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Concentration and purification of soluble pectin from mandarin peels using crossflow microfiltration system

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Abstract

A crossflow microfiltration process was developed to concentrate and purify soluble pectin extracted from the mature citrus peel. The extracted pectin solution was concentrated with the crossflow microfiltration using a 0.2 μm regenerated cellulose membrane. As a result of crossflow microfiltration, the galacturonic acid content of pectin increased from 68.0 to 72.2% while the recovery yield of pectin decreased from 10.5 to 9.9%. In addition, the volume of ethanol used for the recovery of pectin could be reduced to 25%. To purify pectin further, the concentrated pectin extracts was washed using the fed-batch type diafiltration system. By diafiltration, the galacturonic acid content of pectin increased from 72.2 to 75.6%. The yield of pectin, however, decreased from 9.9 to 9.4% at six volumes of diafiltration. The diafiltration process was also effective to remove flavonoids, polyphenols and carotenoids, which were impurities in pectin products. Therefore, the crossflow microfiltration system could be efficiently used for concentration and purification of pectin.

Keywords: Crossflow microfiltration; Mandarin peel; Soluble pectin; Diafiltration

1. Introduction

Pectin, a major biopolymer of fruits, is composed primarily of linear polymers of D-galacturonic acid units joined in α-D-(1 → 4) glycosidic linkages. Pectin is mainly exploited by the food industry as a gel-forming agent, a stabilizer, and an emulsifier (Pilnik, 1990). Recently, their pharmacological activities such as anti-metastasis (Platt & Raz, 1992), immunostimulating (Yamada, Ra, Kiyohara, Cyong, & Otsuka, 1989), cholesterol decreasing (Kay & Truswell, 1977), and anti-ulcer activities (Kiyohara et al., 1994) were reported.

Crossflow microfiltration is a process for separating particles with diameters between 0.1 and 10 μm from suspensions by passing the liquid through a porous membrane. The crossflow microfiltration process has found widespread use in the food and dairy industry (Trägårdh, 1995), biotechnology (e.g. cell separation from fermentation broth) and the treatment of oil and latex emulsions (Mueller, Cen, & Davis, 1997; Redkar & Davis, 1993; Wakeman & Akay, 1995). However, until now there has been no report on the study regarding the concentration or purification of pectin using crossflow microfiltration. Pectin has rather been considered to be the major contributor to the flux decline and to cause the difficulties in cleaning the membrane when using the crossflow microfiltration for purification purpose. The pretreatment with enzymes that degrade pectin was required to increase the flux when fruit juices and other liquid foods containing pectin were purified with the microfiltration, ultrafiltration and reverse osmosis (RO, Alvarez, Alvarez, Riera, & Coca, 1998; Szaniawski & Spencer, 1997). In this study, a crossflow microfiltration process was developed to concentrate pectin solution by exploiting its unfilterable nature against the microfilter.

Currently, an industrial process for pectin production from mandarin peels based on traditional method would require large amounts of ethanol for purification of pectin, and it results in higher operating costs. Therefore, in this study crossflow microfiltration process was developed to concentrate the pectin efficiently thereby decrease

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the amounts of ethanol needed for the precipitation of pectin. The optimum conditions of crossflow microfiltration were determined, and the yield and purity of pectin were evaluated.

2. Materials and methods

2.1. Raw material preparation

Mature Cheju mandarin was obtained from a Cheju mandarin farm in Korea. The peels were separated manually from fruits and chopped into pieces of 0.5 cm width. The chopped peels were dried under the sun and stored at −70 °C.

2.2. Pectin extraction

The procedure for pectin extraction from citrus peels shown in Fig. 1 was modified from the previous method (Kim, Kim, Lee, Kim, & Kim, 2000). To remove soluble pigments and dust, the dried citrus peel samples were washed with 0.03 N hydrochloric acid solution at a solid/solvent ratio of 1:10 at 60 °C and agitated for 30 min. After wash of citrus peel, the residue of citrus peel was hydrolyzed with 0.04 N hydrochloric acid solution at a solid/solvent ratio of 1:2.5 at 90 °C for 20 min. For extraction of pectin from citrus peel, the hydrolyzed residues were extracted with hot water at 90 °C and agitated for 30 min at solid/water ratio of 1:10. The slurries were filtered through the cheesecloth. The resulting filtrate was centrifuged at 7000 rpm for 20 min, and then filtered under vacuum through a 11 μm rated filter paper (Whatman International Ltd, Maidstone, England) to remove solid particles. The resulting pectin extracts was used for the crossflow microfiltration.

