

Original article

Process optimisation of the bitter melon (*Momordica charantia*) concentrated juice preparation for a freeze-dried powder

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Summary Bitter melon (*Momordica charantia*), a commonly consumed vegetable in South Asia, eastern-South Asia, China, Japan, Korea peninsula, Caribbean Sea isles etc., is used as an adjunct in the management of diabetes mellitus. In this study, in order to find the effective ways of producing bitter melon freeze-dried powder, the concentrated juice of bitter melon was produced through enzymatic process (EP), ultra filtration (UF), reverse osmosis and vacuum concentration technology. Results indicated that total saponins, total sugar and pH value in bitter melon juice were almost unchanged after EP and after UF ($P \geq 0.05$). However, UF decreased the turbidity and improved transmittance of juice ($P < 0.05$). When compared with the vacuum concentration alone, the combined process of reverse osmosis followed by vacuum concentration had 3.33 folds of production efficiency and 1.725 times of vitamin C content in concentrated juice. With freeze-drying microscope system, the eutectic point of the freeze-dried product of bitter melon juice was detected to be -37.5°C , which was important to optimise lyophilisation parameters. The freeze-drying microscope system was more accurate than the conventional electric resistance method in detecting the eutectic point of freeze-dried product.

Keywords Bitter melon freeze-dried powder, enzymatic process, eutectic point, reverse osmosis, ultra filtration, vacuum concentration.

Introduction

Many traditional medicines derived from plants have been used for the treatment of diabetes throughout the world (Day *et al.*, 1990; Kumar & Clark, 2002). Among these plants is *Momordica (M) charantia* Linn commonly known as the bitter melon or Chinese melon, which belongs to the Cucurbitaceae family (Day *et al.*, 1990). The hypoglycaemic effect of this plant after oral administration has been proved in experimental animal models and in many clinical studies (Okyar *et al.*, 2001; Ojewole *et al.*, 2006; Dans *et al.*, 2007). Dried powder of bitter melon juice is desirable in practical application as the solid form is more convenient than liquid form; however, not enough is known in literature about the process and parameters in production of bitter melon freeze-dried powder, especially the eutectic point of freeze-dried product of bitter melon juice, which are important in design of the parameters of freeze-drying process. Because the eutectic point is a temperature point representing the minimum melting temperature

(melting starting point) of a solid phase of some mixtures when temperature is increased, or the maximum frozen point (complete freezing point) of solution when the temperature is decreased. It is important to cool the material below its triple point, the lowest temperature at which the solid and liquid phases of the material can coexist. This ensures that sublimation rather than melting will occur in the following steps of the freezing dry process.

The necessity for detecting the eutectic point of liquid solution in freezing dry process is as follows. First of all, the freezing dry is carried out under the vacuum condition, it is necessary for all the liquid solution including product to be frozen completely before sublimation, otherwise the partial unfrozen liquid solution will vaporise quickly under the vacuum condition, which causes the dried product shrinkage, and makes the gases dissolving in the solution bubble up, bringing the product out of the drying chamber. Therefore, during the freezing stage, the liquid solution must be frozen at a temperature point below the eutectic point, only at this temperature the solution will be completely frozen into solid phase, and then the sublimation could be initiated. Second, during the sublimation stage, the temperature

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of the solid phase must not be over the eutectic point, otherwise the product will melt rather than sublimate, which will make the resolvability of the dried product bad and cause the colour of the dried product deep.

Usually, the freezing temperatures are between -50 and -80 °C. The eutectic point is the most critical in the whole freeze-drying process, because the product can be spoiled if the temperature is too high (much higher than the eutectic point). Or freezing-dry cost is too expensive if the temperature is too low (much lower than the eutectic point).

Accuracy of electric resistance method for detecting eutectic point is susceptible to the concentration of freeze-dried product (Han & Bischof, 2004). And freeze-drying microscope method can visually determine the eutectic point, which is not susceptible to the concentration of freeze-dried product.

