

REVIEW

Immunological Functions of Soy Sauce: Hypoallergenicity and Antiallergic Activity of Soy Sauce

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Soy sauce is a traditional fermented seasoning of East Asian countries and is available throughout the world. In Japanese soy sauce (*shoyu*), soybeans and wheat are the two main raw materials, used in almost the same quantity. Proteins of the raw materials are completely degraded into peptides and amino acids by microbial proteolytic enzymes after fermentation, and no allergens of the raw materials are present in soy sauce. In contrast, polysaccharides originating from the cell wall of soybeans are resistant to enzymatic hydrolyses. These polysaccharides are present in soy sauce even after fermentation and termed *shoyu* polysaccharides (SPS). Soy sauce generally contains about 1% (w/v) SPS and SPS exhibit potent antiallergic activities *in vitro* and *in vivo*. Furthermore, an oral supplementation of SPS is an effective intervention for patients with allergic rhinitis in two double-blind placebo-controlled clinical studies. In conclusion, soy sauce would be a potentially promising seasoning for the treatment of allergic diseases through food because of its hypoallergenicity and antiallergic activity.

[Key words: allergy, allergen degradation, antiallergic activity, quality of life, *shoyu* polysaccharides (SPS), soy sauce]

Soy sauce is traditionally used in Japan and several oriental countries, and is a liquid seasoning that is currently used in cooking worldwide (1). The daily consumption of soy sauce in Japan is estimated at about 30 ml per person according to data from the Japan Soy Sauce Brewers Association, 1988. Recent studies suggest that soy sauce contains certain bioactive components in addition to taste and aroma compounds, and various biological activities of soy sauce have been reported, including anticarcinogenic (2, 3), antimicrobial (4), antioxidative (5–7), and antiplatelet (8) activities, and the inhibition of an angiotensin I-converting enzyme (9). Therefore, soy sauce is considered to be not only a traditional seasoning but also a functional seasoning. In this review, new immunological functions of soy sauce are described with respect to allergy, such as the hypoallergenicity and antiallergic activity of soy sauce.

I. JAPANESE SOY SAUCE (*SHOYU*)

Shoyu is the Japanese name for soy sauce, a commonly used liquid seasoning. Many varieties of *shoyu* are produced in Japan and other oriental countries. Their characteristics depend on the various types and different ratios of raw materials used, the types of microorganisms employed, and the

conditions of fermentation. Naturally brewed *shoyu* is prepared as shown in Fig. 1 (1, 10–12). Moistened soybeans are cooked under both high pressure and temperature, while the wheat is roasted and crushed. These two materials are mixed with a small amount of seed mold, *Aspergillus oryzae* or *A. sojae*. This mixture is placed on a large porous plate through which temperature- and moisture-controlled air is passed to provide the appropriate conditions for mold growth and enzyme production. The mold-cultured material is termed *koji*, and the *koji* is mixed with high NaCl in water to make a mash called *moromi*. The *moromi* containing about 15% (w/v) NaCl is stored in large tanks for several months at room or an elevated temperature. The *moromi* is fermented with lactobacilli and yeasts and then well aged. The aged *moromi* is pressed, and the liquid part (raw soy sauce) is pasteurized to obtain the final product, soy sauce (*shoyu*).

In Japan, five typical types of soy sauce are produced as recognized by the Japanese Agricultural Standard (JAS). *Koikuchi* is dark in color and made from approximately equal weights of soybeans and wheat. *Usukuchi* is also made from a mixture containing approximately equal weights of soybeans and wheat. Sometimes, rice is added to these materials in *usukuchi*. The principles of *usukuchi* preparation are almost the same as those of *koikuchi* preparation except that all of the processes are directed towards producing a lighter color in the final product. *Shiro* is very light in color and is made mostly from wheat with very little soybean. In

