Physiochemical Properties of Polysaccharides Extracted from Tofu Processing Wastewater

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ABSTRACT: Yield, gelation, viscosity, emulsifying properties, and sugar composition of polysaccharides extracted from tofu processing wastewater under acidic conditions at different temperature levels and incubation times were determined. Optimum extraction for water-soluble polysaccharides was at pH 1.5 and 100 °C over an incubation period of 6 hours. Extraction ratio (%) and yield of high-molecular-weight polysaccharides were higher at pH ranges of 1.5 to 3.0 with extraction temperatures of 80 to 100 °C. Most water-soluble polysaccharides extracted under strong acidic conditions and above 80 °C remained fluid, while most extracted within the range of 45 to 80 °C gelled. Pure polysaccharides exhibited better emulsifying properties than water-soluble polysaccharides. Apparent viscosities of water-soluble polysaccharides were dependent on sugar concentration.

Keywords: tofu processing wastewater, extraction ratio, polysaccharides, apparent viscosity, emulsifying

Introduction

TOFU SERVES AS AN IMPORTANT SOYbean [*Glycine max* (L.) Merr.] product that has been widely used in a variety of dishes by some Asians for many centuries. It is a highly digestible and nutritive product (Schroder and others 1973) and also serves as an inexpensive source of protein. As a result of the economic importance of the oil and protein constituents of soybeans, most scientific investigations have been concerned primarily with those constituents rather than with the accompanying carbohydrates.

Oligosaccharides, especially sucrose, raffinose, and stachyose, have long been recognized as constituents of soybeans (Aspinall and others 1967; Mulimani and others 1997). Soybean oligosaccharides exhibit important physiological functions in humans (Koga and others 1993). Some reports have been published on soybean polysaccharides (Morita 1965; Yamaguchi and others 1996). Interesting results on soybean polysaccharides and water-soluble soybean polysaccharides and their extractions under different conditions have also been reported (Morita 1965; Yamaguchi and others 1996; Yoshi and others 1996; Furuta and others 1998). Some of these findings have revealed that soybean polysaccharides are composed of arabinogalactans and galacturonic acid, regardless of their solubility and the fact that soybean cotyledon meal contains complex polysaccharides that yield L-arabinose, D-xylose, L-fucose, L-rhamnose, D-galactose, and D-galacturonic acid on hydrolysis. Very few reports have been published on the extraction of water-soluble soybean polysaccharides under acidic, neutral, and/or alkaline conditions, while the extraction of polysaccharides from tofu processing wastewater has yet to be reported.

Recently, the use of water-soluble polysaccharides extracted from soybean okara as emulsifiers or viscoelastic reagents has been found to be economical in the food industry (Yoshi and others 1996). This work, therefore, was undertaken to investigate and establish an optimum level for the extraction of water-soluble polysaccharides and high-molecular-weight polysaccharides from tofu processing wastewater, and to investigate the extent to which the application of heat in the range of 45 to 100 °C at varying pH levels (1.5 to 6.0) can affect the yield, sugar composition, gelation, and apparent viscosity of water-soluble polysaccharides extracted from tofu processing wastewater under acidic conditions. The emulsifying properties and heat stability of pure polysaccharides and water-soluble polysaccharides extracted are investigated. Also an attempt is made in this study to extract polysaccharides from tofu processing wastewater by using isopropanol and deionized water reaction mixture.

The word "wastewater" as used in this study refers to water oozed during pressing after soybean soaking and hot-grinding.

Materials and Methods

THE WASTEWATER DISCHARGED JUST after soybean soaking and hot-grinding was obtained from a small-scale tofu processing factory located at the outskirts of Wuxi, P. R. China. The enzyme AS1398 (origin *Bacillus subtilis*; type proteinase; cellulase activity e"1 $\times$ 10^5 µ/g) was obtained from Genecor Bio-Products Co. Ltd., Wuxi, P. R. China. The catalyst 1-methylimidazole used in the acetylation of monosaccharides and standard dextrans were bought from the Shanghai branch of Sigma Chemical Co. Some of the dextrans obtained from ICN Biochemicals were given to us as a gift. All other chemicals used were of reagent-grade and obtained either from other laboratories or from the Chemical Department of Southern Yangtze University.