The water content of materials is a key parameter for energy cost when freeze-drying is applied. Therefore, concentration process is necessary for reducing partial water of solution before the freeze drying is applied. Moreover, vast amounts of liquid food are industrially concentrated in order to reduce storage, packaging, handling and transportation costs. Vacuum evaporation is the predominant method used by the food industry to produce liquid food concentrates (Petrotos & Lazarides, 2001); however, as can be seen in this study, the single process of vacuum concentration will greatly damage the nutritional components in the product, and the process of reverse osmosis followed by vacuum concentration will lead to a satisfactory retention of active components. The more the clarity of the juice, the easier is the concentration process of the juice. Thus, enzyme hydrolysis and ultra filtration (UF) are advisable before concentration process.

Therefore, in this study, a pilot-plant-scale production process of concentrated juice of bitter melon prepared for freeze-dried powder was presented. A detailed focus includes (i) the enzymatic hydrolysis process optimisation of bitter melon juice; (ii) clarification effect of the juice treated with UF; (iii) physicochemical index comparison of products produced with two concentration processes: the vacuum concentration alone and the combined process of reverse osmosis followed by vacuum concentration; and (iv) determination of eutectic point of the freeze-dried product based on freeze-drying microscope system.

Materials and experimental methods

Materials

Bitter melon used in the study was produced in Shouguang, Shandong province, China. Pectinex 5XL (polygalacturonase [EC 3.2.1.15], 4500 PECTU mL⁻¹) was purchased from Novozymes (China) Biological

Technology Limited Corporation, Beijing, China. FSW (endo-cellulase [EC 3.2.1.4] and acid resistant proteinase [EC.3.4.23] preparation, 30000 u g⁻¹) and 10CZM (endo-cellulase [EC 3.2.1.4] and pectinesterase [EC 3.1.1.11] preparation, 30 000 u g⁻¹) pectin enzymes were from Tianjin Jiayi Enzyme Agent New Technology Company, Tianjin, China. GFM enzyme (polygalacturonase [EC 3.2.1.15], pectinesterase [EC 3.1.1.11] and pectinlyase [C 4.2.2.10] preparation, 500 000 u g⁻¹) was from Shenzhen Jinfumei Biological Technology Development Company, Shenzhen, China.

Preparation of bitter melon juice

Fresh bitter melon with about 70% of ripening degree was washed and its seeds were removed. Then the flesh of bitter melon was crushed to produce the freshly squeezed juice, and then the juice was hydrolysed with pectin enzyme. The optimal parameters of enzyme hydrolysis were determined in L₉(3³) orthogonal test by setting pectin enzyme concentration in the juice, temperature and reaction time as independent variables and turbidity and transmittance ratio as functions. After enzyme hydrolysis, the bitter melon juice was filtered in a ceramic film UF equipment (NCM-TCSY-1.5; Hefei Great Wall New Century Membrane Technology Cop., Hefei, Anhui, China), and the UF was conducted under 0.15 Mpa of operating pressure at ambient temperature (25–30 °C). Ceramic unit was stainless membrane module with effective filtration area of 1.5 m² per set. The membrane pore size was 200 nm.

Preparation of concentrated juice

After filtration, the juice was concentrated with the vacuum concentration process alone or with the combined process of reverse osmosis followed by vacuum concentration.

The reverse osmosis was conducted under 2.2 Mpa of operating pressure at ambient temperature. The operating velocity of juice was 1 m s⁻¹. The reverse osmosis equipment had polyamide film unit with 4.8 m² of effective filtration area (TFC-3838-HR; Kaimi Technology Cop., Nanjing, Jiangsu, China). As can be seen in the Results and Discussion, the final concentration of the juice in the reverse osmosis was fixed at 10 °Brix, and after several trails we found the suitable initial concentration of the juice for freeze drying was 24 °Brix, because it was easier to form a kind of uniform diameter granules during freeze drying for the concentrated juice with concentration of 24 °Brix. Therefore, after the total soluble solid of the juice treated with the reverse osmosis increased from 2.9 to 10 °Brix, the juice was further concentrated with the vacuum concentration process until the total soluble solid increased to 24 °Brix. The vacuum concentration was conducted at 55 °C and