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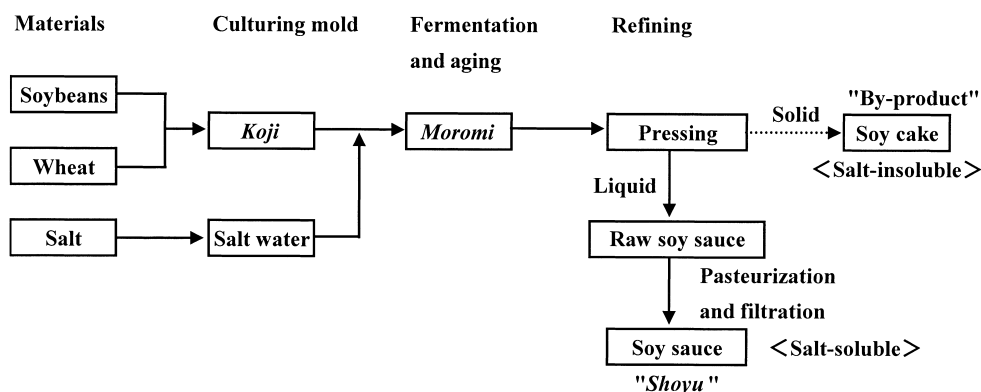


FIG. 1. Brewing process of soy sauce from raw materials to final product *shoyu*.

contrast, *tamari* is extremely dark in color and is made mostly from soybean with very little wheat. *Saishikomi* is made from the *moromi* mixed with both the *koji* and raw soy sauce instead of the more commonly used salt water. Of all the types of *shoyu* consumed in Japan, the two major types, *koikuchi* and *usukuchi*, account for about 95% of the total consumption. Although *koikuchi shoyu* has a strong aroma and a dark color during cooking, *usukuchi shoyu* is used mainly for cooking in order to preserve the original color and flavor of food.

II. DEGRADATION OF WHEAT ALLERGEN IN SOY SAUCE

IgE-mediated hypersensitive responses to wheat have long been emerging as an important public health problem (13). An α -amylase inhibitor from the salt-soluble fraction of wheat flour and other proteins in the salt-insoluble fraction were identified as allergens (14–19), and their IgE-binding epitope was also determined (20–23).

Soy sauce possesses microbial proteolytic activity during fermentation, which degrades soybean proteins including a major soybean allergen (Gly m Bd 30K), into peptides and amino acids after fermentation (24, 25). Although it has been demonstrated that the allergenicity of soybeans in soy sauce is reduced, another major material of soy sauce, wheat, has not been investigated for IgE-mediated allergy. Therefore, by immunoblotting, inhibition ELISA and direct ELISA using sera from five children with wheat allergy, we have shown the degradation of wheat allergens in both salt-soluble and salt-insoluble fractions from each brewing process between raw materials and the final soy sauce (26). In this section, the reduction of wheat allergens in soy sauce is explained from two aspects: solubilization of salt-insoluble protein and degradation of salt-soluble proteins during fermentation.

Immunoblotting The proteins of raw materials were almost totally degraded into peptides and amino acids in soy sauce during fermentation. However, it was necessary to exclude the possibility that IgE-binding wheat proteins remain in soy sauce after fermentation, using immunological methods. For this purpose, immunoblotting analyses were performed using pooled sera from the five children with wheat allergy (26). Either salt-soluble fractions such as raw mate-

rials, raw soy sauce after pressing, and the final soy sauce after pasteurization or salt-insoluble fractions such as raw materials and the soy cake after pressing were immunostained with the pooled sera. Several immunostained proteins in raw materials were detected as allergens in both fractions, but no allergens were detected in raw soy sauce, soy cake, and the final soy sauce (26).

Inhibition ELISA To examine the degradation of wheat allergens during the brewing of soy sauce, it was further determined whether each salt-soluble fraction of soy sauce retained its IgE-binding ability by inhibition ELISA using the pooled sera from the five children with wheat allergy (26). When 0.1 mg/ml wheat flour pancreatic hydrolysate was used as a positive wheat allergen (21, 22), the ELISA inhibition reached about 60–70%, and thus the pooled sera were determined to be an appropriate source of antibody in this assay. As shown in Fig. 2, ELISA inhibition generally decreased from the raw material to the *moromi* stage. However, the inhibition slightly increased from treated materials to the *koji* stage, and then extensively decreased during the *moromi* stage. Finally, the inhibition reached zero in raw soy sauce and remained zero up to the final soy sauce.

