2004). In the present study pectin vield from apple peels was found to be from 2.2% to 4.9% which was higher with the use of microwave heating technique than the pectin yield obtained with Bunsen burner heating technique (1.35% to 4.65%) (Fig.4). Pectin from orange peels also exhibited the higher yield with microwave heating technique (4.25% to 9.35%)as compared to the pectin yield resulted by the use of Bunsen burner heating technique (3.45% to 8.45%) (Fig.5).

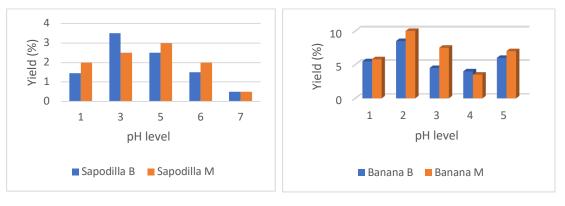
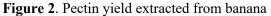


Figure 1.Pectin yield extracted from Sapodilla.



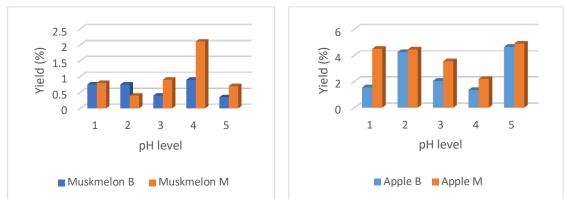


Figure 3. Pectin yield extracted from Muskmelon. Figure 4. Pectin yield extracted from Apple

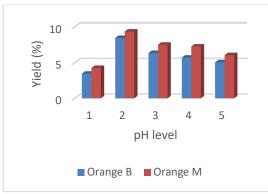


Figure 5. Pectin yield extracted from Orange

| | Table 3.An | alysis of variand | ce (mean squar | es) of yield for | different fruits | | |
|---------------------------------|-----------------------|-------------------|----------------|------------------|------------------|---------------------|--|
| Source of Variation | Degrees of Freedom | Mean Squares | | | | | |
| | | Sapodilla | Banana | Muskmelon | Apple | Orange | |
| pH level | 4 | 5.9105** | 23.599** | 0.95887** | 8.8789** | 20.607** | |
| Heating Method | 1 | 0.0919** | 8.427** | 0.91875** | 9.9188** | 8.748** | |
| pH level x Heating method | 2 | 0.6530** | 2.615** | 0.49687** | 1.9369** | 0.129 ^{NS} | |
| Error | 20 | 0.0059 | 0.054 | 0.00111 | 0.0154 | 0.046 | |
| Total | 29 | | | | | | |

S = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

| Heating Method | | | | | | | |
|-------------------|------------|------------|------------|------------|------------|----------|--|
| Iviciiiou | 1 | 3 | 5 | 6 | 7 | | |
| В | 1.45±0.02e | 3.50±0.05a | 2.50±0.05c | 1.50±02e | 0.50±0.01f | 1.89±27B | |
| Μ | 2.00±0.05d | 2.50±0.05c | 3.00±0.08b | 2.00±0.05d | 0.50±0.02f | 2.00±22A | |
| Mean | 1.73±0.13C | 3.00±0.23A | 2.75±0.12B | 1.75±0.11C | 0.50±0.01D | | |

Table 4. Heating method \times pH interaction mean \pm SE for Sapodilla

B: Heating in Bunsen burner, M: Heating in microwave

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.

The effect of pH was also evaluated with heating methods by extracting pectin from five sources with pH range from 1 to 7. For sapodilla highest yield was 3.5% obtained at pH 3 with heating on Bunsen burner and with microwave heating the highest yield was 3% at pH 5 which was closed to the yield obtained at pH 3 by heating on Bunsen burner. Sapodilla showed lowest pectin yield at pH 7 (Fig.1). The highest

yield of pectin obtained from banana at pH 3 were 10% and 8.5% with microwave heating and heating on Bunsen burner while the lowest yield was found to be 0.5% at pH 7 by heating on Bunsen burner and 4% at pH 6 by microwave heating (Fig.2). Muskmelon peels exhibited highest pectin yield 0.9% and 2.1% at pH 6 but the lowest recorded yield was 0.35% at pH 7 and 0.4% at pH 3 by heating on Bunsen burner and microwave heating respectively (Fig. 3). Highest pectin yield from apple peels were 4.65% and 4.9% at pH 7 while the lowest pectin yield was 1.35% and 2.2% at pH 6 by heating on Bunsen burner and microwave heating respectively (Fig. 4). The highest pectin yield from orange peels was 8.45% and 9.35% at pH 3 and the lowest yield obtained at pH 1 which were found to be 3.45% and 4.25% by heating on Bunsen burner and heating on microwave heating respectively (Fig.5). These results of current study showed that heating methods and pH are the factors influencing the yield of pectin from different sources of pectin investigated.

