

## **CHAPTER 2**

### **LITERATURE REVIEW**

The literature review has been divided into three sections. In the first section composition of peel and possible uses of peel are discussed. In the second part, the articles related to extraction of naringin and in the third section the articles related to the recovery of pectin are briefly reviewed.

#### **2.1 Composition of peel**

On an average, kinnow peel contains 22.45% total solids, 12.50°B TSS, 0.38% acidity, 41.57 mg/100g ascorbic acid, 6.23% total sugars, 5.99% reducing sugars, 0.67% ash, 13.65 mg/100g carotenoids, 7.43 mg/100g  $\beta$ -carotene, 1.85% pectin, 0.77% fat, 0.420 mg/g naringin and 4.69  $\mu$ g/g limonin (Aggarwal and Sandhu 2003, Premi *et al.* 1994). However, author and other members of the group have observed that naringin content in kinnow peels is 6-8 g/kg of wet peels when extracted with boiling water. The naringin content in dropped fruit peels and immature fruit peels is on the higher side.

Potential non-juice products of kinnow fruit from kinnow processing waste are same as that of other citrus processing waste. From other citrus fruits in general, these are candied peel, condiments, peel seasoning, marmalade, baked products, peel fiber, pellets, animal feed, fuel grade alcohol, folded oil, d-limonene, dried pulp, molasses, purees and bases, seed oil, seed meal, limonoids, limonoid glucosides, naringin, peel oil, juice recovery (pulp wash), and pectin (Braddock 1999, Kimball 1991).

#### **2.2. Extraction of Naringin**

The following methods have been reported for extraction of naringin from citrus peels. (i) solvent extraction (ii) supercritical carbon dioxide extraction (iii) ultrasonic assisted

extraction and (iv) adsorption on certain adsorbents and ion exchange resins and subsequent desorption with solvent and recovery.

**(i) Solvent extraction:**

Naringin was extracted with water from grapefruit albedo by Crandall and Kesterson (1976). The extract was precipitated with  $\text{Ca}(\text{OH})_2$  to remove pectin substances. The mixture was filtered, and the liquid was acidified with HCl to bring pH to 4, to obtain a complete precipitation. The precipitated solution was vacuum filtered and dried. Naringin recovery ranged from 2.45-5.3 g per kg of wet albedo.

The hesperidin and naringin were extracted by El-Nawawi (1995) from orange peel and grapefruit peel, respectively, by treating the peel with  $\text{Ca}(\text{OH})_2$ . The yield of naringin was reported 6g/kg of orange peel. A hot water leach was used to recover naringin and pectin successively. The highest yields of naringin were obtained using 1:3 ratio of peel-leach water at 88-90°C for 5 min.

A process through which bergamot peel, which is a waste from citrus industry, can be exploited as a raw material for the production of both pectin and naringin has been described by Tripodo *et al.* (2007). In this process, total pectin yield was between 10.66 % and 17.64 %, and its characteristics were close to that of industrially produced pectin from lemon peel. Naringin yield was in the range of 1.66 -3.80 %.

Naringin was extracted by Sudto *et al.* (2009) from pomelo (*Citrus grandis* Osbeck). In this process, the pomelo peel was extracted with methanol to get the methanolic extract. Naringin was extracted using four methods: (i) Water extraction/crystallisation with dichloromethane addition (from crude methanolic extract) (ii) Hot water extraction/crystallisation (from crude methanolic extract) (iii) Hot water extraction/crystallisation (from peels) (iv) Hot water extraction/crystallisation with dichloromethane addition (from peels). The highest yield (20 g/kg dry peel of >98% purity) of naringin was obtained with method (i).

**(ii) Supercritical carbon dioxide extraction:**

Naringin the major flavonoid from the peel of *Citrus paradisi* L was extracted by Giannuzzo *et al.* (2003). The highest yield (14.4 g/kg) of naringin was obtained using supercritical carbon dioxide modified with 15% ethanol at 95 bars and 58.6°C. The supercritical fluid extraction consumes less solvent and provides a shorter extraction time than conventional extraction methods.