Sample and pre-treatment

Tofu wastewater (25 L) was centrifuged (5000 $\times$ g for 30 min) using a tripod centrifuge produced by Zhang Jiagang Centrifuge factory, Zhang Jiagang, Jiangsu Province, P. R. China. The supernatant was discarded and the centrifugate was oven-dried for 18 hours at 60 °C. The oven-dried centrifugate was milled into a fine powder using a portable electrically operated laboratory milling machine (Shanghai, Shenbo Instruments Company, Shanghai, P.R. China). The powder (52.0 g) was put into airtight containers and stored in desiccators.

Table 1—Sugar composition of PPTW.

Crude protein (%)	6.97
Ash (%)	1.80
Sugars (%)	-
Rhamnose	1.3
Arabinose	3.9
Xylose	0.5
Glucose	28.8
Galactose	65.4

Extraction and purification of tofu wastewater polysaccharides

The oven-dried sample was defatted at 40 °C with 5 times the weight of n-hexane. The extraction of protein from the sample was carried out according to the process for isolating soybean proteins (Morita 1965). The defatted sample (crude protein, 27.77%; ash, 2.4%; as dried matter) was extracted 3 times with 8 times the volume of deionized water at 50 °C for 1 hour, after the pH had been adjusted to 7.0 with 5 *M* NaOH. The residue (crude protein, 16.41%; ash, 2.0%; as dried matter) was obtained as precipitate after centrifugation (5000 × g for 20 min). The remaining protein was hydrolyzed twice with AS1398 enzyme after 5 times the weight of a mixture of isopropanol and deionized water in a ratio of 1:4 was added to the precipitate followed by homogenization. The enzyme was added to the homogenate in a bioreactor (Shanghai, Shenbo Instruments Company, Shanghai, P.R. China) at 1.5% of the weight of the solid matter after the suspension was maintained at constant pH and temperature of 7.0 and 38 °C, respectively, and incubated for 4 hours while continuously stirring to hydrolyze the remaining protein. The hydrolyzed and dissolved proteins were removed by centrifugation (5000 × g for 10 min). The resulting precipitates were oven-dried at 60 °C for 18 hours (crude protein, 6.97%; ash, 1.1%; total carbohydrate, 21.5%; as dried matter) and is referred to in this study as pure polysaccharide from tofu processing wastewater (PPTW).

wastewater from tofu processing
↓centrifuge (5,000 x g for 30 min)
insoluble
↓ defatted
↓ extracted with water x 3
↓ pH 7.0, 50°C
↓
centrifuge
↓
insoluble
↓homogenized (isopropanol-water
↓mixture, 1:4) and hydrolyzed with AS1398 x 2
↓ (pH 7.0, 38°C, 4 h)
insoluble
↓oven-dried
↓ 60°C for 18 hours
PPTW
↓distilled water added
↓pH adjusted with 5 N HCl
heat extraction
↓ centrifuge (5000 x g for 10 min)
soluble
↓neutralized with 5 N NaOH, filtered (0.45 μm)
↓
SPTW
↓3 x volume of ethanol added
ethanol precipitation
↓
insoluble
↓washed with 80% v/v EtOH
insoluble
↓dried at 60°C for 15 hours
HM-SPTW

Figure 1—Flow chart of extraction and purification of polysaccharides from tofu wastewater.

Extraction of water-soluble polysaccharides from PPTW

Deionized water was added to PPTW to adjust the concentration to 10% and the pH values were adjusted to 1.5, 3.0, 4.5, or 6.0 with 5 *M* HCl. Each suspension (20 g) was put into 50-mL heat-resistant tubes with screw caps (Wujin Menghe Huangshan Glass Instruments Factory, Wujin, P.R. China) for heating at 45, 60, 80, or 100 °C and incubated for 0.5, 1.5, 3.0, or 6.0 hours. After cooling to room temperature, the solutions were neutralized with 5 *M* NaOH and centrifuged (5000 × g for 10 min); the supernatant was filtered through a 0.45-¼m membrane and is referred to as SPTW.

Recovery of high-molecular-weight polysaccharides from SPTW

To 10 mL of SPTW, 3 times the volume of 99.5% special-grade ethanol (C_2H_5OH) was added; precipitates were removed by centrifugation (3500 × g for 10 min), washed with 3 times the volume of 80% (v/v) ethanol, dried at 60 °C, and were referred to as HM-SPTW. The yields of PPTW, SPTW, and HM-SPTW were determined according to the method of Dubois and others (1956). A summary of the extraction procedure is given in Figure 1.