Direct ELISA Although the degradation of wheat allergens in salt-soluble fractions was confirmed by inhibi-

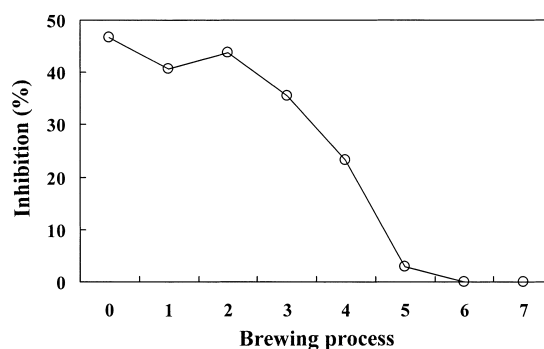


FIG. 2. Inhibition ELISA of salt-soluble fractions obtained from each brewing process. For details of ELISA procedure, see Ref. 26. Lanes of salt-soluble fractions: 0, raw materials; 1, treated materials; 2, *koji*; 3, *moromi* at 10 d; 4, *moromi* at 48 d; 5, *moromi* at 67 d; 6, raw soy sauce after pressing; 7, final soy sauce after pasteurization. Reproduced with permission from the publisher.

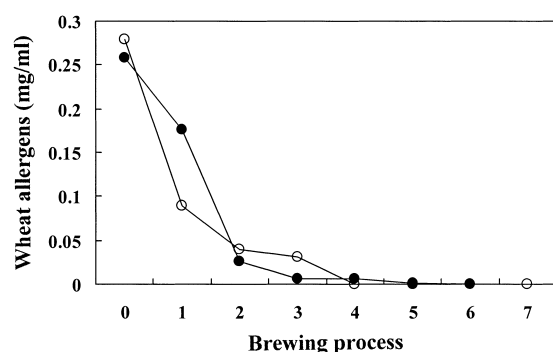


FIG. 3. Direct ELISA of salt-soluble and salt-insoluble fractions obtained from each brewing process. For details of ELISA procedure, see Ref. 26. Symbols (concentration of wheat allergens): open, salt-soluble fractions; closed, salt-insoluble fractions. Lanes of fractions: 0, raw materials; 1, treated materials; 2, *koji*; 3, *moromi* at 10 d; 4, *moromi* at 48 d; 5, *moromi* at 67 d; 6, raw soy sauce (salt-soluble) and soy cake (salt-insoluble) after pressing; 7, final soy sauce (salt-soluble) after pasteurization. Reproduced with permission from the publisher.

tion ELISA, the salt-insoluble fraction was not subjected to this assay because the fraction contained a high concentration of urea. To further examine the degradation of wheat allergens in both fractions, the IgE-binding ability of each fraction was detected by direct ELISA using the pooled sera from the five children with wheat allergy, after each fraction was directly coated as an antigen on a microplate (Fig. 3). As shown in Fig. 3, the concentrations of wheat allergens in both fractions gradually decreased during the brewing. Interestingly, the concentrations of wheat allergens in the salt-insoluble fraction greatly decreased from treated materials to the *koji* stage, and the concentrations of wheat allergens in the salt-insoluble fraction were lower than those in the salt-soluble fraction in both the *koji* and the *moromi* on day 10 (lanes 2 and 3 in Fig. 3). These results indicated that salt-insoluble proteins in treated materials were solubilized to salt water during the *koji* stage by enzymatic degradation. Next, in the *moromi* stage, both the resultant salt-solubilized and initial salt-soluble wheat allergens were degraded extensively, which also corresponded to the results of inhibition ELISA (Fig. 2). Similarly, the decrease in the concentrations of wheat allergens in the salt-insoluble fraction continued in the *moromi* stage. After the *moromi* pressing, the residual concentrations of wheat allergens in both fractions of soy sauce (raw soy sauce and soy cake) were both evaluated as zero, and the final soy sauce contained no wheat allergens after pasteurization.