0.005 Mpa. The vacuum concentration equipment was type L8200 (Kaimi Technology Cop.).

Eutectic point

We determined the eutectic point with the freeze-dry microscope system (type: CX41-12N₂; Shanghai Dongfulong Science & Technology Cop. Ltd, Shanghai, China). The system is a fully integrated freeze-drying microscope that enables critical events such as collapse and melting to be observed *in situ*. The system comprises a compound microscope, focused onto a sample that sits in a vacuum-tight temperature-controlled stage, which acts as a 'micro-freeze-dryer'. The freezing and drying behaviour of the sample can be determined visually, and the system is controlled through a PC, which provides image- and data-capture and archiving functions. The system was operating in the condition of liquid nitrogen refrigeration and electric heating. The freezing speed was $-20\text{ }^{\circ}\text{C}$ per min in freezing period, and the temperature-increasing speed was at $+1\text{ }^{\circ}\text{C}$ per min in sublimation period. The sample of bitter gourd juice was 2 μL and put into the freeze-drying chamber under the microscope. The temperature of the chamber was kept at $-45\text{ }^{\circ}\text{C}$ until the sample was frozen completely. In sublimation period, the system recorded five pictures per min. Because the eutectic point is the temperature representing the minimum melting point (at which the melting starts) of a solid phase of mixture, and the dried layer consists of ice and bitter gourd materials. The first bright point that appeared on the dried layer of the product was indeed a liquid water drop that was formed from the melting ice when the temperature reached at the eutectic point. Therefore, the eutectic point was detected when a bright point appeared between the dried layer and ice layer of the sample.

Physicochemical analysis

Transmittance of bitter gourd juice was measured using a Varian Cary 50 spectrophotometer under 630 nm, expressed with percentage, t (%) (Meydev *et al.*, 1977). Total sugar was directly titrated with Fehling's solution (Dubois *et al.*, 1956). Vitamin C content was measured using iodine titration (Suntornsuk *et al.*, 2002). Saponins content was measured using macro-resin absorption (Gao *et al.*, 2009). And turbidity was determined with Turbidimeter (WGZ-200) (Zhou *et al.*, 2009).

Statistical analysis

All experiments were repeated in triplicate. Results were expressed as mean values \pm standard error of mean (SEM). Differences in mean values were analysed using ANOVA. Statistical analysis was carried out by using the software MicroCal Origin 7.5 (Microcal Software, Inc.,

Northampton, MA, USA) based on a significance level of 95% ($P = 0.05$).

Results and discussion

Enzymatic hydrolysis

The clarifying effect of pectinase varies with the difference of pH values and pectin concentrations in different juices. In this study, four pectinases such as Pectinex 5XL, FSW, 10CZM and GFM were compared in terms of turbidity and transmittance of bitter gourd juice treated with 0.01% of pectinase concentration at $50\text{ }^{\circ}\text{C}$ for 2 h. The results (Table 1) indicated that the transmittance and turbidity of bitter gourd juice treated with GFM were 96.1% and 3.4 Nephelometric Turbidity Units (NTU), respectively, which was the best effect under the experimental condition ($P < 0.05$). This observation might be ascribed to the fact that GFM is a commercial preparation of three enzymes (polygalacturonase, pectinesterase and pectinlyase) and has higher enzyme activity ($500\ 000\ \text{u g}^{-1}$). And polygalacturonase (PG, EC 3.2.1.15) is a member of the pectinase family that acts on α -1-4 linkages of polygalacturonic acid in pectin, a cementing substance in plant cell wall, causing structural degradation. In most industrial applications, fungal PGs prove to be the most useful because of higher enzyme activity and optimum activity at a lower pH range, suited to most fruit and vegetable processing applications (Dziedzic, 1991). Therefore, the GFM pectinase was selected as the exclusive pectinase in our experiment.

Orthogonal test, $L_9(3^3)$, with three factors, such as enzyme concentration in the juice, temperature and reaction time, and three levels ($45, 50$ and $55\text{ }^{\circ}\text{C}$ for temperature, 0.005, 0.01 and 0.015% (w/w) for enzyme concentration, and 1.5, 2 and 2.5 h for time) was used to determine the optimal condition of enzymatic hydrolysis. In order to keep the nature quality of bitter gourd juice, the pH values in juices were unadjusted (pH = 5). According to the orthogonal test, the optimal condition of enzymatic process (EP) was reached at $50\text{ }^{\circ}\text{C}$ for 2 h

