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Physiochemical, Clarification, and Antioxidant Properties of Aqueous Extract of Xiao-Chai-Hu-Tang as Affected by Membrane Process

ZER-RAN YU[1], MEI-SU LIN[2], YIH-MING WENG[2] AND BE-JEN WANG[2]*

[1.] *Graduate Institute of Natural Healing Sciences, Nanhua University, Chiayi, Taiwan, R.O.C.*
[2.] *Department of Food Science, National Chiayi University, Chiayi, Taiwan, R.O.C.*

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ABSTRACT

Xiao-chai-hu-tang (XCHT) is a traditional Chinese herbal medicine which has been demonstrated to exhibit antioxidant and hepatoprotective functions. To reduce the unpleasant dark brown color and precipitates during storage, the performances of membrane process on the clarification and antioxidant capability of XCHT was evaluated. A membrane system equipped with either a microfiltration membrane (0.45 μm) or one of four ultrafiltration membranes (UK 200, UK 50, UP 20 and UK 10) was used. Our results indicated that less total solid was filtered through the membranes and thus a higher degree of clarification degree was achieved in permeates. Higher concentrations of active compounds, such as total phenolic compounds, saikosaponins, glycyrrhizin and baicalin, were detected in permeate using membranes of smaller pore-size of membranes. Antioxidant capabilities, including scavenging of free radicals, reducing power and total antioxidant activity of permeates, increased as compared to those of feed, particularly of permeates obtained with a UK 10 ultrafiltration membrane. Taken together, membrane process clarified aqueous herbal extract and selectively partitioned bioactive components in permeates, thus enhancing the feasibility of XCHT to be a commercial health drink product.

Key words: membrane process, clarification, antioxidant, Xiao-chai-hu-tang, health herbal drinks

INTRODUCTION

Xiao-chai-hu-tang (XCHT), a traditional Chinese medicine for curing chronic hepatitis, is an aqueous extract from Bupleuri Radix, Pinelliae Tuber, Scutellariae Radix, Zizyphi Fructus, Ginseng Radix, Glycyrrhizae Radix, and Zingiberis Rhizome[1]. Pharmacokinetic studies show that XCHT possesses antiviral activity[2] and hepatoprotective activity against CCl_4- or H_2O_2-induced hepatic injury[3,4] and hepatitis B virus[2,5].

XCHT contains many bioactive components such as saikosaponins, glycyrrhizin, and baicalin[6]. Those compounds were reported to exhibit antioxidant capabilities and protect liver from chemical-induced injury[7,8]. Saikosaponins, a group of triterpene glycoside compounds, possess scavenging activity against reactive oxygen species and inhibit peroxidation in primary cultured rat hepatoctes[9]. Glycyrrhizin, a triterpene glycoside, exhibits superoxide radical scavenging activity and reduces lipid peroxidation[10]. Baicalin, a flavonoid compound, displays the scavenging activity against hydroxyl, DPPH and alkyl radicals in a dose-dependent manner[7]. Baicalin also effectively inhibits lipid peroxidation induced by Fe^{2+}-ascorbic acid[7] and showed antifibrotic effects in a rat liver model[4].

XCHT is an aqueous extract from dried stems and roots of herbal plants and it appears in dark brown color and frequently forms precipitates during storage. In order to gain consumer acceptance of the commercial XCHT products, the intensity of dark color should be reduced and the formation of precipitates be minimized. Moreover, it is also equally important that the consumer-appealing oriented manufacture procedures should not lower the contents of bioactive compounds and functions of the original extract. Among many processing techniques, the membrane process is a relatively simple separation technology and an appropriate candidate to meet the requirements[11,12].

The membrane process, including microfiltration and ultrafiltration, separates sample based on the molecular weight and/or particle size. Generally, microfiltration is a membrane process for separating colloidal and suspended

* Author for correspondence. Tel: +886-5-2717626;
Fax: +886-5-2777922; E-mail: bejen@mail.ncyu.edu.tw

particles in the range of 0.05-10 microns; thus, microfiltration is suitable for biomass clarification and recovery. Ultrafiltration is a selective fractionation process and widely applied to concentrate suspended solids and solutes of molecular weight greater than 1,000 Da[13]. However, the performance of membrane process might influence the distribution of functional components in permeates and retenates and those composition changes might in turn alter the physiochemical characteristics and functionalities[14,15]. Therefore, the aims of this study were to determine the parameters of membrane process and to investigate the physiochemical and functional properties of XCHT treated with various membranes.

