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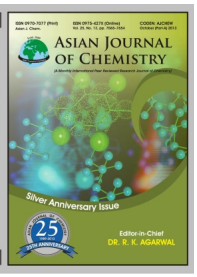

Optimized Extraction Conditions of Polysaccharides from Different Plants

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The optimal conditions for the extraction of polysaccharides from the *Laminaria Japonica* using in the hot-water method including alkalinity acidity, extracting time, temperature and sample to water ratio were examined. The contents of polysaccharides in *Laminaria japonica*, *Salicomia herbacea*, *Hizikia fusiformis* and *Gelidium amansii* were also compared. The optimal conditions were as follows: 90 ºC, neutral solution, dried sample to water ratio of 1/40 and heating time of 3 h. Under these conditions, the maximum amount of polysaccharides extracted from *Laminaria japonica* was 18.41 g/100 g dried sample. Among the four plants, *Hizikia fusiformis* had the highest polysaccharide content of 21.75 g/100 g dried sample, whereas *Salicomia herbacea* had the lowest.

Key Words: *Laminaria japonica*, Extraction condition, Polysaccharide.

INTRODUCTION

Laminaria japonica, a kind of brown seaweed, is a common type of seafood in China, Korea and Japan. *Laminaria japonica* is distributed widely and tends to form a seaweed ecosystem. This species has been applied as a traditional medicine in China for more than 1000 years and has a high productivity of approximately 500,000-700,000 tons in dry weight per year from the Chinese coasts[1]. During past decades, more and more attentions were focused on it allowing for its multiple functional compounds and their bioactivities which will no doubt make a contribution to the potential and promising applications in the area of food, materials and pharmaceuticals. Polysaccharides, existing primarily in the cell walls, are regarded as the main active compounds. The composition of polysaccharides varies according to season, age and geographic location[2]. The main polysaccharides in *L. japonica* are fucoidan, laminarin and alginic acid.

Salicomia herbacea, which is used as a traditional medicine to treat nephropathy, diarrhea, hepatitis or constipation in Korea as well as common daily food for some people living in coastal areas, is considered a promising medicine against obesity, constipation and hepatitis for its multiple bioactivities[3]. The polysaccharide from *S. herbacea* was found to be an active compound with an immunomodulatory function[4]. The major activities of this plant highlight its potential as a functional remedy. *Hizikia fusiformis* is also a nutrient plant cultivated in South Korea, Japan and China. It is known to be rich in dietary fiber and essential minerals. *Gelidium amansii*, which is used to produce agar, is a type of economically red algae cultivated in the shallow coast of East and Southeast Asian counties.

Many extract methods including water extract, enzyme hydrolysis[5] and ultrasonic-assisted extraction[6], have been applied to extract polysaccharides from range of plants. Each method has its specific advantages and disadvantages. Enzyme hydrolysis and ultrasonic-assisted extraction methods are energy saving but have a lower target yield. The aim of this paper was to determine the optimal extract conditions using water extract by considering four factors: alkalinity acidity, temperature, sample to water ratio and heating time. The polysaccharides content in *Laminaria japonica*, *Salicomia herbacea*, *Hizikia fusiformis* and *Gelidium amansii* after extraction under optimal conditions was compared.

EXPERIMENTAL

The seaweed species, *Laminaria japonica*, *Salicomia herbacea*, *Hizikia fusiformis* and *Gelidium amansii*, were purchased from a supermarket in Korea. The standard glucose and phenol were obtained from Sigma Aldrich (Seoul, South Korea). Ethyl ether, acetone, ethyl ether and sulphuric acid were supplied by Duksan (Gyeonggi-do, South Korea). A lambda 750 UV spectrometer was purchased from Perkin Elmer.

Extraction of polysaccharides preparation: 2.00 g of dried and shattered *L. Japonica* was weighed to dip into water at certain ratios ranging from 1/20-1/60 g/mL (sample/water).

Five groups of the mixture were heated at different temperature (60, 70, 80, 90 and 100 ºC) with constant stirring for set times (Table-1). Vacuum filter was used to obtain the filtrate and the residue was discarded. To obtain the crude polysaccharide, ethanol was applied at an ethanol to filtrate ratio of 3/1 for precipitation. The sediments were washed twice with acetone and ether sequentially. The depurated sediments were dried at the temperature of 60 ºC and the polysaccharides were obtained for further analysis.