Limonoids and naringin were extracted by Yu *et al.* (2007) from grapefruit (*Citrus paradisi Macf.*) seeds by using supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction technique. Extraction was completed in two stages. In the first stage, less polar limonin was extracted using SC-CO<sub>2</sub> while in the second stage extraction of highly polar limonin and naringin were completed using SC-CO<sub>2</sub> modified with ethanol as co-solvent. The highest yield of naringin (0.2 mg/g seeds) was achieved in 40 min at 41.4 MPa pressure, 50°C and 20% ethanol concentration. In all the experiments, the mobile phase flow rate was kept constant at 5.0 ml/min.

Naringin and hesperidin were extracted by Suetsugu *et al.* (2013) from a peel of *Citrus Junos* using supercritical carbon dioxide with the polar solvent. The operating parameters were pressure 30 MPa, temperature ranging from 80 to 160°C and ratio of water and ethanol as a co-solvent was varied from 0 to 100%. The highest yield of naringin was obtained with temperature 160°C, pressure 30 MPa and water to ethanol ratio 20:50.

**(iii) Ultrasonic-assisted extraction:**

Polyphenols (flavanone glycosides) were extracted by Khan *et al.* (2010) from orange (*Citrus sinensis* L.) peels using ultrasonic assisted and soxhlet extraction. The results showed that the quantities of naringin and hesperidin from ultrasonic-assisted extraction (70.3 and 205.2 mg/100 g of fresh weight, respectively) were considerably higher than those obtained by Soxhlet extraction (50.9 and 144.7 mg/100 g fresh weight, respectively).

Naringin was extracted by Tang *et al.* (2011) from pomelo peels using ultrasonic assisted extraction and macroporous resin purification technology. The naringin content in the mature pomelo peels was found to be 2.20% on dry basis. The purity of obtained naringin was 77.26%.

Naringin was extracted by Kong *et al.* (2013) from *Citrus grandis* Tomentosa peels. The operating parameters are ultrasonic extraction temperature, the ratio of solvent to raw material, extraction time and ultrasonic power. The highest yield was obtained when temperature, a ratio of solvent to raw material, ultrasonic time and ultrasonic power were 40°C, 20:1, 50 min, and 280 W, respectively. The extraction yield of naringin was 82.11%.

There are some disadvantages of these methods, which includes time-consuming, a bulk amount of solvent wastage, toxic solvents residues, high yielding cost, low efficiency or purity, expensive equipment, high production costs, and unsuitable for large-scale industrial production (Geng *et al.* 2009, Fu *et al.* 2006). Alternatively, the method of adsorption technology using macroporous resins is one of the most frequently used method for the separation of flavonoids, due to its appropriate adsorption capacity, simpler operation, high efficiency, low toxicity, high selectivity, more friendly to environmental protection, and easy regeneration (Zhou *et al.* 2013, Pompeu *et al.* 2010, Kim *et al.* 2007). Also, macroporous resins are having many special characteristics such as high mechanical strength, good acid and alkali resistance, high specific surface area, and long lifetime (Wen *et al.* 2016, Zhe *et al.* 2015, Wu *et al.* 2015, Long *et al.* 2014).

#### **(iv) Adsorption on certain adsorbents and ion exchange resin followed by desorption**

Adsorption of flavonoids or naringin from citrus peels using polymeric adsorbent resins followed by desorption reported for recovery of naringin is reviewed in next paragraphs.

A process described by Calvarano *et al.* (1996) for obtaining naringin from bergamot peels with hot water extraction followed by ultrafiltration and resin adsorption. Amberlite XAD-16

resin (macroporous cross-linked polystyrene) was selected for naringin adsorption from water extract. Naringin yield varied between 73.8 to 93.3%. Ethanol was used for desorption of naringin from resin saturated with naringin.

X-5 resin exhibited higher adsorption and easier desorption of naringin, among five kinds of macroporous resins through static adsorption and desorption experiments (Jiang *et al.* 2006). Effects of concentration, pH value and flow rate of naringin extract on the adsorption of naringin on X-5 resin were studied. Meanwhile, the effect of these factors on desorption of naringin from the X-5 resin was also investigated. The experimental results showed that the adsorption isotherm of naringin by X-5 resin could be described by the Langmuir isotherm equation. The static maximum adsorption capacity of resin was found to be 32.6 mg/g with naringin concentration at 2.7 g/L, while the dynamic adsorption capacity of naringin is 23.8 mg/g with naringin extract flow rate at two times that of resin volume per hour.

