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Debittering of Tangerine *Citrus Reticulata* Blanco Juice by β-Cyclodextrin Polymer

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Key words: β-cyclodextrin, β-cyclodextrin polymer, *Citrus reticulata* Blanco, debittering, tangerine, citrus juice

Abstract

The use of insoluble β-cyclodextrin polymer for the reduction of limonin in Thai tangerine juice by batch, column and fluidized bed processes were studied and compared. Direct correlation between complexation of limonin and amount of β-cyclodextrin polymer was observed. The binding was not affected by temperature when tested at cold room and room temperatures. Using 3 g% β-CD polymer at room temperature, limonin reduction by the batch and column processes were 70 and 94% respectively. In a fluidized bed column (50 × 3 cm i.d.) under the condition of 15 g β-cyclodextrin polymer with a feed flow rate of 100 mL/min at room temperature, the initial efficiency of debittering was about 90% and gradually decreased. Increasing the flow rate or amount of the cyclodextrin bed did not improve the efficiency at the present study condition.

Introduction

Excessive bitter taste in citrus product, especially citrus juice, is a major problem in citrus industry worldwide because it reduces the quality and commercial value of the product. The two principal compounds which impart the bitterness in citrus fruits are naringin (flavanone glycoside) and limonin (highly oxygenated triterpene) (Figure 1). Naringin is common in bitter citrus species such as pummelo, grapefruit, sour orange, and pumello hybrid natsudaidai [1]. Naringin is high in young tissues and decreases upon maturation. This flavonoid is an indigeneous component in the membrane and albedo of the fruit and contribute to the bitterness of fresh fruit and juice. Its taste threshold is approximately 20–50 ppm (by HPLC) [1, 2]. Limonin, the other bitter compound, is found in all citrus species and is exceedingly bitter [3] with taste threshold of 5–6 ppm in orange juice [1, 4]. Limonin is the primary cause of 'delayed bitterness' in which the fruit or its juice is not bitter if consumed fresh but gradually become bitter upon storage, even when refrigerated or frozen. This phenomenon is due to the presence of non-bitter precursor of limonin – limonoate A-ring lactone (LARL) – in the segment and juice sac membrane. When the membrane is ruptured during juice extraction, LARL comes in contact with the acidic juice medium and is converted to limonin by limonin D-ring lactone hydrolase [1]. Heat treatment alone can also rapidly convert LARL to limonin [5]. The level of limonin varies with cultivars, degree of ripeness, climatic condition, horticulture practices, and processing and storage conditions.

The negative impact created by bitter taste in citrus juice has made debittering a generally incorporated step in industrial juice processing technology. Several methods to reduce or control the bitter compounds had been developed, mostly in the laboratory scale. They include blended bitter juice with non-bitter juice, addition of sweetener or other chemicals to mask the bitter off-taste, selective breeding of less bitter cultivars, use of plant growth regulators to inhibit the synthesis of naringin or LARL [2, 6, 7], or remove the bitter compounds from the juice by various means. Debittering of the juice by selective or combination removal of naringin and limonin had been widely explored. Some of them are extraction with supercritical carbon dioxide, treatments with bacteria [8] or specific enzymes [9–12]. Using adsorptive and/or ion-exchange resins are the preferred methods due to easy handling and possibility of regeneration for long-term use. Several natural or synthetic hydrophobic and hydrophillic adsorbents were tested and was critically reviewed by Kimball and Norman [13]. Current commercial debittering unit (first installed in the U.S. in 1988) uses styrene-divinylbenzene copolymer as the hydrophobic adsorbing resin due to its high debittering efficiency, easy regeneration, and

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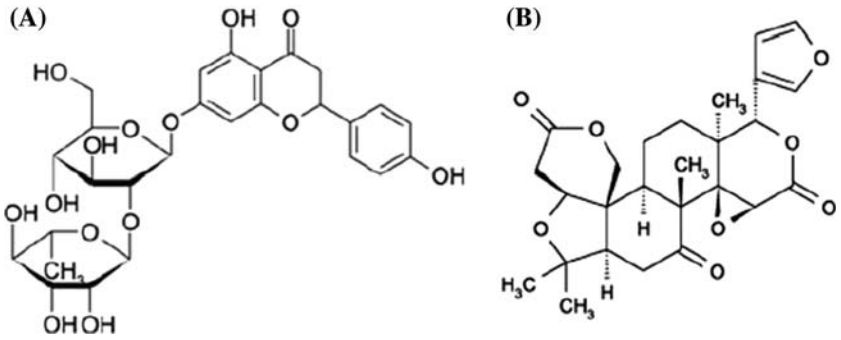