TABLE-1
FACTORS AND LEVELS IN THE TEST OF HOT WATER METHOD

Level	Factors			
	pH	Temp. (ºC)	Time (h)	Ratio of sample and water
1	3	60	2	1/20
2	5	70	3	1/30
3	7	80	4	1/40
4	9	90	5	1/50
5	11	100	6	1/60

UV spectrometer detection preparation: The colorimetric method was used to determine the polysaccharide content. The dried polysaccharides were dissolved in water and diluted to the required concentration[7]. 0.06 g phenol was dissolved in 1 mL water followed by the addition of sulphuric acidwith stirring. 5.00 mL of colour reagent was pipetted into the sample liquid. The mixture was shaken to blend well and placed for 20-30 min in a water bath at 90-100 ºC before taking the readings. It is necessary to make sure the detection with UV spectrometer can finish in a set time after polysaccharides extraction, for the colour would change with time lasting.

Standard curve: Glucose was used to obtain a standard curve. The glucose solutions at concentrations of 0, 25, 50, 75, 100 µg/mL were prepared to test by the sulphuric acid-phenol method.

RESULTS AND DISCUSSION

Effect of alkalinity and acidity of solvent on extracted amount: The pH of the mixtures was set to 3, 5, 7, 9 and 11 using HCl and NaOH to determine the optimal pH for the maximum extraction rate. As shown in the Fig. 1, the extraction rate reached the maximum when the pH is set in the value of 7. We can figure out that if the pH is too low or too high, it can decrease the extraction ratio from *L. Japonica*. This trend happened for the extreme pH may destroy the structure of the polysaccharides existing in the cell wall of the plant. Alkali conditions appear to have a stronger effect on destruction than peracid processing.

Effect of temperature on the extracted amount: The extraction ratio increased with increasing temperature to 90 ºC and decreased with further increases in temperature (Fig. 2). The structures of polysaccharides can be destroyed if the temperature risen to 100 ºC. At the temperatures between 50-70 ºC, the concentration of polysaccharide extracted increased at a relative stable speed. While from the temperature of 80 ºC, the extraction rate changed suddenly and finally ascended to 17.63 % at 90 ºC. This suggests that the higher the temperature is, the greater the level of destruction of the cell wall. Therefore, 90 ºC was fixed as a constant parameter for the subsequent experiments.

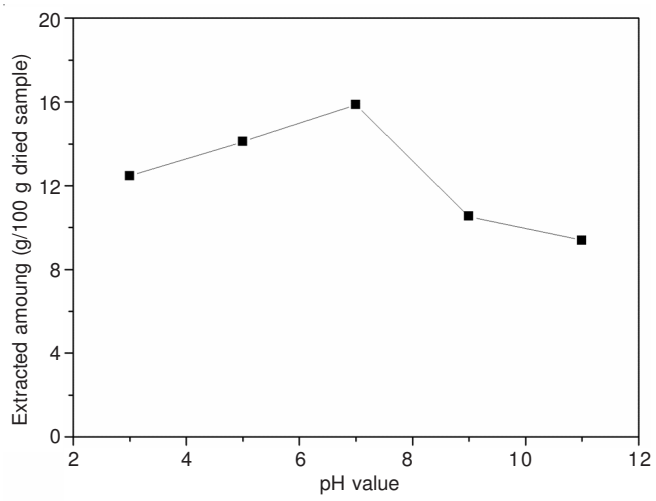

Fig. 1. Effect of pH value on extracted amount. (Temp. = 90 ºC, sample/water ratio = 1/30 g/mL, time = 3 h)

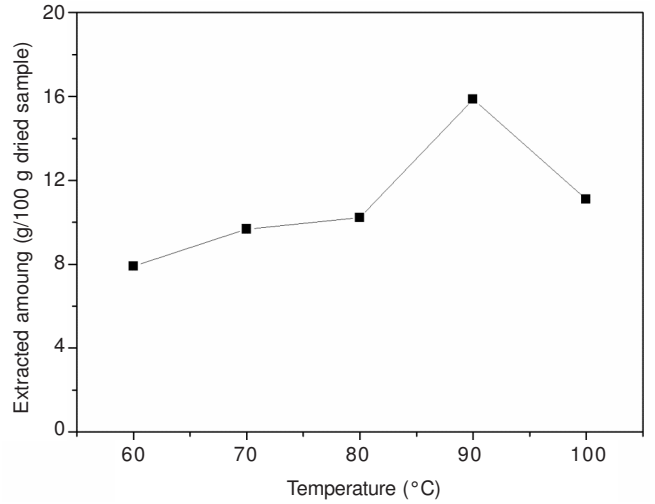

Fig. 2. Effect of temperature on extracted amount. (pH = 7, sample/water ratio=1/30 g/mL, time=3 h)