Table 1 Clarifying effect of different pectinases on the bitter gourd juice

	Control	Sample 1	Sample 2	Sample 3	Sample 4
A(%)	87 ± 4^d	93 ± 4^b	91 ± 6^c	93 ± 6^b	96 ± 4^a
B(NTU)	25 ± 2^a	12 ± 1^d	14 ± 1^c	17 ± 1^b	3.4 ± 0.2^e

A is transmittance, B is turbidity; Control means no enzyme; Sample 1, 2, 3 and 4 were used with Pectinex 5xl ($4500\ \text{PECTU mL}^{-1}$), FSW ($30000\ \text{u g}^{-1}$), 10CZM ($30\ 000\ \text{u g}^{-1}$) and GFM ($500\ 000\ \text{u g}^{-1}$) pectinase, respectively. Values are expressed with mean \pm standard error of mean (SEM). Repetition is 3. The different superscript letters, a, b, c and d in the end of digits each row indicate results were significantly different at confidential level of 95% ($P < 0.05$).

of enzymatic reaction time when GFM enzyme concentration was 0.005% (w/w). This observation is in agreement with the result reported by Ortega *et al.* (2004) that the PG optimum temperature ranged from 50 to 55 °C and the optimum pH for enzyme activity was 4.7 for Pectinex 3XL (with primarily polygalacturonase activity, produced by Novozymes Corp.). Ceci & Lozano (1998) reported that the optimum pH for PG was approximately 4.6. However, the curve for pectinase activity as a function of pH was much broader, being difficult to identify as a single optimal value. In this case, an optimal range of pH 5–6 may be defined. 50 °C was a well-defined breaking point for pure PG where enzymes rapidly decrease their activity. Liu & Luh (1978) reported that the commercial enzymes were more heat-tolerant than purified fractions. This phenomenon was attributable to the thermo-protective action of impurities.

The enzymatic hydrolysis was carried out under the optimal enzymatic hydrolysis condition. And the nutritional components before and after the EP were analysed. The results (Table 2) indicated that total saponins, total sugar and pH value did not change significantly during the EP ($P \geq 0.05$). However, vitamin C decreased significantly ($P < 0.05$), the transmittance of the juice increased from 87.2 to 93.6 and the turbidity decreased from 25 to 8.7.

According to Grassin & Fauquembergue (1996), pectin was the main cause of turbidity. Application of enzyme will reduce the turbidity by degrading the pectin content in the juice. Upon enzyme treatment, pectolytic enzymes break down the pectin molecules, which facilitate the formation of pectin-protein flocs, leaving a high transmittance and low turbidity juice (Yusof & Nurzarina, 1994; Alvarez *et al.*, 1998). The vitamin C loss was primarily caused by the thermal effect.

Ultra filtration

When the velocity for the feed stream that parallels to the film face was kept at 5 m s^{-1} , the permeate flux of

the juice was shown in Fig. 1. As can be seen, the permeate flux almost linearly increased as the operating pressure increased. However, when operating pressure was more than 0.15 Mpa, the slope of permeate flux curve became flat. Similar results were observed by Li *et al.* (2006) in clarification and sterilization of apple juice with a cross-flow pilot scale UF system using ceramic tubular membranes. Therefore, the operating pressure in pilot-plant-scale experiment was fixed at 0.15 Mpa.

Under the operating pressure of 0.15 MPa, the permeate flux of bitter gourd juice vs. operating time was shown in Fig. 2. After a rapid decrease at the beginning, the permeate flux decreased slowly. The permeate flux was up to 137 L per (h.m^2) at the starting period, but it was still kept at 110 L per (h.m^2) when the operating time was at 160 min, indicating that the ceramic film had a good productivity under the lower operating pressure, which is similar to the observation by Li *et al.* (2006) in clarification of apple juice with a cross-flow UF system using ceramic membranes.

