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Deodorization of Soybean Proteins by Enzymatic and Physicochemical Treatments

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To utilize soy protein isolate (SPI) more widely, a convenient and effective method for deodorizing it is required. This paper reports a new deodorizing method using various types of solid adsorbents made of polystyrene, polymethacrylate, and zeolite, as well as charcoal. Treatment of the SPI solution with them decreased the hexanal content in the solution, whereas the content of linoleic acid was not much decreased. A brominated polystyrene adsorbent (SEPABEADS SP207) and a zeolite adsorbent (HSZ-360HUD) removed hexanal most effectively, although 30−40% of the total hexanal remained. A model experiment showed that their hexanal adsorption capacity was much higher than the hexanal content in the SPI solution and that an excess amount of hexanal added to the SPI solution was mostly removed by them. These results suggest that hexanal in the SPI solution can be classified into two types. Hexanal of type I may be free or bound weakly on the surface of proteins and is removable by the adsorbents, whereas hexanal of type II may be bound tightly inside proteins and is unremovable by the adsorbents. Despite the considerable amount of hexanal remaining in the SPI solution even in the most successful cases, the SPI solution was well deodorized as shown by the sensory test. Accordingly, type I hexanal may be closely related to the soybean odor. Removal of hexanal by the adsorbents was not much improved by α-chymotryptic digestion of SPI. Type II hexanal might be in similar states even in the chymotryptic digests.

KEYWORDS: Adsorbent; hexanal; deodorization; soybean; soy protein isolate

INTRODUCTION

Soybean is a major source of plant oil, and a large quantity of defatted soy proteins is available economically. Soybean proteins have high nutritional quality and good functionality for foods (*1*). Although the defatted soy proteins have been considered to be a prominent protein resource to overcome a food shortage, they are not utilized for foods and are yet treated as waste matter and are mainly fed to domestic animals. One of the reasons preventing their effective and wide utilization is an unfavorable offensive odor. It was reported that one of the major compounds of the soybean odor is hexanal, which is considered to be generated from linoleic acid through peroxidation by lipoxygenase, followed by decomposition of linoleic 13-hydroperoxide by hydroperoxide lyase (*2*−*4*). For the purpose of deodorizing soy proteins, enzymes such as aldehyde dehydrogenase (*4*, *5*) and lipase (Trumbetas, J. F.; Franzen, R. W.; Loh, J. P. Removal of odor and offensive taste from protein foods using lipases. Eur. Patent EP 572139 A2, 1993), microorganisms (*6*), ion exchange resins (Nakamura, M.; Nishitani. T.; Mihashi, T.; Tomizawa, A. Method for deodorizing soybean proteins with ion exchangers. Jpn. Patent JP 94276955 A2, 1994), and ultracritical carbonic dioxide gas (*7*) have been applied. However, none of these is satisfactory for the purpose. On the other hand, soybean species lacking lipoxygenase have been bred, but the generation of hexanal has not yet been successfully eliminated (*8*−*10*). Recently, it has been reported that the odor of the soy protein isolate (SPI) is greatly diminished when oil-body-associated proteins (*11*, *12*) are removed from SPI (*13*).

In this study, we aim to establish a convenient and effective method for deodorizing SPI with various solid adsorbents. The efficiency of deodorization was evaluated by measuring hexanal and linoleic acid and by a sensory test as well. Furthermore, we also describe the effect of proteolytic digestion on the removal of hexanal with the adsorbents. These results suggest a new concept for states of hexanal in SPI and the relationship between the states and the soybean odor.

MATERIALS AND METHODS

Proteins. SPI (Fujipro R, lot 97.06.14.018) was supplied by Fuji Oil Co. (Osaka, Japan). The SPI sample was prepared according to the method described (*13*). Defatted soybean flour was prepared by solvent extraction of soybean flakes with *n*-hexane at 40 °C. The defatted soybean flour was mixed with water (1:15, w/w) and stirred for 1 h at pH 7.5, followed by centrifugation at 10000*g* for 10 min. Defatted soybean extract was prepared in the supernatant by removing insoluble

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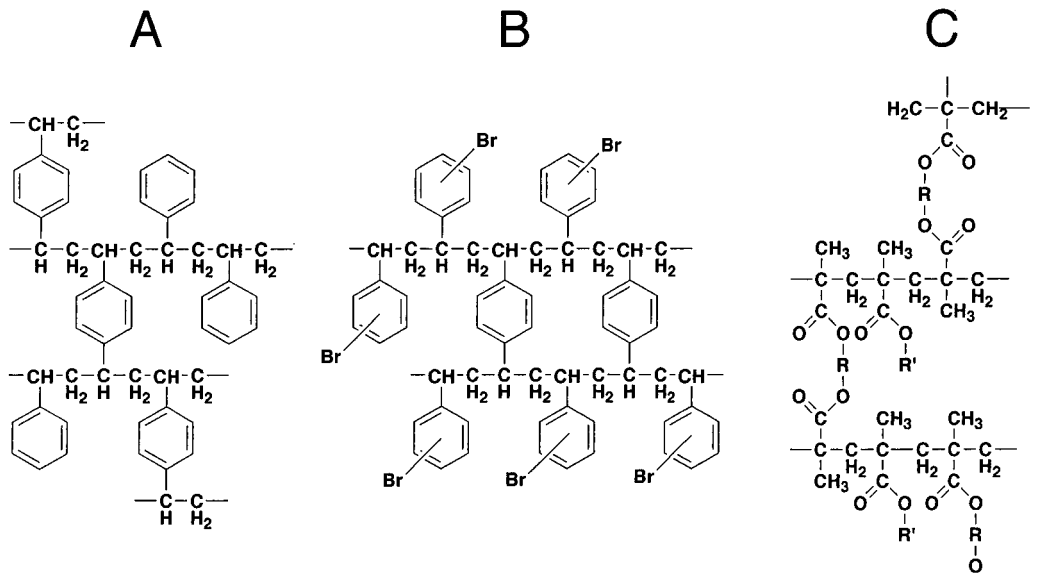

