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Optimized extraction, quality characterization of Nagpur mandarin (*Citrus reticulata*) peel pectin

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ABSTRACT

Nagpur mandarin (*Citrus reticulata* Blanco) is composed of 50-55 g peel per 100 g fresh fruit which is discarded as waste during processing. Pectin was extracted from fresh peel of Nagpur mandarin at ICAR- Central Citrus Research Institute, Nagpur, Maharashtra, India (2016-17) using four different methods. Pectin yields varied from 1.70-2.80% on dry weight basis. The extraction condition using pre-treatment (Blanching at 74ºC for 10 mins), double extraction method from fresh peel gave better yield. The isolated pectin contained 69.35% anhydrouronic acid (AUA), equivalent weight of 694.44, methoxyl content 6.57%, 56.33% degree of esterification and jelly grade 160. Purification using alcohol precipitation followed by acetone wash was effective to yield pectin of high purity. According to the values of methoxyl content and degree of esterification, pectin isolated from Nagpur mandarin peels can be classified as high methoxyl content (HMP) and has potential industrial use in manufacture of value added products.

Keywords: Citrus pectin, Double extraction, High methoxyl content, Pre-treatment

Citrus fruits production occupies third position after mango and banana. The fruits are mostly consumed as fresh and a small part of produce goes for processing. There are two parts of citrus fruit: the pulp and peel (Devi *et al*. 2014). In the present scenario, the peel is either used as animal feed or discarded as waste in the environment without any treatment creating disposal problems (Kulkarni and Vijayanand 2010). Pectin can be extracted from peels and is one of the valuable by-products. Pectin is biopolymers which can be obtained from fruit waste, viz. peels (Devi *et al*. 2014). Pectin is abundantly present in the epicarp, mesocarp and endocarp of citrus fruits (Aina *et al*. 2012). Currently, commercial pectins derived from citrus peel are extracted at low *p*H and high temperature. Depending on the molecular weight, acetyl content and degree of esterification, the pectin possesses different functional properties (Aina *et al*. 2012). High methoxyl pectin forms gel in presence of high sugar and acid concentration and contain more than half of carboxyl groups in the form of methyl esters. On the other hand, low methoxyl pectin has less than half of carboxyl groups as methyl esters (Shaha *et al*. 2013).

Pectin found many applications in the food industry. It is used as a gelling agent, stabilizing agent in preparation of jams, jellies, marmalades and also in pharmaceutical preparation (Georgiev *et al*. 2012, Devi *et al*. 2014). It also plays an important role in cosmetic industry. Citrus pectin and its modified derivatives are reported in preventive effect of cancer growth and metastasis (Ramachandran *et al*. 2011). Although having great potential, the processing technology has not developed so far yet and limited research reports are there for extraction of pectin from its peel. The present study was conducted to explore the possibility of re-utilizing the mandarin peel waste for production of dry pectin and to investigate the effect of different extraction conditions on the composition and physico-chemical characteristics of extracted pectin and to establish suitable extraction condition in order to obtain better yield of high quality pectin while maintaining its desirable properties.

MATERIALS AND METHODS

Present experiment was conducted in processing laboratory of ICAR-Central Citrus Research Institute, Nagpur, Maharashtra, India (2016-17). Mature Nagpur mandarin (*Citrus reticulata* Blanco) fruits were collected from local orchard, sorted and washed with continuous flow of water to remove the dirt, weighed and used for further analysis. Fruit diameter was taken using vernier calliper (MITUTOYA, Japan). Juice content was determined after extraction of juice by juice reamer (Philips India Ltd, Calcutta) and expressed in %. Total soluble solids of juice were determined using thermostat based refractometer DR6000 Series (A. Kruss Optronic, Germany) with scale range of and 0.00-95.00% Brix at 25ºC, moisture content of fresh peels was determined from known weight of

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sample in infrared moisture meter MOC-120H (Shimadzu Corporation, Japan). Titratable acidity (%), ascorbic acid (mg/100 ml), pectin as calcium pectate, crude fibre, crude fat, total ash content and acidity as citric acid in the fresh peels were determined according to the method described by Ranganna (1987) and results were reported as %.