#### **Selection of an adsorbent:**

The following macroporous adsorbents XAD-4, D380, ADS-17, ADS-21 and AB-8 (Geng *et al.* 2016), XAD-16 and SP825 (Dong *et al.* 2015), XAD-7 and XAD-4 (Deka and Saikia, 2015), p-phenylenediamine (PPDA), p-aminophenol, and p-aminobenzoic acid (PANB) (Ye *et al.* 2014), XAD 16 (Geerkens *et al.* 2015), HPD100B, HPD300, AB-8, and HPD722 (Li *et al.* 2013), and XAD-7, XAD-4, AB-8, and ADS-17 (Yao *et al.* 2015) are reported for the extraction of flavonoids

However, non-ionic polystyrene divinylbenzene polymeric adsorbents are being commercially exploited for debittering of citrus juices. All thirty-four commercial debittering units operating worldwide used food grade polystyrene divinyl benzene cross-linked polymeric resins (Shaw *et al.* 2000). Polymeric adsorbents are highly porous structures whose internal surfaces can adsorb and desorb a wide variety of different species based on the process and conditions they are applied. These are used globally in a number of applications

across various industries for their wide variations in functionality, surface area and porosity (Ribeiro *et al.* 2002, Barkakati *et al.* 2010).

In the present work, indigenous resin Indion PA-500 and PA-800 were used. These are polystyrene divinylbenzene based cross-linked polymeric adsorbent resins. The properties of polystyrene divinylbenzene polymeric resin suitable for naringin adsorption as mentioned by Manlan *et al.* (1990) are high cross-linking >16%, density about 1.2 g/ml for proper submergence of resin beads, large specific surface area > 500 m<sup>2</sup>/g of dry adsorbent and low mean pore diameter about 50-60 °A.

The pore size distributions of indigenous Indion PA-500 and PA-800 adsorbents (resins) were reported excellent by the manufacturer for the removal of the large organic molecules from polar solvents (Product information brochure). Naringin is a large molecule having molecular weight 580 that can be adsorbed from polar solvent like Kinnow peel boiled water. The properties of the resins suited for our purpose and therefore selected for studies after confirmation with preliminary experiments that the resins adsorb naringin from kinnow peel boiled water (KPBW), and naringin is desorbed from these both with ethanol. The resins are very cheap in comparison to the XAD-16 resin. The properties of the resins are reported by the manufacturer, are presented in Appendix Table A1.

### **2.3. Extraction of Pectin**

The most of the commercial pectins are extracted from citrus peels and apple pomace, which are by-products of juice production. The pectin content in these by-products ranges from 20 to 40 g/100 g on dry weight basis (Kulkarni and Vijayanand, 2010).

The two methods are reported in previous studies for pectin extraction, (i) acid extraction and (ii) enzymatic extraction. The acid extraction is commercially used for extraction of pectin.

Although our work regarding pectin extraction is focused only on the recovery of pectin from spent kinnow peel boiled water remaining after naringin adsorption from column study, the literature review on pectin recovery from citrus peels by acid extraction is discussed in detail.

### **Acid Extraction Method:**

In this method, pectin extraction from raw material is carried out by boiling the material in dilute acids. The mineral acids (such as hydrochloric, sulfuric, and nitric acid) or organic acid (citric) can be used to extract the pectin from the different materials. The extracted pectin is then precipitated by adding acidified alcohol (usually ethanol or isopropyl alcohol) or aluminium chloride. The precipitated pectin is then filtered off from alcohol water mixture and purified by repeated washing with acidified alcohol and dried at low temperature under vacuum and then quickly ground (Sharma *et al.* 2006).

### **Extraction of pectin from citrus peels**

#### **(i) Orange peels (*Citrus sinensis* (L.))**

Pectin extraction was studied by Ma *et al.* (1993) from orange peel using hydrochloric acid under four variables to obtain optimum conditions for production of good quality of pectin. The optimised conditions were pH 4.2, temperature 95°C, and time 8 h.