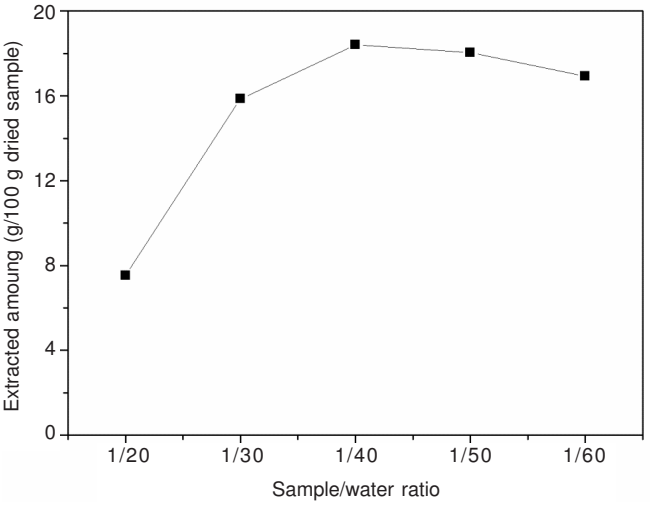

Fig. 3. Effect of sample/water ratio on extracted amount. (pH = 7, temp. = 90 ºC, time = 3 h)

Effect of sample/water ratio on extracted amount: The extraction rate was also related to sample/water ratio. Large volumes of solvent are not only uneconomical but can also influence the extract efficiency. A series of extractions were carried out at different sample/water ratios (1/20, 1/30, 1/40 1/50 and 1/60 g/mL) to evaluate its effect (Fig. 3). With increasing amount of water, the leaching-out rates is elevating until the sample/water ratio reaches to 1/40 g/mL and decreased with further increases in rate. Therefore, the ratio of 1/40 g/mL was considered as the optimal ratio choice for next testes.

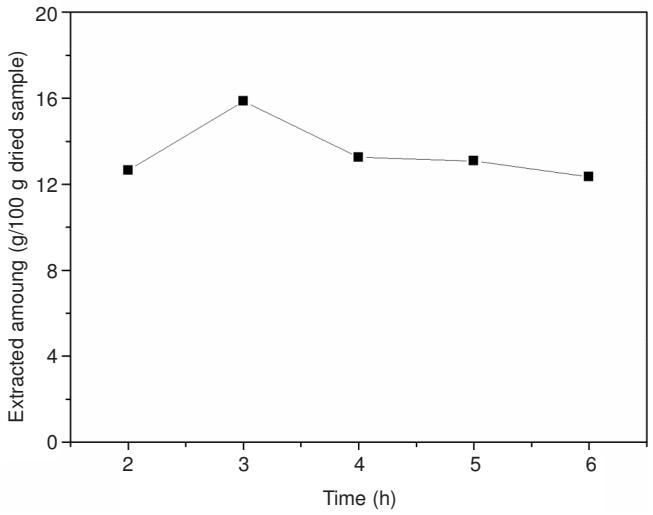

Fig. 4. Effect of heating time on extracted amount. (pH = 7, sample/water ratio = 1/40 g/mL, time = 3 h)

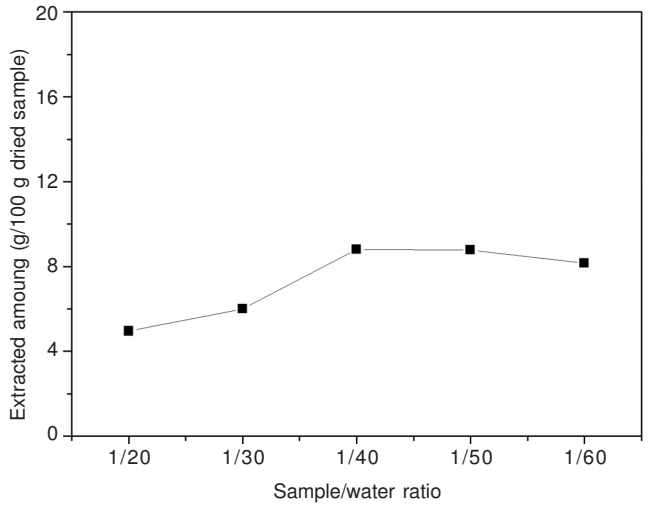

Fig. 5. Effect of sample/water ratio with ultrasonic-assisted method on extracted amount (pH = 7, temp. = 50 ºC, time = 25 min, 75 W)