The fresh peels were shredded to small pieces using shredder [Bajaj Processpack Ltd, Noida (UP)]. Pectin was extracted using four different extraction methods, viz. T_1- Single extraction; T_2- Double extraction; T_3- Pre-treatment with single extraction; T_4- Pre-treatment with double extraction. The basic extraction procedure was based on method given by Pirshahid *et al*. 2005 with some modifications. In single extraction (T_1) - Water was added to the shredded peel in 1:4 (Peel: Water) ratio and *p*H was adjusted to 1.5 by with dilute HCl solution. The mixture was heated at 90ºC for 60 min in the water bath. The hot extract was filtered through muslin cloth and the filtrate was allowed to cool. In the double extraction (T_2) - The hot extract obtained after the single extraction was filtered through muslin cloth and the filtrate was allowed to cool. Again water was added to the residue in 1:2 ratio and the mixture was heated at 90ºC for 60 min in the water bath. The hot extract was filtered through muslin cloth both the extracts were combined together and allowed to cool. In pre-treatment with single extraction (T_3) the shredded peels were pre-treated (blanched at 74ºC for 10 min). In this treatment one part of freshly shredded peels were stirred with 2.5 part of heated tap water for 10 min at 74ºC. After draining, another 2.5 part of tap water at room temperature were added to peel and stirred for 5 min to bring temperature of this leach to normal. The mixture was again drained and pressed to remove excess water. Water was added to the pre-treated peel in 1:4 ratio and *p*H was adjusted to 1.5 by with dilute HCl solution. The mixture was heated at 90ºC for 60 min in the water bath. The hot extract was filtered through muslin cloth and the filtrate was allowed to cool. In pre-treatment with double extraction (T_4) of extraction peels were pre-treated and further treatment T_2 were followed. The hot extracts so obtained were combined together for purification process. The purification process was carried out by the method given by Yapo (2011) with some modifications. The aqueous extract containing pectin was coagulated using equal quantity of ethanol and was allowed to stand for 30 min. The precipitate was separated through filtration to remove maximum ethanol. Precipitate was washed twice with acetone (Aina *et al*. 2012) and filtered again. Pectin so obtained was dried in vacuum dryer at 40ºC and grind to powder. Finally pectin was packed in air tight bottles.

The dried pectin obtained from the various extraction methods from fresh Nagpur mandarin peel was subjected to the following qualitative and quantitative test to characterize them. The pectin was compared to the commercial pectin. The method of Ranganna (1987) is used for estimation of ash content, methoxyl content of pectin, alkalinity of ash and expressed in %. Equivalent weight is used to calculate the anhydrouronic acid content and the degree of esterification. Jelly grade was measured following test jelly method under standard conditions (TSS 68ºB, *p*H 3.2).When the data of equivalent weight, methoxyl content and alkalinity of ash of pectin is known, its AUA was calculated. The method described by Jain *et al*. (1984) is used for estimating degree of esterification (DE). The results obtained are means of three replications and reported as means ± standard deviation. The experimental data was statistically analyzed by multiple range tests (Tukey's HSD test).

RESULTS AND DISCUSSION

Physico-chemical composition of Nagpur mandarin: Nagpur mandarin fruit juice contains 26.00 mg/ml of ascorbic acid and 0.51% titratable acidity. Similar result was also obtained by Marti *et al*. (2009) and reported 20-60 mg/ml of ascorbic acid content in juices of mandarin/ lemons. In processing industries, juice content is one of the important factor (Mena *et al*. 2011). The average juice content of Nagpur mandarin fruit weighing 173.70 g was found to be 41.64%. Total soluble solids (TSS) content and fruit diameter were 9.80ºBrix and 7.40 cm respectively. Verma *et al*. (2012) obtained acidity, fruit diameter and TSS in the range of 0.72-1.53%, 4.59-6.71 cm and 8.50-11.83ºBrix in Nagpur mandarin grown in Jhalawar, Rajasthan.