Pectin was extracted by Wang *et al.* (2005) from orange peel using hydrochloric acid. The pectin extraction rate was found to be high when the ratio of solution/material was in the range of 10-15, pH 2-3 for 45 min, and at a temperature around 80°C. The yield was 70.68% (based on initial pectin present) with the feasible conditions of solution/material ratio 10:1, pH 2 at 80°C, and extraction time 45 min.

A process design was developed by Casas-Orozco *et al.* (2015) for the industrial production of pectin from orange peels under optimum experimental extraction conditions (90°C, 75 min, pH 1.5). Total production of 337,610 kg/year of pectin was obtained in the designed facility, with a batch size of 3383 kg of peel and a cycle time (3.61 h).

Pectin was extracted by Hosseini *et al.* (2016) from sour orange peel with operating conditions temperature (75-95°C), time (30-90 min), and liquid-solid ratio (20-40 v/w). The highest extraction yield (17.95±0.3%) was obtained at a temperature of 95°C, time of 90 min, and a liquid-solid ratio of 25 (v/w). The DE values for the pectin ranged from 17% to 30.5%, indicating that the pectin was low methoxyl.

**(ii) Lemon peels (*Citrus limon* L.)**

Low methoxyl pectin (LMP) was prepared from lime peel by de-esterification with acid, sodium hydroxide and ammonia by Padival *et al.* (1979). The suitable conditions for good pectin yield were found with de-esterification (pH 0.5, 60°C/ 9 h and 70°C/5 h), sodium hydroxide (pH 10.5, 5°C/1-2 h), and ammonia (25°C, pH 10.5/2-3 h, pH 11.7/1-2 h). Low methyl pectin was found to contain 4.5% methoxyl groups and molecular weight of 40,000–70,000.

The pectin was extracted by Koubala *et al.* (2008) from lime peel using HCl (pH 1.5) at 85°C for 1 h, deionized water at 75°C for 1 h, and ammonium oxalate (0.25%) at pH 4.6 (adjusted by using oxalic acid) at 85°C for 1 h. The extracts were precipitated with ethanol. The extraction yields of pectin with HCl, deionised water, ammonium oxalate extracts were 198±2, 67±5, 226±9 mg/g dry peel respectively was observed. The DM (%), acetylation (%), MW (kDa), and intrinsic viscosity (mL/g) of HCl extraction pectin were 49±1.2, 4±0.3, 123±5, and 560±5 respectively, 82±1.7, 6±0.3, 252±5, and 414±6 with deionized water respectively, and 77±1, 3±0.2, 305±5, and 857±5 with ammonium oxalate (on alcohol insoluble residue basis) respectively.

The pectin was extracted from dried and fresh wet lemon peel by Lerotholi *et al.* (2012). It was found that the extraction temperature, time, pH, and size of the peel are most significant variables affecting the pectin yield. Drying the peels increased the pectin yield as expected



and had no drastic effect on the GalA (Galacturonic acid) but it reduced the DE of the extracted pectin.

**(iii) Kinnow peels** (*Citrus nobilis* × *C. deliciosa*)

Pectin was extracted by Singh and Dhillon (2007) from dried kinnow peels using 0.05 N HCl at 90°C. The yield was about 14.80% (w/w) with DE 54.23%. At lower temperature DE was higher and but the yield was lower, however, with an increase of conc. of acid pectin yield was increased, but the DE decreased.

**(iv) Citrus unshiu** (*C. Marcovitch* × *C. nobilis Loureiro*)

Pectin was extracted by Kurita *et al.* (2008) from the peel of citrus *unshiu* (Kara Mandarin) by two methods: (i) pretreatment of citric acid at different pH in the range of 5.8 to 8.0 and temperatures 50, 60 and 80°C for 2 h. (ii) Without any pretreatment. Samples from both methods were extracted with conc. HCl at pH 2.2. The precipitation of the extracts was done with ethanol. The final pectin contains a unique chemical composition of low methylation and high neutral sugar contents. The neutral sugar content of the citric acid treated pectin (53.2%) was much higher than that of untreated pectin (44.6%). The GalA content in the citric acid treated pectin (26.6%) was considerably lower than that in the untreated pectin (34.3%). The yield of citric acid treated pectin, and untreated pectin on a dry basis was 8.5±0.08%, 7.4±0.06% respectively.

