Microstructural Changes in Winged Bean and Soybean During Fermentation into Miso

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MISO was prepared from winged bean, being substituted for soybean. Microstructural changes of winged bean miso at various stages of the manufacturing processes were studied by means of light and transmission electron microscopies. Soybean was also studied for comparison.

After steaming, winged bean and soybean cells were shrunken and intracellular spaces enlarged and cell wall structure was degraded, showing layered structures and aggregated lumps in intraspaces between cell walls during fermentation into miso. At the end of the fermentation, the PAS reaction with cell walls almost disappeared. Surprisingly the thick cell walls of winged bean degraded as completely as those of soybean. However, seed coat tissues were not digested. Lipid bodies were broken and fused into oil drops located inside and outside of cells and then degraded, showing wavy and scaly patterns during fermentation.

Protein bodies lost their membranes and coagulated after steaming, and then were degraded as aging progressed. Generally, degradation of the gross cellular structures seemed to be faster in soybean miso than in winged bean miso. Partly degraded gross structures of raw beans remained in the miso after two months of aging.

Key words: Winged bean, Soybean, Miso, Cell wall, Protein body, Lipid body, Fermentation, Light microscopy, Transmission electron microscopy, Microstructure
Preparation of specimens for microscopy

Cotyledonary tissues of raw and steamed beans were cut into small pieces with a razor blade, fixed with 5% glutaraldehyde solution and then with 1% osmium tetroxide solution (both in phosphate buffer containing 5% sucrose, pH 6.7), dehydrated with a graded alcohol series (40 to 100%), exchanged with propylene oxide-Epon resin series (50 to 100%) and finally embedded in Epon resin.

With miso paste, a small piece (2 to 3 mm³) on the tip of a stainless steel needle was dipped into a lukewarm agar solution and the beaded specimen was then processed for electron microscopy in the phosphate buffer containing blade, fixed with 100% ethanol for the transmission electron microscope (TEM, JEM EX-1200).

Results

LM micrographs of winged bean, soybean, WB miso and SB miso at various stages of miso manufacturing processes are shown in Figure 2. The characteristic winged bean cell wall structure with pit-pairs (Fig. 2A1) became indistinct after steaming; the steamed cells were shrunken and the intracellular spaces were enlarged as compared to the raw seeds (Fig. 2B1). On the other hand, the cells of steamed soybean were plasmolyzed and lipid bodies were broken and fused into large oil drops located inside and outside of the cells (Figs. 2A2, 2B2). Lipid body fusion into oil drops in steamed winged beans was usually minor (Fig. 2B1), but was clearly observed in later stages of processing, such as after inoculation with starter (Figs. 2C1, 2D1) and in the first period of aging (Fig. 2E1). After inoculation, mycelia from koji (cereal grains on which abundant conidiospores of Aspergillus oryzae have been grown; rice is most popularly used) were recognized in WB miso and SB miso (Figs. 2D1, 2D2). The PAS reaction with cell walls gradually weakened during processing and appeared in network spaces between degraded cellular substances (Fig. 2G2). The seed coat tissues remained undigested in both WB miso and SB miso (Figs. 2G1, 2F2). Generally, the degradation of the gross cellular structures seemed to be faster in SB miso than in WB miso.

LM micrographs of WB-R miso and SB-R miso at major stages of processing are shown in Figure 3. Some lipid body fusion was noted in the steamed winged beans (Fig. 3A1) but not to the extent seen in soybeans (Fig. 3A2). Almost the same changes found in WB miso and SB miso were recognized in the miso pastes fermented after mixing with rice koji. Because SB miso after one to two month aging is widely sold in the Japanese market, LM micrographs of one month aging SB-R miso (Figs. 3C1, 3C2) and two month aging WB-R miso (Figs. 3D1, 3D2) are shown. At lower magnification (Figs. 3D1, 3D2), heterogeneity of miso paste is clearly observed and it is noted that gross cell structures were still retained.

TEM micrographs of WB miso and SB miso at different stages of miso making are shown in Figures 4 and 5. In the latter figure, the micrographs are at higher magnification than those in Figure 4 and were selected to show the changes in cell wall structure and degradation process of the oil droplets. The protoplasts in raw beans (Figs. 4A1, 4A2) were weak and plasmolyzed on steaming (Figs. 4B1, 4B2). As miso making progressed, cracks between cell walls and cell membranes became indistinct (Figs. 4C1, 4G1) and layered structures (Figs. 4H, 5A2) and aggregated lumps in intraspaces between cell walls (Figs. 4C1, 4G2, 5A1, 5A2, 5B1) were often seen.

