Microencapsulation of Linoleic Acid with Low- and High-molecular-weight Components of Soluble Soybean Polysaccharide and Its Oxidation Process

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Soluble soybean polysaccharide (SSPS) was fractionated into its low- (LMW) and high-molecular-weight (HMW) components to test their antioxidative and emulsifying properties. Linoleic acid was emulsified with an aqueous solution of SSPS, HMW, a mixture of LMW or HMW with maltodextrin, or maltodextrin alone. The emulsions prepared with SSPS, HMW and the mixture of HMW with maltodextrin were stable. These emulsions were spray-dried to produce microcapsules. The encapsulated linoleic acid was oxidized at 37°C and at various levels of relative humidity. Linoleic acid encapsulated with the mixture of LMW with maltodextrin or HMW was stable to oxidation, and this stability increased as the weight fraction of LMW in the mixture was increased. The LMW components also had high DPPH-radical scavenging activity. These results indicate that LMW played an important role in suppressing or retarding the oxidation of linoleic acid encapsulated with SSPS. The oxidative stability of linoleic acid encapsulated with a mixture of the LMW and HMW components was high at low and high relative humidity, but not at intermediate levels of relative humidity.

Key words: linoleic acid; microencapsulation; oxidation; soluble soybean polysaccharide

Soluble soybean polysaccharide (SSPS) is a watersoluble polysaccharide extracted from soybean cotyledons and includes ca. 9% proteinous or peptidyl constituents.1,2) SSPS has a high dietary fiber content, high solubility in water, relatively low viscosity, a strongly adhesive and film-forming property, and shows a variety of functions such as dispersion stabilizing, emulsifying and anti-sticking effects.2) It may therefore be applicable for use not only in the food but also in the chemical industry. Microencapsulation can suppress or retard the oxidation of an unsaturated fatty acid or its acyl-glycerol.3–6) SSPS has been found to be a good wall material for the microencapsulation of linoleic acid, and the oxidation of linoleic acid encapsulated with SSPS was significantly suppressed.7) The process for microencapsulation of a liquid lipid into a powdery matrix of a wall material consists of two steps: one involves emulsification of the lipid with an aqueous solution of the wall material to produce an O/W emulsions, and the other involves rapid dehydration of the resulting emulsion. Spray-drying is commonly used for such dehydration.

It has been shown that the diameter of oil droplets in an emulsion8) and the presence of an amphiphilic antioxidant at the interface between an oil droplet and the dehydrated layer of the wall material9) affects the oxidative stability of the encapsulated linoleic acid. Elucidation of the reason why SSPS would be a good wall material for microencapsulation would be helpful for designing a wall material that can suppress or retard the oxidation of an unsaturated lipid by its microencapsulation.

In this context, SSPS was fractionated into its low- and high-molecular-weight components to identify their antioxidative and emulsifying properties. The oxidation of linoleic acid encapsulated with SSPS, a mixture of the low- or high-molecular-weight components with maltodextrin, a mixture of the low- and high-molecular-weight components or maltodextrin alone was observed at various levels of relative humidity. The emulsifying and emulsion-stabilizing abilities of the low- and high-molecular-weight components of SSPS were also measured.

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Materials and Methods

Materials. SSPS was a product of Fuji Oil Co. (Soyafibe® S-DN; Osaka, Japan). Maltodextrin with a dextrose equivalent of 2-5 was purchased from Matsutani Chemical Industries (Osaka, Japan). Linoleic acid (>90% purity), methyl palmitate (>95%) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). All other chemicals of analytical grade were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Preparation and characterization of the low- and high-molecular-weight components of soluble soybean polysaccharide. SSPS was fractionated into the low- and high-molecular-weight components by first being dissolved in distilled water at a concentration of 5% (w/v). Aliquots of the solution (3 ml each) were taken in test tubes, and ethanol was added to each solution to produce a volume ratio of 1:1. The mixture was then subjected to vortex mixing and centrifuged to recover the supernatant. The precipitate was dissolved in distilled water. The foregoing procedure was repeated twice more. The combined supernatant was rotary-evaporated to recover the low-molecular-weight component (abbreviated LMW). The precipitate after the third centrifugation was collected and dried as the high-molecular-weight component (HMW). The classification of LMW and HMW is based on the results of a gel permeation chromatographic analysis which will be shown later. The recovery of LMW and HMW was 17% (w/w) and 76%, respectively.

