Hydrolysis Kinetics of Okara and Characterization of Its Water-soluble Polysaccharides

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As part of a study of re-use of waste okara, the kinetic mechanism of okara hydrolysis and the properties of the water-soluble polysaccharides (WSP) extracted were investigated. Okara was hydrolyzed by being autoclaved at pH 4.5 in two volume of water with or without a chelator such as hexametaphosphate. Okara hydrolysis proceeded by surface degradation mechanism without a chelator. Characterization of WSP suggested that the “egg-box regions” in okara were susceptible to degradation by a chelator and that the “non-egg-box regions” contained WSP linked with hydrophobic proteins. WSP with a molecular weight of more than 10^5 and the amount of protein bound to the polysaccharides seemed to govern their emulsifying characteristics.

Key words: okara; hydrolysis; water-soluble polysaccharides; emulsifying characteristics

Okara, a by-product of tofu and soybean protein manufacturing, is treated as industrial waste. Over one million tons of soybeans are consumed yearly in Japan. About 1.4 million tons of okara are produced yearly since 1 kg of soybean produces 1.4 kg of okara (about 85% moisture).

Several groups have studied the use of okara in foods, forage, and media for bacterial growth. Recently, Maeda† has proposed a means of extracting water-soluble polysaccharides (WSP) from okara, and has used them as an emulsifier or viscoelastic reagent. Powdery WSP were obtained from okara by acid hydrolysis at pH 4.5, filtration, deodorization, and spray drying. Filtered cakes of okara could be prepared by dehydration in a drum dryer, and they were suitable for use as forage. For use of WSP as an emulsifier in the food industry, extraction of WSP from okara should be more economical than the preparation of other emulsifiers such as gum arabic.

Okara fibers are irregular in shape, and consist of cellulose–hemicellulose frames embedded in the amorphous matrices of pectic substances. Okara contains about 50% carbohydrates on a dry basis, which is about 34% fibrous and the remainder pectic substances. Labavitch et al.2) and Misaki et al.3) have suggested that the pectic fraction of okara is mainly galacturonic acid bound to galactan and arabinan. Many investigators have reported that pectin can be readily extracted from plant cell walls under moderate conditions.4,5) Treatment with heat or a chelator has been investigated in examinations of the mechanism of degradation of pectic substances in the cell wall.6,7) Yamaguchi et al.8) have reported the extraction of pectic substances from okara with a chelator, hexametaphosphoric acid (HMP).

Calcium is important in the regulation of cell wall hydrolysis because of its mediation between pectin molecules.9) The unesterified domains of pectin can self-associate to form aggregates with a calcium-dependent mechanism, giving the structure called an “egg-box,”10,11) in which the mechanical strength of the cell walls is increased.12) Details of the structure formed from okara pectin and fibrous cellulose are unknown.

In this study, the kinetic mechanism of okara acid hydrolysis with or without a chelating agent was investigated so that WSP could be extracted easily from okara, and the emulsifying properties of the WSP extracted were characterized. The structure of okara fiber was considered in terms of the results we obtained.

Materials and Methods

Materials and chemicals. Frozen okara (81% moisture) was the product of Fuji Oil Co., Ltd., Osaka. Calcium chloride, sodium hexametaphosphate (HMP), and other reagents of analytical grade were purchased from Wako Pure Chemicals, Osaka. Pullulans used as standards for molecular weights were from Showa Denko, Tokyo.

Acid hydrolysis of okara and preparation of WSP powder. Frozen okara was thawed at room temperature. Thirty grams of wet okara was placed in a stainless steel chamber (diameter, 10 cm; height, 6 cm), to which 2 volumes of distilled water was then added. After CaCl2, HMP, or EDTA was added to the okara slurry, the pH was adjusted to 4.5 with 1 N hydrochloride. The chamber was covered with aluminum foil and autoclaved at 120°C for 5–60 min (Tommy, SS-25). Forty grams of hydrolyzed okara was placed in a dehydrator on filter paper, and compressed at 10 kg/cm² with a constant temperature of 50°C. The filtrate (water-soluble polysaccharides, or WSP) was lyophilized (Tokyo Rika, FD-50) at room temperature for 30 h to form WSP powder.