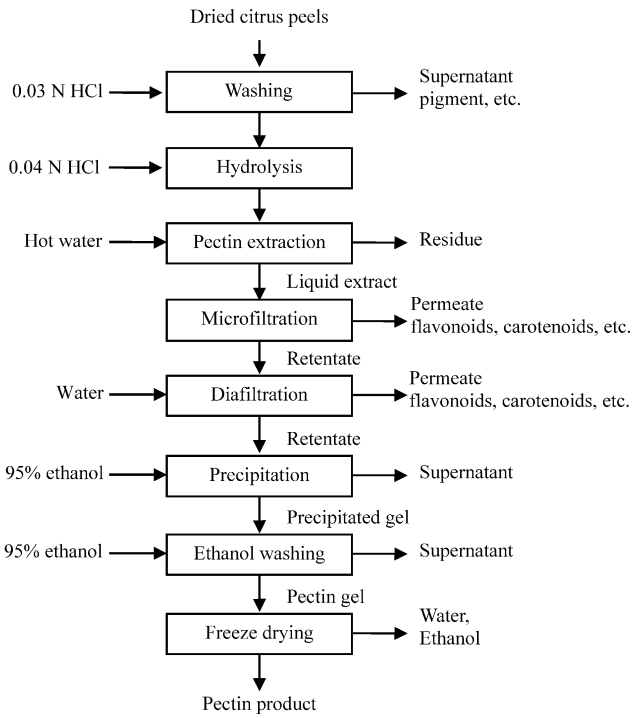

Fig. 1. Flow chart of pectin extraction procedure from citrus peels.

2.3. Crossflow microfiltration step

To concentrate the pectin extract, the pectin extract was applied to the crossflow microfiltration system. Details of the experimental set-up are shown in Fig. 2. The crossflow microfiltration was conducted in a flat plate membrane module (Sartorius, Götingen, Germany). The equipment consisted of a 10 l stainless steel storage tank with a double cooling jacket, a rotary lobe feed pump and membrane holding device. A regenerated cellulose commercial membrane cassette (Sartorius, Götingen, Germany) with a surface area of 0.1 m^2 and a nominal pore size of 0.2 μm was used. The crossflow microfiltration of pectin extracts was operated in a batch mode.

2.4. Diafiltration step

After concentration of the pectin extracts, the diafiltration system was applied for further purification of concentrated pectin solution. The diafiltration was operated in a fed-batch mode. The membranes were carefully cleaned with 1 N NaOH solution after experiment, and rinsed with RO water. The flux of RO water at a transmembrane pressure (TMP) of 1 bar and 25 °C was measured before experiment.

2.5. Recovery of pectin

Each pectin solution obtained from the each step of extraction, concentration and purification was precipitated by adding two volumes of 95% ethanol at 4 °C for 60 min with gentle stirring to break up the gelatinous lumps and to obtain a homogeneous mixture. Each precipitate was filtered

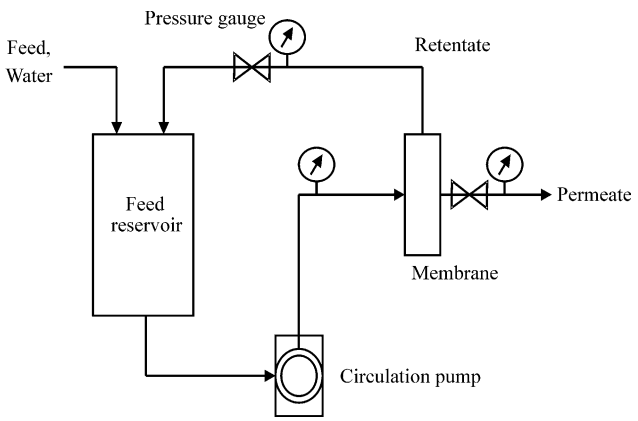

Fig. 2. Experimental set-up for crossflow microfiltration system.

through cheesecloth and washed with 95% ethanol. Finally, each precipitated pectin gel was freeze-dried.

2.6. Analysis

2.6.1. Yield of pectin

The yield of pectin was determined by measuring the dry weight of pectin precipitated with ethanol on the basis of 100 g of dried citrus peel.