MATERIALS AND METHODS

I. *Chemical Reagents*

Ferric chloride, Folin-Ciocalteu's reagent, gallic acid, peroxidase, 1,1-diphenyl-2-picryl hydrazyl (DPPH), sodium dihydrogen phosphate (NaH_2PO_4) and sodium hydrogen phosphate (Na_2HPO_4) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Potassium ferricyanide ($K_3Fe(CN)_6$), sodium carbonate anhydrous (Na_2CO_3) and trichloroacetic acid (TCA) were from Showa Chemical Industry Co., Ltd. (Tokyo, Japan). Acetonitrile, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and hydrogen peroxide (H_2O_2) were from Merck Co. (Darmstadt, Germany). Baicalin, glycyrrhizin, saikosaponin a, saikosaponin c and saikosaponin d were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

II. *Preparation of XCHT Aqueous Extracts*

XCHT composed of seven different plants, including Bupleuri Radix, Scutellariae Radix, Glycyrrhizae Radix, Zizyphi Fructus, Pinelliae Tuber, Zingiberis Rhizome, and Ginseng Radix was purchased from a local herb pharmacy (Chiayi, Taiwan). The preparation of XCHT was carried according to the formulation described by Sakaguchi *et al.*[1]. It contained Bupleuri Radix, Pinelliae Tuber, Scutellariae Radix, Zizyphi Fructus, Ginseng Radix, Zingiberis Rhizome, and Glycyrrhizae Radix at the weight ratio of 7 : 5 : 3 : 3 : 3 : 2 : 1[16]. All the ingredients were pooled, grounded and sieved with a 0.42 m/m screen. The powdered herbs (100 g) were extracted with distilled water (900 mL) at 95°C in a temperature-controlled shaking water bath for 1 h and then filtered with filter paper (Whatman No. 1, Springfield Mill, Maidstone, England). The filtrate was stored at -20°C until further used.

III. *Membrane Process*

The membrane system was implemented either with 0.45 μm microfiltration membrane or one of various molecular weight cut-off (MWCO) ultrafiltration membranes (UK 200, UK 50, UP 20 and UK 10). UK 200 (cut-off size 200 kDa), UK 50 (cut-off size 50 kDa) and UK 10 (cut-off size 10 kDa) are made of polysulfone and UP 20 (cut-off size 20 kDa) membrane is made of aromatic polyamide. All the membranes used in this study are disc membranes and from Toyo Roshi Kaisha, Ltd. (Tokyo, Japan). This experiment was conducted at room temperature (25°C). The aqueous extracts of XCHT (300 mL) were filtered through a Model PC351 stirred cell (Corning Ltd., Corning, NY, USA) of 76 mm diameter membrane. A transmembrane pressure of 5 kg/cm^2 (480.35 kPa) was supplied by a compressed nitrogen cylinder and a stirred rotation of 600 rpm. Permeate was collected by retriever (Retriever 500, Isco Ltd., Lincoln, Neb, USA) and the weight of permeate obtained was monitored during the operation time to calculate flux. The physiochemical characteristics of permeates and retenates were analyzed after treated with various membranes at volume concentration ratios (VCR) equaled to 4, where VCR is the ratio of the initial feed volume to the volume of retenate remaining at any time during the microfiltration process. VCR of 1 refers to the fresh feed.

IV. *Analysis of Fouling Layer Resistance*

Fouling layer resistance was calculated according to conventional filtration theory[17]. The volumetric flux through a membrane can be characterized by:

$$\frac{1}{Am} \times \frac{dV}{dt} = \frac{\Delta P}{\mu\,(Rm + Rf)} \quad (1)$$

where *Am* denotes the surface area of membrane, *V* the permeate volume, *t* the time, *ΔP* the transmembrane pressure, μ the permeate viscosity, *Rm* the resistance of the bare membrane and *Rf* the fouling layer resistance.

When all solids are retained on the membrane surface, fouling layer resistance (*Rf*) can be expressed as

$$Rf = \alpha p \times C_B \times \frac{V}{Am} \quad (2)$$

where αp denotes the specific resistance and C_B represents the concentration of bulk solids. After substituting Eq. (2) into Eq. (1) and rearranging, the equation becomes:

$$\frac{t}{V} = Rm \times \frac{\mu}{\Delta P \times Am} + V \times \frac{\alpha p \times C_B \times \mu}{Am^2 \times \Delta P} \quad (3)$$

Thus, the slope of the linear region of a *t/V* versus *V* plot can be used to determine the fouling layer specific resistance ($\alpha p\ C_B$). All specific resistance measurements were made in triplicate.

V. *Determination of Physiochemical Characteristics*

Total solids, viscosity and color changes of permeates and retenates were determined according to AOAC 990.20[18]. The viscosities of the solutions were measured at 20°C using a Brookfield synchro-lectric viscometer with a LV No. 2 spindle at 60 rpm (Model LVT, Brookfield Engineering Laboratories, Ltd. Stoughton, MA, USA). Color changes

during processing were determined using a color difference meter (Σ90 Color Measuring System, Nippon Denshoku, Tokyo, Japan) and expressed as Hunter L, a and b values.