It can be observed in Fig. 2 that permeate flux declined sharply within the initial phase of 10–20 min and becomes gradual thereafter. Our observation in cross-flow UF was similar to the phenomena in dead-end microfiltration of mosambi (a sweet orange) juice using low-cost ceramic membrane (Nandi *et al.*, 2009) that permeate flux declined sharply within the initial phase of 5–10 min and becomes gradual thereafter. The results are typical behaviours in UF processes when membrane fouling occurs. Fouling is caused by increased solute concentration at the membrane surface, which leads to concentration polarisation (CP). The CP is the major culprit in decreasing the permeate flux. A gel layer is formed on the membrane surface (Marshall *et al.*, 1993). The membrane fouling is one of the most important challenges faced in membrane UF operation. Different methods are available to reduce membrane fouling including cleaning with backwash and pulsating flow (Ben Amar *et al.*, 1990), alkaline solution (Nandi *et al.*, 2009) and acid injection (Mondor *et al.*, 2000),

Table 2 Physicochemical indexes of bitter gourd juice before and after enzymatic process (EP) and after ceramic film ultra filtration (UF), as well as after vacuum concentration alone (V) or after vacuum concentration combined with reverse osmosis (VR)

	T (%)	Tu (NTU)	TSS (°Brix)	pH (–)	TS (g per 100 mL)	Vc (mg per 100 mL)	TSA (mg per 100 mL)
Before	87 ± 2 ^c	25.0 ± 0.5 ^a	2.9 ± 0.2 ^a	5.1 ± 0.1 ^a	0.76 ± 0.09 ^a	18.76 ± 0.02 ^a	56 ± 2 ^a
After EP	94 ± 3 ^b	8.7 ± 0.4 ^b	2.9 ± 0.3 ^a	5.0 ± 0.1 ^a	0.73 ± 0.07 ^a	13.89 ± 0.03 ^b	56 ± 2 ^a
After UF	99 ± 2 ^a	1.2 ± 0.3 ^c	3.0 ± 0.5 ^a	5.0 ± 0.1 ^a	0.73 ± 0.07 ^a	12.86 ± 0.05 ^b	54 ± 2 ^a
V	–	–	24.0 ± 0.3 ^b	4.9 ± 0.1 ^a	6.00 ± 0.03 ^b	49.50 ± 0.04 ^c	413 ± 1 ^b
VR	–	–	24.3 ± 0.4 ^b	5.0 ± 0.1 ^a	6.04 ± 0.06 ^b	85.39 ± 0.05 ^d	426 ± 1 ^c

T is transmittance; TU is turbidity; TSS is total soluble solid; TS is total sugar; Vc is vitamin C, and TSA is total saponins. The vacuum concentration alone (V) is extended for 75 min, and vacuum concentration combined with reverse osmosis (VR) is extended for 22.5 min. Values are expressed with mean ± standard error of mean (SEM). Repetition is 3. The different superscript letters, a, b, c and d, in the end of digits each column indicate results were significantly different at confidential level of 95% ($P < 0.05$).

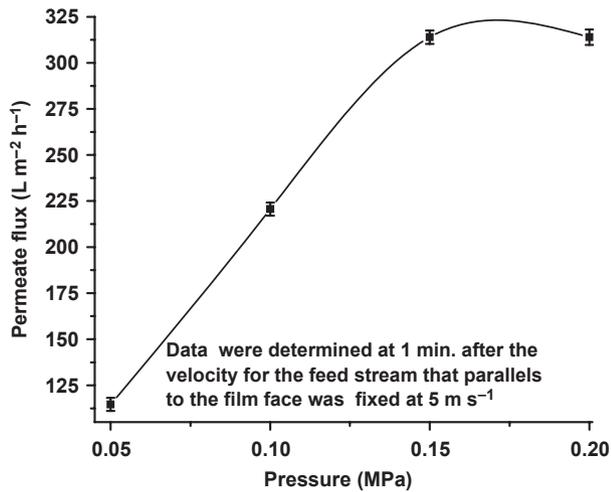


Figure 1 Relationship of operating pressure and permeate flux of bitter gourd juice. Data were determined at 1 min after the velocity for the feed stream that parallels to the film face was fixed at 5 m s⁻¹.

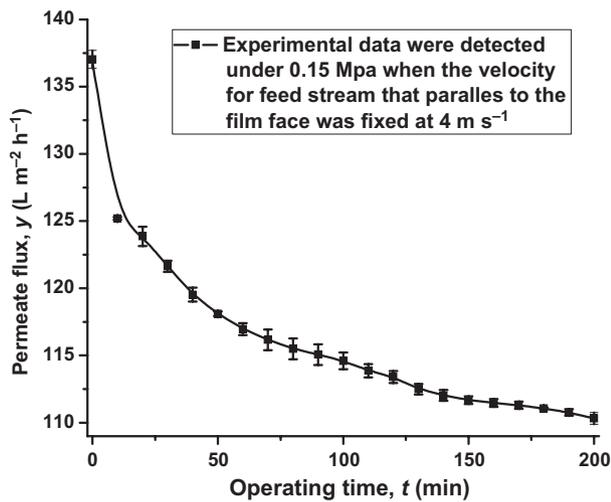


Figure 2 Relationship between flux of bitter gourd juice vs. operating time. Experimental data were detected under 0.15 Mpa when the velocity for feed stream that parallels to the film face was fixed at 4 m s⁻¹.