2.6.2. Purity of pectin

The purity of each prepared pectin was determined by measuring the content of galacturonic acid. The concentration of galacturonic acid was determined using the method of Taylor and Buchanan-Smith (1992), which is a modified method of McComb (1952). Standard solutions of galacturonic acid were prepared by adding 0–100 μg of D-galacturonic acid monohydrate (Sigma Chemical Co. St Louis, USA) into a test tube which contained up to 200 μl of water. Three milliliters of concentrated sulfuric acid (98%) was added into the standard solutions prepared, and then 100 μl of 0.1% carbazole (Sigma Chemical Co. St Louis, USA) reagent in ethanol was added. The mixture was incubated at 60 °C for 1 h in a water bath. After incubation the mixture was equilibrated at room temperature. The absorbance of the mixture was measured at 530 nm with a UV–VIS spectrophotometer (CARY 300 Bio, Varian, Palo Alto, USA). To determine the concentration of galacturonic acid, each pectin sample was dissolved in deionized water at a concentration of 250 mg/l and treated according to the method explained above. After measuring the absorbance, the galacturonic acid content in the sample pectin was calculated using a standard curve prepared by standard solutions.

2.6.3. Permeate flux

During the crossflow microfiltraton, the permeate flux of extracted pectin solution was determined by measuring the permeate volume collected for 10 s.

2.6.4. Viscosity

Viscosities of concentrated pectin extracts were measured at the shear rate range of $0 \sim 500\ s^{-1}$ using a rheometer (RS 150, Haake Inc., Karlsruhe, Germany) at 25 °C.

2.6.5. Determination of impurities in pectin extracts

The total content of flavonoids, polyphenols and carotenoids in concentrated pectin extracts was measured by spectrophotometric scanning from 190 to 900 nm. Petrus and Dougherty (1973) reported that the total flavonoids content was measured ultraviolet absorption at 280 nm and the total polyphenolic content was measured at 325 nm. Carotenoids were determined from absorption peaks at 425, 443 and 465 nm.

3. Results and discussion

3.1. Effect of transmembrane pressure on crossflow microfiltration

To optimize the condition of microfiltration with pectin extracts, the effect of permeate flux on the TMP was measured. TMP is defined as the average of inlet and outlet gauge pressures with the permeate at atmospheric pressure. The permeate flux was determined by measuring the permeate volume collected for 10 s. The TMP provided the maximum flux was determined as the optimum TMP. As shown in Fig. 3, permeate flux was maximum at 1.25 bar of TMP and declined above 1.25 bar of TMP. Therefore, the optimum TMP for the maximal permeate flux was 1.25 bar ($P_{in} = 2.5$ bar and $P_{out} = 0$ bar).

In crossflow microfiltration of pectin extracts, the behavior of permeate flow-pressure showed a bell-shape (Fig. 3). This bell-shaped permeate flow characteristic was also shown in the ultrafiltration of various fruit juices (Kirk, Montgomery, & Kortekaas, 1983; Sulaiman, Sulaiman, & Yih, 1998). On contrary, for protein solution, the permeate flux continued to increase, and the permeate flux had constant value above the optimum TMP (Cheryan, 1977; Omosaiye & Cheryan, 1979; Setti, 1976). The profile of permeate flux was determined according to the nature of the polarized gel layer formed on the top of the membrane by the feed material. Because the proteins are spherical or globular molecules, the spaces within a protein gel layer are never completely closed, thus allowing the permeate to pass through to give a flux plateau above the optimum TMP. On the other hand, pectin is the aggregates of chain-like

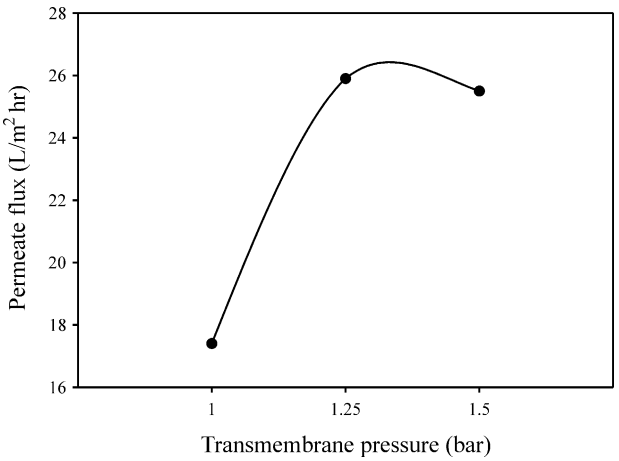

Fig. 3. Changes of permeate flux with increases in the transmembrane pressure during concentration of pectin extracts with crossflow microfiltration. Transmembrane pressure (TMP) is the average pressure across the membrane. The permeate flux was determined by measuring the permeate volume collected for 10 s.

molecule, which typically consists of galacturonic acid bridged by hydrogen bonds (Pilnik & Voragen, 1970). Therefore, at over optimum TMP, these bridges could be collapsed and the network of chains could close off the membrane. This high compressibility of pectin molecule would explain the decrease of permeate flux when pressure increased above the optimum TMP in our data.