VI. *Determination of Active Compounds*

The concentration of total phenolic compounds was determined spectrophotometrically using Folin-Ciocalteu's reagent by the method of Singleton and Rossi[19]. The absorbance at 760 nm was measured after thoroughly mixing 100 μL samples or gallic acid (as the standard), 500 μL Folin-Ciocalteu's reagent, 400 μL sodium carbonate (75 g/L) and 5 mL ddH_2O, and then keeping the mixture in the dark for 30 min. The concentrations of baicalin, glycyrrhizin, and saikosaponins (saikosaponin a, c and d) were analyzed on a high performance liquid chromatographer (HPLC L-7000, Hitachi, Tokyo, Japan) equipped with an Inertsil ODS-3 C18 column (250 × 4.6 mm). For the analysis of baicalin and glycyrrhizin, the elution solution consisted of (A) 25 mM NaH_2PO_4 (pH 2.5) and (B) acetonitrile. With the initial elute composition set at 30% B, the programmed gradient elute was gradually shifted to 100% B in 25 min, and held for 10 min before returning to the initial condition. Detection wavelength was 277 nm for baicalin and 252 nm for glycyrrhizin[20]. For the analysis of saikosaponins, the programmed gradient elution using acetonitrile-water from 40 : 60 (v/v) to 50 : 50 (v/v) in 10 min at a flow rate of 1.0 mL/min and detection wavelength of 203 nm were used[21]. Standard curves established with baicalin, glychrrhizin, and saikosaponins (a, c, and d) were used for the calculation. All of these determinations were repeated three times.

VII. *Antioxidant Characteristics*

Total antioxidant activity (TAA) was determined by the method of Arnao *et al.*[22] with some modifications. ABTS (0.25 mL, 100 μM), peroxidase (0.25 mL, 4.4 U/mL) and H_2O_2 (0.25 mL, 50 μM) and distilled water (1.5 mL) were mixed and kept in the dark for 1 h to form $ABTS^{\cdot+}$ radicals. One milliliter of permeate or rertenate (0.5, 1, 3, 5, and 10 mg/mL) were added and the absorbance at 734 nm was measured. TAA (%) was calculated according to the equation: TAA (%) = [1-($A_{sample\ at\ 734\ nm}$/ $A_{blank\ at\ 734\ nm}$)] × 100. IC_{50} represents the required concentration to inhibit 50% lipid peroxidation.

The scavenging of DPPH radicals was carried out following the method of Shimada *et al.*[23]. One milliliter of permeate or retenate (0.5, 1, 3, 5 and 10 mg/mL) was mixed with 5 mL of DPPH solution (0.1 mM) and shaken vigorously. The mixture was kept at room temperature for 50 min before the absorbance at 517 nm was read. The scavenging activity was determined by comparing the absorbance with that of the blank (100%) containing only DPPH and solvent. The scavenging effect (%) was calculated according to the equation: scavenging effect (%) = [1-($A_{sample\ at\ 517\ nm}$/ $A_{blank\ at\ 517\ nm}$)] × 100. SC_{50} represents the required concentration to scavenge 50% of DPPH radicals.

The reducing power of permeates and retenates was measured according to the method of Oyaizu[24]. One milliliter of permeate or retenate (0.5, 1, 3, 5, 10 mg/mL), phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide (1 mL, 10 mg/mL) were incubated at 50°C for 20 min. After incubation, trichloroacetic acid (1 mL, 100 mg/mL) was added to the mixture and then the mixture was centrifuged at 650 *g* for 10 min. The absorbance of the supernatant (2 mL) mixed with distilled water (2 mL) and ferric chloride (0.4 mL, 1 mg/mL) was measured at 700 nm after setting for 10 min. Reducing power was expressed as OD_{700nm}. OD_{700}= 1 represents the required concentration of feed, permeates or retenates, when the reading at 700 nm equals to 1.

VIII. *Statistical Analysis*

Data were expressed as mean ± SD determined from triplicate analysis. Student's *t*-test was used to compare the difference between feed and treatment groups. ANOVA procedures analyzed the variance among the samples. A p value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Flux reduction due to concentration polarization and membrane fouling resulted from the accumulation of feed components in the membrane pores and on the membrane surface is the major problems associated with membrane process[25]. As membrane pore size increases, the flux generally improves due to lower flow resistance. This work examined the effect of various MWCO size membranes on permeate flux and fouling operated at transmembrane pressure (TMP) 5.0 kg/cm^2 and 25°C. Figure 1 shows the plots of the permeate flux versus time for XCHT treated with various cut-off size membranes. Resembled to other observations[12], permeate flux increased as membrane pore size increased due