Each component was analyzed by HPLC with a Shimadzu LC-10ATvp instrument (Kyoto, Japan) fitted with a Shodex OHpack SB-806MHQ GPC column (Showa Denko, Tokyo, Japan; 8 mm i.d. and 300 mm). The eluent used was distilled water and its flow rate was 0.6 ml/min. The effluent was monitored with an SPD-10Avp UV detector (Shimadzu, Kyoto, Japan) at 215 nm and with a YRU-880 midget RI detector (Shimamuratech, Tokyo, Japan).

The contents of saccharides and proteinous or peptidyl constituents in the HMW and LMW components were estimated by the phenol-H2SO4 \(^{8}\) and Lowry \(^{11}\) methods, using glucose and bovine serum albumin as respective standards.

The radical-scavenging activities of HMW, LMW and original SSPS were estimated by the DPPH method. \(^{12}\) HMW, LMW or SSPS was dissolved in 60% (v/v) ethanol at 0.182–0.917 g/l. Five milliliters of the solution was put into an amber vial, and 0.5 ml of DPPH dissolved in ethanol at a concentration of \(5 \times 10^{-4}\) mol/l was added to the vial. The headspace of the vial was filled with nitrogen gas, and the vial was then tightly sealed. The vial was vigorously shaken and then incubated for 20 min at 25°C. The activities were measured by the decolorization of DPPH at 516 nm with a Shimadzu UV-1200 spectrophotometer.

Encapsulation of linoleic acid by spray-drying. SSPS, maltodextrin, HMW and a mixture of HMW or LMW with maltodextrin at a weight ratio of 1:1 were used as wall materials. LMW was mixed with HMW to produce weight fractions of 0.09, 0.18 and 0.36, and these mixtures were also used as wall materials. Microcapsules of linoleic acid with a wall material were prepared according to our previous methods. \(^{7}\) The wall material (45 g) was dissolved in 300 ml of distilled water, and 9 g of linoleic acid was added to the solution. The weight fraction of linoleic acid to the wall material was fixed at 0.2 throughout this study. The mixture was emulsified with a rotor/stator homogenizer (Polytron PT20SK, Kinematica, Lucern, Switzerland) for 1 min at a power setting of 8 to produce an O/W emulsion. The median diameter of the oil droplets in the emulsion was determined with an SALD3000 laser diffraction particle size analyzer (Shimadzu, Kyoto, Japan).

The emulsion was fed into an LB-8 spray dryer (Ohkawara, Yokohama, Japan) to produce microcapsules under the following operating conditions: feed rate of the emulsion = 3.0 kg/h, speed of the centrifugal atomizer = 3 \(\times\) 10\(^4\) rpm, flow rate of air = 7.5 m\(^3\)/min, and temperatures of the air at the inlet and outlet of the dryer = 200°C and 100–110°C, respectively.

Stability of the O/W emulsion. A portion of the foregoing emulsion was measured for its stability by the turbidimetric method. \(^{13}\) The emulsion was kept at 25°C while gently stirring by a magnetic stirrer. A portion of the emulsion (40–100 μl) was occasionally sampled and appropriately diluted with 0.1% (w/v) sodium dodecyl sulfate. The absorbance at 500 nm was then measured with the UV-1600 spectrophotometer.

Oxidation process of the encapsulated linoleic acid. Microcapsules (20 mg) were weighed in a flat-bottom glass cup (1.5 cm i.d. and 3 cm height); about 15 cups were prepared for each sample. The cups were placed in a desiccator where the relative humidity was regulated to 12%, 44% or 75% with a saturated KCl, K\(_2\)CO\(_3\) or NaCl solution. The desiccator was stored in the dark at 37°C. At appropriate intervals, a cup was removed from the desiccator, and the amount of unoxidized linoleic acid was determined by gas chromatography, using methyl palmitate as the internal standard. \(^{5}\)