Surface degradation during okara hydrolysis. The degree of hydrolysis was measured as follows. Hydrolyzed okara was washed with 1 liter of distilled water, filtered under reduced pressure through a Buchner funnel (11.5 cm in diameter), dried under reduced pressure at 80°C for 15 h, and weighed. The conversion of hydrolysis was defined as 1 – W/Wo, where W/Wo is the ratio of the dry weight of hydrolyzed okara (W) and the okara at the start (Wo). On the assumption that okara fibers consist of particles, the hydrolysis rate of okara with surface degradation can be represented as

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Abbreviations: HMP, hexametaphosphate; Ms, mean molecular weight; WSP, water-soluble polysaccharides; CaCl2, okara hydrolysis with CaCl2; HMP, okara hydrolysis with HMP; NA, okara hydrolysis with no additives.
\[-\frac{dW}{dt} = kW^{2.3}\]  
(1)

where \(k\) is the rate constant of okara hydrolysis, and \(W^{2.3}\) is the apparent surface area of the particles. Equation (1) can be integrated to give

\[(W/W_0)^{1/3} = 1 - kt^{3/2}\]  
(2)

Equation (2) shows that \((W/W_0)^{1/3}\) decreases linearly with hydrolysis time \(t\) if the hydrolysis is by surface degradation. \((W/W_0)^{2/3}\) is the apparent dimensionless diameter of hydrolyzed okara particles.

**Molecular weight of WSP and fluorescence of the polysaccharide solution.** Ten milliliters of a 1% (w/v) WSP solution was put on a gel filtration column (Tosoh, TSK-GMPWXL, 7.8 mm x 30 cm) equilibrated with 20 mM potassium phosphate buffer (pH 7) containing 0.1 M NaSO_4. The merging peaks were detected with an Elmer ERC-7512 refractometer or a Shimadzu RF-535 fluorescence monitor (excitation at 280 nm and emission at 350 nm). Chromatograms were recorded on an integrator (JASCO, 807-TI).

WSP contained several percents of proteins, which influencing the emulsifying ability of WSP. For this reason, lyophilized WSP (4 mg) was dissolved in 4 ml of 0.1 M potassium phosphate buffer (pH 7), and the fluorescence intensity was measured at 350 nm (excitation at 280 nm) for estimation of the protein content.

**Measurement of uronic acid in WSP.** The uronic acid in WSP was measured by the m-phenylphenol method with galacturonic acid as the reference standard.

**Emulsifying properties.** Emulsifying properties were assessed by the method of Pearce and Kinella. For preparation of an emulsion, 1 ml of corn oil and 3 ml of 1% WSP in 0.067 M sodium phosphate buffer of pH 7.4 were homogenized with a Polytron at drive setting 8 for 1 min at 20 C. The emulsifying ability and stability were measured as described by Matsudomi et al.

**Results and Discussion**

**Kinetics of okara hydrolysis.**

Figure 1 is a plot of the apparent dimensionless diameter, \((W/W_0)^{1/3}\), of the hydrolyzed okara particles against hydrolysis time. The changes in \((W/W_0)^{1/3}\) differed depending on the additive. When okara hydrolysis was in the absence of any additive, \((W/W_0)^{1/3}\) decreased linearly with time. The straight line in Fig. 1 is for the line of regression \((W/W_0)^{1/3} = 1 - 2.71 \times 10^{-3} t\) \((r^2 = 0.904)\) of \((W/W_0)^{1/3}\) vs. hydrolysis time (see Eq. (2)) for hydrolysis without additives. The volume-mean diameters of okara particles measured with a laser-particle analyzer (Helo & Rodos) before and 30 min after hydrolysis began were 97 and 89 \(\mu\)m, respectively. This ratio of okara particle diameter (0.918) was concordant with the value (0.92) estimated from the line of regression in Fig. 1, although the irregular shapes of the okara fibers prevented accurate measurement of their particle diameters. These results suggest that okara fibers are hydrolyzed by surface degradation in the absence of a chelator and CaCl_2.