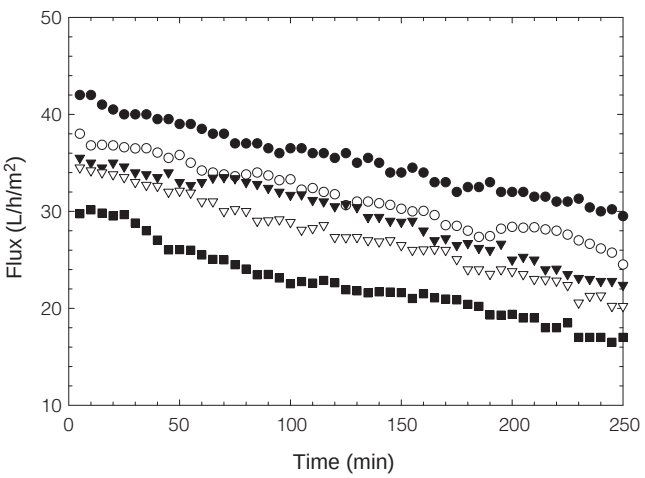

Figure 1. Permeation flux versus time for XCHT clarified using various membranes. (●: 0.45 μm membrane, ○: UK200 membrane, ▼: UK 50 membrane, ▽: UP 20 membrane, ■: UK 10 membrane).

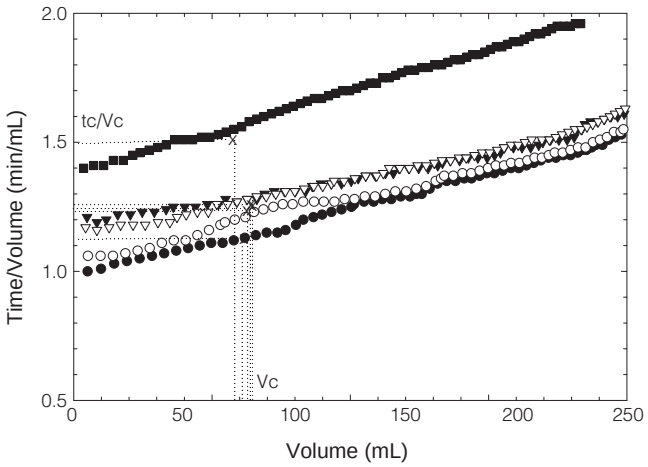

Figure 2. Typical time/volume versus time for XCHT clarified using various membranes. (●: 0.45 μm membrane, ○: UK 200 membrane, ▼: UK 50 membrane, ▽: UP 20 membrane, ■: UK 10 membrane).

Table 1. Consolidation time (tc, min) and resistance ($\alpha_p C_B$, $m^{-2} \times 10^{-6}$) of fouling layers formed on a 0.45 μm microfiltration membrane and UK200, UK50, UP20, UK10 ultrafiltration membranes during clarification of Xiao-chai-hu-tang

Membrane Type	t_C (min)	$\alpha_P C_B$ ($m^{-2} \times 10^{-6}$)
0.45 μm	67.2	2.13
UK 200	75.0	3.62
UK 50	76.8	3.63
UP 20	77.4	3.83
UK 10	93.6	4.78

to lower flow resistance. Figure 2 is the typical time/volume (t/V) versus volume (V) curves for the example given in Figure 1. For most solutions, the fouling layer formed usually rapidly formed on the membrane surface, leading to a low liquid flux[25,26]. Following the initial formation, plots of typical time/volume (t/V) versus volume (V) were linear[27]. The slopes in Figure 2, representing the fouling layer specific resistance ($\alpha p C_B$) were calculated. Moreover, the fouling layer consolidation time (t_C) was also estimated based on the V_C and t_C/V_C values at the beginning of linear relationship. Table 1 lists the t_C and $\alpha p C_B$ values for XCHT treated with various MWCO membranes. The analytical results show that consolidation time of XCHT clarified with 0.45 μm microfiltration membrane and ultrafiltration membrane UK 200, UK 50, UP 20, and UK 10 were 67.2, 75.0, 76.8, 77.4 and 93.6 min, respectively ($p < 0.05$). The fouled consolidation filtrated with microfiltration membrane was faster than that filtrated with other ultrafiltration membranes. However, the resistance to the fouling layer treated with microfiltration membrane ($\alpha p C_B$ = 2.13 $m^{-2}*10^{-6}$) was lower than those treated with ultafiltration membranes, for which reistances ($\alpha p C_B$) were 3.62, 3.63, 3.83, and 4.78 $m^{-2}*10^{-6}$ for UK 200, UK 50, UP 20, and UK 10 membrane, respectively. This behavior resembled other observations[12,28], which demonstrated that elevated pore size caused less flow resistance and thus achieved the benefit of higher permeation flux.