Statistical analysis was applied for better evaluation of effects of heating method and pH on yield of pectin. The analyzed results indicated that heating method and pH level have significant impacts on the pectin yield obtained from five fruits at 1 % level of significance. Analysis of variance (ANOVA) was applied which showed positive effects of interaction of heating method and pH level on the yield of pectin extracted from each fruit peels (Table 3).

The interaction effect of heating method and pH level for sapodilla peels pectin using applying Tukey's test is represented in table 4. The overall means of factors investigating in the current study showed that pH 3 and microwave heating have significant impact (P<0.05) on pectin yield from sapodilla peels. The alphabetical order was used to indicate the effectiveness on pectin yield. The letter A was used for the highest effect on pectin yield and subsequent letters were used to show lesser effect. The lowest yield was resulted with pH 7

and heating with Bunsen burner according to the overall means. Previous study on the pectin from same source also showed highest yield at pH 3 and lowest at yield at pH 7 (Siddiqui, 2018). So the results showed the inverse relationship of pectin yield from acidic to basic medium which is in accordance with the previous studies (Tiwari, 2017; Zaid , 2016). The current investigation also showed significant effect on pectin yield from sapodilla peels by microwave heating method which also authenticate the previous studies on pectin of same source (Siddiqui., 2018) and from other source (Mosayebi, 2015; Wang., 2007).

For banana peels pectin, the effect of interaction of heating method and pH level using Tukey's test is shown in table 5. Like sapodilla alphabetical order is used for effectiveness on pectin yield. Letter A is used for highest effect on pectin yield while the subsequent letters refer the subsequent lesser effect on pectin yield. Results showed that pH 3 and microwave heating have significant effect (P<0.05) on pectin yield while the lowest yield extracted from banana peels was obtained by heating on Bunsen burner at pH 6. The previous study also showed highest pectin yield from banana peels at pH 3 (Swamy,2017). The current and the previous studies favored acidic medium for higher pectin yield from banana peels. The significant effect on pectin yield from banana peels was also observed previously by microwave heating technique (Siddiqui, 2018) while the power of microwave was found to have direct relationship with the pectin yield from banana peels (Swamy, 2017).

| Heating | 5 I | | | | | |
|---------|------------|------------|------------|-------------|------------|------------|
| Method | 1 | 3 | 5 | 6 | 7 | |
| В | 5.50±0.5d | 8.50±0.19b | 4.50±09e | 4.00±0.03ef | 6.00±0.09d | 5.70±0.42B |
| М | 5.80±0.1d | 10.00±26a | 7.50±0.13c | 3.50±0.09f | 7.00±0.13c | 6.76±0.57A |
| Mean | 5.65±0.08C | 9.25±36A | 6.00±.67CD | 3.75±0.12 | 6.50±0.24B | |

Table 5.Heating method × pH interaction mean±SE for Banana.

B: Heating in Bunsen burner, M: Heating in microwave

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.

| Heating Method | 5 | | | | | |
|-------------------|-------------|-------------|------------------|------------|------------|------------|
| Method | 1 | 3 | 5 | 6 | 7 | |
| В | 0.75±0.01cd | 0.75±0.01cd | 0.40±0.01e | 0.90±0.03b | 0.35±0.01e | 0.63±0.06B |
| М | 0.80±0.01c | 0.40±0.01e | $0.90{\pm}0.02b$ | 2.10±0.05a | 0.70±0.01d | 0.98±0.16A |
| Mean | 0.78±0.01B | 0.58±0.08D | 0.65±0.11C | 1.50±0.27A | 0.53±0.08D | |

Table 6.Heating method × pH interaction mean±SE for Muskmelon.

B: Heating in Bunsen burner, M: Heating in microwave

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.

The investigated effect of variables on muskmelon peels pectin showed significant effect (P<0.05) on pectin yield by microwave heating at pH 6 and lowest impact was observed by heating on Bunsen at pH 7 (0.53±0.08) and pH 3 (0.58±0.08). The same alphabetical order representing the order of effectiveness on pectin yield from muskmelon peels. Where highest letter A indicated the highest yield while the lowest yield was indicated by the lowest used letter D (table 6). In contrast, significant effect at pH 5 and while the lowest effect at the same pH 7 was found in the previous study buts significant effect with microwave heating was observed (Siddiqui., 2018) as observed in the current study.

In the present study the interaction effect of heating method and pH on apple peels pectin is represented in table 7. Where the alphabetical order represented the impact of heating method and pH level on pectin yield. In case of apple

peels pectin, the highest impact (P < 0.05) on pectin yield was also observed by microwave heating method as indicated by letter A. Unlike pectin from sapodilla, banana and muskmelon, the apple pectin showed significant effect (P<0.05) on yield was exhibited at pH 7. The second highest yield obtained at pH 3 indicated by letter A which is closed to the pectin yield at pH 7. As discussed in the previous study that both alkaline and acidic media with accelerated temperature can promote release and hydrolysis of protopectin (Siddiqui., 2018) but the previous study favor acidic medium for higher pectin yield (Ziari,, 2010). The lowest yield was found to be at pH 6 as observed in case of banana peels pectin. Previously reported positive effect on pectin yield from apple pomace (Sandarani, 2017) and apple peels (Siddigui, 2018) by the use of microwave assisted extraction as compared to conventional extraction method is also confirmed by the present study.