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**Figure 1** Processes of preparation of four kinds of miso

| Winged Bean (WB) | Water soaking at 70°C for 5 hrs. | Steaming at 1 Kg/cm² for 50 min and kept at 30°C for 47 hrs | Inoculation mixed with starter* | WB-koji | Mixing | Aging | WB-miso
|------------------|---------------------------------|---------------------------------------------------|-------------------------------|----------|-------|-------|--------|
| Rice (R)         | Water soaking (almost same conditions as above) | Steaming | Inoculation | Mixing | Steamed WB: 2500g | Rice-koji: 875g | salt: 536g | water: 1644g | Aging | WB-R miso
| Soybean (SB)     | Water soaking at 20°C for 20 hrs | Steaming at 1 Kg/cm² for 50 min and kept at 30°C for 47 hrs | Inoculation mixed with Starter* | SB-koji | Mixing | Aging | SB-miso
| Rice (R)         | Water soaking (almost same conditions as above) | Steaming | Inoculation | Mixing | Steamed SB: 2500g | Rice-koji: 875g | salt: 517g | water: 125g | Aging | SB-R miso

* Starter used was conidiospores of mycelia of Aspergillus oryzae (trade name: Yamazaki)
Microstructural Changes of Winged Bean and Soybean in Miso-making

Figure 2. LM micrographs of winged bean, soybean, WB miso and SB miso at various stages of miso making.
P8, protein body; CW, cell wall; O, oil drops; M, mycelia of koji; PAL, palisade cells of seed coat; SA, area which is strongly positive to PAS reaction; White arrows, pit-pairs. Magnification in all micrographs is the same as in Fig. A1.
MISO IN JAPAN

MISO IN JAPAN has many variations depending on regions and people just as is found for cheese in Europe, but can be classified into three groups based on the starting materials, namely, miso prepared only with soybean, miso made with soybean and rice and miso manufactured from soybean and wheat. The production and consumption of miso made from soybean and rice is the largest in the Japanese market, even though this type has a variety of minor deviations in formula, salt concentration, heating conditions for raw materials and degree of mashing of the mixture of soybean and rice. In our studies winged bean was substituted for soybean in the all-soybean and soybean plus rice types of miso.

Because the thick cell walls of winged bean became negative to the PAS reaction, they apparently are utilized effectively as a carbohydrate source in miso making as is true with soybeans. However, the seed coats were not digested even after three months of aging. Cell wall structure seems to be less lignified than seed coat tissues.

Discussion

Magnifications of A and B are the same as A. Those of D1 and D2 are the same.

Figure 3. LM micrographs of winged bean, soybean, WB-R miso and SB-R miso in miso making.

A1, A2: Cotyledonary cells of steamed winged beans and soybean, respectively.
B1, B2: Paste of winged bean and soybean after mixing with rice koji, respectively.
C1, C2: Paste after one month aging of winged bean and soybean, respectively.
D1, D2: Paste after two month aging of winged bean and soybean, respectively.
CW, cell wall; O, oil drops; PAL, palisade cells of seed coat; M, mycelia of koji;
SA, area which is strongly positive for PAS reaction.

Magnifications of B and C are the same as A. Those of D1 and D2 are the same.

found. These layers and lumps may be intermediate structures of degrading cell walls.

After steaming, lipid bodies were extensively broken and fused into oil drops in soybean and only slightly in winged bean. In the comparison of lipid bodies in winged bean (Figs. 4Al, 4Bl) with those in soybean (Fig. 4A2), the latter were electron dense when stained by osmium tetroxide, whereas the former were electron translucent in spite of being fixed in the same way at the same time. After fusing, the oil drops in winged bean at first seemed to be electron dense, but degraded portions of oil drops became electron translucent (Figs. 4Fl, 4Fl, 5Cl, 5D1), showing wavy or scaly patterns (Figs. 4Fl, 4F2, 4Fl, 5Cl, 5D1, 5D2) and finally disappeared. In soybean, small fused oil drops (Fig. 5C2) were found up to two to three months of aging, located around coagulated protein bodies. The membranes of protein bodies were broken after steaming and coagulated protein parts were also degraded (Figs. 4D1, 4D2, 4E1, 4G1, 4H2). As shown in Fig. 4E2 hyphae of Koji mycelia were sometimes observed in cells.