3.2. Effects of volume concentration factor on crossflow microfiltration

Table 1 shows the changes of the pectin yield, viscosity and average flux in the pectin solution concentrated four and six times at 1.25 bar of TMP. Volume concentration factor (VCF) is the amount that the feed stream has been reduced in volume from the initial volume. The VCF calculated as

VCF = Total starting feed volume added to the operation/

Current retentate volume

In the pectin solution concentrated six times, the yield of pectin slightly decreased by 0.6% compared to non-concentrated pectin solution, however, the viscosity increased drastically to 201 cP. Due to its high viscosity, operation of a crossflow microfiltration with the concentrated pectin solution was difficult. In addition, the recovery of pectin and cleaning of the membrane were troublesome. In the pectin solution concentrated four times, the viscosity of the pectin solution was only 44% of that of pectin solution concentrated six times. Due to this moderate viscosity, concentrating four times was more suitable for the crossflow microfiltration. The yield of pectin was the same as that of the pectin solution concentrated six times. The average permeate flux of the pectin solution concentrated four times, however, was 18% higher than that of the solution concentrated six times. Thus, the optimal VCF of extracted pectin solution was four.

Table 1
Changes in the pectin yield, viscosity and average flux of the extracted pectin solution by crossflow microfiltration

Volume concentration factor[a]	Yield of pectin[b] (%)	Viscosity[c] (cP)	Average flux (l/m^2 h)
Non-concentrated	10.5 ± 0.3	10.3 ± 0.1	–
4	9.9 ± 0.0	89.0 ± 1.6	31.5
6	9.9 ± 0.0	201.0 ± 3.2	25.9

All data are averages of three experiments ± SD.
[a] Volume concentration factor (VCF) is the amount that the feed stream has been reduced in volume from the initial volume.
[b] The yield of pectin was determined on the basis of 100 g of dried citrus peel.
[c] Viscosity of pectin solution was measured at a low shear rate (0 ~ 500 s^{-1}) for 60 s using a rheometer at 25 °C.

3.3. Concentration effects on ethanol volume required

The yield of pectin, galacturonic acid content and ethanol volume required for precipitation of pectin in the pectin solution concentrated four times and non-concentrated pectin solution were compared in Table 2. When the extracted pectin solutions were concentrated four times, the pectin yield decreased about 0.6% while galacturonic acid contents increased about 4.2%. In addition, only a quarter of ethanol was used for pectin recovery from concentrated pectin extracts. Therefore, it was suggested that crossflow microfiltration could successfully increase the purity of pectin and reduce the amount of ethanol for precipitation of pectin without significant loss of pectin.

3.4. Effects of diafiltration on the purity of pectin

For further purification of concentrated pectin solution, the pectin solution concentrated four times was applied to the diafiltration system. Fig. 4 shows the changes of galacturonic acid content and pectin yield in purified pectin solution after diafiltration process with the various volumes of diafiltration. After diafiltration, the yield of pectin decreased from 10.5 to 9.4%, while the galacturonic acid content increased from 68.0 to 75.6%. Thus, it could be concluded that the pectin solution was purified further by diafiltration process, probably due to removal of small water-soluble oligosaccharides. When the volume of diafiltration was larger than two times of the original feed volume, the galacturonic acid content and the yield of pectin were not drastically changed. Therefore, considering the process time and energy consumption during diafiltration process, the optimum volume of diafiltration was two times of the feed volume.

Table 2
Changes in the pectin yield, galacturonic acid content and required ethanol volume for pectin precipitation by concentration of pectin extract with crossflow microfiltration

Volume concentration factor[a]	Yield of pectin[b] (%)	Galacturonic acid[c] (%)	Required ethanol volume ratio[d]
Non-concentrated	10.5 ± 0.3	68.0 ± 0.7	4
4	9.9 ± 0.0	72.2 ± 1.0	1

All data are averages of three experiments ± SD.
[a] Volume concentration factor (VCF) is the amount that the feed stream has been reduced in volume from the initial volume.
[b] The yield of pectin was determined on the basis of 100 g of dried citrus peel.
[c] The content of galacturonic acid in pectin product was analyzed by the Taylor and Buchanan-Smith method.
[d] Required ethanol volume was the amount of ethanol used for pectin precipitation.