Figure 1. Chemical structures of DIAION and SEPABEADS adsorbents: (A) polystyrenic adsorbents, DIAION HP20 and SEPABEADS SP825; (B) brominated polystyrenic adsorbent, SEPABEADS SP207; (C) polymethacrylic adsorbent, DIAION HP2MG. Characteristics of the adsorbents are shown in **Table 1**.

Table 1. General and Adsorptive Properties of Zeolite Adsorbents and Synthetic Polymer Adsorbents (DIAION and SEPABEADS Adsorbents)[a]

	Zeolite	
	HSZ-690HOD3A	HSZ-360HUD
Na_2O (wt %)	<0.05	<0.10
SiO_2/Al_2O_3 (mol/mol)	200	15
surface area (m^2/g)	420	600
crystal size (μm)	0.1–0.5	0.2–0.4
main particle size (μm)	5–7	6–8
hydrophobicity	++	+

	Synthetic Adsorbents			
	DIAION		SEPABEADS	
	HP20	HP2MG	SP825	SP207
pore volume (mL/g)	1.2	1.2	1.6	1.1
surface area (m^2/g)	600	470	1000	630
pore radius (Å)	200–300	150–300	50–60	100–120
hydrophobicity	++	+	++	+++

[a] The values were cited from the manufacturers' specifications. Relative strength of hydrophobicity: +++, high; ++, medium; and +, low.

materials. The defatted soybean extract was mixed with 4 volumes of water and kept at pH 4.5. Precipitates were collected by centrifugation at 10000*g* for 10 min. SPI was prepared by suspending the precipitates in water at pH 7.5 and spray-dried. According to the manufacturer, the moisture content in the SPI preparation was 4.9%, the protein content estimated by Kjeldahl method was 90.0% (dry), and the ash content was 4.3% (dry). An SPI solution of 50 mg/mL in 20 mM Tris-HCl buffer was used at pH 8.0, at which the solubility of the SPI protein was maximum, in all experiments unless otherwise mentioned. α-Chymotrypsin (lot ELS3559) was purchased from Wako Pure Chemical Industries (Osaka, Japan).

Chemicals. Adsorbents examined were as follows: zeolite beads, HSZ-360HUD (or HSZ-360) and HSZ-690HOD3A (or HSZ-690); polystyrene beads, DIAION HP20 and SEPABEADS SP825; polymethacrylate beads, DIAION HP2MG; brominated polystyrene beads, SEPABEADS SP207; and charcoal (washed with HCl). The zeolite adsorbents were supplied by Tosoh Co. (Tokyo, Japan). Their general and adsorptive properties are shown in **Table 1**. The DIAION and SEPABEADS adsorbents were supplied by Mitsubishi Chemical Co. (Tokyo, Japan). Before use, the adsorbents were immersed in ethanol for 30 min and then immersed in water for 30 min. The water-immersed adsorbents were used immediately without drying. **Figure 1** shows the structures of the adsorbents. All other chemicals were of reagent grade and purchased from Nacalai Tesque (Kyoto, Japan).

Measurement of Hexanal. Hexanal was reacted with 2,4-dinitrophenylhydrazine (DNPH) and was converted to a derivative of 2,4-dinitrophenylhydrazone, which was subjected to high-performance liquid chromatography (HPLC) (*14*). Fifty milligrams of DNPH was dissolved in a 100 mL solution containing ethanol/HCl/water = 90:2:8 (v/v/v), which was termed a DNPH solution. One milliliter of the sample solution was mixed with 1 mL of the DNPH solution and allowed to stand at 45 °C for 30 min. After centrifugation at 5000*g* for 10 min at 4 °C, 0.2 mL of the supernatant was mixed with 0.8 mL of acetonitrile, and the mixture was centrifuged at 5000*g* for 10 min at 4 °C. Thirty microliters of the supernatant was subjected to HPLC on a YMC ODS-AMC18 column (i.d. 4.6 mm × 300 mm; Kyoto, Japan) equilibrated with 70% acetonitrile, at 50 °C in a Hitachi HPLC 7000 system. The DNPH derivative of hexanal was eluted with a linear gradient of acetonitrile from 70 to 100% conducted over 15 min from time 0 at a flow rate of 1 mL/min and was monitored by absorbance at 360 nm. The standard solutions of hexanal (0.5−10 ng/mL) in 20 mM Tris-HCl buffer (pH 8.0) were prepared, being treated in the same way as for the SPI solution, and the calibration curve of hexanal was obtained.