Sorption isotherm of water on soluble soybean polysaccharide or maltodextrin. SSPS, HMW or...
maltodextrin, which had been dried at 150°C for one day and then cooled over phosphorus (V) oxide, was weighed (5 to 10 mg) in a platinum cell. The cell was stored at 37°C for 3 days in a desiccator whose relative humidity (shown in parentheses) was regulated with a saturated aqueous solution of LiCl (12%), CH3COOK (23%), MgCl2 (33%), K2CO3 (44%), NH4NO3 (55%), NaCl (75%), KCl (86%) or K2SO4 (96%). The cell was set in a Shimadzu TGA-50H thermogravimeter (Kyoto, Japan). The sample in the cell was heated from room temperature to 150°C at a rate of 5°C/min and then held at that temperature for 30 min. The amount of water sorbed was calculated from the initial weight and that after heating. The measurement was triplicated for each sample and relative humidity, the amount being averaged.

Results and Discussion

Properties of the low- and high-molecular-weight components

Figure 1 shows chromatograms for LMW, HMW and original SSPS. The concentrations of SSPS, HMW and LMW applied to HPLC were 0.20% (w/v), 0.16% and 0.04%, respectively. The concentrations of HMW and LMW were roughly determined from their contents in SSPS. SSPS showed two peaks at retention times of 10 min and 13 min. We have previously reported that SSPS contained roughly three components having approximate molecular weights of 5.5 × 10^5, 2.5 × 10^4 and 5.0 × 10^3, the content of the second component being lower than those of the other two. Therefore, the earlier- and later-eluted components would correspond to the first and third components, respectively. The major peaks in the chromatograms for HMW and LMW were observed at retention times of 10 min and 13 min, respectively. HMW and LMW were classified in this study from the results of the HPLC analyses. LMW exhibited high and low responses to the UV and RI detectors, respectively. The high response of LMW to the UV detector would indicate that it contained proteinous or peptidyl constituents.

The contents of saccharide and proteinous or peptidyl constituents, which were determined by the phenol-H2SO4 and Lowry methods, respectively, are listed in Table 1. HMW mainly consisted of saccharide, while LMW contained more proteinous or peptidyl constituents than original SSPS. HMW would have consisted of rhamnogalacturonan and homogalacturonan. The contents of saccharide and proteinous or peptidyl constituents, which were determined by the phenol-H2SO4 and Lowry methods, respectively, are listed in Table 1. HMW mainly consisted of saccharide, while LMW contained more proteinous or peptidyl constituents than original SSPS. HMW would have consisted of rhamnogalacturonan and homogalacturonan.

Figure 2 shows the radical-scavenging activities of HMW, LMW and SSPS. LMW exhibited the highest activity, HMW the lowest, and the activity of SSPS was intermediate. The results shown in Table 1 and Fig. 2 indicate that the activity of SSPS would have originated from LMW. Since the amino acid composition of LMW was not measured, we cannot specify...
what type of protein or peptide would respond to the activity. It has been reported that soy protein hydrolysates possessed scavenging activity toward DPPH, the activity of the smaller hydrolysate being stronger. Since SSPS was produced by extraction from soybean cotyledons, LMW seems to have had an amino acid composition similar to that of the hydrolysate.

**Oxidation process of linoleic acid encapsulated with the low- or high-molecular-weight component mixed with maltodextrin**

Figure 3 shows the oxidation process of linoleic acid encapsulated with various wall materials at 37°C and at 12%, 44% and 75% relative humidity. The weight fraction of LMW or HMW in the mixture with maltodextrin was 0.5. The oxidation of linoleic acid encapsulated with the mixture of LMW with maltodextrin was significantly retarded at any relative humidity. The mixture of HMW with maltodextrin was ineffective for suppressing the oxidation of linoleic acid encapsulated with it as well as with maltodextrin alone.