Allergen degradation As determined by inhibition ELISA (Fig. 2), the concentrations of salt-soluble wheat allergens slightly increased after the *koji* stage, and then decreased to zero during the *moromi* stage. By direct ELISA (Fig. 3), the degradation of salt-insoluble allergens was determined to be faster than that of salt-soluble allergens between the *koji* and the *moromi* stages. These results indicated that the wheat allergens of raw materials were degraded as follows. At either the *koji* or the *moromi* stage, the salt-soluble allergens of raw materials were degraded well, and most of the salt-insoluble allergens were hydrolyzed to become salt-soluble by the action of microbial enzymes and were thereafter extensively degraded at these stages. As for

soy sauce, it has been reported that the allergenicity of soybeans is greatly reduced in soy sauce after fermentation (24, 25). Therefore, both types of allergens of wheat and soybeans, which are raw materials of soy sauce, are considered to be degraded completely in the *moromi* stage, and soy sauce would be useful as a seasoning for both wheat- and soybean-associated allergic patients.

III. ANTIALLERGIC ACTIVITY OF SHOYU POLYSACCHARIDES (SPS)

Allergic responses can be divided into four general categories on the basis of the mechanism of immunological involvement (27–29). Type I allergy, which causes various symptoms of asthma, rhinitis, atopic dermatitis and anaphylactic shock, is the most common (27–29). The pathological mechanism of the type I allergy has been explained as the degranulation of mast cells and the release of chemical mediators such as histamine, leukotrienes and prostaglandins from these cells (27–29). Mast cell degranulation occurs in response to immunological stimuli in which the antigen-IgE antibody reaction predominates on the cell membrane (27–29).

Hyaluronidase, which is one of the mucopolysaccharide-splitting enzymes, has been implicated in allergic reactions, the migration of cancer cells, inflammation and an increase in the permeability of the vascular system (30–36). Kakegawa and coworkers (37–39) have reported a good correlation between the inhibitory effect of hyaluronidase and that of histamine release from mast cells in response to acidic anti-allergy agents. Rat basophilic leukemia (RBL-2H3) cells release histamine when exposed to various stimuli *in vitro* (40, 41). RBL-2H3 cells have the same function as mast cells and basophils, which are involved in allergic type I reactions (31–34, 40, 42–47).

As mentioned above, proteins of the raw materials are completely degraded into peptides and amino acids by microbial proteolytic enzymes after fermentation (26), and no allergens of the raw materials, soybeans and wheat, are present in soy sauce (24–26). In contrast, polysaccharides originating from the cell wall of soybeans are resistant to the enzymatic hydrolyses, and these polysaccharides are contained in soy sauce even after fermentation (48, 49). In 1972, Kikuchi and Yokotsuka (48) purified polysaccharides from soy sauce, and investigated their properties in detail. The cell wall polysaccharides of soybean, one of the main raw materials of soy sauce, contain a large amount of galacturonic acid and are only slightly hydrolyzed by mold enzymes during the *koji* and *moromi* stages (48, 49). Although it has been about 30 years since it was reported that polysaccharides such as pectic substances are present in soy sauce (48, 49), the biological activities of polysaccharides have not been investigated as yet. Therefore, we obtained polysaccharides from either the dialysate or ethanol precipitate of raw soy sauce, and the polysaccharides were termed *shoyu* polysaccharides (SPS) (50). In this section, the anti-allergic activity of SPS originating from soy sauce *in vitro* and *in vivo* is described (50).

As a first screening *in vitro*, the inhibitory effects on hyaluronidase are usually measured to evaluate antiallergic ac-

tivity (30–36). In previous studies (30–36), extracts of many vegetables, fruits and herbs were evaluated for inhibitory effects, and several antiallergic constituents were determined. Notably, in 1992, Sawabe *et al.* (31) showed that pectic substances such as those in apple and citrus inhibit both activated hyaluronidase and histamine release from mast cells. This was the first report on the antiallergic activity of polysaccharides. In 1997, it was also reported that alginic acids from brown algae have inhibitory effects (33).