Measurement of Linoleic Acid. Linoleic acid was reacted with 5% HCl in methanol, converted to a methylated derivative, and subjected to gas chromatography (GC). Six milliliters of the sample solution was added to 12 mL of chloroform/methanol mixture (1:1, v/v), shaken vigorously, and allowed to stand for 30 min. After 3 mL of the chloroform layer was transferred into a tube and evaporated at an ordinary pressure, 0.3 mL of methanol was added to the tube and the wall rinsed. An equal volume of 5% HCl in methanol was added to the tube and mixed well. Then the tube was sealed and allowed to stand at 95 °C for 2 h. After extraction with 0.2 mL of hexane, 1 μL of the hexane layer was injected into a 10% Silar 10C-Chromosorb W column MESH 80−100 (i.d. 3.2 mm × 2100 mm) in a Shimadzu GC-9APTF GC system (Kyoto, Japan). Methylated linoleic acid was vaporized with a linear gradient of temperature (4 °C/min) from 160 to 240 °C with a flow rate of N_2 gas (60 mL/min) and monitored by a flame ionization detector (FID). The standard solutions of linoleic acid (0.02−0.2 μg/mL) in 20 mM Tris-HCl buffer (pH 8.0) were prepared, being treated in the same way as for the SPI solution, and the calibration curve of linoleic acid was obtained.

Treatment of SPI by Adsorbents. Two grams at the dry-weight base of the adsorbent was added to 40 mL of the SPI solution in a 100

mL glass beaker (diameter 5 cm) and stirred for 15 min at 25 °C; the top of the beaker was sealed with a sheet of laboratory film (Parafilm, American National Can, Menasha, WI). Two milliliters of the solution was taken out and centrifuged at 5000*g* for 10 min to precipitate the adsorbent. The supernatant was used for the subsequent analysis.

Binding Capacity of the Adsorbents to Hexanal. Two grams of each absorbent was added to 40 mL of 20 mM Tris-HCl buffer (pH 8.0) containing various concentrations (0−225 μM) of hexanal. In the same way, 2 g of each adsorbent was added to 40 mL of the SPI solution containing various concentrations (0−113 μM) of hexanal. After the hexanal-containing solution had been stirred with adsorbents for 15 min at 25 °C, 2 mL of the mixture was centrifuged at 5000*g* for 10 min. The supernatant was subjected to subsequent analysis of hexanal.

α-Chymotryptic Digestion of SPI and Its Effect on the Removal of Hexanal by the Adsorbents. SPI (50 mg/mL) in 20 mM Tris-HCl buffer (pH 8.0) was digested by various concentrations (0−48 nM) of α-chymotrypsin in the presence of 0.05% NaN_3 at 37 °C for 12 h, and the SPI solution was treated by the adsorbents in the same way as described above. Hexanal was extracted three times with an equal volume of the chloroform/methanol mixture (1:1,v/v), followed by evaporation at an ordinary pressure at 15−20 °C. The residual hexanal was dissolved in 20 mM Tris-HCl buffer (pH 8.0) and subjected to subsequent analysis of hexanal.

Analytical Studies. SDS-PAGE of the chymotryptic digests of SPI was performed using a 4−20% gradient gel system (Multigel 4/20, Daiichi Pure Chemicals, Tokyo) under reducing conditions (*15*). Ten microliters of the SPI solution (50 mg/mL in 20 mM Tris-HCl buffer, pH 8.0) was mixed with 90 μL of 20 mM phosphate buffer (pH 6.8). Thirty microliters of the mixture was added to 30 μL of 625 mM Tris-HCl buffer (pH 6.8) containing 2% SDS, 15% glycerol, 5% 2-mercaptoethanol, and 0.001% bromophenol blue (BPB). Then, the solution was treated at 100 °C for 10 min and applied to SDS-PAGE. Proteins were stained with Coomassie brilliant blue. The molecular mass marker kit (Daiichi-II, Daiichi Pure Chemicals) containing rabbit muscle myosin (200 kDa), *Escherichia coli* β-galactosidase (116 kDa), bovine serum albumin (66 kDa), rabbit muscle aldolase (42 kDa), bovine erythrocyte carbonic anhydrase (30 kDa), and horse muscle myoglobin (17 kDa) was a product of Daiichi Pure Chemicals.