Table 2 showed the physiochemical properties of permeates and retenates processed with various MWCO membranes at VCR= 4. As described in previous studies[14,29], membrane selectivity influences the components distribution in permeate and retenate. In the present study, the total solid concentration of feed was 72.9 mg/mL. The concentrations

Table 2. Physiochemical properties of feed, permeates and retenates of XCHT processed with the 0.45 μm, UK 200, UK 50, UP 20 and UK 10 membranes

	Total solids	Apparent viscosity	Color		
	(mg/mL)	(cPs)	L	a	b
Feed	72.9 ± 0.6	6.4 ± 0.6	5.5 ± 0.1	0.7 ± 0.1	-0.5 ± 0.1
Permeate					
0.45 μm	49.6* ± 1.2	4.8* ± 1.2	72.0* ± 1.2	2.0* ± 0.1	41.5* ± 1.2
UK 200	43.1* ± 1.3	4.8* ± 1.3	75.0* ± 1.3	2.4* ± 0.1	43.0* ± 1.3
UK 50	42.1* ± 0.9	3.6* ± 0.9	76.4* ± 0.9	2.6* ± 0.1	43.7* ± 0.9
UP 20	40.0* ± 0.6	3.6* ± 0.6	76.9* ± 0.6	2.8* ± 0.1	44.0* ± 0.6
UK 10	38.1* ± 0.7	3.1* ± 0.7	80.7* ± 0.7	3.3* ± 0.1	44.4* ± 0.7
Retenate					
0.45 μm	151.9* ± 3.3	37.9* ± 3.3	4.1* ± 0.1	0.6 ± 0.1	-0.6 ± 0.1
UK 200	153.2* ± 2.5	49.0* ± 2.5	4.0* ± 0.1	0.3* ± 0.1	-0.7 ± 0.1
UK 50	153.6* ± 4.2	49.1* ± 4.2	4.0* ± 0.1	0.1* ± 0.1	-0.9* ± 0.1
UP 20	156.8* ± 5.6	49.9* ± 5.6	3.9* ± 0.1	0.1* ± 0.1	-1.1* ± 0.1
UK 10	166.7* ± 5.5	55.5* ± 5.5	3.5* ± 0.1	0.1* ± 0.1	-1.1* ± 0.1

Data are mean ± SD (n = 3).
* Significantly different ($p < 0.05$) compared to the feed.

of total solids decreased in permeates (38.1-49.6 mg/mL) but increased in retenates (151.9-166.7 mg/mL) processed with all types of MWCO membranes (Table 2). However, total solids in permeates consistently decreased as membrane pore size decreased. The viscosities of permeates (3.1-4.8 cPs) decreased but those of retenates (37.9-55.5 cPs) increased compared to the feed (6.4 cPs). This is similar to the observation of total solid content indicates that more total solids could not pass through the membrane and thus retained in retenate causing the viscosities increase in retenate[30].

The color indexes (L, a and b values) of all types of permeates were increased as compared to those of the feed. The L, a and b values for permeates were 72.0-80.7, 2.0-3.3 and 41.5-44.4, respectively. On the other hand, the L, a and b values for the feed were 5.5, 0.7 and -0.5, respectively. Low L values indicate a dark color. The results indicate that permeates were clarified by the membrane process and became light yellow. In addition to the reduction of total solid and apparent viscosity, which might lead to less precipitate formation, the intensity of dark color was successfully reduced by the membrane process.

Since XCHT is the hot water extract of Chinese herbs, it contains both high molecular weight compounds (HWC) and low molecular weight compounds (LMC)[7]. LWC, including baicalin, glycyrrhizin, and saikosaponins, have been isolated and demonstrated their various pharmacological effects[6,31]. Membrane process might affect the distribution of low molecular bioactive components between permeates and retenates[11]. Table 3 showed the total polyphenols, saikosaponins, glycyrrhizin and baicalin in the feed, and in the permeates and retenates of XCHT treated with the 0.45 μm, UK 200, UK 50, UP 20 and UK 10 membranes. Generally speaking, significantly higher amounts ($p < 0.05$) of all analyzed major active compounds were detected in permeates than in the retenates, and the contents of those low molecular bioactive compounds in permeates increased as the membrane pore size reduced. The highest saikosaponins and glycyrrhizin contents were detected in the XCHT permeates prepared with UK 10 ultrafiltration membranes (0.66 and 1.08 mg/g XCHT, respectively). Higher concentrations of baicalin in permeates were obtained when XCHT prepared with UP 20 and UK 10 ultrafiltration membranes. The results indicate that the membrane process could be used to concentrate the LWC, especially the compounds with bioactive functions, in permeates in the case of XCHT. Health drinks with higher amount of bioactive components are worth developing.