Calculation of extraction ratio

The extraction ratio (ER), an indication of the degree of extraction of SPTW and HM-SPTW from PPTW, was calculated by the method of Furuta and others (1998).

Observation of gelation

The degree of gelation of each extracted solution of SPTW was observed after storage at 4 °C for 10 hours. Those solutions that underwent gelation were heated in boiling water for 5 min to determine if the process was reversible.

Viscosity determination

Apparent viscosities (temperature 25 °C; velocity $4.276 \times 10^{-2} \pm 1 \times 10^{-2}$ L/s) of each of the extractable SPTW solutions were determined immediately after extraction and neutralization using the 1101 model T.A. Instruments for Thermal Analysis and Rheology (a subsidiary of Waters Corporation) produced by T.A. Instruments Production Center, New Castle, England.

Determination of emulsifying properties of PPTW and SPTW

The emulsifying properties and stability were determined according to the meth-

Table 2—Extraction ratio of SPTW (%) and gelation of SPTW.[a]

	45 °C				60 °C				80 °C				100 °C			
pH	0.5 h	1.5 h	3.0 h	6.0 h	0.5 h	1.5 h	3.0 h	6.0 h	0.5 h	1.5 h	3.0 h	6.0 h	0.5 h	1.5 h	3.0 h	6.0 h
1.5	23.9*	42.1*	49.6*	57.3*	25.4	47.8* + 61.0*		+74.3*	58.4	67.3* + 70.4*		+78.9*	+70.3	71.1	77.5	83.2
3.0	ND	—	21.6	34.6	ND	20.5	48.3	56.8	ND	30.4*	34.5*	36.8	40.1*	54.2*	62.5	70.4
4.5	ND	—	15.2	24.3	—	—	29.7	41.9	—	—	43.1	48.6	38.1	44.3	59.7	68.8
6.0	ND	—	—	8.7	—	—	—	10.9	—	—	21.6	30.3	14.3	18.6	49.4	64.5

[a] *, gelation; *+, reversibility; ND, not detectable. ER was determined from the equation ER (%) = S/P × 100, where ER is the extraction ratio, S is the total sugar concentration of SPTW solution measured by the phenol-sulfuric acid method, and P is the PPTW concentration in the suspension before extraction. Each SPTW solution was observed for gelation after storage at 4 °C for 10 hours. Those solutions that underwent gelation were heated in boiling water to identify their gelling reversibility.

Table 3—Yield of HM-SPTW (%).[a]

	45 °C				60 °C				80 °C				100 °C			
pH	0.5 h	1.5 h	3.0 h	6.0 h	0.5 h	1.5 h	3.0 h	6.0 h	0.5 h	1.5 h	3.0 h	6.0 h	0.5 h	1.5 h	3.0 h	6.0 h
1.5	18.9	30.3	38.6	44.7	20.4	40.0	48.2	56.6	21.8	41.3	46.4	58.3	30.2	48.6	55.4	60.9
3.0	ND	—	2.1	5.8	—	6.8	18.4	27.6	ND	30.4	31.6	33.3	38.1	46.3	50.4	56.3
4.5	ND	—	0.8	1.6	—	—	8.3	15.6	ND	—	19.6	23.4	21.6	28.3	30.7	36.1
6.0	ND	—	—	0.4	—	—	—	2.6	—	—	3.1	5.2	4.1	6.6	11.7	18.4

[a] ND, "not detectable"; HM-SPTW was precipitated from the SPTW solution with 80% (v/v) ethanol. The yield of HM-SPTW from PPTW was determined with the same equation as used to calculate ER of SPTW.

ods of Pearce and Kinsella (1978) and Matsudomi and others (1994). For the determination of emulsifying properties, emulsions of oil (pure soybean salad oil produced by East Ocean Oils and Grains Industries, Zhang Jiagang, Jiangsu Province, P.R. China) and 0.1% of each of the polysaccharide components (PPTW, SPTW, and Gum Acacia powder, a commonly used food emulsifier that was used as a control) were formed in a ratio of 1:3, respectively. The solutions were shaken and homogenized (24000 rpm for 1 min) at room temperature using an ULTRA-TURRAX T25 model homogenizer produced by Janke and Kunkel GmbH & Co. KG, IKA Labortechnik, Staufen, Germany. Aliquots (100 μL) were taken from the bottom of the emulsion after standing for 0, 1, 3, 7, 10,15, 20, 25, and 30 min and diluted with 5 mL of 0.1% sodium dodecyl sulfate (SDS) solution. The absorbance of the diluted emulsion was measured at 500 nm. The emulsifying activity was determined from the absorbance measured immediately after emulsion formation (0 min). For the measurement of emulsion stability, each of the emulsions under test were held at constant temperatures of 60 and 80 °C in a temperature-controlled water bath while being gently stirred. Periodically, aliquots of the emulsions were taken for dilution and turbidity measurements, as described above.