Figure 1. Chemical structures of naringin (A) and limonin (B).

stability [13]. Reports for debittering citrus juice with cyclodextrins are quite limited. Konno *et al.* [14] used soluble β-cyclodextrin monomer to encapsulate limonin and naringin in citrus juice. The cyclodextrin was still present in the finished product and could result in rejection by some countries although the use of cyclodextrin as processing aids was generally recognized as safe (GRAS). Shaw and colleagues used insoluble β-cyclodextrin polymer to remove limonin and naringin from navel orange and grapefruit. In the batch process, the insoluble cyclodextrin could be removed by filtration. They showed that β-cyclodextrin polymer could reduce the bitter flavonoids and limonoids by about 30–50% without significantly affect the soluble solids, total acid, or ascorbic content of the juice [15, 16]. Flavor evaluation also showed that debittered juice was more acceptable [15]. Recently, Fontananova *et al.* [17] innovatively immobilized *O*-octyloxycarbonyl-β-cyclodextrin onto polymeric membrane for naringin removal.

Tangerine is one of the most grown citrus in Thailand. Although most of the fruits are consumed fresh, its juice (fresh or packaged) has become increasingly popular and gain a significant market share. With this growing potential, processing and packaging technologies to enhance the quality of the beverage for consumer acceptance are essential. This study reports the use of β-cyclodextrin polymer to debitter Thai tangerine *Citrus reticulata* Blanco juice by batch, packed bed and fluidized bed at the laboratory scale. A low cost and simple process is aimed for small scale orange juice producers.

Experimental

Materials

Insoluble β-Cyclodextrin polymer was a gift from Cerestar, U.S.A., Inc., Indiana, U.S.A. Reagents for analytical analysis were of HPLC grade.

Sample preparation

Juice from Thai tangerine fruits purchased from local markets or commercial tangergine juice (Tipco Foods Thailand) were kept at −20 °C before used. To optimize the efficiency and capacity of the adsorptive process, all samples were centrifuged to remove pulps prior to further analysis.

Extraction of limonin

Limonin in the juice was extracted by solid phase extraction method (SPE) using C-18 column. The column was activated consecutively with acetonitrile and water before 3–6 mL juice was passed through the column at gravitation flow (1.0–1.5 mL/min). This was followed by excessive amount of water to remove sugar. Limonin was eluted from the column with acetonitrile, filtered through a 0.45 μm nylon filter membrane before determination of limonin content by HPLC.

Determination of limonin content

The limonin content was determined by reverse-phase HPLC using analytical 5 μm Inertsil ODS-3 C-18 column (150 mm × 4.6 mm i.d.) (GL Sciences Inc., Japan) following the method of Mozaffar *et al.* [18]. HPLC analysis was performed with Hewlett Packard Series 1050, Japan. The chromatographic condition was 40 °C column temperature, pressure 70 bar, flow rate of 1.0 mL/min, absorbance at 214 nm and 37% acetonitrile as mobile phase.

Debittering processes

Batch process

A $3 \times 3 \times 2 \times 2$ factorial design experiment was erformed by varying the amount of β-cyclodextrin polymer (1,3, and 5 g%), temperature (~6 °C and ~30 °C), contact time (0.5, 1.0 and 1.5 h), and mixing speed (moderate and high). At the end of the experiment, the polymer was removed by filtration through 170 mesh screen and the treated tangerine juice was quantitated for limonin content. Optimum condition was determined by Duncan's Mutiple Range Test.

Column process

The process was conducted at room temperature (~30 °C). Three grams of β-cyclodextrin polymer

(0.03 g polymer/mL bed volume) were suspended in water for 30 min and packed in a glass column (12 × 1.2 cm i.d.) at flow rate of 0.35 mL/min monitored by a peristatic pump. The column was equilibrated with water before application of the juice (100–300 mL) using the same flow rate. Five milliliter fraction of debittered juice was collected and subjected to limonin analysis.