Effect of heating time on extracted amount: The heating time plays a major role in the polysaccharides extraction efficiency. In this study, the polysaccharides distributing in the cell of plants require time to dissolve in water. Therefore, the heating time was examined over the range of 2-6 h (Fig. 4). The highest peak was observed after heating for 3 h and the concentration of the polysaccharides turned to decrease with time increasing. The reason of decreasing can be attributed to the decomposition of polysaccharides and impurity dissolution which inhibited more thorough immersion of polysaccharides. Therefore, 3 h was selected for the optimal heating progressing.

Comparison of water extraction and ultrasonic-assisted extraction method: In addition to the water extraction method, ultrasonic-assisted extraction method was also performed to compare the yield of polysaccharides. The experiment procedure was carried out under the condition of 50 ºC, 25 min, 75 W and neutral solvent[6,8] (Fig. 5). A range of different sample to water ratios were installed from 1/20-1/60 g/mL. The tendency of the change showed extraction amount increased until 1/40 g/mL, while the amount began to decline at higher ratios. The highest extracted amount was 8.8 g/100 g. But compared with the water extraction in ratio optimizing study, the yields of the polysaccharides were far behind. Although the ultrasonic-assisted treatment is simple, less energy consuming, the efficiency was relatively lower. According to these results, water extraction was considered a better choice in terms of the greater yield.

Comparison of polysaccharides concentration in different plants: As shown in the column chart (Fig. 6), the polysaccharides extracted amount from the different plants varied considerably. *Salicomia herbacea* and *Glidium amansii* have similar polysaccharides content, the polysaccharides content of *Salicomia herbacea* amounted to 4.31 g/100 g and *Glidium amansii* contained 5.51 g/100 g. On the other hand, the polysaccharides amount in *Hizikia fusiformis* and *Laminaria japonica* were relatively higher than the two mentioned above. Among these four plants, *Hizikia fusiformis* had the maximum polysaccharides amount of 21.75 g/100 g dried sample while the polysaccharides level in *Salicomia herbacea* was the lowest.

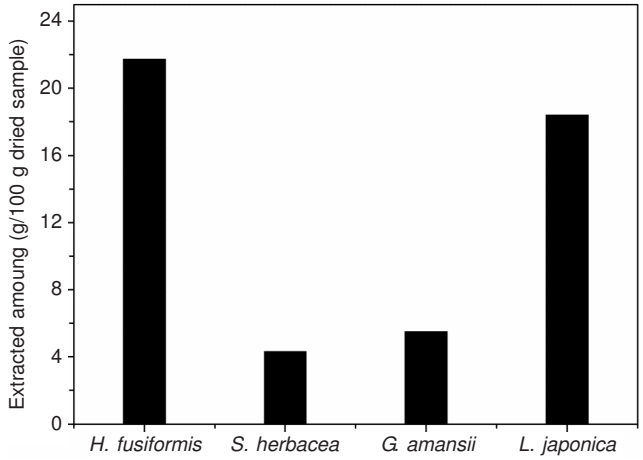

Fig. 6. Comparison of polysaccharides content in different plants (pH = 7, sample/water ratio = 1/40 g/mL, time = 3 h, temp. = 90 ºC)

Conclusion

Due to the multiple bioactivities and extended medical applications of polysaccharides extracting from different edible nature plants, the most efficient method and conditions for the extraction acting as the most important preparation for further separation and activities study were urgent to figure out. It is noted that the standard curve displayed using glucose was not so accurate for determining all types of monosaccharide degraded from polysaccharides in nature. Based on the whole

experiment, the optimal parameters were 90 ºC, neutral solution, 1/40 of ratio of dried sample to water and heating time of 3 h. According to the results, *Hizikia fusiformis* had the maximum polysaccharides amount of 21.75 g/100 g dried sample while the polysaccharides level in *Salicomia herbacea* was the lowest. The study provided efficient hot water extraction condition and exhibited the rough polysaccharides content distributing in different plants. Further studies on polysaccharides separation by HPLC from *Laminaria japonica* are under process.

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