Chemical methods of analysis

Crude protein and ash contents were measured by AOAC (1984) methods. Total sugar was measured by the phenol-sulfuric acid method (Dubois and others 1956), using glucose ($C_2H_2O_6$) as a standard. After hydrolysis and conversion of monosaccharides produced into alditol acetate derivatives, the quantitative and qualitative neutral sugar components were determined by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis, respectively. The sample was prepared according to the method described by Blakeney and others (1983). Briefly, the sample preparation was carried out as follows: 10 μg of each sample (PPTW, SPTW, and HM-SPTW) was treated in a test tube with 125 μL of 72% (w/w) H_2SO_4 and dissolution of the samples in the acid was accelerated by agitation in a vortex mixer. The test tube was sealed under pressure and placed in a temperature-controlled oven at 121 °C for 1 hour. The remaining processes of hydrolysis, reduction of monosaccharides, and acetylation were carried out according to the method used by Blakeney and others (1983). The pipetted samples, which were stored in 1-mL septrum-capped vials, were used for GC and GC-MS analyses (results obtained for the latter are not shown in this paper). GC was carried out with a Shimadzu GC-14A under the following conditions: OV1701 column (30 × 0.25 mm) with column temperature of 195 to 240 °C, nitrogen gas carrier (1 mL/min), FID (Flame Ionization Detector) temperature of 230 °C, and injection temperature of 250 °C.

Molecular weight determination of PPTW, SPTW, and HM-SPTW by SE-HPLC

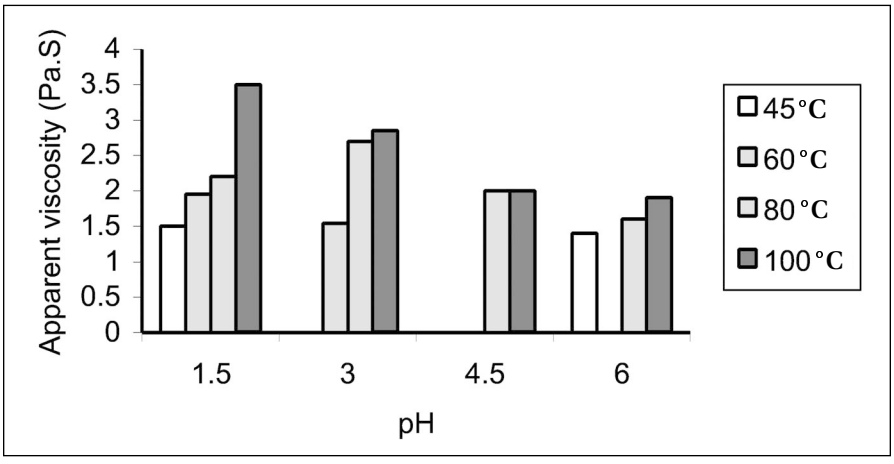
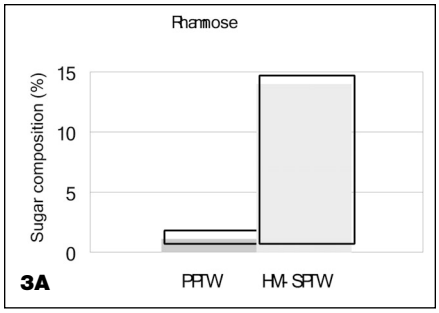
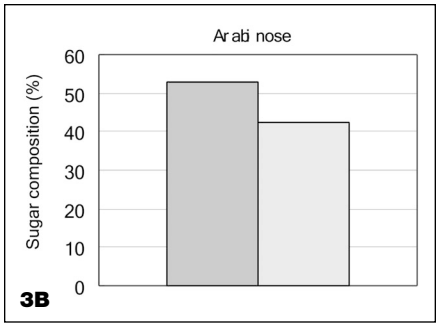