Proximate and physico-chemical composition of Nagpur mandarin peel: Nagpur mandarin fresh peel contained moisture content 76.84%, crude fat 1.79%, fibre 4.98%, acidity in terms of citric acid 0.35%, ash 0.68% and pectic substance as calcium pectate 2.88% (Table 1). M' Hiri *et al*. (2015) and Bejar *et al*. (2011) reported moisture content of 76.015% and 74.804% while analyzing peels of orange *Maltease* and *citrus sinensis*. Moisture content in *C. sinensis* varities studied ranged from 68.53-77.37% (Lagha- Benamrouche and Madani, 2013). M' Hiri *et al*. (2015) and Bejar *et al*. (2011) also found fat content of 0.800 and 0.955 g/100g and ash content as 3.170 and 3.313 g/100g in peel of orange *Maltease*.

Characterization of pectin

Pectin yield: The yield of pectin extracted from Nagpur mandarin using different treatments ranged between 1.70-2.80% of the fresh peel (Table 2). Maximum recovery of pectin (2.80%) and minimum recovery (1.70%) was obtained after T_4 and with T_1 methods. T_4 treatment was

Table 1 Proximate composition of Nagpur mandarin peel

Parameter (%)	Observed values
Moistuure content	76.84 ± 1.536
Crude fibre	4.98 ± 0.151
Crude fat	1.79 ± 0.101
Total ash	0.68 ± 0.036
Pectic substances (as calcium pectate)	2.88 ± 0.010
Acidity as citris acid	0.35 ± 0.010

Data presented are in means ± standard deviation (n=3)

Table 2 Quantitative and qualitative parameters for characterization of pectin extracted by four different methods

Treatment	Yield (%)	Ash content (%)	Equivalent weight	Anhydrouronic acid (%)
T1	$1.70^{c} \pm 0.114$	$2.20^{a} \pm 0.092$	$877.19^{a} \pm 2.506$	$60.90^{bc} \pm 1.606$
T2	$2.70^{a} \pm 0.066$	$2.80^{a} \pm 0.366$	$877.19^{a} \pm 2.977$	$59.15^{c} \pm 0.936$
T3	$2.10^{b} \pm 0.100$	$2.40^{a} \pm 0.567$	$781.25^{b} \pm 1.034$	$64.06^{b} \pm 1.204$
T4	$2.80^{a} \pm 0.053$	$1.75^{a} \pm 0.125$	$694.44^{c} \pm 0.806$	$69.35^{a} \pm 0.863$
Commercial pectin	-	1.35	833.34	57.73
Tukey HSD at 1%	0.3102	1.2401	7.354	4.2569

Data presented are in means ± standard deviation (n=3). Statistical note: Means (n=3) within a column followed by different letters are significantly different at $P < 0.01$ according to the Tukey HSD multiple range test, *Means with superscripts having the same letter are not significantly different.

found to be more appropriate method for pectin extraction according to yield.

Pectin colour: Colour is an important parameter affecting the appearance of the gel. The higher colours having little effect on the final appearance of a food product would be preferred. The colour of pectin obtained is slightly off-white and pectin extracted using pre-treatment method was found brighter than others. Pectin are usually light in colour and factors like surface contamination, environmental factors, types of fruits used and human error might contribute to the discrepancy of the colour (IPPA 2009).

Equivalent weight: The value for equivalent weight was found to be highest (877.19) in pectin extracted by T_1 and T_2 treatment (Table 2). Pectin extracted T_4 had the lowest value (694.44). However, the equivalent weight of the commercial pectin was 833.34. The equivalent weights obtained were used in the calculations of anhydrouronic acid (%AUA) and degree of esterification (% DE).

