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Kurzmitteilung

Miso from peas *(Pisum sativum)* and beans *(Phaseolus vulgaris)* of domestic origin
Fermented foods from agricultural Products in Europe. II

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Miso aus Erbsen (Pisum sativum) und Bohnen (Phaseolus vulgaris) aus heimischer Herkunft

Summary: Miso is a fermented soybean paste widely used in Japan as a soup base or as a seasoning agent. Koji (cereal grains with the mold *Aspergillus oryzae*) serves as enzyme source. Peas *(Pisum sativum)* and beans *(Phaseolus vulgaris)* of German origin can be used as substitutes for soybeans in the preparation of miso-like products. The legumes (peas, beans and soybeans for comparison) are washed, soaked in boiled water, dehulled and cooked for 35 min at reduced pressure. After grounding the seeds are mixed with salt, koji and mugi miso as starter and incubated at 45 °C for 14 days. During fermentation the glucose content increases up to 8–10 days and subsequently drops down. Crude protein decreases during incubation while dry matter increases. The pH value of all three miso types decreases during the fermentation period. Most of the 40 test persons characterize odor and flavor of the three misos as aromatic or sour; pea miso is often recorded to have a sweet-like odor and flavor. A legume-like taste of the final products has not been recorded.

Zusammenfassung: Miso ist eine fermentierte Sojabohnenpaste, die in Japan weite Verwendung als Suppeneinlage und Würzmittel findet. Bei der Herstellung wird Koji (mit dem Schimmelpilz *Aspergillus oryzae* durchwachsenes Getreide) als Enzymquelle eingesetzt. Erbsen *(Pisum sativum)* und Bohnen *(Phaseolus vulgaris)* heimischer Herkunft können die Sojabohnen als Substrat ersetzen. Dabei werden die Körner (Erbsen, Bohnen und Sojabohnen zum Vergleich) gewaschen, eingeweicht, geschält und 35 Minuten lang bei vermindertem Druck gekocht. Anschließend werden die Körner zermahlen und mit Salz, Koji und Mugi-Miso als Starter vermischt und 14 Tage lang bei 45 °C inkubiert. Während der Fermentation steigt der Glukosegehalt bis zu einem Maximalwert nach 8–10 Tagen an und fällt dann ab. Der Rohproteingehalt fällt während der Inkubation, während die Trockenmasse ansteigt. Bei allen drei Miso-Arten sinkt der pH-Wert während der Fermentation ab. Geruch und Geschmack der Endprodukte werden von den meisten der 40 Testpersonen als aromatisch-säuerlich bezeichnet; Erbsen-Miso riecht und schmeckt für viele Personen auch etwas süßlich. Der typische Leguminosengeschmack fehlt immer.

Key words: Fermented foodstuffs – miso – koji – peas *(Pisum sativum)* – beans *(Phaseolus vulgaris)*

Schlüsselwörter: Fermentierte Lebensmittel – Miso – Koji – Erbsen *(Pisum sativum)* – Bohnen *(Phaseolus vulgaris)*

Introduction

Mixtures of legumes and cereals are widely used for human consumption because they supply in their amino acid content. Thus, soybeans are low in methionine and cysteine

and rich in lysine, whereas rice and wheat are rich in methionine and low in lysine (5). The utilization of legumes is affected by the presence of several antinutrients (e.g., inhibitors of proteases and amylases, flatus factors, metal chelates, antivitamins). Proper processing of cereal-legume mixtures is essential to minimize or eliminate these antinutrients before comsumption (3). Consequently, a variety of fermented foods is prepared from legumes (mainly soybeans) and cereals (rice or barley) with strains of *Aspergillus oryzae* as source of enzymes. One of these products is miso, which is one of the most important fermented foodstuffs in Japan. Miso is prepared by fermenting soybeans with salt and koji, a starter containing various strains of *A. oryzae*. The molds produce amylases and proteases which hydrolyze the cereal starch and the proteins in soybeans and cereals. In this way growth factors are formed that are used in a second fermentation by osmiophilic yeasts and lactic acid bacteria (13). Amino acids and their salts (particularly sodium glutamate) are responsible for the typical flavor of miso (11). By varying the ratio of soybeans to cereals, the amount of salt, and the length of fermentation, various types of miso may be produced (4).

In general, miso is a paste of bright yellow to dark brown color, the taste of which is meatlike and varies from salty to sweet. Traditionally, miso is dissolved in water and used as a base for hot breakfast soups or as a flavoring agent for fish, meat, fruits, and vegetables (13). The basic principles of miso fermentation from soybeans have been extensively studied by Shibasaki and Hesseltine (9, 10). The composition of the various types of miso are given by Shibasaki and Hesseltine (11).

So far, there are only few studies concerning the substitution of soybeans by other leguminous crops. Robinson and Kao (8) used chickpeas *(Cicer arietinum)* and horse beans *(Vicia faba)* and compared the chemical composition of the resulting products with those of soybean miso. Chickpeas were also used in India for miso preparation (according to 12). In Europe peas *(Pisum sativum)* and beans *(Phaseolus vulgaris)* are widely used crops. Thus, it seemed to be interesting to determine whether these legumes can replace soybeans in several fermented foods of Oriental origin. In a previous study (7) peas proved to be a useful substrate for the preparation of tempeh, and Indonesian food. This was the reason for a study on the applicability of peas and beans in the production of miso. For the purpose of comparison, soybeans were also used as substrate.

Materials and methods

1) *Legumes:* Peas ("Belinda") and beans ("Flageolet") were purchased from Davertmühle (48308 Senden, FRG). The soybeans of US origin came from F. Rapunzel (87764 Legau, FRG).