Sensory Test. The odor of the SPI solution treated by the adsorbents was examined by sensory test. The test was performed by 22 untrained volunteers (graduate and undergraduate students in our laboratory; 13 males and 9 females) at the ages of 20−26. Two grams at the dry weight base of the adsorbent was added to 40 mL of the SPI solution in a 100 mL glass beaker (diameter 5 cm) and stirred for 15 min at 25 °C; the top of the beaker was sealed with a sheet of laboratory film (Parafilm). The volunteers smelled the SPI solution immediately after uncovering the top of the beaker in a dark room kept at 25 °C. The time taken for smelling each sample was set for up to 15 s. Before and after the volunteers smelled the sample solution, they were allowed to smell the control (the SPI solution that had not treated with the adsorbents) and water. After smelling the sample solution, each volunteer gave three kinds of marks according to the strength of the residual soybean odor: 0, not smelled; +1, slightly smelled; or +2, strongly smelled. Then, the next sample solution was presented to the volunteer. The sample solutions were presented to the volunteers in the order of those treated with charcoal, SP207, HP20, SP825, HP2MG, HSZ-360, and HSZ-690. The efficiency of the adsorbents in the deodorization was represented as a percentage of the sum of the marks given by 22 volunteers against 44 points given for the control solution without treatment with the adsorbents. The same tests were done by changing the order of the sample solutions presented to the volunteers in the order of those treated with HP2MG, SP825, HSZ-690, HP20, SP207, HSZ-360, and charcoal and in the order of those treated with charcoal, HP2MG, HSZ-360, SP825, HP20, HSZ-690, and SP207. The sensory tests were performed for the α-chymotryptic digests of SPI in the same system as done for the SPI solution. SPI (50 mg/mL, 40 mL) in 20 mM Tris-HCl buffer (pH 8.0) was digested by α-chymotrypsin (48 nM) at 37 °C for 12 h in the presence of 0.05% NaN_3. After the digests had been cooled by incubation at 25 °C for 10 min, they were treated with 2 g of adsorbents with stirring at 25 °C for 15 min and then supplied to the sensory test.

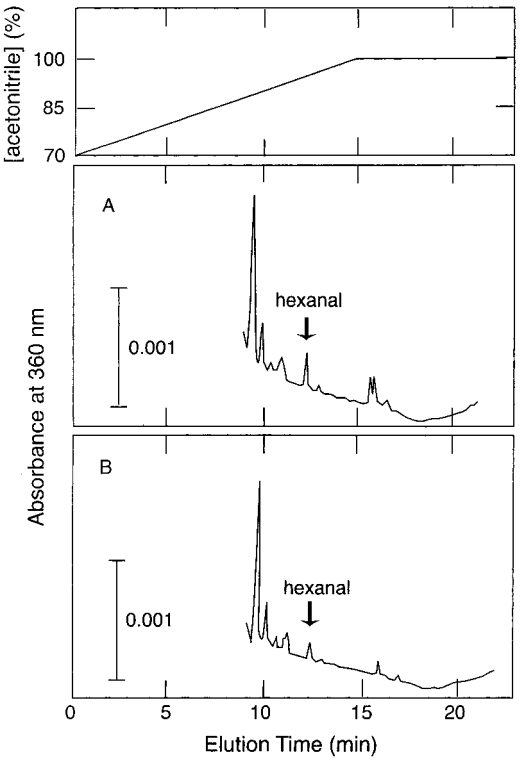

Figure 2. HPLC of the SPI solution for the hexanal determination: (A) SPI solution without adsorbent treatment; (B) SPI solution treated with SEPABEADS SP207. The DNPH derivative of hexanal was eluted at 12 min.

RESULTS

Elimination of Hexanal in the SPI Solution by Treatment with the Adsorbents. Figure 2 shows the HPLC patterns of the SPI solutions prepared for determining hexanal. The DNPH derivative of hexanal in the SPI solution without the adsorbent treatment was eluted at 12 min (**Figure 2A**), and the concentration of hexanal detected was determined to be 1.3 μM (130 ng/mL) from the calibration curve. When the SPI solution was treated with a brominated polystyrene adsorbent, SEPABEADS SP207, the peak for hexanal was remarkably decreased to 0.4 μM (40 ng/mL), and other peaks appearing from 15 to 20 min almost disappeared (**Figure 2B**). The efficiency of various adsorbents in the removal of hexanal is shown in **Figure 3**, where the vertical axis shows the percentage of the hexanal concentration after the adsorbent treatment against the control value (1.3 μM). In all cases, the amount of the residual hexanal decreased. Particularly, zeolite HSZ-360HUD and SEPABEADS SP207 adsorbents removed hexanal most effectively, and SEPABEADS SP825 showed fairly good efficiency. The efficiencies of charcoal, DIAION HP20, DIAION HP2MG, and HSZ-690HOD3A were similar, and 55−60% of hexanal remained. It is noted that hexanal was not eliminated completely even with the most successful adsorbents, and more than one-third of hexanal remained. As to the zeolite adsorbents, HSZ-360HUD is less hydrophobic than HSZ-690HOD3A, and the former is more effective than the latter in eliminating hexanal. As to the synthetic adsorbents, the efficiency seems to be increased with the increase in hydrophobicity of the adsorbents.