Methoxyl content: Methoxyl content is an important factor in controlling pectin setting time and also in determining the ability of the pectin in formation of gels (Shaha *et al*. 2013). The methoxyl content using T_3 treatment and T_4 treatment was higher and found to be 7.13% and 6.57% respectively (Fig 1). The commercial pectin has methoxyl content of 5.30%. Methoxyl content of pectin extracted by different treatments have values below 7.0% indicating that the pectin is of low ester characterisation and also good in terms of quality.

Anhydrouronic acid content: Suitability of pectin for use in jams, jellies, etc. is indicated by anhydrouronic acid (AUA) content. It also determines the purity. AUA should not be less than 65% (Devi *et al*. 2014). In the present study, AUA content was found to be highest (69.34%) when extracted by T_4 treatment and lowest (59.15%) in

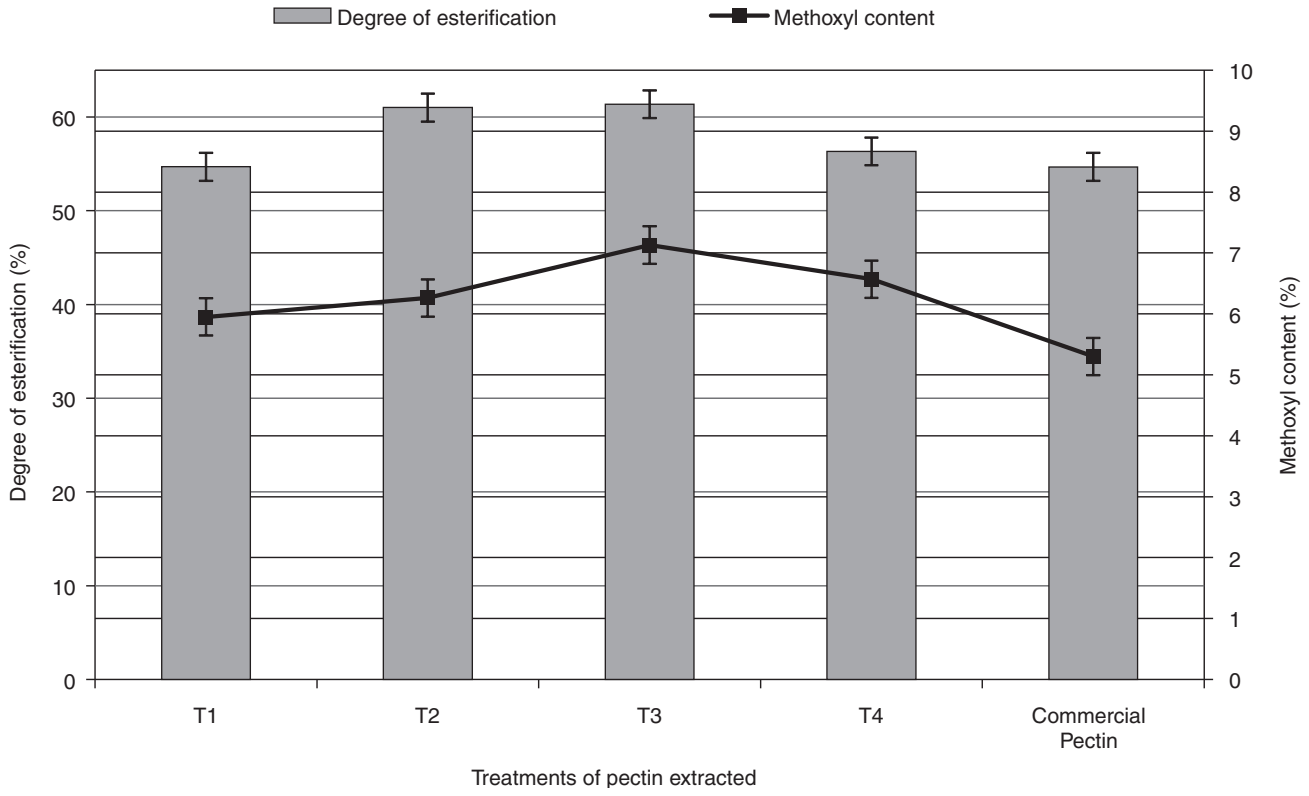

Fig 1 Degree of esterification and methoxyl content of pectin of Nagpur mandarin peel extracted using different treatments.