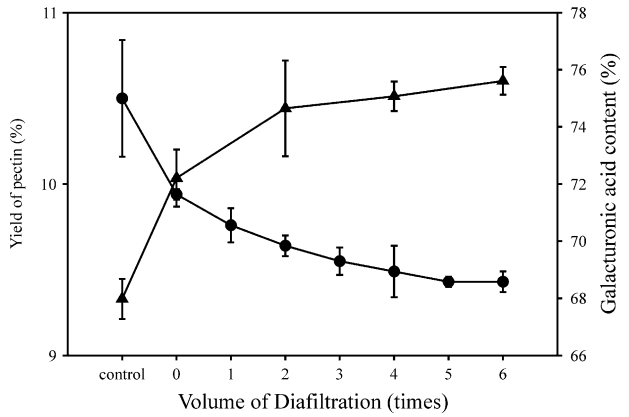

Fig. 4. Changes of pectin yield and galacturonic acid content during diafiltration. The pectin solution concentrated four times was applied to the fed-batch diafiltration system. The yield of pectin was determined on the basis of 100 g of dried citrus peel. The content of galacturonic acid in pectin product was analyzed by the Taylor and Buchanan-Smith method. Control was non-concentrated pectin. (●), yield of pectin; (▼), galacturonic acid content.

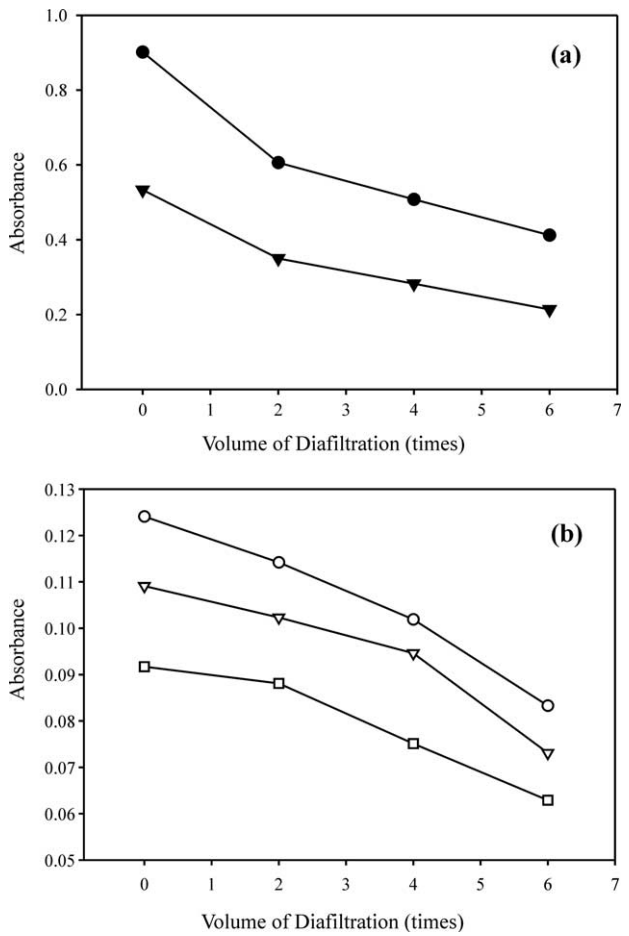

Fig. 5. Removal of flavonoids, polyphenols and carotenoids content during diafiltration of pectin extracts. The pectin extracts was purified by fed-batch method of diafiltration. During diafiltration, the changes in the content of flavonoids, polyphenols, and carotenoids of washed pectin solution were detected by spectrophotometer. (a) the flavonoids detected at 280 nm (●) and polyphenol detected at 325 nm (▼). (b) the carotenoids detected at 425 nm (○), 443 nm (▽) and 465 nm (□).

3.5. Removal of impurities by diafiltration

The flavonoids and polyphenols are impurities and the carotenoids are major pigment in pectin products. Petrus and Dougherty (1973) reported that flavonoids and polyphenols showed the absorbance at 325 and 280 nm of UV spectrum and carotenoids showed at 465, 443 and 425 nm of visible spectrum. Therefore, the residual content of flavonoids, polyphenols and carotenoids was determined by measuring the absorbance at each wavelength. The content of flavonoids and polyphenols in pectin solution decreased about 34% at two volumes of diafiltration, and finally decreased about 57% at six volumes of diafiltration (Fig. 5). The content of carotenoids in pectin solution decreased about 8% at two volumes of diafiltration and decreased about 33% at six volumes of diafiltration. Therefore, both the flavonoids and carotenoids in the pectin extracts could be efficiently removed by the diafiltration.

4. Conclusions

The crossflow microfiltration effectively concentrated pectin extracts, which saved 75% of ethanol consumption required for precipitation of pectin. During the diafiltration, undesirable impurities (flavonoids, polyphenols and carotenoids) were effectively removed from concentrated pectin extracts. Therefore, the crossflow microfiltration and diafiltration process can be used to purify water soluble pectin from waste citrus peels.

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