Elimination of Linoleic Acid in the SPI Solution by Treatment with the Adsorbents. Figure 4 shows the GC patterns of the SPI solutions prepared for determining linoleic acid. The methylated linoleic acid in the SPI solution without the adsorbent treatment was eluted at 9 min (**Figure 4A**), and

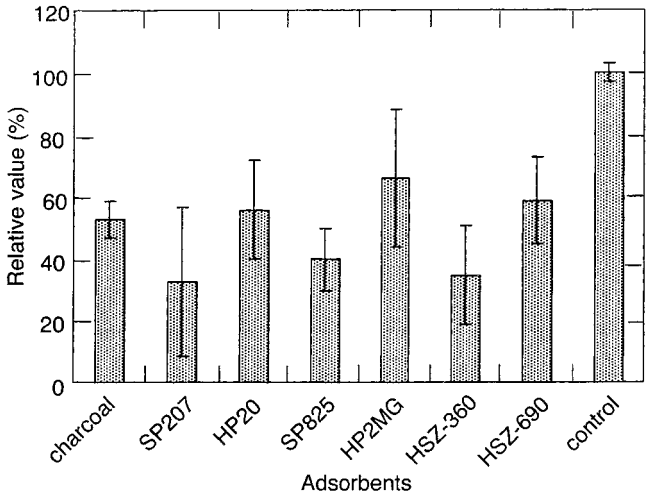

Figure 3. Removal of hexanal from the SPI solution by the adsorbents. The relative value of the hexanal content in the SPI solution treated with the adsorbent is shown. The content (1.3 μM) in the control SPI solution was set to 100%. The measurement was replicated five times.

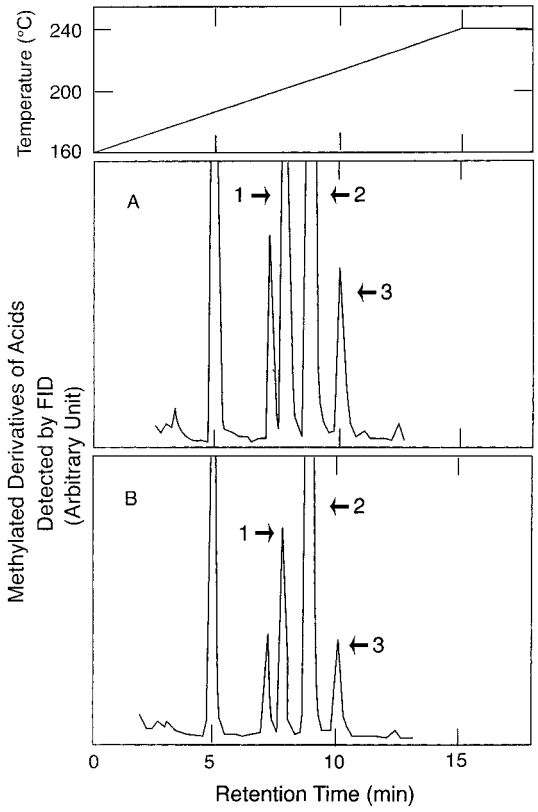

Figure 4. GC of the SPI solution for the determination of linoleic acid: (A) SPI solution without adsorbent treatment; (B) SPI solution treated with charcoal. Oleic acid (peak 1), linoleic acid (peak 2), and linolenic acid (peak 3) were eluted at 8, 9, and 10 min, respectively.

its concentration was determined to be 430 μM (120 μg/mL) from the calibration curve. Peaks of oleic acid and linolenic acid were also observed at 8 and 10 min, respectively. After the treatment of the SPI solution with charcoal, 360 μM (100 μg/mL) of linoleic acid was detected in the solution (**Figure 4B**). **Figure 5** shows the effect of the adsorbents in eliminating linoleic acid from the SPI solution, where the vertical axis shows the percentage of the concentration of linoleic acid detected after the adsorbent treatment against the control value (430 μM). Charcoal was the most effective in eliminating linoleic acid,

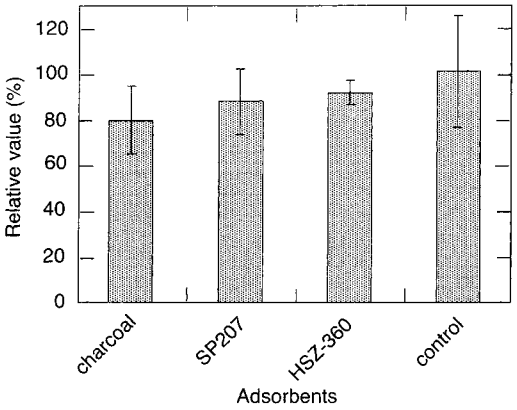

Figure 5. Removal of linoleic acid from the SPI solution by adsorbents. The relative value of the linoleic acid content in the SPI solution treated with the adsorbent is shown. The content (430 μM) in the control SPI solution was set to 100%. The measurement was replicated five times.