Among those bioactive components, saikosaponins possessed scavenging activity against free radical and inhibited peroxidation of rat liver homogenate[32] while glycyrrhizin exhibited antioxidant effects upon $FeCl_2$-ascorbate-induced lipid peroxidation in mice liver homogenate and upon superoxide radical scavenging activity[10]. Baicalin was shown to scavenge DPPH radical in a dose-dependent manner. Moreover, baicalein down-regluated PDGF-beta receptor and prevented liver fibrosis induced by CCl_4[2].

Higher content of bioactive compounds in permeates might imply stronger antioxidant capabilities. Table 4 listed the antioxidant characteristics of XCHT in permeates and retenates. The total antioxidant activity, expressed as the ability to scavenge $ABTS^{\cdot+}$, of permeates and retenates demonstrated that clarification significantly boosted the antioxidant activity. The concentrations of 2.0, 1.3-1.9, and 2.1-2.7 mg/mL were needed to scavenge 50% $ABTS^{\cdot+}$ radicals for feed, permeates, and retenates (Table 4), respectively.

Table 3. The contents of polyphenol, and bioactive compounds of feed, permeates and retenates of XCHT processed with the 0.45 μm, UK 200, UK 50, UP 20 and UK 10 membranes

	Polyphenol (mg GA/g)	Saikosaposins (mg/g)	Glycyrrhizin (mg/g)	Baicalin (mg/g)
Feed	0.16 ± 0.01	0.46 ± 0.01	0.89 ± 0.01	0.048 ± 0.001
Permeate				
0.45 μm	0.21* ± 0.01	0.47 ± 0.01	0.90 ± 0.01	0.051* ± 0.001
UK 200	0.25* ± 0.01	0.47 ± 0.01	0.93* ± 0.01	0.066* ± 0.001
UK 50	0.31* ± 0.01	0.52* ± 0.01	1.02* ± 0.01	0.070* ± 0.001
UP 20	0.36* ± 0.01	0.53* ± 0.01	1.06* ± 0.01	0.078* ± 0.001
UK10	0.45* ± 0.01	0.66* ± 0.01	1.09* ± 0.01	0.080* ± 0.001
Retenate				
0.45 μm	0.14 ± 0.03	0.43 ± 0.03	0.77* ± 0.03	0.040* ± 0.003
UK 200	0.09* ± 0.03	0.36* ± 0.03	0.76* ± 0.03	0.036* ± 0.003
UK 50	0.09* ± 0.04	0.33* ± 0.04	0.67* ± 0.04	0.033* ± 0.004
UP 20	0.05* ± 0.06	0.32* ± 0.06	0.64* ± 0.06	0.030* ± 0.006
UK 10	0.02* ± 0.06	0.32* ± 0.06	0.61* ± 0.06	0.025* ± 0.006

Data are mean ± SD (n = 3).
* Significantly different ($p < 0.05$) compared to the feed.

Permeates treated with a UK 10 ultrafiltration membrane exhibited the best antioxidant activity.

Free radicals in the biological systems initiate lipid peroxidation; therefore, the depletion of free radicals by free radical scavengers inhibits the peroxidation. With IC_{50} in the range of 2.8-3.8 mg/mL for permeates and 5.6-6.3 mg/mL for retenates, the results indicated that more free radical scavengers remained in permeates, particularly permeates clarified with a UK 10 ultrafiltration membrane.

Reducing power, the capability of donating a hydrogen or electron, is one of antioxidant mechanisms. The reducing power (absorbance at 700 nm) increased in permeates (OD_{700} = 1.36-1.68) but decreased in retenates (OD_{700} = 0.83-0.96) as compared with feed (OD_{700} = 1.06) (data not shown). A higher reading indicates a stronger reducing power. In Table 4, the reducing power was expressed by the concentrations when the absorbance at a wavelength of 700 nm equaled to 1. The concentrations of 4.6, 2.7-3.3, and 4.7-5.4 mg/mL were needed to reach OD_{700} = 1 for feed, permeates, and retenates, respectively. In general, those data showed that clarification improved the reducing power of XCHT. Among the various MWCO membrane treatments, permeates treated with a UK 10 ultrafiltration membrane exhibited the best reducing power.

Ultrafiltration is widely applied to concentrate suspended solids and solutes of molecular weight greater than 1,000 Da. Our results revealed that the highest amounts of bioactive compounds contents were detected in permeate obtained with UK 10 ultrafiltration membrane. Furthermore, as the bioactive contents increased in permeates treated with UK 10 membrane, the best inhibitory effect on total antioxidant activity, the highest free radical scavenging activity and reducing power were also observed in UK 10 membrane-treated permeates.