pectin extracted by T_2 treatment (Table 2). The result was in accordance with Georgiev *et al*. (2012).

Degree of esterification: Degree of esterification (DE) affects the rate at which the gel formation takes place. More is the degree of esterification more is the rapid setting of gel. Fig 1 depicts the degree of esterification of pectin extracted from Nagpur mandarin peel. The degree of esterification of pectin extracted using T_3 treatment was found to be highest (61.36%) followed by T_2 (61.01%). The degree of esterification of commercial pectin was found to be 54.68% (Table 2). The degree of esterification of pectin was 72.40% when extracted from golden delicious pomace and 56.9% from golden delicious partially ripe apple respectively, whereas that of mango peel was 76% (Shrirangarajan and Shrikhande 1977). From the results obtained, it can be concluded that the pectin extracted is high methoxyl pectin (HMP) since it has degree of esterification higher than 50%. DE depends on species, tissue and maturity (Devi *et al*. 2014).

Jelly grade: Jelly grade is defined as the number of part of sugar require for one part of pectin to produce jelly of required consistency under standard condition (TSS 68ºB, *p*H 3.2). Jelly grade of pectin extracted using T_2 and T_3 treatments was found lower (140) and that for treatment T_1 and T_4 contained 170 and 160 respectively. The jelly grade of commercial pectin was 150. Jelly grade of pectin extracted from golden delicious pomace was 160 and from golden delicious partially ripe apple were 170. Srirangarajan and Shrikhande (1977) reported the jelly grade of pectin was 200 when obtained from mango peel.

Ash content: Ash content was found minimum (1.75%) in the pectin extracted using T_4 and found maximum (2.8) using T_2 method. Ash content of commercial pectin was 1.35% (Table 2). This concludes that the pectin extracted by T_4 has fewer impurities. The ash content of pectin extracted from golden delicious pomace was 1.84%, from mandarin pomace was 0.45% and from mandarin peel was 0.50%.

It may be concluded from the present investigation that highest pectin yield of 2.80% was obtained using pre-treatment, double extraction method. The anhydrouronic acid was above 58% which indicated that the pectin was pure. When compared with commercial pectin, Nagpur mandarin peel pectin can be classified as high methoxyl pectin because of its high degree of esterification, methoxyl content and jellying capacity. From the results obtained, it can be said that Nagpur mandarin peels yielded pectin in sufficient amount and can be considered in commercial production of pectin alongside with other citrus sources.

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REFERENCES

Aina V O, Barau M M, Mamman O A, Zakari A, Haruna H, Hauwa Umar M S and Abba Y B. 2012. Extraction and characterization of pectin from peels of lemon (*Citrus limon*), grapefruit (*Citrus paradisi*) and sweet orange (*Citrus sinensis*). *British Journal of Pharmacology and Toxicology* **3**(6): 259–62.

Bejar A K, Ghanem N, Mihoubi D, Kechaou N and Mihoubi N B. 2011. Effect of infrared drying on drying kinetics, color, total phenols and water and oil holding capacities of orange (*Citrus sinensis*) peel and leaves. *International Journal of Food Engeneering* **7**(5): 1–25.

Devi W E, Shukla R N, Abraham A, Jarpula S and Kaushik U. 2014. Optimized extraction condition and characterization of pectin from orange peel. *International Journal in Engineering and Advanced Technology* **2**(2): 1–8.