These layers and lumps may be intermediate structures of degrading cell walls.
Figure 4. TEM micrographs of winged bean, soybean, WB miso and SB miso making.
A1, A2: Cotyledonary cells of raw winged bean and soybean, respectively. B1, B2: Steamed WB and SB cotyledonary cells. C1, C2: WB and SB paste after mixing with koji starter. D1, D2: WB and SB paste after inoculation. E1, E2: WB-R koji and SB-R koji. F1, F2: WB and SB paste after one month aging. G1, G2: WB and SB paste after two month aging. H1, H2: WB and SB paste after three month aging. CW, cell wall; O, oil drops; P, coagulated protein; PB, protein body; L, lipid body; M, hypha of koji mycelia; IS, intracellular space; Magnification of all micrographs as indicated in Fig. Bl.
Figure 5. TEM micrographs of WB miso and SB miso at high magnification.
A1, A2: Intracellular space adjacent to cell walls after steaming of winged bean and soybean, respectively. B1: Intracellular space adjacent to cell walls after two month aging of winged bean. C1, C2: Degrading oil drops after two month aging of winged bean and soybean, respectively. D1, D2: Degrading oil drops after three month aging of winged bean and soybean, respectively.
IS, intracellular space; O, oil drops; CW, cell wall; P, coagulated protein.
Bars shown in each micrograph equal 1 μm.

as judged from the microstructure of the latter (Saio and Watanabe, 1973). The digestion is probably caused by hydrolytic enzymes such as cellulase, pectinase and amylases present in the koji.

Measurements of pH, acidity, protein and carbohydrate digestibilities and lightness of color of miso paste were carried out by the laboratory of Nagano Miso Co. Ltd., using the same samples (Kobayashi et al. in preparation). Results on protein and carbohydrate digestibilities showed that SB miso digested faster than WB miso but both types of miso reached almost the same level of digestion after three months of aging. The Y values of lightness were much higher for SB miso than WB miso in the beginning but became somewhat higher for WB miso after three months of aging, which seemed mainly due to nonenzymatic browning between liberated amino acids and sugars.

WB miso and WB-rice miso prepared in these experiments, were acceptable in taste, texture of body, and color, according to testing by Nagano Miso Co Ltd. and the National Food Research Institute. Their flavor was somewhat different from that of usual SB miso, but the preference depends on individual tastes, as Japanese are used to eating soybean foods, whereas people of other countries such as Southeast Asia, Latin America and Africa prefer other pulses. Trials to make WB miso as a special product of Okinawa are progressing.

Microstructural changes in steamed soybeans for miso making (Shibazaki and Asano, 1968, Saio and Watanabe, 1973) and chopped soybeans in miso paste (Shibazaki and Asano, 1968) by LM were reported, but there are no reports on soybean paste by LM and TEM. Consequently, our studies provide new information on the microstructural changes in soybeans and winged beans that occur during the complex fermentation involved in manufacturing miso.

References


Microstructural Changes of Winged Bean and Soybean in Miso-making


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Discussion with Reviewers

W.J. Wolf: You state that winged beans are more difficult to cook than soybean, yet the same cooking conditions were used for both legumes. Does this cause overcooking of soybeans and could such overcooking be responsible for the greater fusion of the lipid bodies in soybeans as compared to winged beans? It would be interesting to measure tenderness and structure of the seeds as a function of cooking time and determine whether lipid body fusion parallels tenderness.

Authors: In this experiment, the temperature and time of soaking beans were different but cooking time was the same in both. As the cooking condition used is normal for SB-miso making, it may be under-cooking for winged bean, which might result in the delay of microstructural changes as compared to soybean. I also think that the experiment you suggested would be interesting.

D.J. Gallant: In a recent work yet unpublished on cytochemical studies of lupine seeds, we have found a negative correlation between the content of storage components and the thickness of cotyledon cell walls. It might be the same in your case. Could you explain why ripe winged beans which have comparable content of storage components as soybean have much thicker cell walls?

Authors: We have no answer to your question. We also found thick cell walls in some lupine seeds but not in wild type soybean seeds. We are now working on other legume seeds.

D.J. Gallant: What kind of polysaccharides and possible antinutritional components such as the alpha-galactosides exist in winged bean cell walls? Is miso from winged bean nutritionally as good as soybean miso?

Authors: We reported in a previous paper (Saio et al., 1983) that winged bean contains extraordinarily high levels of hemicellulose. It might serve a role as dietary fiber, but we did not determine the nutritional value of winged bean miso.

D.J. Gallant: Could you explain why protein bodies were clearer in soybean cotyledons than in winged bean cotyledons (Figure 2 A1-A2, B1-B2)?

Authors: The original micrographs are color prints of sections stained by the PAS reaction. The intensity of PAS staining varies with minor changes in conditions, so that we can not say that the protein bodies of winged bean contain more polysaccharides than those of soybean.

D.J. Gallant: Were the raw materials soaked or hydrated before fixation?

Authors: No, they were not.

D.J. Gallant: In the TEM studies, you noted differences in contrast between lipids in the winged bean and soybean cells. Could they be related to less saturation of storage soybean lipids as compared to winged bean lipids?

Authors: We also think that is one of the reasons for differences in the contrast of lipid bodies between soybean and winged bean, but are still not sure.

D.J. Gallant: You noticed remnant cell walls in winged bean miso after three month aging. Are they organoleptically detected by Japanese people?

Authors: We found remnant seed coats after fermentation. They were darker in color than the paste part and felt rough to the tongue.