etc. In this work, 0.5% NaCl and 3.0% NaOH were used as backwash rinsing agents. At 50 °C the membrane was rinsed with NaCl and NaOH for 50 and 10 min, respectively, the permeate flux was recovered to about 97%. This observation was similar to the result reported by Li *et al.* (2006) when the mixed solution of 0.5% (w/w) hypochlorite sodium solution and 4% (w/w) NaOH solution was used as a cleaning agent at 50 °C, the flux recovery rate could be up to 98% in comparison with the initial pure water permeability of the membrane module. Also it was similar to the observation by Nandi

et al. (2009) that the membrane regained more than 98% of pure water flux of the fresh membrane by using alkaline (NaOH) solution combined with ultrasound.

After UF, the transmittance of the juice increased from 93.6 to 98.5 and turbidity decreased from 8.7 to 1.2; however, the hypoglycaemic and nutritional components, i.e., total saponins, mg per 100 mL (TSA) and Vc, pH, total soluble solute, total sugar (Table 2) did not change significantly ($P \geq 0.05$). Therefore, ceramic membrane was satisfactory for UF of bitter gourd juice in the pilot-plant-scale experiment. This observation was similar to the result of clarification of apple juice in a ceramic membrane system reported by Li *et al.* (2006) that pH, total acidity and total soluble solute did not change significantly and turbidity decreased from 10 to 0.20. The different effect of clarification between this study and Li *et al.* (2006) might primarily be ascribed to the difference of pore size, which was 200 nm in this study and 100 nm in the study by Li *et al.* (2006).

It is known that the vitamin C is highly sensible to thermal effects. In Table 2 is presented a significant reduction in the content of this vitamin during EP, but no effect is detected during the UF. In this work, the EP was carried out at 45–55 °C, but the temperature during UF was kept at 25–30 °C. For this reason, the vitamin C loss occurred during the process with major thermal effect.

Combination of reverse osmosis and vacuum concentration

The permeate flux decreased with the increase in total soluble solid concentration of the bitter gourd juice in the process of reverse osmosis (Fig. 3). After the total

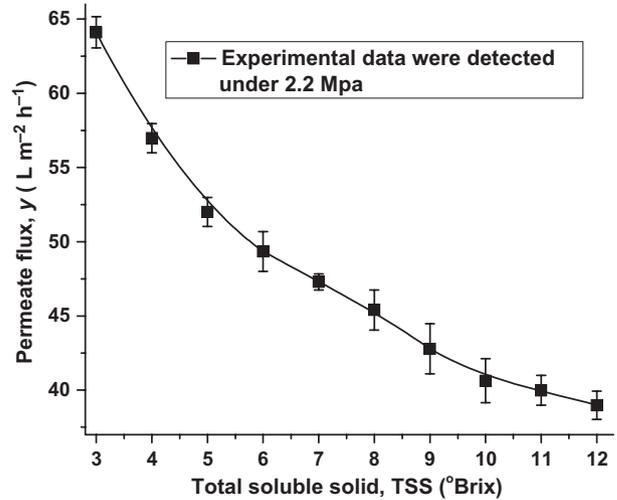


Figure 3 Relationship between permeate flux and total soluble solid of bitter gourd juice in reverse osmosis process. Experimental data were detected under 2.2 Mpa.

soluble solid increased above 10 °Brix, the permeate flux was too slow. And the permeate flux of bitter gourd juice at 10 °Brix was 63.3% of the initial permeate flux in the reverse osmosis process, which was coincidentally close to the Gold Section Scale (61.8%). Therefore, the final concentration of the total soluble solid was fixed at about 10 °Brix in the reverse osmosis process, and then the juice was further treated with the vacuum concentration at 55 °C under 0.005 Mpa until the concentration reached 24 °Brix.