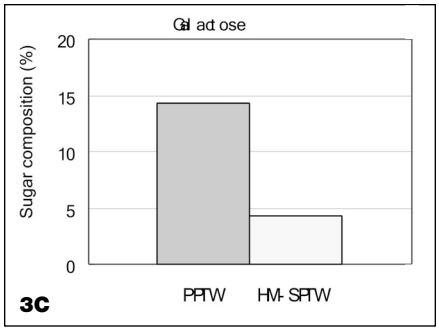
Figure 2—Apparent viscosity of SPTW

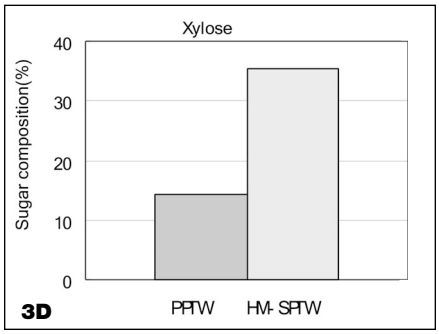
Table 4—Molecular weights of PPTW, SPTW, and HM-SPTW extracted with 0.1 M $NaNO_3$ as calculated from the SE-HPLC analysis.

	Molecular weight (Da)		
Peak	A (PPTW)	B (SPTW)	C (HM-SPTW)
1	16000	716000	960000
2	<1000	330000	<1000
3	-	<2000	-
4	-	<1000	-









Figures 3 A-E—Sugar composition (%) of PPTW and HM-SPTW

Size exclusion–high-performance liquid chromatography (SE-HPLC) was performed on a Waters Associates (Milford, Mass., U.S.A.) liquid chromatography system equipped with a model 510 pump, WISP model 712 injector, and a model 2410 refractive index detector. The detector signal was recorded and integrated by a data module integrator (Waters 740). An Ultrahydrogel guard column followed size-exclusion Ultrahydrogel linear (7.8-mm i.d. × 300 mm) and 2 Ultrahydrogel 120 columns (120 Å pore size). Elution was with 0.1 M $NaNO_3$ at a flow rate of 0.4 mL/min at 40 °C. The samples were solubilized in 0.1 M $NaNO_3$ solution at 35 °C for 30 min. After solubilization, the samples under test were centrifuged (1500 × g for 10 min) and filtered through a 0.45-µm filter under vacuum. The clear supernatant solution (20 µL) was injected into the SE-HPLC column. The calibration equation was obtained by linear regression of a plot of retention time against log molecular weight of standards. The standard plot was obtained by injecting 5 dextran standards with varying molecular weights (1×10^4 Sigma Company; 7.2×10^4 ICN Biochemicals; 2×10^5 ICN Biochemicals; 5×10^5 Sigma Company; 2×10^6 Sigma Company) into the SE-HPLC unit.

Results and Discussion

Sugar composition of PPTW

The protein removal rate in the isopropanol-water reaction mixture was comparatively higher than those obtained in similar studies where only deionized water was used (Furuta and others 1998). In addition to the effect of the enzyme, this result could be attributed to the ability of proteins to dissolve in isopropanol-water reaction mixture while polysaccharides remained insoluble. The sugar composition of PPTW as obtained from GC analysis is shown in Table 1. These results confirm earlier findings on the polysaccharide components of soybeans (Morita 1965; Aspinall and others 1967; Furuta and others 1998).