**Oxidation process of linoleic acid encapsulated with mixtures of the low- and high-molecular-weight components at various weight fractions**

To evaluate the effect of the weight fraction of LMW in a wall material on the oxidation of encapsulated linoleic acid, the acid was microencapsulated with mixtures of LMW and HMW, and then oxidized at 37°C and at various levels of relative humidity (Fig. 4). In this experiment, HMW was used as the basic wall material instead of maltodextrin, because HMW has the same antioxidative property as maltodextrin, as shown in Fig. 3. At any relative humidity, the oxidation of linoleic acid encapsulated with the wall materials containing the higher LMW weight fractions was more retarded. The weight fraction of LMW in SSPS was 0.17. The oxidation process of linoleic acid encapsulated with SSPS was similar to that of linoleic acid encapsulated with the mixture containing the 0.18 LMW weight fraction at 12% and 44% relative humidity. However, the process with the former was much faster than that with the latter at 75% relative humidity.

**Stability of O/W emulsions containing a wall material**

Figure 5 shows the stability of O/W emulsions containing an aqueous phase of SSPS, maltodextrin, HMW, a mixture of HMW and maltodextrin, or a mixture of LMW and maltodextrin at a concentration of 13% (w/v). Among the wall materials tested, SSPS showed the highest emulsion stability. The emulsions prepared with the aqueous solution of HMW or the mixture of HMW with maltodextrin were also stable for 2 days. The absorbance of the emulsions prepared with the aqueous solution of the mixture of LMW and maltodextrin rapidly decreased in the early stage, indicating the instability of the emulsions.

Figure 6 shows the stability of O/W emulsions which were used for preparing microcapsules with different weight fractions of LMW. The emulsions with the higher LMW weight fraction tended to be more unstable. The inset to Fig. 6 shows the relationship between the median diameter of the oil droplets in the emulsion immediately after preparation and the LMW weight fraction in the mixture. There was no significant effect of the LMW weight fraction on the median diameter, although the median diameter of the oil droplets in the emulsion prepared with the mixture containing the 0.36 LMW weight fraction was slightly larger.
We have previously shown that SSPS produced a fine O/W emulsion and that the oxidation of linoleic acid encapsulated with SSPS was slower than that of non-encapsulated linoleic acid.⁷) SSPS therefore possessed emulsifying, emulsion-stabilizing, and antioxidative activities. The results shown in Figs. 3 to 6 indicate that the emulsifying and emulsion-stabilizing activities originated from HMW, while the antioxidative property was due to LMW which had high radical-scavenging activity.

**Effect of relative humidity on the oxidation of encapsulated linoleic acid**

Figure 3 shows that linoleic acid tended to be oxidized more at higher relative humidity, except for the case of linoleic acid encapsulated with the mixture of LMW and maltodextrin. Figure 4 also shows that the oxidation process of linoleic acid encapsulated with the mixture of LMW and HMW depended on the relative humidity. Since the relative humidity affected the oxidative stability of encapsulated linoleic acid, the sorption isotherms of water on SSPS, HMW and maltodextrin at 37°C were measured (Fig. 7). The isotherm for each wall material could be expressed by the Guggenheim-Anderson-de Boer equation:

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q = \frac{q_m c k p}{(1 - kp)(1 + (1 - (c - 1)k p)}
\]

where \( q \) is the amount of water sorbed, \( p \) is the relative humidity, \( q_m, k \) and \( c \) are parameters, and \( q_m \) indicates the amount of water for monolayer coverage. The \( q_m, k \) and \( c \) values estimated for SSPS, HMW and maltodextrin were 0.0727 g/g, 0.846 and 12.4, 0.0708 g/g, 0.939 and 7.67, and 0.0573 g/g, 0.866 and 23.8, respectively. The isotherms indicate that the wall materials were not fully covered with water molecules at 12% relative humidity, but that they were fully covered with water molecules at 44% relative humidity.
It has been reported that limonene in orange oil encapsulated with maltodextrin was scarcely oxidized at low and high relative humidity, but was susceptible to oxidation at intermediate levels of relative humidity.\(^{16}\) These results are similar to those in the present work. The susceptibility of encapsulated limonene was interpreted to be based on the glass transition of the wall material and the collapse of the matrix of the wall material in the rubber state.\(^{16}\) Glass transition seems to have played a role in the oxidation of linoleic acid encapsulated with SSPS, HMW or maltodextrin, although we did not measure the glass transition of the wall materials. Since the wall material adsorbed much more water at high levels of relative humidity, the mobility of LMW would have been increased to facilitate its antioxidative ability for linoleic acid.

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**References**