Fluidized process

Slurry of 15, 20 or 25 g of β-cyclodextrin polymer was packed into a 50 × 3 cm i.d. fluidized column. The sample juice was counter-flowed by pump into the column at the flow rate of 75, 100 or 120 mL/min. At intervals, 100 mL juice was collected for determination of limonin content.

Statistical analysis

Data analysis was conducted using ANOVA (SAS Program V.6.12) at a significant level of $p \leq 0.05$.

Results and discussion

Direct correlation between efficiency of limonin complexation and amount of β-cyclodextrin polymer in the batch process was observed (Table 1). The result also indicated that binding was already saturated at 30 min and temperature did not have apparent effect on the binding ability. Applying Duncan Multiple Range Test, the optimum condition for the batch process was 5 g% polymer, 30 min contacting time, at either cold room (~6 °C) or room temperature (~30 °C). At this condition, the limonin reduction was approximately 80% (from initial 7.4 ppm limonin juice). However, 3 g% of β-cyclodextrin polymer still gave acceptable juice with limonin not exceeding the bitter threshold level (<5 ppm). If the batch contain very high level of limonin, larger amount of the polymer should be considered. Moderate or high mixing speed of the process gave about the same debittering result (at 95% confidence level) (Table 2). However, it was observed that some deterioration of beads occurred at high mixing speed. Thus gentle mixing is recommended.

Using 3 g% β-cyclodextrin in a packed bed column (12 × 1.2 cm i.d. glass column; 10 mL bed volume) and condition stated in experimental, much better debittering result (94% limonin reduction) was observed (data not shown). However, in order to maintain similar contact time as the batch process (30 min) for reason of comparison, the flow rate used in the experiment was extremely slow (0.35 mL/min) and impractical. Considering the high stability of the β-cyclodextrin polymer, much higher flow rate could be used. Shaw and Wilson [19] reported the use of 1.2–1.8 g% β-cyclodextrin polymer, room temperature, 60 min contacting time for limonin reduction in grapefruit juice. The reduction was around 50 and 90% for batch and column process, respectively. In the column experiment, they used the flow rate of 3 mL/min. Figure 2 shows the maximum volume for a 10 ppm limonin sample juice that could be debittered through the column and still maintain the limonin level below 5 ppm (limonin taste threshold level) was 240 mL or 24 bed volume.

To scale up the debittering process, a fluidized bed was performed. A glass column of 50 × 3 cm i.d. was used. The minimum fluidizing velocity was determined to be 90 mL/min. Using 15 g β-cyclodextrin polymer and varying the flow rate at 75, 100, and 120 mL/min, it was shown that the flow rate above (120 mL/min) or below (75 mL/min) the minimum fluidizing velocity gave less debitter efficiency and was unsuitable (Figure 3). At 75 mL/min feed rate, complete fluidization may not yet reached as can be seen from the fluctuated complexation level. At too high flow rate (120 mL/min), rapid passing through the bed rendered decreased contact between limonin in the juice and the polymer, thus reducing the debittering efficiency. Figure 4 shows the result of fluidization by varying bed concentration (15, 20 and 25 g β-cylodextrin polymer) at 100 mL/min flow rate. All concentrations of the tested bed showed very rapid limonin reduction (~90%) in the first 25 mL product. The efficiency gradually decreased. Increasing the amount of β-cyclodextrin polymer did not increase the capacity and efficiency of the process. Considering the capacity of polymer, 15–20 g bed significantly produced better debittering results and use of 15 g polymer should be appropriate. It is possible that maximum complexation of limonin in the sample juice was already attained at this bed concentration and lower amount of β-cyclodextrin could be used. In a separate experiment, it was found that reducing the β-cyclodextrin polymer to 11 g (equivalent to 1.25 g%) gave similar limonin reduction as 15 (result not shown). Acceptable