It was indicated that SPS from raw soy sauce have the same potent inhibitory effect on the activation of hyaluronidase as the antiallergic medicine disodium cromoglycate (50). In contrast to the above pectic substances (31), interestingly, polysaccharides prepared from treated materials of soy sauce such as soybeans and wheat, have no inhibitory effects (50). Therefore, antiallergic SPS originate from partially degraded polysaccharides of the materials in the *koji* stage, and are stable even during the *moromi* stage. Soy sauce contains about 1% (w/v) SPS, the novel antiallergic substances similar to disodium cromoglycate (50).

Furthermore, we examined the antiallergic activities of SPS by assaying histamine release from RBL-2H3 cells, which had been induced by the antigen. SPS that inhibit hyaluronidase also significantly inhibit histamine release from RBL-2H3 cells (50). The inhibitory effect of SPS on histamine release is concentration-dependent, and was estimated to be about one-tenth that of ketotifen, an antiallergic drug (46). However, the cytotoxicity of SPS is at least 10-fold lower than that of ketotifen (50). Therefore, SPS from soy sauce would be safe and is expected to act as an antiallergic seasoning for foods.

Finally, we evaluated the antiallergic effect of orally administered SPS by inducing passive cutaneous anaphylaxis (PCA) reaction in the ears of mice, one of the animal models for a type I allergy. Although many antiallergic substances inhibit hyaluronidase and histamine release from basophils or mast cells *in vitro* (31–34), there were no studies on the effects of these substances on the PCA reaction *in vivo*. SPS have a significant suppressive effect on the PCA reaction when 12 mg/kg body weight per day was administered orally for 3 d (50). The effective concentration of SPS is lower than that of antiallergic substances described previously (51–54). For example, the minimum effective oral doses for suppressing the PCA reaction were reported as follows: 100 mg/kg of persimmon leaf extract (51), 125 mg/kg of *Perilla frutescens* extract (52, 53), and 100 mg/kg of buckwheat grain extract (54). In contrast, 12 mg/kg of SPS per day corresponded to only about 1 ml/kg of raw soy sauce per day (50).

IV. CLINICAL STUDIES ON SPS

Allergic rhinitis is a common allergic disease in industrialized nations, characterized by sneezing, watery rhinorrhea, itching in the nose, eyes, and palate, and nasal congestion (55). In many instances, patients also experience headaches and fatigue, with a significant effect on their quality of life (QOL). Recently, the incidence of both perennial allergic rhinitis (PAR) (56–61) and seasonal allergic rhinitis (SAR) (62–66) has been increasing worldwide, leading to a marked

increase in direct and indirect treatment costs. Despite steady advances in conventional therapies for allergy symptoms, natural products such as phytochemicals and herbal extracts have been widely used by consumers as alternatives to prescription drugs without definitive clinical evidence.

However, few placebo-controlled clinical trials have examined the efficacy and safety of these natural products. In randomized, double-blind, placebo-controlled clinical studies of PAR using an 11-herb mixture (Biminne) (58), and SAR using grapeseed extract (64), *Perilla frutescens* extract (65), and a Chinese herbal medicine (containing 18 herbs) (66), it was demonstrated that Biminne, the *P. frutescens* extract, and the Chinese herbal medicine are significantly effective, but grapeseed extract is not effective.

Improvement of allergic symptoms of patients with PAR PAR causes major discomfort in affected patients, seriously degrades work and learning performances as well as the quality of sleep, and has perturbing physical, psychological and social consequences (56–61). Therefore, effective management should be directed not only towards rapid and adequate relief of the clinical symptoms but also towards a sustained improvement in the patients' daily living. QOL is a concept comprising physical and psychological characteristics that can be used to assess problems in the social aspect of a person's lifestyle. Generic questionnaires make it possible to measure these factors.

In a 4-week randomized, double-blind, placebo-controlled parallel group study (67), patients with PAR were treated with 600 mg of SPS (n=11) or a placebo (n=10) each day. After 4 weeks of treatment with SPS, symptom scores for runny nose, sore throat, and eye itching significantly decreased from the baseline within the group ($p<0.05$), but no changes in these scores were observed after 4 weeks treatment in the placebo group (67). The total symptom score, calculated from the sum of individual scores, showed a significant difference between the two groups after 4 weeks of treatment ($p<0.05$) as shown in Fig. 4.