Extraction ratio and gelation of SPTW solutions

At 100 °C, the mean ER at pH 1.5 was 75.5% with the lowest ER (70.3%) obtained after 0.5 hour and the highest ER (83.2%) after 6.0 hours of incubation (Table 2). It can also be seen from Table 2 that at all levels of extraction, ER was dependent on incubation time, with higher extraction ratios being obtained with an increase in incubation time. Also, values obtained for ER were found to be decreasing with an increase in pH values, irrespective of temperature and incubation time, as higher ER values were obtained at pH 1.5 than those obtained at pH levels of 3.0, 4.5, and 6.0. However, results that indicated high yields of SPTW extraction at low pH and low temperature levels suggest that SPTW is bound to plant cell wall material like pectins by such polyvalent cations as Ca^{2+}, which are extractable under acidic conditions. The optimum extraction condition obtained for the extraction of SPTW is at pH 1.5 and 100 °C over an incubation period of 6.0 hours. The SPTW solutions were stored at 4 °C for 10 hours after neutralization to determine their ability to gel. Most of the solutions remained fluid, while some underwent gelation. Those solutions extracted below 100 °C underwent gelation, whereas those extracted mostly below 60 °C and at pH 1.5 showed irreversibility in their gel formation. These results have some similarities with those obtained by Furuta and others (1998) and are indicative of the fact that the degree of gelation and gelation characteristics of SPTW solutions are related to polyvalent cations that can be separated from the cell walls and soluble polysaccharide solutions during neutralization, thereby promoting the occurrence of gelation. Also, for solutions exhibiting irreversibility in their gel formation, it is possible that unhydrolyzed protein in PPTW may have remained unaffected by relatively low extraction temperatures, thereby resulting in the formation of stronger protein-polysaccharide interaction and, consequently, increasing the tendency of gelling macromolecules to cross-link, thus resulting in increased gel strength.

Yield of HM-SPTW

The yield of HM-SPTW was dependent on the yield of SPTW. The relationship between yields obtained for HM-SPTW and extraction conditions followed the same trend

as those of SPTW with similar optimum extraction conditions of pH 1.5 and 100 °C over an incubation period of 6.0 hours (Table 3). This shows that higher recovery rates of SPTW yielded higher yields of HM-SPTW under similar extraction conditions.

Apparent viscosities of extracted SPTW solutions

Mean apparent viscosities of SPTW solutions obtained over different incubation periods at similar pH and temperature levels are shown in Figure 2. Interesting results were obtained with regard to apparent viscosities of SPTW samples. Those extracted at 100 °C exhibited higher viscosities as viscosity determinations were carried out immediately after neutralization of extracted solutions. Based on the understanding that viscosity is concentration-dependent, results obtained were expected since higher extraction temperatures yielded higher extraction ratios leading to higher neutral sugar concentrations and, therefore, higher viscosities.

Sugar composition of HM-SPTW

The HM-SPTW was precipitated from the extracted SPTW. Its sugar composition is shown in Figure 3A to 3E. Similar sugars appeared in PPTW and HM-SPTW with significant variations in their weight ratios. This also confirms that HM-SPTW contains neutral sugar fractions. However, the rhamnose and xylose contents of HM-SPTW were much higher than those of PPTW, indicating that some neutral sugar polysaccharides decomposed during extraction, resulting in the formation of higher rhamnose and xylose contents.

Emulsifying characteristics of PPTW and SPTW

Yoshi and others (1996) reported the suitability of water-soluble polysaccharides extracted from soybean okara as emulsifiers for beverages. This prompted our investigation of the emulsifying properties and stability of PPTW and SPTW. Emulsifying properties are usually attributed to the flexibility of solutes (namely, the ability to go into solution and adsorb to interfaces) and exposure of hydrophobic domains. This means that the molecular size and the hydrophobic hydrophilic balance (HLB) are some of the parameters that come into play in determining the emulsifying property and stability of solutes. PPTW has a lower molecular weight compared with SPTW, which renders the former to have a higher capacity to adsorb easily in the emulsion system. Also, PPTW is less soluble, which increases its tendency to have high affinity for both oil and water, thereby enhancing its emulsifying property and stability. These results are clearly shown in Figure 4 and 5. On the other hand, SPTW has a higher molecular weight than PPTW and also has higher affinity for water than for oil, which renders it to exhibit lower emulsifying property and stability compared with PPTW. Also, the content of protein-polysaccharide conjugates in PPTW is higher than in SPTW, which may have also served as a major contributing factor to the higher emulsifying property and stability exhibited by the former. Also, PPTW exhibited a slightly higher emulsifying property and stability than the control (Gum Acacia powder, a commonly used emulsifier in foods), while the latter (Gum Acacia powder) also exhibited slightly higher emulsifying property and stability than SPTW. These two polysaccharide fractions (PPTW and SPTW) can serve as cheap sources of emulsifiers for different food preparations such as beverages and confectionery products.

