Analysis of Pectin in Different Citrus Fruits and Evolutionary Relationship

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Abstract. Pectin is a polysaccharide present in fruit cell walls can be extracted from fruit wastes obtained after processing. The objective of the present investigation was to study the potential of citrus fruit peels to be a source of pectin as it is of great importance in food and medical industry. This study aimed at comparing the characteristics of different pectin and to see which one is more suitable for industrial application. Pectin was extracted using alcohol precipitation method from peels of grapefruit, mousambi, and orange so that the use of pectin can be applied to a wider range. According to the study, the pectin extracted by mousambi on wet basis (17.1%) was higher in comparison to the pectin extraction of orange and grapefruit. The pectin can be used in many fields like food and medicines so the improvement in quality and production is very important. MicroRNAs have the main function of guiding the base pairing with target mRNA to negatively regulate its expression for gene silencing via mRNA clevage. In the present study we also found the phylogenetic relationship among the citrus fruits by multiple sequence alignment of microRNAs stem-loop sequence from the miRbase database of the citrus fruits and after getting phylogenetic tree we got the common ancestors. In recent years, the center of production has moved to Europe and to citrus- producing countries like Mexico and Brazil. Further changes of structure and location of the industry continue, but are constrained by the need for large capital investment to setup Plant of economic size, and the need for a large-scale source or sources of raw material.

Keywords: Citrus fruit · pectin · phylogenetic tree

1 Introduction

The term pectin was coined for the first time by Henry Cannot. He described it and isolated it in 1825. Long before when the pectin industry was not well developed the makers of jam used to make simple extract of pectin from waste fruit material such as

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apple cores or surplus orange pith. Earlier, pectin rich fruit extractwere mixed withfew fruits which does not have well jam setting property wellstrawberry, gooseberry or with red currant, for example, apple peels extract and cores were substituted for “difficult to set” jams and jellies. For the first time production of liquid pectin on commercial scale was recorded in 1908 in Germany, and in short span of time it spread rapidly in United States and patent obtained by Douglas (1913). The concentration of pectin is maximum found in the middle lamella of cell walls, and minimum in the plasma membrane. Pectin is an important constituent of majority of plant tissues but very less sources for its commercial production. Due to variations in different parameters pectin does not have same gelling ability Therefore detection of a large quantity of pectin in a fruit alone is not in itself enough to qualify that fruit as a source of commercial pectin. Pectin is found in most plant, but it is most concentrated in citrus fruits (orange, lemon, mousambi etc.) Usually pectin contains 65% galacturonic acid and may show three kinds of form: the linear homo polygalacturonan, ramified and rhamno galacturonan I and II [1]. Citrus fruit contain pectin but significant amount of pectin is present in citrus peel which is available as a by-product.The present study was conducted to investigate the effect of temperature, time and pH on the yield of pectin from citrus fruit peel, and to investigate the chemical features of the peels from the citrus fruits. Pectin was extracted using acid extraction method by using citric acid.

1.1 microRNA – Non Coding Genes

microRNAs are short non-coding RNAs that regulates the gene expression post-transcriptionally. microRNAs (miRNAs), which are 20–24 nucleotides in length, play a important role in regulating molecules found in plants and animals. Derived from stem loop hairpin primary miRNA transcripts (pri-miRNAs), miRNAs regulate the target genes negatively through homology-directed cleavage or translation inhibition of mRNAs [3, 4]. Recently microRNAs have also been found to play vital role in regulating the phytohormone response pathways in plants specially by affecting their metabolism and perception [4]. The unravelling role of the miRNAs differentially expressed miRNAs helps tounderstand their role in the regulation of the synthesis and storage of healthy and important nutrients in fruit. Some examples of citrus genus are lemon, orange, grapefruit and they have high economic and nutritional values. In the present study we selected the common miRNA precursor sequences (stem-loop sequence) of *Citrus clementina, Citrus reticulata, Citrus cinesis, Citrus trifoliata, Vitis vinifera from* miRbase database and carried out the phylogenetic analysis to see common ancestors for future studies on citrus fruits.

2 Materials and Method

2.1 Sample Collection

Fresh citrus fruit (mousambi, orange, and grapefruit) were obtained from local market. The fruits were peeledup and the pulp was discarded. The peelwas taken for further uses. Peels were washed in order to remove dirt, dust and pesticides residues. They were

Fig. 1. Dried Peel Oforange

Fig. 2. Dried Peel Of Mausambi

Fig. 3. Dried Peel Of Grapefruit

cut into small pieces for easy drying. Now the peels were treated with absolute ethanol for 30 min in order to remove oil from peel then washed with water to remove excess moisture. The peels were subjected to sunlight in open place for 9–10 h. The dried peels were crushed using mortar and pestle, then stored in tightly closed container at room temperature (Figs. 1, 2, 3, 4 and 5).