Improvement of allergic symptoms of patients with SAR SAR also causes major discomfort in affected patients, for example in Japan, with allergy to Japanese cedar (*Cryptomeria japonica*) pollen in spring (62–66). SAR may occur at any age and affects between 10% and 30% of children (62). SAR is episodic and can cause fatigue, absenteeism, and irritability, adversely affecting the child's adjustment and achievement in school (62). Furthermore, children with SAR may subsequently develop PAR (62).

In an 8-week randomized, double-blind, placebo-controlled parallel group study (68), patients with SAR due to Japanese cedar pollen were treated with 600 mg of SPS (n=25) or a placebo (n=26) each day. After 4 weeks of treatment with SPS, scores of symptoms such as sneezing, nasal stuffiness, and hindrance of daily life were significantly different ($p<0.05$) from those in the placebo-treated groups (68). The total symptom score, calculated from the sum of individual scores, showed a significant difference ($p<0.05$) between the two groups after 4 to 8 weeks (Fig. 5).

In the two double-blind placebo-controlled clinical studies (67, 68), the patients with PAR received SPS after past sensitization with allergens (67), whereas the patients with SAR due to Japanese cedar pollen received it before sensi-

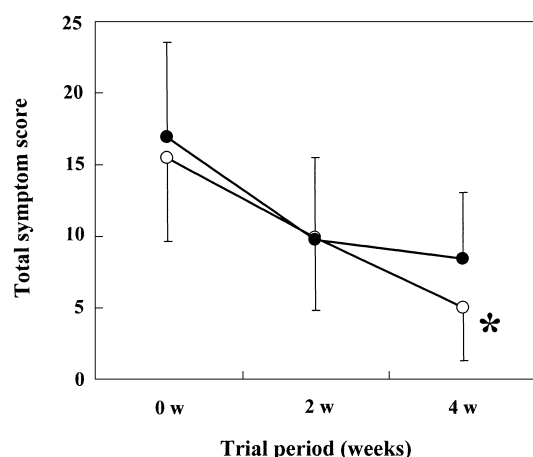


FIG. 4. Total symptom scores in SPS- and placebo-treated groups of patients with PAR. For details of clinical study, see Ref. 67. Twenty-one patients were finally enrolled in the study (11 in the SPS group and 10 in the placebo group). The severity of three nasal symptoms (sneezing, runny nose, and stuffiness) and five non-nasal symptoms (sore throat, eye itching, headaches, skin itching, and hindrance of daily life) was recorded by the investigator on discussion with the patients, on day 0 (baseline), after 2 weeks, and after 4 weeks (at the end of the study). The symptom score was rated using a 5-point scale: 0, absent (symptoms not present); 1, mild; 2, moderate; 3, severe; 4, very severe. The total symptom score was calculated from the sum of the above eight symptom scores. Differences in total symptom score were evaluated between groups by the Mann–Whitney U test. Symbols: open, SPS groups; closed, placebo groups. * $p < 0.05$ was considered statistically significant, and data are presented as the mean $\pm$ SD.

