CHAPTER III

MATERIAL AND METHODS

3.1 Preparation of fermented liquor and rice wine

The fermented liquor (FL) was prepared by a traditional batch fermentation process. For experiments 3.2.1 and 3.2.2, imported Thai glutinous rice (Three Ladies Brand Sanpatong Sweet Rice, Pacific Eastern Trading Corp, Los Angeles, CA), which was purchased at a local grocer in Atlanta, Georgia, was used. For experiments 3.2.3 and 3.5, glutinous rice from Sanpatong, ChiangMai, Thailand was used. The rice was washed 2-3 times until the water was clear and then soaked in 2 parts of water for approximately 2-3 hours. The rice was placed in a traditional hand-woven steaming basket which was placed in an aluminum steaming pot, and then the rice was steamed for approximately 0.5 hours. When the rice was cool, it was mixed with 2% w/w starter culture (Hang Tai Products, Hong Kong, for experiment 3.2.1 and 3.2.2 and products from Lumpang, Thailand, for experiments 3.2.3 and 3.5) and 50% w/w deionized water. The mixture was stored in 5-gal glass carboy jars under aerobic fermentation at 30 ^{O}C to ensure conversion of starch to fermentable sugars. After 4 days, deionized water was added to adjust the soluble solids to 24 ^{O}Brix and the containers were kept under anaerobic conditions for a total of 10 days. For experiments 3.2.1 and 3.2.2, the fermentation vessels (see Appendix A) were fitted with "S" locks (Vinquiry, Windsor, CA) to allow CO_2 to escape while preventing air from entering. The fermented liquor was racked from the Lees by siphoning with a 3/8 inch inner diameter plastic hose. For experiment 3.2.3 and 3.5, the fermentation

vessel was 2 L glass jar covered with plastic lid. The rice wine (RW) was obtained by filtering the fermented liquor through 10 mesh sieve. Samples of FL and RW were stored at 3 ^{O}C for less than 1 week prior to analysis. The characteristics of FL and RW were determined as described in 3.4.

Generally, Thai rice wine starter culture is mixed culture of fungi (*Amylomyces rouxuii and Rhizopus oryezae*) and yeast (*Saccharomycopsis fibuligera*, *Saccharomyces cereviseae* and *Candida utilis*). It also contains rice flour and herbs (garlic, ginger, galingale, liquorices, pepper and long pepper (Lotong, 1998).

3.2 Microfiltration experiments

3.2.1 Effect of solid-liquid separation methods on microfiltration performance and microfiltered rice wine characteristics

3.2.1.1 Solid-liquid separation methods

The cloudy fermented liquor was subjected to three different solid-liquid separation methods prior to microfiltration. The first batch (10 L) was immediately poured through two layers of 18 mesh cheesecloth. The second batch (10 L) was allowed to sediment for 3 hours before decanting the clear liquid. The final batch (10 L) was subjected to centrifugation at 3000xg for 30 min. The liquid obtained from these three separation methods was called rice wine (RW). The pretreated samples were kept at 3 ^{O}C for less than 1 week prior to subsequent microfiltration experiments.

3.2.1.2 Microfiltration

All microfiltration experiments were conducted at 20 ^{O}C in a 2 L batch stainless-steel microfiltration unit equipped (Amicon Model 2000, Millipore, USA) with a magnetically coupled stirring unit (0-340 rpm) immediately above the membrane surface (see Figure B1 in Appendix B). The ΔP was controlled with a pressure gauge connected to a laboratory-grade compressed nitrogen tank, which was fed into the top of the microfiltration unit. This allowed ΔP from 68.9 to 689.5 kPa. The stirring speed and ΔP used in this experiment were 100 rpm and 276 kPa, respectively. Hydrophilic PVDF (polyvinylidene difluoride) membranes were used with average pore sizes of 0.45 μm (Durapore®, Millipore, USA). These were placed at the bottom of the unit and had an effective filtration area of 0.0133 m^2. All experiments were conducted in duplicate. The permeate (microfiltered rice wine, MRW) was collected and weighed every 2 min. Filtration rate, α, and compressibility were determined as described in 3.3. The characteristics of feed (RW) and permeate (MRW) were determined as described in 3.4. After each use, the microfiltration unit was sanitized by cleaning with 250 ppm sodium hypochlorite (2 L), followed by sterile deionized water (2 L) at 20 ^{O}C and 276 kPa ΔP. A new membrane was used for each 2 L batch.