Molecular weights of PPTW, SPTW, and HM-SPTW

The molecular weight distributions of PPTW, SPTW, and HM-SPTW were determined by SE-HPLC and are shown in Figure 6 and Table 4. The molecular weights for all samples were calculated according to the standard equation below:

$$\mathrm{Log}M_W = -0.459_{RT} + 13.012$$
$$r = 0.09984$$

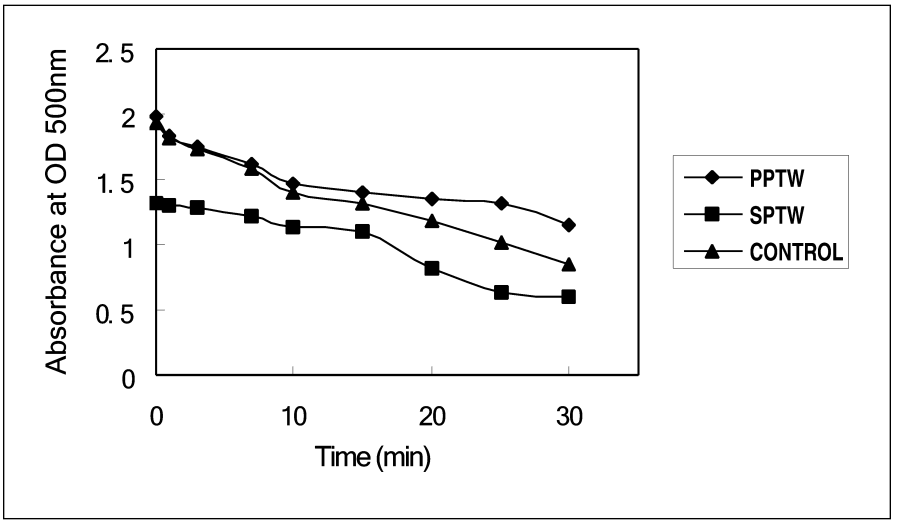


Figure 4—Emulsifying Properties of PPTW, SPTW and Control (Gum Acacia Powder)

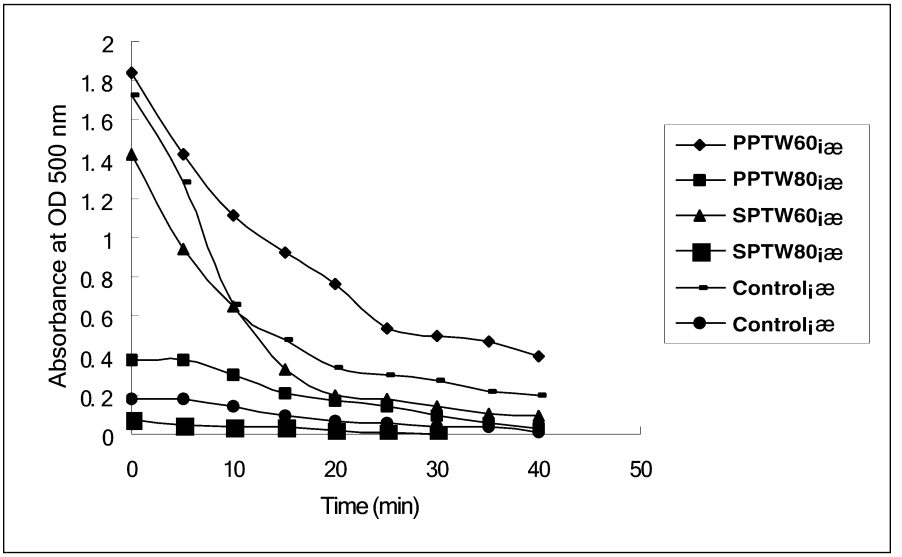


Figure 5—Heat stability of PPTW, SPTW and Control (Gum Acacia powder)emulsions stabilized by 0.1% SDS solutions and heated at 60 °C and 80 °C

where M_W = molecular weight and RT = retention time in minutes.

Interesting results were obtained for the molecular weights of PPTW, SPTW, and HM-SPTW. Under normal circumstances, hydrolysis is expected to lead to the extraction of low-molecular-weight compounds with respect to the original compound. By implication, the molecular weights of SPTW and HM-SPTW would have been in descending order with regard to that of PPTW. On the contrary, the molecular weights of these extracted polysaccharide fractions were in ascending order: PPTW < SPTW < HM-SPTW (Table 4). This does not in any way indicate that our results are wrong since hydrolysis by itself can be a complex mechanism. It is possible that during the drying process there was an occurrence of the phenomenon of gelatinization, which may have resulted in a reassociation of molecules, thereby generating higher-molecular-weight polysaccharides.