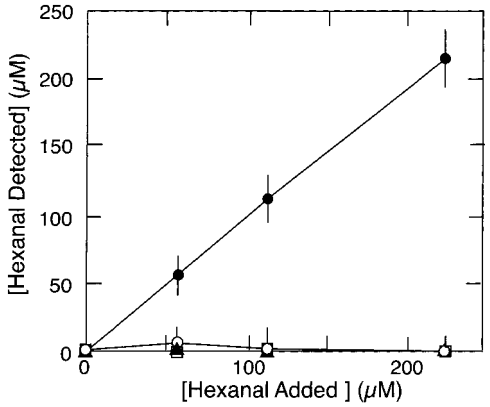

Figure 6. Effect of adsorbents on the removal of hexanal added to the 20 mM Tris-HCl buffer (pH 8.0) solutions containing various concentrations of hexanal: (●) control; (▲) charcoal-treated; (○) SEPABEADS SP207-treated; (□) zeolite HSZ-360HUD-treated. The relative area of the hexanal peak eluted in HPLC was plotted against hexanal concentration added to the buffer solution. The measurement was replicated five times.

and the residual amount was 80% of the control. The residual linoleic acid decreased slightly to 85% with HSZ-360HUD and SEPABEADS SP207, but linoleic acid was hardly removed with other adsorbents. It should be noted that the residual oleic acid and linolenic acid were also decreased to 75−80% by the treatment with charcoal, but others were not effective in the elimination of oleic and linolenic acids (figures not shown).

Binding Capacity of the Adsorbents to Hexanal. A model experiment was conducted to examine the hexanal-binding capacity of the adsorbents charcoal, HSZ-360HUD, and SEPABEADS SP207. An excess amount of hexanal (56−225 μM) compared with that detected with the SPI solution (1.3 μM, **Figure 2**) was added to 20 mM Tris-HCl buffer, and the buffer solution containing hexanal was treated by the absorbents. All adsorbents eliminated hexanal completely from the buffer solution (**Figure 6**), indicating that the binding capacity of the adsorbents is >225 μM hexanal.

Elimination of Hexanal Added to the SPI Solution by Treatment with the Adsorbents. Another model experiment was conducted to examine the relationship between the hexanal-binding capacity of the adsorbents (charcoal, HSZ-360HUD, and SEPABEADS SP207) and the states of hexanal in the SPI solution. After an excess amount of hexanal (23−113 μM) was added to the SPI solution, the solution was treated by the adsorbents (**Figure 7**). The hexanal level in the SPI solution

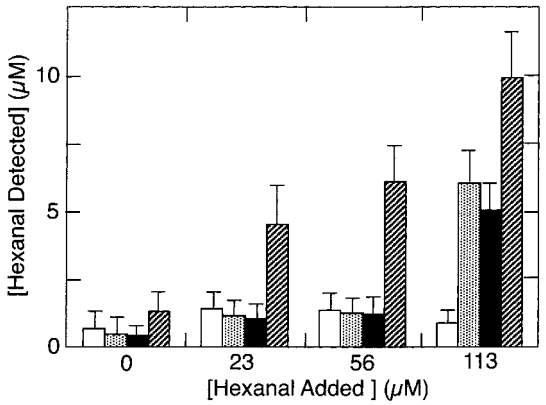

Figure 7. Effect of adsorbents on the removal of hexanal from SPI solutions to which hexanal had been added externally: (white bars) charcoal-treated; (black bars) SEPABEADS SP207-treated; (dotted bars) HSZ-360HUD; (hatched bars) control. The measurement was replicated five times.

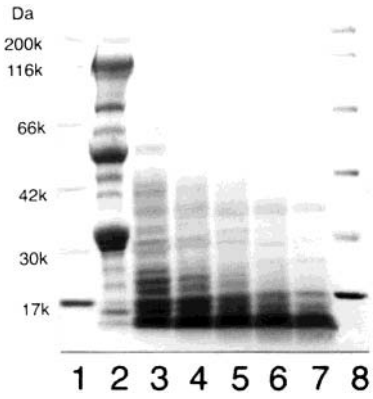

Figure 8. SDS-PAGE of α-chymotryptic digests of SPI: (lanes 1 and 8) molecular mass marker proteins; (lanes 2–7) SPI digests obtained in the digestion at pH 8.0 and 37 °C for 12 h with the concentrations of α-chymotrypsin indicated (lane 2, 0 nM; lane 3, 2.4 nM; lane 4, 4.8 nM; lane 5, 9.6 nM; lane 6, 24 nM; lane 7, 48 nM).

without the adsorbent treatment increased with an increase of hexanal added. However, it should be noted that the hexanal level detected was much lower than the hexanal added. When 113 μM hexanal was added to the SPI solution, only 10 μM hexanal was detected in the SPI solution, suggesting that almost all hexanal added externally was bound tightly to the SPI protein and not removed by the adsorbents. When hexanal at not more than 56 μM was added to the SPI solution, it was mostly removed by the adsorbents, and 1.3 μM hexanal remained. The residual amount of hexanal slightly increased in comparison with that observed for the SPI solution without externally added hexanal. When 113 μM hexanal was added, charcoal removed the added hexanal mostly to the residual level of 1.3 μM, but HSZ-360HUD and SEPABEADS SP207 did not remove well the added hexanal, and the residual level was 5.0−6.0 μM.