Pectin extraction was done by M.Kratchanova *et.al* [5] method. The purification of pectin was done by Omar *et.al* method [6]. Pectin was dried using method of Pushpita Sari *et.al* method [7].

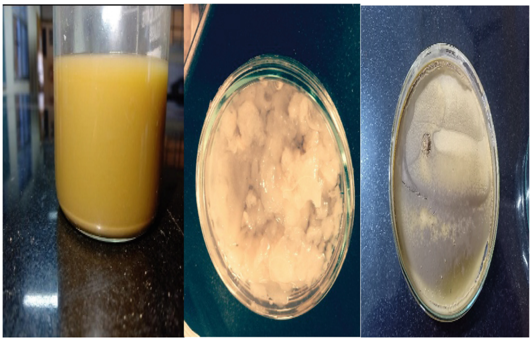

Fig. 4. Coagulated pectin after ethanol addition, stained and dried extract using citric acid

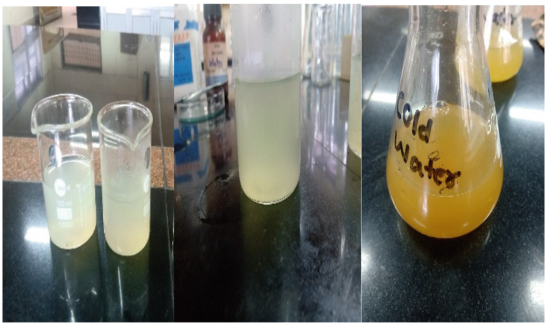

Fig. 5. Pectin solubility in cold and hot water

Percentage yield determination was done by Elizabeth *et.al.* Method [8]. Equivalent weight determination was done by Ranggana method [9]. Ash content of pectin was determined by Ranggana method [9]. Solubility of dry pectin in cold and hot waterwas done by Mohammed. H method [10]. Methoxyl content was determined by Ranggana and Norziah, Fang and Karim method [15].

2.2 miRNA Sequence Alignment and Phylogenetic Analysis

miRNA 166, miRNA 167, miRNA 168, miRNA 171, miRNA 396, miRNA 156, miRNA 164 were selected for all the species viz. *Citrus clementina, Citrus reticulata, Citrus cinesis, Citrus trifoliata, Vitis vinifera* and their stem-loop sequence was downloaded-frommiRbase (https://www.mirbase.org/) and multiple sequence alignment was carried out keeping all the parameters to default. MSA data was used to create phylogenetic tree using the online software tool CLUSTALOMEGA (https://www.ebi.ac.uk/Tools/msa/clustalo/).

3 Results and Discussion

3.1 Qualitative Test for the Samples

The pectin colorobtained from three samples is brown while According to IPPA (2009), pectin is light in color. This is due to environmental factors, surface contamination also due to amount of alcohol used for purification process (Tables 1 and 2).

shows the result that the equivalent weight in mg/mole found to be in grapefruit 312.5 orange 211.11, and mousambi 434.76. The highest equivalent weight was found in mousambi which is 434.76, while the methoxy content determined from the fruits are in orange 2.54, and grapefruit 3.88. The percentage of moisture content obtained from the three was 87.7, 80.6, and 90.22%. The percentage yield of pectin on wet basis are mousambi 17.1%, orange 16.3% and in grapefruit 15.3%. While on dry basis the percentage yield of mousambi was 3.1%, pin 2.833 and in grapefruit was 3.56%.

Table 1. Result of the qualitative test for the three samples

Parameter	Sweet lime (Mousambi)	Orange	Grapefruit
Colour	Brown	Brown	Brown
Solubility in cold water	Insoluble, from suspension	Insoluble, from suspension	Insoluble, from suspension
Solubility at 85–90 °C for 15 min.	The mixture dissolves	The mixture dissolves	The mixture dissolves
Solubility of pectin suspension in cold alkali	The pectin suspension forms yellow precipitate	The pectin suspension forms yellow precipitate	The pectin suspension forms yellow precipitate
Solubility of pectin suspension in hot alkali	The pectin suspension dissolved and turned milky	The pectin suspension dissolved and turned milky	The pectin suspension dissolved and turned milky