| Heating pH Level | | | | | | Mean |
|------------------|------------|------------|------------|------------|-------------|------------|
| Method | 1 | 3 | 5 | 6 | 7 | |
| В | 3.45±0.06h | 8.45±0.13b | 6.35±0.15d | 5.70±0.10e | 5.05±0.14f | 5.80±0.44b |
| М | 4.25±0.06g | 9.35±0.10a | 7.50±0.08c | 7.25±0.20c | 6.05±0.13de | 6.88±0.45A |
| Mean | 3.85±0.18E | 8.90±0.21A | 6.93±0.27B | 6.48±0.36C | 5.55±0.24D | |

Table 7.Heating method \times pH interaction mean \pm SE for Orange.

B: Heating in Bunsen burner, M: Heating in microwave

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.

| Tuble officiality method primiteration mean=52 for reprict | | | | | | | | |
|------------------------------------------------------------|-------------|-------------|------------|------------|-------------|------------|--|--|
| Heating | | Mean | | | | | | |
| Method | 1 | 3 | 5 | 6 | 7 | | | |
| В | 1.55±0.02f | 4.25±0.02c | 2.05±0.03e | 1.35±0.01f | 4.65±0.16ab | 2.77±0.37B | | |
| Μ | 4.50±0.03bc | 4.45±0.10bc | 3.55±0.08d | 2.20±0.05e | 4.90±0.06a | 3.92±0.26A | | |
| Mean | 3.85±0.66C | 4.35±0.06B | ±0.34D | 1.78±0.19E | 4.78±0.09A | | | |

Table 8. Heating method × pH interaction mean±SE for Apple.

B: Heating in Bunsen burner, M: Heating in microwave

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.

The investigated overall interaction effects of boiling method and pH level on pectin yield from orange peels pectin showed significant effect (P<0.05) on pectin yield by microwave heating as observed in previous described fruits peels pectin (sapodilla, banana, muskmelon and apple) at pH 3 as observed in case of banana and sapodilla peels pectin. The lowest yield was shown by heating on Bunsen burner techniques as observed by previous discussed fruits pectin (sapodilla, banana, muskmelon and apple) at pH 1 (table 8). Recorded highest pectin yield from orange peels was also resulted in acidic pH (2 to 2.5) with temperature and duration of 70°C and 30 minutes respectively (Khan, 2015). Previous investigation on pectin from orange peels also found better yield by microwave heating as compared to the conventional method (Alwan, 2016).

The overall interaction effect of pH level and heating techniques showed significant impact on pectin yield of each extracted pectin. Microwave assisted extraction which is preferable heating method because of large handling capacity, less duration of processing with good purity (Sandarani, 2017) showed significant effect on pectin yield from five sources in the current investigation as observed in the earlier studies of pectin (Mosayebi, 2015; Siddiqui., 2018; Wang The mechanism demonstrated 2007). previously in case of orange pectin extraction, is that when orange peels were subjected to microwave irradiation, there is inactivation of enzyme pectin esterase responsible for interaction with pectin substance in the orange and reduction of their solubility and peels destruction of orange skin cells which ultimately

improve the pectin extraction Further more disintegration of parenchyma cells increases surface area to improve the water absorption capacity of the plant cells which contributes to minimize extraction time and energy (Sandarani, 2017). The present study showed highest pectin

yield in acidic medium from all the sources except apple pectin where highest yield was found at pH 7 but followed by pH 3 and yield at these pH levels were found to be closed to each other. So it may be due to any experimental effect. But overall effect of pH on pectin yield was found to show highest yield in acidic medium as observed in the earlier works (Siddiqui, 2018; Tiwari., 2017; Zaid, 2016). The scientist also justified this relation of low acidic pH with high yield by stating that at low pH high concentration of hydrogen ions causing conversion of hydrated carboxylate groups to less hydrated carboxylate groups thus the loss of carboxylic groups associated with reduction of repulsion of polysaccharide molecules which accelerating the gelatinous ability of pectin that promotes precipitation of pectin. Different acids are used in pectin extraction such as nitric, oxalic, phosphoric, acetic, citric, lactic, malic, tartaric (organic), hydrochloric, phosphoric and sulfuric acids. But in the current study hydrochloric acid was preferred because it was found to show highest yield as compared to citric and nitric acids in case of guava, papaya, citrus fruits, banana, and cocoa pods in acidic medium and temperature ranged from 1 to 3 and 60°C-85°C (Sandarani, 2017). Another reason of using low strength (0.1N) HCl in the present

study was to maintain environment friendly (Siddiqui, 2018)

4. Conclusions

It is concluded from the current investigation that pectin market can be improved by utilizing the new sources for pectin production on commercial scale.

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