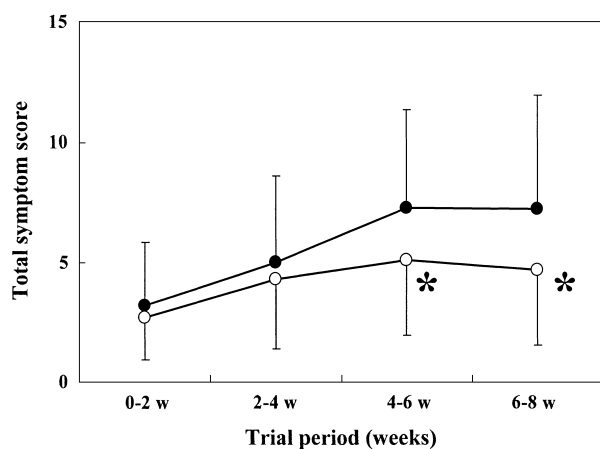


FIG. 5. Total symptom scores in SPS- and placebo-treated groups of patients with SAR due to Japanese cedar pollen. For details of clinical study, see Ref. 68. Fifty-one patients were finally enrolled in the study (25 in the SPS group and 26 in the placebo group). The severity of three nasal symptoms (sneezing, runny nose, and stuffiness) and three non-nasal symptoms (eye itching, tears, and hindrance of daily life) was recorded daily by the patients, using a 5-point scale: 0, absent (symptoms not present); 1, mild; 2, moderate; 3, severe; 4, very severe. The total symptom score was calculated from the sum of individual symptom scores. These scores were evaluated as 2-week averages at 0–2 w, 2–4 w, 4–6 w, and 6–8 w. Differences in total symptom scores were evaluated between groups using the Mann–Whitney U test. Symbols: open, SPS groups; closed, placebo groups. * $p < 0.05$ was considered statistically significant, and data are presented as the mean $\pm$ SD.

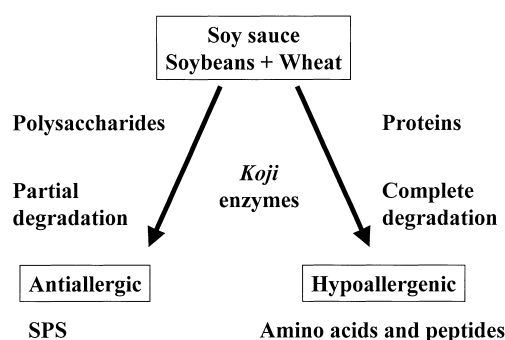


FIG. 6. Hypoallergenicity and antiallergic activity of soy sauce (*shoyu*).

zation with the allergen (68). It was demonstrated that SPS statistically significantly improved the QOL of patients with both PAR and SAR. However, SPS did not affect the levels of histamine, specific IgE, and Th1/Th2 in serum, which is not consistent with the findings of an animal study (50). The discrepancy might be explained in part by differences in species, allergen, or period of SPS administration. Furthermore, routine blood tests, including complete blood cell counts, hepatic and renal function tests, and concentrations of proteins and lipids, showed the safety of SPS at a daily dose of 600 mg for 8 weeks (68). The SPS supplementation (600 mg of SPS per day) corresponded to 60 ml of soy sauce per day. This alternative treatment with SPS originating from Japanese soy sauce might reduce therapy costs for allergic diseases.

V. CONCLUSION

In Japanese soy sauce (*shoyu*), soybeans and wheat are the two main raw materials. The proteins of the two raw materials have been completely degraded into peptides and amino acids by microbial proteolytic enzymes such as the *koji* enzymes, after the *moromi* fermentation. We firstly clarified that wheat allergens are completely degraded into amino acids and peptides to negate their IgE-binding ability during the fermentation of soy sauce, and that no allergens are thus contained in soy sauce (26). There is a considerable degradation of wheat allergens during brewing, particularly the *koji* (mold cultivation, enzyme production, and solubilization from salt-insoluble to salt-soluble proteins) and then *moromi* (enzymatic degradation of both salt-soluble and salt-insoluble proteins) stages. These results *in vitro* are quite informative for wheat-allergic patients as well as pediatricians, physicians, and dermatologists. For further details, it will be necessary to confirm the hypoallergenicity of soy sauce by a clinical study.

In contrast, polysaccharides originating from the cell wall of soybeans are resistant to enzymatic hydrolyses. These polysaccharides (SPS) are present in soy sauce even after fermentation, and SPS have potent antiallergic activities *in vitro* and *in vivo*. Furthermore, an oral supplementation of SPS was found to be an effective intervention for patients with allergic rhinitis in two double-blind placebo-controlled clinical studies. Therefore, soy sauce would be a potentially promising seasoning for the treatment of allergic diseases

through food because of its hypoallergenicity and antiallergic activity, as illustrated in Fig. 6. The mechanism of the antiallergic activity of SPS, the purification of SPS, and its application as a food supplement are currently under investigation in our laboratory.

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