**(v) Citrus pomelo** (*Citrus maxima*)

The influence of acid concentration (HCl), temperature, and extraction time on pectin extraction was investigated from dried peels of *citrus grandis* using hot water by Mai and Luyen (1989). The extraction conditions used were acid conc. (0.72 to 2.31%), temperatures (47.8 to 72.2°C, time (5 to 9.34 h). The optimal values for extraction were found to be acid conc. 0.72%, temperature 50°C, and time 9 h.

Pectin extraction was studied by Xiao-feng *et al.* (2011) from Guanxi honey pomelo peel with dilute hydrochloric acid. The effect of process parameters such as temperature, solid/liquid ratio, extraction time and pH for pectin extraction and quality parameters of pectin on extraction was investigated. The process parameters were found to be a little effect on DE of the pectin, but the remarkable effect on GalA content and the largest effect on viscosity average molecular mass. The obtained product contained DE in the range of 63.5-63.8% and GalA content in the range of 74.2-88.5%.

Pectin was extracted by Sotanaphun *et al.* (2012) from *Citrus maxima* fruit peel with water at different pH (2, 3 and 4.5) and temperatures (30, 50, 80 and 100°C) for 3 h. Amberlite XAD-16 was used to remove phenolic compounds before concentration and precipitation. Pectin was precipitated with ethanol. The optimum extraction conditions were temperature 80°C and pH 4.5. The yield, GalA content, and DE of the obtained pectin were 7.23±0.19%, 74.12±2.07%, and 76.30 ±3.38% respectively.

**(vi) Citrus depressa**

Polysaccharides were extracted by Tamaki *et al.* (2008) with hot HCl solution at 85°C for 1 h from the endocarp of *Citrus depressa*, and the extract was precipitated with ethanol. The obtained pectin was (Highly Methylated Pectins) HMP and contained total carbohydrates 89.3%, uronic acid 79.2%, ash 4.1%, moisture 8.8%, and DM 66.2%.

**(vii) Galgal Peel (*Citrus pseudolimon* Tan.)**

The recovery of pectin was studied by Attri and Maini (1996) from galgal fruits peels by using extractants HCl, HNO<sub>3</sub>, citric acid, and tartaric acid. The extracts were precipitated with 0.75% aluminium chloride (AlCl<sub>3</sub>), and ethanol. The extractant to peel ratio, and extraction time were varied. The best extractant was found be 0.1 N HCl, with an extraction time of 60 min, and peel to acid ratio of 1:10. Ethanol precipitation gave better yield than aluminium chloride precipitation.

It has been concluded from above that acid extraction followed by ethanol precipitation may be suitable for pectin recovery from spent kinnow peel boiled water remaining after naringin extraction.

#### **2.4. Conclusions from literature search**

From the above discussion, the silent findings were observed.

##### **(A) Salient Findings**

1. The methods reported for recovery of naringin from citrus peels include
  - (i) Solvent extraction after that precipitation and crystallization
  - (ii) Supercritical CO<sub>2</sub> extraction
  - (iii) Ultrasonic-assisted extraction
  - (iv) Adsorption on certain adsorbents and ion exchange resins followed by desorption (adsorption-desorption method).
2. Methods other than adsorption-desorption are either costly or not eco-friendly.
3. Only two studies for recovery of naringin were reported with peel boiled water using polymeric resin (XAD-16 from bergamot peel boiled water).
4. It is notable that acid extraction of citrus peel is used for pectin production commercially and most of the authors used the following extraction parameters in the range pH 1-3, temperature 60-90°C and extraction time 1-3 h.

##### **(B) Gaps**

From the literature survey, it is clear that these are several aspects still to be investigated.

These are as following

1. No study is reported for recovery of naringin from kinnow peels
2. The modelling studies have not been reported for adsorption of naringin from citrus peel boiled water

3. The modelling studies have not been reported for desorption of naringin from exhausted resin
4. Only one study that too with bergamot peel is reported for recovery of pectin from the citrus peel boiled water after recovery of naringin. However the quality of pectin extracted is not reported in this study.