Table 1. Percentage limonin reduction for batch process

Process time (min)	% Limonin reduction					
	Room temperature (~30 °C)			Cold room temperature (6 °C)		
	1 g% CD	3 g% CD	5 g% CD	1 g% CD	3 g% CD	5 g% CD
30	42.73 ± 0.65^{a}	68.06 ± 0.88^{b}	78.49 ± 0.93^{d}	42.37 ± 0.31^{a}	63.82 ± 0.05^{e}	80.60 ± 0.07^{d}
60	43.85 ± 0.98^{a}	71.80 ± 1.48^{c}	80.71 ± 0.48^{d}	43.46 ± 1.39^{a}	66.69 ± 0.79^{f}	80.96 ± 0.24^{d}
90	43.56 ± 0.59^{a}	70.44 ± 0.59^{c}	80.41 ± 0.40^{d}	43.19 ± 1.78^{a}	66.50 ± 1.08^{f}	80.15 ± 1.53^{d}

Note: Means with the same letter in the column and row are not significantly different at $p \leq 0.05$, $n = 3$.

Table 2. Effect of stirring speed on % limonin reduction

Processing time (min)	% Limonin reduction	
	Speed No 3	Speed No 5
0	0	0
30	68.06 ± 1.12	70.80 ± 1.11
60	71.83 ± 1.50	71.04 ± 1.64
90	70.44 ± 0.38	69.21 ± 0.51

Note: Magnetic stirrer: model MR3003 Heidolph, Germany.

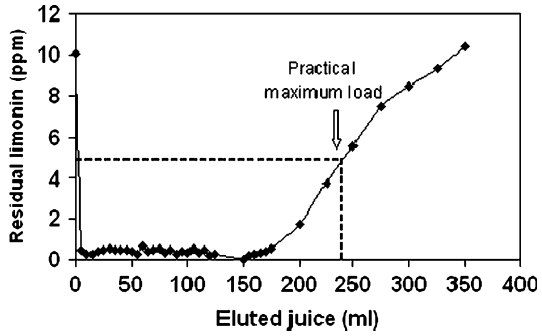

Figure 2. Limonin removal by 3 g% β-cyclodextrin polymer in a packed bed column. The initial limonin concentration was 10.11 ppm. Column size was 12 × 1.2 cm i.d. and the flow rate was 0.35 mL/min.

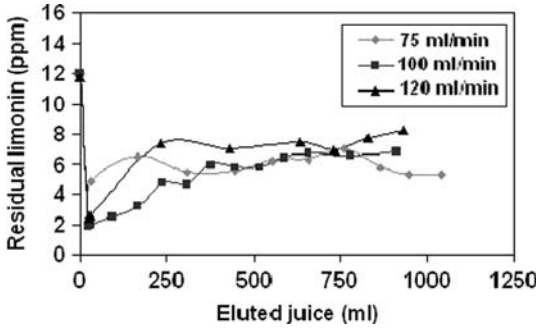

Figure 3. Effect of flow rate on limonin reduction in a fluidized bed column. The amount of β-cyclodextrin polymer was 15 g packed into a 50 × 3 cm i.d. glass column. The initial concentration of limonin was 12 ppm.

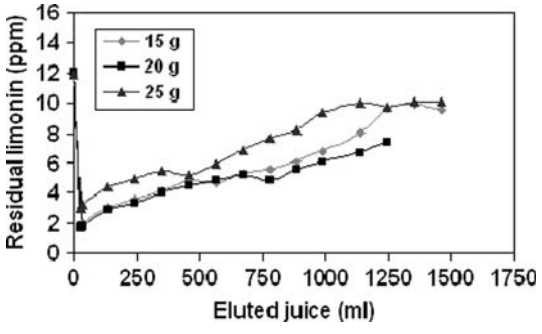

Figure 4. Effect of β-cyclodextrrin polymer concentration on limonin reduction in a fluidized bed column. The flow rate was 100 mL/min and the initial concentration of limonin was 12 ppm.

amount of the debittered juice (limonin below 5 ppm) was about 900 mLfor these columns (50–60% reduction). Twenty five grams of polymer gave the least favorable result, both efficiency and maximum practical load wise.

The present study suggested that insoluble β-cyclodextrin polymer is a good candidate for debittering of citrus juice, considering its complexing efficiency with limonin, cost, physical and chemical stability and its GRAS status for processing aids. In addition, β-cyclodextrin polymer is easily regenerated with 2% NaOH. Shaw and Wilson [19] showed that the polymer could be regenerated for 19–21 cycles without losing its efficiency.

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