Georgiev Y, Ognyanov M, Yanakieva I, Kussovski V and Kratchanova M. 2012. Isolation, characterization and modification of citrus pectins *Journal of Bioscience and Biotechnology* **1**(3): 223–33.

IPPA. 2009. International Pectin Producers Association. Retrieved from: *www.interscience.com*.

Jain R K, Ghankrokta S S and Agrawal J D. 1984. Isolation and characterization of pectin from apple pomace. *Indian Food Packer* **38**: 65–70.

Kulkarni S G and Vijayanand P. 2010. Effect of extraction conditions on the quality characteristics of pectin from passion fruit peel (*Passiflora edulis f. Flavicarpa* L.). *LWT- Food Science and Technology* **43**: 1026–31.

Lagha- Benamrouche S and Madani K. 2013. Phenolic contents and antioxidant activity of orange varieties (*Citrus sinensis* L. and *Citrus aurantium* L.) cultivated in Alg eria: Peels and leaves. *Industrial Crops and Products* **50**: 723–30.

Marti N, Mena P, Canovas J A, Micol V and Saura D. 2009. Vitamin C and the role of citrus juices as functional food. *Natural Product Communications* **4**(5): 677–700.

Mena P, Garcia-Viguera C, Navarro-Rico J, Moreno D, Bartual J, Saurab D and Mart N. 2011. Phytochemical characterisation for industrialuse of pomegranate (*Punica granatum* L.) cultivars grown in Spain. *Journal of the Science of Food and Agriculture* **91**: 1893–1906.

M'Hiri N, Ioannou I, Ghoul M and Boudhrioua M. 2015. Proximate chemical composition of orange peel and variation of phenols and antioxidant activity during convective air drying. *Journal of New Sciences, Agriculture and Biotechnology* **9**: 881–90.

Pirshahid P A, Thisayakom K, Giwanon R, Phoonsiri C, Kajsongkram T, Chawanorasest K, Suntorntanasart T and Trangwacharakul S. 2005. Semi-Pilot Scale Production of Pectin from Lime Peel Waste for Cholesterol Lowering Agent. *(In)* III WOCMAP on Medicinal and aromatic plants- Traditional Medicine & Nutraceuticals. Eds. Palaniswamy, U. R., Gardner, Z. E. and Craker L E. 6, Acta Hort. 680, ISHS. 10.17660/Acta Hortic.2005.680.17.

Ramachandran C, Wilk B J, Hotchkiss A, Chau H, Eliaz I and Melnick S J. 2011. Activation of human T-helper/inducer cell, T-cytotoxic cell, B-cell, and natural killer (NK)-cells and induction of natural killer cell activity against K562 chronic myeloid leukemia cells with modified citrus pectin. *BMC Complementary and Alternative Medicine* **11**: 59.

Ranganna S. 1987. *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*, 2nd ed., pp 8, 9, 10, 25, 33, 34, 40, 44, 105. Tata- McGraw Hill Publishing Company Limited, New Delhi.

Shaha R K, Nayagi Y, Punichelvana A P and Afandi A. 2013. Optimized extraction condition and characterization of pectin from Kaffir lime (*Citrus hystrix*). *Research Journal of Agriculture and Forestry Sciences* **1**(2): 1–11.

Srirangarajan A N and Shrikhande A J. 1977. Characterization of mango peel pectin – A research note. *Journal of Food science* **42**: 279–80.

Verma S, Bhatnagar P and Yadav A. 2012. Physico-chemical, yield and yield attributing characteristics of Nagpur mandarin (*Citrus reticulata* Blanco) orchards surveyed in Jhalawar district of Rajasthan. *Asian Journal of Horticulture* **7**(2): 437–41.

Yapo B M. 2011. Pectic substances: From simple pectic polysaccharides to complex pectins-A new hypothetical model. *Carbohydrate Polymers* **86**: 373–85.