Table 2. Parameters characterized for the three samples

Sl.No.	Percentage (%)	Sweet lime (mousambi)	Orange	Grapefruit
1	Percentage yield of pectin in wet basis	17.1	16.3	15.8
2	Percentage yield of pectin on dry basis	3.56	2.833	3.1
3	Equivalent weight (mg/mol)	434.7	211.12	312.5
4	Methoxyl content	3.88	1.9	2.54
5	Ash content	34.00	30.00	35.00
6	pH	2.4	4.2	3.1

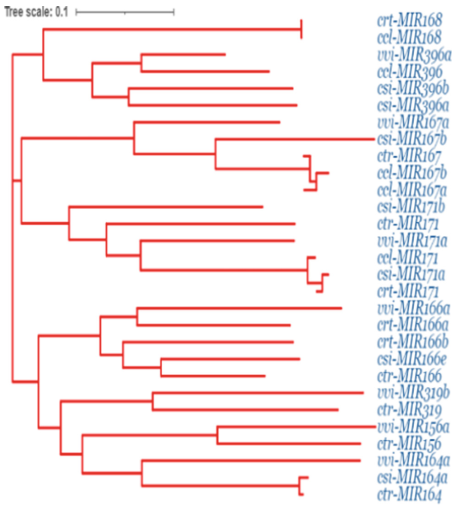

Fig. 6. Cladogram phylogenetic tree between family rutaceae and vitaceae

Grapefruit had the highest percentage yield of pectin. The aqueous solution of pectin is slightly acidic and the peels of mousambi, orange and grapefruit are good source of pectin which is extracted by using ethyl alcohol precipitation.

3.2 Phylogenetic Tree

The phylogenetic tree was constructed ITOL Software (https://itol.embl.de/) between the different species of two families Rutaceae and Vitaceae showing the evolutionary relationship (Fig. 6).

4 Discussion

All the experiment with respect to temperature, pH and time of extraction were performed to know the ideal conditions for maximum yield of pectin. The highpectin yield was observed at pH 2.5 confirming the behaviour of weak acid properties of the pectin. Decreasing the pH results in increasing the degree of dissociation of the carboxylic groups, dipole-dipole produced, induced dipole and hydrogen bonding interactions with each other or with neighbouring molecules. On lowering the pH, the probability to solubilize some insoluble pectic substances such as protopectin increases, and because of thisstrongacids are preferred.The results coincide with those obtained by [11], which Achieved pectin yields of 10.22% (pH of 1.5) and 1.63% (pH of 2.0), the results found are similar to findings of [16]. The Pectin yield increases at low pH and longer extraction times The increased yield of pectin with the polarity of the precipitating solvent, that is, higher for methanol, followed by ethanol and 1-propanol respectively. These results agree with the trend observed by [12] who also obtained higher yields with ethanol than with 1-propanol.Methanol has the property of high chemical affinity with pectin, due maximum rate of formation of methyl esters from the carboxylic groups of galacturonic acid (GalA).

It was observed that the extraction time (100–120 min) was good for extraction of pectin and highest yield was recovered through mousambi (17.1%) in accordance with [17]. The phylogenetic tree gave the evolutionary relatedness of different species.

5 Conclusion

The pectin extracted from mousambi (17.1%) on wet basis and on dry basis (3.56%) was higher in comparison to pectin content of pineapple and grapefruit. Highest equivalent weight was recorded with mousambi (434.76 mg/mole) whereas the least equivalent weight was estimated with pineapple (211.3 mg/mole). Among precipitating agent (95% ethanol, methanol, isopropanol), 95% ethanol was the best for extraction of pectin. The results obtained from this study suggested that selected citrus fruits (mousambi, orange and grapefruit) are good source of pectin which were extracted using ethanol precipitation. The Cladogram phylogenetic tree among two families of citrus fruits gives the phylogenetic tree that well produced greatly supported clade that has all the members of citrus fruits thus supporting their monophyletic arrangements thus supporting results of [13, 14]. It gives idea of further future investigation of role of miRNAs in citrus plants in research on various parameters such as drought and salt tolerance.

Author's Contribution. Author 1 has done all the experiments, collected and compiled all data and carried out the research work. Author 2 has assisted in collection of data and implementation of ideas. Author 3 and Author 4 has helped in formulation of research goals and development of methodology. Corresponding author is responsible for ensuring that the allresearch work and descriptions are correct and accurate and all the authors agree to it.

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