The effects of CaCl_2, EDTA, and HMP on the acid hydrolysis of okara fibers are also shown in Fig. 1. When a chelator was added, \((W/W_0)^{1/3}\) decreased at the early stage of hydrolysis, and reached a plateau. When 1% CaCl_2 was added, hydrolysis progressed more slowly. We assumed that the structure of okara fibers might be similar to that of plant cell walls. In the case of adding CaCl_2, the cross-linkage of galacturonic (uronic) acid in okara pectin may have increased the strength of the okara fibers. On the other hand, it seems likely that HMP or EDTA would remove Ca\(^{2+}\) from the egg-box regions in WSP of okara pectin, and thereby speed hydrolysis. These results suggest that okara hydrolysis in the presence of a chelator occurs with two steps; at an early stage, hydrolysis proceeds in the egg-box regions, and later, the reaction shifts into the non-egg-box regions. Ca\(^{2+}\) is important in interacting with pectic acid polymers to form Ca\(^{2+}\) cross-linkages that increase the resistance of okara fibers to hydrolysis. Therefore, during okara hydrolysis, chelation of Ca\(^{2+}\) helps in the extraction of WSP.

**Effects of hydrolysis conditions on the properties of WSP.**

The properties of WSP (mean molecular weights (Mw), galacturionate content, and protein content) were examined under three hydrolysis conditions: in the presence of 1% CaCl_2 (condition CaCl_2), in the presence of 2% HMP (condition HMP), and with no additives (condition NA).

a) **Mw of WSP.** The Mw of WSP under the three hydrolysis conditions was estimated by gel permeation chromatography. Figure 2 shows changes in Mw during hydrolysis. The Mw was influenced by the additive, if any. The Mw of WSP under condition NA increased gradually, reaching about 5.0 \times 10^3 after 50 min of hydrolysis. Under condition HMP, the Mw increased in the first 10 min, and then decreased. These results suggest that the removal of Ca\(^{2+}\) ions from the egg-box regions by a chelator speeded

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**Fig. 1.** Effects of CaCl_2, EDTA, and HMP on Okara Hydrolysis. The straight line is a line of regression (Eq. (2)) for hydrolysis data in the absence of any additive. ▲ 1% CaCl_2; ▼ 1% EDTA; ▶ 2% EDTA; ■ 0.5% HMP; ◆ 2% HMP; ● with no additives.

**Fig. 2.** Effects of Hydrolysis Time on the Mean Molecular Weight of WSP from Okara. ▲ 1% CaCl_2; □ 2% HMP; ○ with no additives.
hydrolysis at the start of the reaction, promoting degradation of extracted WSP. Probably, the addition of CaCl₂ stabilized the egg-box region, because the Mw of WSP under condition CaCl₂ was still less than $10^5$ even after 50 min of hydrolysis. The changes in Mw during hydrolysis indicated that the okara had a complicated fibrous structure. The mechanism of pectin breakdown in okara is still obscure unknown. The soluble polymer may be entangled in the okara fiber matrix.

b) Galacturonate content in WSP. Pectin, a galacturonate chain, is made soluble by acid hydrolysis, and can be measured with uronic acid. The galacturonate content in WSP reflected the degree of degradation of the okara pectic fiber. Therefore, the content of uronic acid (galacturonate) in WSP was measured after hydrolysis for various times. The Mw and the galacturonate content in WSP are plotted in Fig. 3 as a function of conversion by hydrolysis. The galacturonate content increased in proportional to the hydrolytic conversion under all three conditions. The Mw also was proportional to conversion. These findings suggest that the galacturonate content may be related to the Mw of WSP, and that the different galacturonate content for each hydrolysis condition may result from the distribution of the breakdown point of homogalacturonan in okara pectin.17,18) It seemed that pectins with a low Mw could be extracted at the stage when hydrolysis has just started because of the presence of entangled pectin polymers in the okara fibers. The entangled pectin polymers probably are untied by chelation when conversion has proceeded.