Table 4. Antioxidant characteristics of feed, permeates and retenates of XCHT processed with the 0.45 μm, UK 200, UK 50, UP 20, and UK 10 membranes

	Total antioxidant activity	DPPH scavenging	Reducing power
	IC_{50}	SC_{50}	OD_{700}=1
Feed	2.10 ± 0.11	5.31 ± 0.26	4.64 ± 0.17
Permeate			
0.45 μm	1.97 ± 0.21	3.82* ± 0.12	3.30* ± 0.12
UK 200	1.93 ± 0.18	3.51* ± 0.13	3.21* ± 0.13
UK 50	1.60* ± 0.15	3.42* ± 0.09	3.02* ± 0.09
UP 20	1.35* ± 0.08	2.86* ± 0.06	2.85* ± 0.06
UK 10	1.30* ± 0.09	2.81* ± 0.07	2.73* ± 0.07
Retenate			
0.45 μm	2.16 ± 0.33	5.64 ± 0.36	4.71 ± 0.33
UK 200	2.36 ± 0.13	5.72* ± 0.23	5.13* ± 0.25
UK 50	2.44* ± 0.24	5.78* ± 0.33	5.25* ± 0.42
UP 20	2.50* ± 0.36	6.10* ± 0.24	5.40* ± 0.56
UK 10	2.71* ± 0.25	6.32* ± 0.39	5.46* ± 0.55

IC_{50}: the required concentration (mg/mL) to inhibit 50% lipid peroxidation;
SC_{50}: the required concentration (mg/mL) to scavenge 50% of DPPH radicals;
OD_{700}=1: the required concentration (mg/mL) when the reading at 700 nm equals to 1.
Data are mean ± SD (n = 3).
* Significantly different ($p < 0.05$) compared to the feed.

CONCLUSIONS

According to the performance of one microfiltration membrane and four ultrafiltration membranes tested, all permeates become less viscous and lighter in color, which indicates that membrane processing clarifies the darkness of herbal extract and reduces the formation of precipitates. Furthermore, all the antioxidant capabilities of permeates increased as compared to the original feeds. Among the five different permeates, XCHT permeates treated with UK 10 ultrafiltration membrane results in the permeate containing the highest contents of bioactive components and thus exhibits the best total antioxidant activity, the highest free radical scavenging activity and reducing power. Therefore, the UK 10 membrane is well-suited for the concentration of bioactive compounds of XCHT and is the most feasible membrane for developing health drinks with good consumer appealing properties. Additionally, our findings suggest that membrane process is applicable for other commercially manufactured herbal products to enhance the overall appearance while maintaining the biofunctions.

REFERENCES

1. Sakaguchi, S., Tsutsumi, E. and Yokota, K. 1993. Preventive effects of a traditional Chinese medicine (sho-saiko-to) against oxygen toxicity and membrane damage during endotoxemia. Biol. Pharm. Bull. 16: 782-786.
2. Chang, J. S., Wang, K. C. and Liu, H. W. *et al.* 2007. Sho-saiko-to (Xiao-Chai-Hu-Tang) and crude saikosaponins inhibit hepatitis B virus in a stable HBV-producing cell line. Am. J. Chin. Med. 35: 341-351.
3. Sugihara, N., Furuno, K., Kita, N., Murakami, T. and Yata, N. 1992. Distribution of quinidine in rats with carbon tetrachloride-intoxicated hepatic disease. J. Pharmacobiodyn. 15: 167-174.
4. Sun, H., Che, Q. M., Zhao, X. and Pu, X. P. 2010. Anti-fibrotic effects of chronic baicalein administration in a CCl_4 liver fibrosis model in rats. Eur. J. Pharmacol. 631: 53-60.
5. Tseng, Y. P., Wu, Y. C., Leu, Y. L., Yeh, S. F. and Chou, C. K. 2010. *Scutellariae radix* suppresses hepatitis B virus production in human hepatoma cells. Front. Biosci. E 2: 1538-1547.
6. Ohtake, N., Nakai, Y. and Yamamoto, M. *et al.* 2004. Separation and isolation methods for analysis of the