Digestion of SPI by α-Chymotrypsin. SPI was digested by various concentrations (2.4−48 nM) of α-chymotrypsin at pH 8.0 for 12 h at 37 °C (**Figure 8**). Major protein bands of 34, 50, and 110 kDa almost disappeared in the digestion at 9.6 nM α-chymotrypsin. We prepared three different chymotryptic digests with α-chymotrypsin at 0, 2.4, and 9.6 nM and treated them with the adsorbents zeolite HSZ-360HUD and SEPABEADS SP207. The residual level of hexanal after the treatment with the adsorbents was not changed significantly even by the chymotryptic digestion, despite the considerable fragmentation of SPI (**Figure 9**).

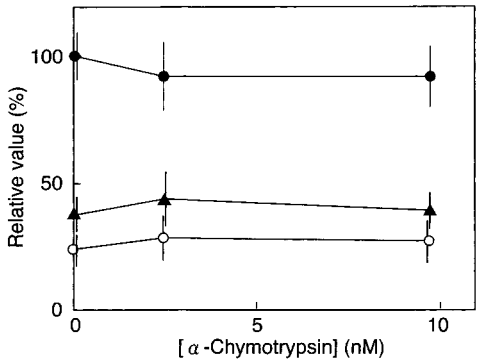

Figure 9. Effects of adsorbents on the removal of hexanal from the α-chymotryptic digests of SPI treated with (●) control, (▲) SEPABEADS SP207, and (○) zeolite HSZ-360HUD. The measurement was replicated five times.

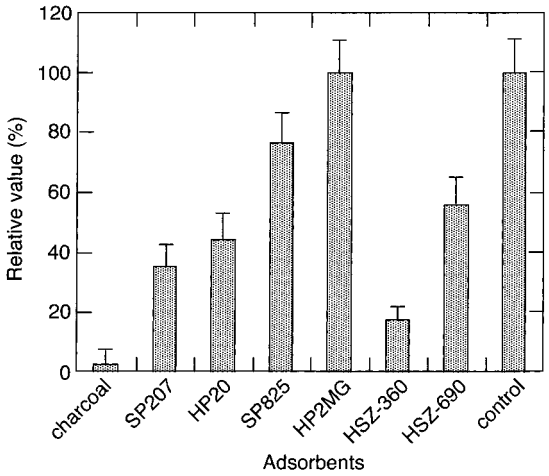

Figure 10. Efficiency of adsorbents in deodorization of SPI and the α-chymotryptic SPI digests as examined by a sensory test. The efficiency of the adsorbents in the deodorization is represented as the percentage in the sum of the marks of 22 persons against the control of 44 points. The test was replicated three times. Sample solutions: (dotted bars) SPI solution; (black bars) α-chymotryptic SPI digests.

Sensory Test. In the sensory test, the SPI solution treated with charcol gave the lowest score, and almost all volunteers did not perceive the soybean odor (**Figure 10**). Zeolite HSZ-360HUD gave a fairly low score, and only a few volunteers perceived the odor. The sensory level of the SPI solutions was considerably improved by the treatment with SEPABEADS SP207, DIAION HP20, and HSZ-690HOD3A, but that of the SPI solutions treated with SEPABEADS SP825 and DIAION HP2MG was only slightly improved. The order of the effectiveness of the adsorbents in the sensory test was charcol > HSZ-360 > SP207 > HP20 > HSZ-690 > SP825 > HP2MG. This order is different from that in the elimination of hexanal: SP207 > HSZ-360 > SP825 > charcoal > HP20 > HSZ-690 > HP2MG (**Figure 3**). This discrepancy suggests that hexanal is not the only cause of the soybean odor and that there might be other causes. The effect of α-chymotryptic digestion of SPI was also examined. The strength of the odor was shown to be slightly decreased when SPI was digested by α-chymotrypsin. Thus, the digestion has a positive effect on the deodorization of SPI by the adsorbents, although it is small. The order of the effectiveness of the adsorbents in the deodorization of the α-chymotryptic digests was the same as that of SPI. This suggests that the odor compounds of SPI were not effectively removed by the adsorbents even after α-chymotryptic digestion.

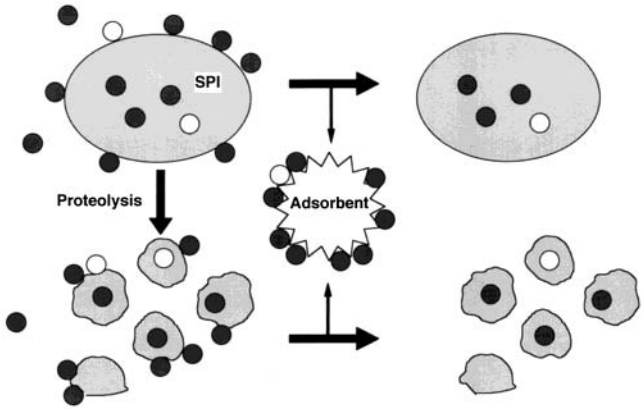

Figure 11. Schematic representation of the deodorization of SPI by the adsorbents. Some adsorbents examined can trap hexanal molecules that are free or bound weakly on the surface of the SPI proteins but cannot trap those bound strongly inside of the proteins. The strongly bound hexanal molecules cannot be trapped by the adsorbents even after digestion of the proteins. Hatched circles and open circles represent hexanal and other odor compounds, respectively.