3.2.2 Effect of transmembrane pressure, membrane pore size and stirring speed on microfiltration performance and microfiltered rice wine characteristics

Prior to microfiltration, FL was allowed to sediment for 3 hours, then the sediment was discarded and the RW was collected. The microfiltration experiments were as described in 3.2.1. The details on membrane pore size, ΔP, and stirring speed used in each experiment are shown in Table 3.1. The MRW was collected and weighed every 2 min. Filtration rate, α, and compressibility were determined as described in 3.3. The characteristics of RW and MRW were determined as described in 3.4.

Table 3.1 Summary of operation parameters used for experiment 3.2.2

Effects of	Operation parameters used		
	Membrane pore size (μm)	Stirring speed (rpm)	ΔP (kPa)
Membrane pore size and transmembrane pressure	0.10	100	138
			276
			414
			552
	0.22	100	138
			276
			414
			552
	0.45	100	138
			276
			414
			552
Stirring speed	0.22	0	138
			552
		100	138
			552
	0.45	0	138
			552
		100	138
			552

3.2.3 Effect of suspended solid size, size distribution and concentration on specific cake resistance

3.2.3.1 Sample preparation

Rice wine samples having suspended solid of different average particle size and concentration were prepared by filtration of FL through 18 mesh cheesecloth and then 35 mesh standard sieve (Retsh, Haan, Germany) for particle size < 500 μm, or 100 mesh standard sieve for particle size < 100 μm, or sedimentation of FL for 3 hr for particle size < 10 μm. Each supernatant was centrifuged using Universal 32R centrifuge (Hettich, Tuttlingen, Germany) at 3,600 xg and 25 ^{O}C for 30 min. The supernatant was microfiltered as explained in 3.2.3.2. The cake was resuspended in the filtered supernatant and followed by determination of suspended solid content by following the method described in 3.4, and particle size and particle size distribution by using a laser diffraction particle size analyzer (Mastersizer 2000, Malvern, UK). Then each sample was diluted with the filtered supernatant to obtain the suspended solid content in the range of 0.04 to 1.0% w/w.

To obtain samples of different particle size distribution, FL was filtered through 35, and then 325 mesh standard sieves. The retentate ("R") contained suspended solid of particle size in the range of 45-500 μm. The filtrate ("F") contained suspended solid of particle size < 45 μm. Both fractions were centrifuged using Universal 32R centrifuge (Hettich, Tuttlingen, Germany) at 3,600 xg and 25 ^{O}C for 30 minute. The supernatant was microfiltered as explained in 3.2.3.2. Each sediment was resuspended in the filtered supernatant and followed by determination of suspended solid content, and then diluted with filtered supernatant to obtain the

suspended solid content of 1% w/w. The suspension obtained from "R" was called "L" fraction, while the suspension obtained from "F" was called "S" fraction. The "S" fraction was mixed with the "L" fraction. The weight ratios of "S" fraction to "L" fraction were 0:100, 75:25, 50:50, 25:75, and 100:0. The mixtures were diluted with the filtered supernatant to obtain the suspended solid content of 0.2, 0.5, and 1%w/w. The particle size and particle size distribution of these mixtures were determined with a laser diffraction particle size analyzer (Mastersizer 2000, Malvern, UK).

3.2.3.2 Microfiltration

All microfiltration experiments were conducted without stirring at 25 ^{O}C in a 0.5 L batch acrylic resin microfiltration unit (locally made in Bangkok, Thailand) immediately above the 0.45 μm cellulose acetate membrane (Sartorius, Goettingen, Germany) having 0.0033 m^2 effective filtration area (Figure B2 in Appendex B). The ΔP was controlled at 200 kPa with a pressure gauge connected to a laboratory-grade compressed nitrogen tank, which fed into the top of the microfiltration unit. After each use, the microfiltration unit was rinsed with 1 L sterile deionized water at 25 ^{O}C and 200 kPa. A new membrane was used for each run. All experiments were conducted in duplicate. The permeate was collected and weighed every 2 min. Filtration rate and α were determined as described in 3.3.

3.3 Determination of filtration rate, specific cake resistance, and cake compressibility

The permeate (MRW) from 3.2.1.2 and 3.2.2 were collected and weighed every 2 min through the course of the microfilration process. The permeate volume was calculated from permeate weight and density, which was determined as described in 3.4. The filtration rate was determined from the change in volume with time. The cake filtration theory was applied to evaluate filtration performance in terms of α. Under constant ΔP conditions, the inverse filtration rate is a function of α as shown in Equation 2.13. Therefore, $1/J$ was plotted against V and the slope "s" was determined. Then α for each ΔP was calculated from Equation 2.14. Finally, the compressibility of the cake layer (n) was determined from Equation 2.15.