active principles of Sho-saiko-to (SST) oriental medicine. J. Chromatogr. B 812: 135-148.
7. Gao, Z., Huang, K., Yang, X. and Xu, H. 1999. Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of *Scutellaria baicalensis* Georgi. Biochim. Biophys. Acta 1472: 643-650.
8. Mu, Y., Liu, P. and Du, G. *et al.* 2009. Action mechanism of Yi Guan Jian decoction on CCl_4 induced cirrhosis in rats. J. Ethnopharmacol. 121: 35-42.
9. Matsuda, H., Murakami, T., Ninomiya, K., Inadzuki, M. and Yoshikawa, M. 1997. New hepatoprotective saponins, bupleurosides III, VI, IX, and XIII, from Chinese Bupleuri Radix: Structure-requirements for the cytoprotective activity in primary cultured rat hepatocytes. Bioorg. Med. Chem. Lett. 7: 2193-2198.
10. Jeong, H. G., You, H, J. and Park, S. J. *et al*. 2002. Hepatoprotective effects of 18 β-glycyrrhetinic acid on carbon tetrachloride-induced liver injury: Inhibition of cytochrome P450 2E1 expression. Pharmacol. Res. 46: 221-227.
11. Girard, B. and Fukumoto, L. R. 1999. Apple juice clarification using microfiltration and ultrafiltration polymeric membranes. LWT Food Sci. Technol. 32: 290-298.
12. Giorno, L., Mazzei, R. and Oriolo, M. *et al*. 2005. Effects of organic solvents on ultrafiltration polyamide membranes for the preparation of oil-in-water emulsions. J. Colloid Interface Sci. 287: 612-623.
13. Postlethwaite, J., Lamping, S. R. and Leach, G. C. *et al.* 2004. Flux and transmission characteristics of a vibrating microfiltration system operated at high biomass loading. J. Membrane Sci. 228: 89-101.
14. Rassis, D. and Saguy, I. S. 1995. Kinetics of aseptic concentrated orange juice quality changes during commercial processing and storage. Int. J. Food Sci. Technol. 30: 191-198.
15. Yu, Z. R., Hung, C. C., Weng, Y. M., Su, C. L. and Wang, B. J. 2007. Physiochemical, antioxidant and whitening properties of extract from root cortices of mulberry as affected by membrane process. LWT Food Sci. Technol. 40: 900-907.
16. Sakaguchi, S., Tsutsumi, E. and Yokota, K. 1993. Preventive effects of a traditional Chinese medicine (Sho-Saiko-To) against oxygen toxicity and membrane damage during endotoxemia. Biol. Pharm. Bull. 16: 782-786.
17. Riedl, K., Girard, B., Lencki, R. W. 1998. Interactions responsible for fouling layer formation during apple juice microfiltration. J. Agric. Food Chem. 46: 2458-2464.
18. AOAC. 1990. The Official Methods of Analysis of the Association of Official Analytical Chemists. 15th ed. The Association of Official Analytical Chemists. Arlington, VA, USA. AOAC Official Method 990.20.
19. Singleton, V. L. and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Amer. J. Enol. Vitic. 16: 144-158.
20. Ong, E. S. and Len, S. M. 2003. Pressurized hot water extraction of berberine, baicalein and glycyrrhizin in medicinal plants. Anal. Chim. Acta. 482: 81-89.
21. Park, I. S., Kang, E. M. and Kim, N. 2000. High-performance liquid chromatographic analysis of saponin compounds in *Bupleurum falcatum*. J. Chromatogr. Sci. 38: 229-233.
22. Arnao, M. B., Cano, A. and Acosta, M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 73: 239-244.
23. Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem. 40: 945-948.
24. Oyaizu, M. 1986. Antioxidative activity of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. Nippon Shokuhin Kogyo Gakkaishi 35: 771-775.
25. Vladisavljevi'c, G. T., Vukosavljevi'c, P. and Bukvi'c, B. 2003. Permeate flux and fouling resistance in ultrafiltration of depectinized apple juice using ceramic membranes. J. Food Eng. 60: 241-247.
26. Todisco, S., Pena, L., Drioli, E. and Tallarico, P. 1996. Analysis of the fouling mechanism in microfiltration of orange juice. J. Food Process Pres. 20: 453-466.
27. Lencki, R. W. and Riedl, K. 1999. Effect of fractal flocculation behavior on fouling layer resistance during apple juice microfiltration. Food Res. Intl. 32: 279-288.
28. Chamchong, H. and Noomhorm, A. 1991. Effect of pH and enzymatic treatment on microfiltration and ultrafiltration of tangerine juice. J. Food Process. Eng. 14: 21-34.
29. Evans, P. J. and Bird, M. R. 2006. Solute-membrane fouling interactions during the ultrafiltration of black tea liquor. Food Bioprod. Process. 84: 292-301.
30. Padilla-Zakour, O. and McLellan, M. R. 1993. Optimization and modeling of apple juice cross-flow microfiltration with a ceramic membrane. J. Food Sci. 58: 369-374.
31. Taira, Z., Yabe, K. and Hamaguchi, Y. *et al.* 2004. Effects of Sho-saiko-to extract and its components, baicalin, baicalein, glycyrrhizin and glycyrrhetic acid, on pharmacokinetic behavior of salicylamide in carbon tetrachloride intoxicated rats. Food Chem. Toxicol. 42: 803-807.
32. Wang, B. J., Liu, C. T., Tseng, C. Y., Wu, C. P. and Yu, Z. R. 2004. Hepatoprotective and antioxidant effects of *Bupleurum kaoi* Liu (Chao et Chuang) extract and its fractions fractionated using supercritical CO_2 on CCl_4-induced liver damage. Food Chem. Toxicol. 42: 609-617.