Conclusions

THIS WORK HAS DEMONSTRATED that "wastewater" discharged during tofu processing contains polysaccharides and that the use of an isopropanol-water reaction mixture during the hydrolysis process can increase the degree of purity of extracted polysaccharides. SPTW and HM-SPTW had similar optimum extraction conditions of pH 1.5 and 100 °C over an incubation period of 6 hours. The possibility of extraction of higher-molecular-weight compounds has also been demonstrated in our study. Emulsifying properties and the stability of PPTW and SPTW were carefully evaluated and results indicate that PPTW has slightly higher emulsifying properties and stability than SPTW. The dependency of viscosity on concentration was demonstrated in our study. Also, the characteristic types of soluble polysaccharides extracted from PPTW include heat reversibility gelation, heat irreversibility gelation, fluidity at 4 °C, suitable viscosity, and emulsifying characteristics. These properties show that PPTW and SPTW could be usefully applied in food processing as gelling agents, thickening agents, emulsifying agents, and stabilizing agents.

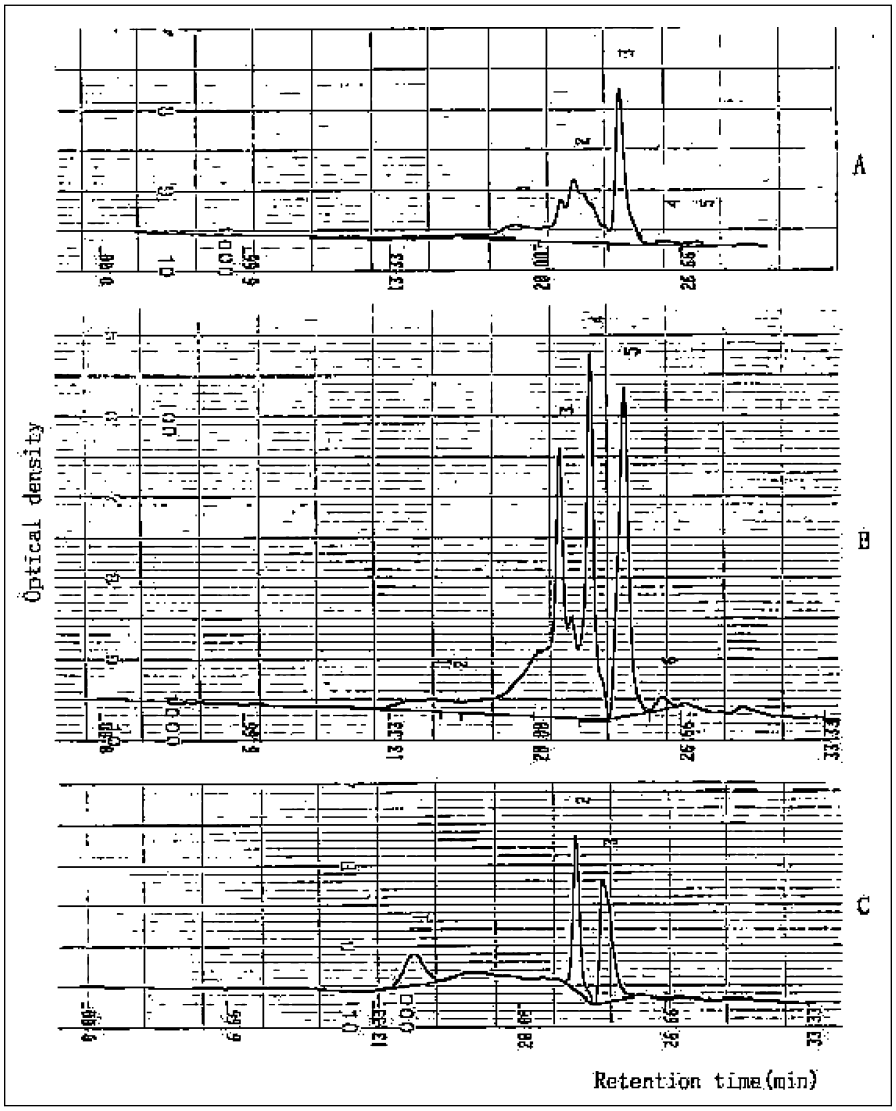


Figure 6—Molecular weight distribution determined by SE-HPLC
A = PPTW, B = SPTW, C = HM-SPTW

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