c) Protein content in WSP. The protein content in WSP and the structure of glycoprotein are important in the emulsification of WSP. To investigate the effects of hydrolysis conditions on the amount of protein in WSP, the fluorescence of WSP was used as an index of the amount of hydrophobic peptides or proteins. Figure 4 shows changes in the fluorescence of WSP during hydrolysis under various conditions. The fluorescence intensity under conditions CaCl₂ and HMP increased with time and reached a plateau that was 2.8 times higher under condition CaCl₂ than under condition HMP after 40 min of hydrolysis. Under condition NA, the fluorescence reached a maximum in about 20 min.

d) Overall characterization of WSP. The galacturonate content and the changes in fluorescence intensity of WSP were plotted against the Mw of WSP in Fig. 5. Results grouped themselves into clumps: a high protein content but low Mw under condition CaCl₂, a high Mw but low protein content under condition HMP, and a high Mw and high protein content under condition NA. This pattern suggests that the protein level was higher in the non-egg-box regions than in the egg-box regions. The egg-box region in okara

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Fig. 3. Effects of Conversion by Hydrolysis on the Galacturonate Content and the Mean Molecular Weight of WSP.
The unbroken and dotted lines are lines of correlation for the galacturonate content and the Mw of WSP, respectively. One line is for the reaction in the presence of 1% CaCl₂ or 2% HMP, and the other line is for the reaction with no additives. Galacturonate content: △ 1% CaCl₂; □ 2% HMP; ○ with no additives. Molar weight: △ 1% CaCl₂; □ 2% HMP; ○ with no additives.

Fig. 4. Effects of Hydrolysis Time on the Protein Fluorescence of WSP.
△ 1% CaCl₂; □ 2% HMP; ○ with no additives.

Fig. 5. Characterization of WSP from Okara.
Fluorescence: △ 1% CaCl₂; □ 2% HMP; ○ with no additives. Galacturonate content: △ 1% CaCl₂; □ 2% HMP; ○ with no additives.

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Fig. 6. Structural Model of Okara Fiber.
Okara had two kinds of structures: the egg-box region, which is a region of cross-linkage of galacturonic acid with Ca²⁺, and the non-egg-box region, containing protein.
may be susceptible to degradation by a chelator, because removal of Ca$^{2+}$ chelated by uronic acid makes the okara fiber structure looser. On the other hand, many more polysaccharides bind with proteins in the non-egg-box region than in the egg-box region. With the different features of WSP under these three hydrolysis conditions taken into consideration, the structure of okara fiber was probably as shown in Fig. 6.

**Emulsifying characteristics of okara WSP**

Okara WSPs are useful as an emulsifier for beverages. The emulsifying activity and stability of WSP were plotted against their Mw. The two unbroken lines in Fig. 7 are the regression curves for emulsifying activity and stability, obtained by Kato et al.\(^{19}\) for soy protein-polysaccharide conjugates. The emulsifying activity of WSP was higher as its Mw increased, and its regression line was closely correlated with the one reported by Kato et al.\(^{19}\). However, the emulsifying stability of WSP was below the regression curve. This finding may have arisen from the lower content of protein–polysaccharide conjugates in WSP than that used by Kato et al.\(^{19}\). WSP under condition CaCl$_2$ contained less protein–polysaccharide conjugates than under other two conditions, and its emulsion stability was almost zero.

Figure 8 shows the effect of protein content on the emulsifying characteristics of WSP. The emulsifying activity and emulsion stability reached maxima near or at the same intensity of fluorescence, about 4000. This result suggests that there is an optimum protein content in WSP for the best emulsifying capacity. This relationship between okara hydrolysis and its resulting fluorescence implies that it is required to removed low Mw polysaccharides with a diafiltration, when emulsifiers are to be manufactured from okara.

**Concluding remarks**

This investigation of okara acid hydrolysis at pH 4.5 showed that the structure of okara fiber consists of cross-linked galacturonate polymer with Ca$^{2+}$ between carboxylic groups (the so-called “egg-box regions”) and non-egg-box regions with bound proteins, and that okara hydrolysis without chelating agents proceeds by surface degradation. Calcium seemed to be an important part of the structure of okara pectin. Okara hydrolysis without HMP produced WSP useful as an emulsifier.

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**References**