DISCUSSION

States of Hexanal in the SPI Solution. The hexanal-binding capacity of the adsorbents charcoal, HSZ-360HUD, and SEPABEADS SP207 in 20 mM Tris-HCl buffer (pH 8.0) was determined to be >225 μM (**Figure 6**), and the capacity is much larger than the hexanal concentration (1.3 μM) in the SPI solution. However, hexanal was not eliminated completely from the SPI solution by the adsorbents, and 0.4−0.8 μM hexanal remained (**Figure 3**). When an excess amount of hexanal (23−113 μM) was added externally to the SPI solution, only 4−10 μM hexanal was detected, and almost all hexanal was not detected. This suggests that added hexanal binds too tightly to the SPI protein to be detected. In other words, the method applied here is useful for the determination of free hexanal in the solution, and it is suggested that the SPI protein can accommodate 80−90% of hexanal added and only 10−20% (4−10 μM) may be free to be detected. From these lines of evidence, we propose the states of hexanal in the SPI solution. Hexanal in the SPI solution can be classified into two types (**Figure 11**). Hexanal of type I may be free or bound weakly on the surface of proteins and can be partly removed by the adsorbents. Hexanal of type II may be bound strongly inside proteins and unremovable by the adsorbents. The idea is supported by the results that not more than 56 μM hexanal added to the SPI solution was mostly removed but a nearly constant level of hexanal remained (**Figure 7**). The finding that the remaining amount of hexanal after the treatment with SEPABEADS SP207 was larger than that by charcoal after the addition of 113 μM hexanal to the SPI solution might be explained the difference in the interaction of the adsorbents with hexanal between charcoal and SEPABEADS SP207. Fujimaki et al. showed that pepsin digestion of dried yeast promoted the ether extraction of low molecular weight compounds such as fats and flavor compounds, suggesting the existence of the low molecular weight compounds inside proteins (*16*). However, in this case, the α-chymotryptic digestion of the SPI has no effect on the removal of hexanal by the adsorbents (**Figures 8** and **9**), suggesting that the microenviroment around the unremovable hexanal was not changed after the α-chymotryptic digestion (**Figure 11**). It might be considered that difficulty in the complete removal of hexanal was due to not only the enclosure of hexanal by such proteins as oil-body-associated proteins but also the strong binding of hexanal to the soy proteins.

Correlation between the Sensory Test and the Hexanal Unremovable by Adsorbents. Charcoal, HSZ-360HUD, and SEPABEADS SP207 are the prominent adsorbents against hexanal (**Figure 3**) and also deodorize the SPI solution efficiently (**Figure 10**). However, it is worth remarking that in the treatment of the SPI solution by charcoal, considerable deodorization was attained although a considerable amount of hexanal remained (53%). This may be explained by assumption that the unremovable hexanal which is bound strongly inside proteins is not related to the soybean odor. Finally, it must be emphasized that the SPI used in this study, Fujipro R, was originally deodorized at a higher level and the content of the hexanal was considered to be a very low level. Therefore, there is a possibility that a compound having a lower odor threshold than hexanal such as 2-*n*-pentylfuran, which imparts a characteristic beany odor to an oil (*17*), comprises the soybean odor in the present study. Digestion of SPI by α-chymotrypsin was not so effective in deodorization by the adsorbents. Recently, we have reported that SPI forms coagula during its digestion by various proteases such as subtilisin (*18*). Whereas the coagulating effect of α-chymotrypsin is much weaker than that of subtilisin, the coagulum formation might be why the deodorization effect by the adsorbents was not much improved by the chymotryptic digestion.

CONCLUSION

A new deodorizing method using various types of solid adsorbents has been described. Treatment of the SPI solution with them decreased the hexanal content in the solution. A brominated polystyrene adsorbent (SEPABEADS SP207) and a zeolite (HSZ-360HUD) adsorbent removed hexanal most effectively, whereas 30−40% of the total hexanal remained. However, their hexanal adsorption capacity was much higher than the hexanal content in the SPI solution, and an excess amount of hexanal added to the SPI solution was mostly removed by them. These results suggest two types of hexanal in the SPI solution: type I hexanal may be free or bound weakly on the surface of proteins and is removable by the adsorbents, and type II hexanal may be bound tightly inside proteins and is unremovable by the adsorbents. Despite the considerable amount of hexanal remaining in the SPI solution even in the most successful cases, the SPI solution was well deodorized as shown by the sensory test. Accordingly, type I hexanal may be closely related to the odor. Removal of hexanal by the adsorbents was not much improved by α-chymotryptic digestion of SPI. Type II hexanal might be in similar states even in the chymotryptic digests.

ABBREVIATIONS USED

DNPH, dinitrophenylhydrazine; SPI, soy protein isolate; HPLC, high-performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; GC, gas chromatography; FID, flame ionization detector; BPB, bromophenol blue.

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