3.4 Characterization of fermented liquor, rice wine, and microfiltered rice wine

The chemical and physical characteristics as well as microbial counts of the FL, RW, and MRW were analyzed as listed in Table 3.2. The pH was determined with an Orion Sure-Flow pH electrode (AOAC 960.19, 2005). Titratable acidity was determined by titration with 0.1 N NaOH according to Method 962.12 (AOAC, 2005) and expressed as g citric acid per 100 g sample. Alcohol content was determined from the boiling point using an Ebulliometer (Dujardin-Salleron, Arcueil, France). Total soluble solid content was measured with a refractometer (Atago, Tokyo, Japan) following Vaillant *et al.* (1999). To determine total suspended solid content, sample of known weight was filtered through the weighed membrane (0.22 μm Nitrocellulose, Millipore, Bedford, MA, USA). The cake was dried in hot air oven

(model ED, Winder, Tuttlingen, Germany) at 105 ^{O}C for 24 hours, and then dried cake was weighed and total suspended solid content calculated. Viscosity was measured by capillary viscometry (Cannon-Fenske viscometer No. 150, Kimble Glass, Vineland, NJ, USA) following AOAC 974.07 (2005), while specific gravity was determined with a pycnometer (25 ml, Kimble Glass, Vineland, NJ, USA) following AOAC 920.56 (2005). Particle size distribution of the samples was determined with a laser diffraction particle size analyzer (Mastersizer 2000, Malvern, UK). Enough sample was introduced into water in the flow cell to give an obscuration factor of at least 20%.

Table 3.2 The characteristics of fermented liquor, rice wine and microfiltered rice wine to be determined.

Characteristics	Fermented liquor	Rice wine	Microfiltered rice wine
Physicochemical characteristics			
pH	✓	✓	✓
Titratable acidity	✓	✓	✓
Alcohol content	✓	✓	✓
Total soluble solid content	✓	✓	✓
Total suspended solid content	✓	✓	✓
Particle size and its distribution of suspended solid	✓	✓	✓
Viscosity			✓
Density			✓
Microbiological characteristics			
Yeast count	✓	✓	✓
Total bacteria count	✓	✓	✓
Lactic acid bacteria count			✓

For FL and RW, yeast counts were determined using Petrifilm™ Yeast and Mold Plates (AOAC 997.02, 2005 and Robinson *et al.*, 2000). Total bacterial counts of FL and RW were determined using standard pour plate method (AOAC 966.23, 2005). The aerobic bacteria count of MRW was determined using hydrophobic grid membrane filter method (AOAC 986.32, 2005) using plate count agar (PCA, Difco Laboratories, Livonia, MI, USA). For yeast and lactic acid bacteria counts of MRW, sample (100 ml) was filtered through 0.45 μm hydrophobic grid membrane (Millipore, Bedford, MA, USA) as described by Harrigan (1998), the membrane was then aseptically removed and placed on surface of agar. For yeast count, the agar was made from the Wallerstein nutrient agar (WL nutrient medium, Difco Laboratories, Livonia, MI, USA) and incubated in aerobic condition at 30 ^{O}C for 48 hours (see Appendix C). For lactic acid bacteria count, the deMan, Rogosa, Sharpe agar (MRS agar, Difco Laboratories, Livonia, MI, USA) was used and incubated in anaerobic condition, in order to enhance growth of homo- and hetero-fermentative lactic acid bacteria, at 35 ^{O}C for 3 days (see Appendix D).

3.5 Effect of solid-liquid separation methods on taste of microfiltered rice wine

Prior to microfiltration, FL was preseparated as described in 3.2.1.1 then followed by microfiltration described in 3.2.3.2. Three sets of test were conducted. The 1st set was MRW preseparated by filtration through cheesecloth against MRW preseparated by sedimentation, the 2nd set was MRW preseparated by filtration through cheesecloth against MRW preseparated by centrifugation, and the 3rd set was MRW preseparated by sedimentation against MRW preseparated by centrifugation. For each set, 20 experienced panelists evaluated the difference in taste of MRW

prepared with different preseparation methods using the Duo-Trio test. Three glasses of 10 ml. MRW (at 5 ^{O}C) coded with random three digit number were presented to each judge. The scoresheet was shown in Appendix E.