The vitamin C content in the concentrated juice was well maintained in the process of reverse osmosis followed by vacuum concentration. As shown in Table 2, vitamin C content of the concentrated juice after the combined process was 85.39 mg per 100 mL, 1.725 times of concentration (49.5 mg per 100 mL) in the juice treated with vacuum concentration alone. At the same time, the concentration time decreased greatly from 75 to 22.5 min, indicating that production efficiency of the combined process was 3.33 (75/22.5) folds of the vacuum concentration alone.

As mentioned in the last paragraph of 3.2 UF, the vitamin C loss occurred during the process with major thermal effect. The same is applied to the results for the vitamin C content presented in Table 2, where the major damage is noted during process V that includes concentration under vacuum at 55 °C during 75 min. Process VR, a combined process with RO at ambient temperature, includes only 22.5 min at 55 °C under vacuum for the juice concentration.

Eutectic point

The eutectic point is the minimum melting point (at which the melting starts) of a solid phase of mixture. The first bright point in the dried layer of the product appeared on the fifth picture (Fig. S1) when the temperature in the freeze-drying chamber increased from -45 to -37.5 °C. The bright points were indeed the water drops on the surface of the frozen product. Therefore, the eutectic point of the frozen product of bitter gourd juice was -37.5 °C. When we used the electric resistance method described by Han & Bischof (2004), the eutectic point of freeze-dried product of bitter gourd juice with 24 °Brix was -28 °C when is measured during cooling, and -25 °C if the measurement was carried out during warming. This observation is similar to the results reported by Han & Bischof (2004) that the value of eutectic point for a KCl medium (-14.2 °C) if the measurement was carried out during warming was higher than that (-20.7 °C) when is measured during cooling. However, the values (-28 to -25 °C) detected by the electric resistance method were obviously higher than -37.5 °C detected by the freeze-drying microscopic method. Therefore, the accuracy of electric resistance method was not satisfactory.

The reason might be ascribed to the fact that the electric resistance method has several disadvantages when compared with the microscopic method. First, the electrodes used in the electric resistance method are buried in the sample, which damaged the structure of the sample, that is, the structure of detected sample is not as the same as that of the real frozen product, which might be the primary cause why the detected values of eutectic point are different between the contact method (electric resistance method) and non-contact method (microscopic method); second, in freezing process, the electrodes in the sample can act as nucleus, which can facilitate the crystal process, and thus, elevate the detected eutectic point; and third, in drying process, when the first water drop appeared on the surface of the sample the electric resistance will not change sharply, only if temperature increases enough so that many water drops can connect both electrodes the electric resistance between both electrodes will change sharply. As we know that the eutectic point detected by the electric resistance method is dependent on the sharply change of detecting resistance. That is why the values of eutectic point detected by the electric resistance method were higher than that by the freeze-drying microscopic method.

Conclusions

GFM pectinase can effectively clarify the bitter gourd juice. The optimal condition of EP for bitter gourd juice was 0.005% of GFM pectinase concentration in the juice at 50 °C for 2 h, and the total saponins, total sugar and pH value in bitter gourd juice did not change after EP and after ceramic film UF ($P \geq 0.05$), Vitamin C content of the concentrated juice after combination of reverse osmosis followed by vacuum concentration was 85.39 mg per 100 mL, 1.725 times of concentration (49.5 mg per 100 mL) in the juice treated with vacuum concentration alone. At the same time, the concentration time decreased greatly from 75 to 22.5 min, indicating an increase of 3.33 folds of production efficiency. The eutectic point of the frozen product of bitter gourd juice was -37.5 °C.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. The eutectic point detected with freeze-drying microscope system. In the picture, A is ice layer of the frozen product, B is dried layer of the frozen product, C is background, and D is wall of the freeze-drying chamber; No. 1–8 indicated the order No. of the pictures took in the freeze-drying chamber during sublimation of the product. Because the eutectic point is the temperature representing the minimum melting point (at which the melting starts) of a solid phase of mixture, and the dried layer consists of ice and bitter gourd materials. The first bright point appeared on the dried layer of the product was indeed a liquid water drop that was formed from the melting ice when the temperature reached at $-37.5\text{ }^{\circ}\text{C}$ in the fifth picture.

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