2) *Koji and starter miso:* The koji preparation as source of hydrolytic enzymes consisted of barley grains overgrown with *A. oryzae* (Schwarzwurzel GmbH, 20146 Hamburg, FRG). The yeasts and bacteria necessary for the second fermentation phase were introduced by addition of a mugi miso (prepared from barley and soybeans) that was purchased from Schwarzwurzel (20146 Hamburg, FRG).

3) *Preparation of miso:* From the variety of miso preparations sweet white miso (shiro miso) was chosen because of its short fermentation period of only 14 days (12). 100 g legumes (peas or beans or soybeans) were thoroughly washed and soaked in 300 ml boiled water for 15 h at room temperature. The legumes were subsequently manually dehulled and cooked with 250 ml water for 35 min at reduced pressure. After cooling

down the legumes were coarsely ground, thus enabling a better penetration of the koji enzymes. 20 g salt, 50 ml water, a tablespoonful mugi miso (containing different yeasts and lactic bacteria (11)) and 200 g koji were added. The mixture was transferred into a stoneware pot (2 l) that had been spread with salt and was incubated for 14 days at 45 °C. The addition of a relatively high amount of salt is necessary because it acts as a preservative and inhibits the growth of dangerous anaerobic bacteria, thus enlarging the shelf life of the miso (6, 14). Additionally, sodium chloride favors the development of the flavor and aroma-forming yeasts and bacteria (1).

4) *Determination of biochemical processes during miso fermentation:* Samples were taken from the miso mixtures before and during the incubation at intervals of 2 days. The samples were ground, suspended in distilled water (1:5, v/v) and placed into a water bath at 80 °C for 15 min to stop all enzymatic processes. After that the samples were centrifuged at 4600 rpm. The supernatant was diluted with dist. water in a relation 1:5 (v/v) for protein determination (with the semiquantitative Biuret test set No. 3372 of E. Merck, 64271 Darmstadt, FRG) and 1:1000 (v/v) for the glucose determination (enzymatic test combination Glucose, No. 716251 of Boehringer, 68305 Mannheim, FRG). The pH value was determined with an electric pH meter. The dry matter was estimated by drying 10 g miso preparation at 100 °C to constant weight. All tests were made in duplicate.

5) *Organoleptic evaluation:* The odor and the flavor of the three miso preparations were subject to a panel of 40 judges.

Results and discussion

1) *Consistency of the misos:* All three miso preparations were semi-solid pastes of brown color.

Table 1. Changes of glucose, crude protein, dry matter, and pH value during miso fermentations

	Days of Fermentation							
	0	2	4	6	8	10	12	14
Glucose (%)								
– soybean miso	2.2	6.7	13.3	27.1	34.2	30.1	28.5	26.2
– pea miso	2.6	8.8	21.3	29.8	35.2	41.2	35.2	33.0
– bean miso	2.8	10.2	18.0	26.1	37.5	42.7	39.2	36.5
Crude protein (%)								
– soybean miso	17.3	17.0	16.5	12.8	9.2	8.3	8.0	7.9
– pea miso	10.0	10.0	8.2	7.1	5.8	5.7	5.3	5.1
– bean miso	9.2	8.9	7.2	5.9	5.2	4.8	4.4	4.4
Dry matter (%)								
– soybean miso	49.0	51.2	52.6	54.4	54.5	55.1	56.1	56.5
– pea miso	52.1	52.9	53.9	54.3	54.9	55.0	55.8	55.9
– bean miso	51.5	53.2	53.6	53.7	54.2	55.2	56.7	57.0
pH value								
– soybean miso	7.3	7.0	7.0	6.7	6.3	6.1	5.8	5.5
– pea miso	6.8	6.6	6.5	6.3	5.9	5.7	5.3	5.3
– bean miso	7.0	6.8	6.3	6.2	5.7	5.8	5.6	5.1

2) *Glucose* (Table 1): The amount of glucose present in the three miso types rose continuously during 8–10 days of incubation and subsequently dropped. Bean miso had the highest glucose maximum (42.7 %) after 10 days of fermentation, and soybean miso the lowest (34.2 %) after 8 days.

The amylase of the koji mold is able to hydrolyze the legume starch, yielding mainly glucose, but also maltose (12). Glucose is subsequently metabolized by a second fermentation by lactic acid bacteria and during an alcoholic fermentation by the yeasts of the starter miso. These processes lead to a decrease of the glucose content during the second phase of miso preparation. The different glucose contents of the three miso types reflect the different starch contents of the grains.

A similar increase of the amount of reducing sugars in various miso preparations as compared to original soybeans is described by Robinson and Kao (8) and Abiose et al. (1).

3) *Crude protein* (Table 1): The initial protein content of soybean miso was higher than that of the other types, reflecting the high protein concentration of the raw beans (2). In all three miso types the protein content decreased continuously during the whole incubation period. The relative loss of protein was 54.3 % in soybean miso, 52.2 % in bean miso, and 49.0 % in pea miso.

A corresponding loss of protein during the preparation phase of soybean miso is reported by Abiose et al. (1): the initial protein content of soybeans was 19 % and dropped to 8 % in white miso as final product. The reduction of proteins is due to the action of proteolytic enzymes produced by the koji fungus *A. oryzae.*

4) *Dry matter* (Table 1): During the fermentation the dry matter increased for 15.3 % in soybean miso, 10.8 % in bean miso, and 7.3 % in pea miso. The loss of water is, to a great extent, the consequence of the various hydrolytic processes during the fermentation. According to Chavan and Katam (3), sweet soybean miso has a moisture content of 42.6 % corresponding to the results of this study.

5) *pH value* (Table 1): At the beginning of fermentation the pH value of all three kinds of miso was about 7.0. During incubation it decreased to about 5.3. Increases of organic acids (lactic, succinic, and acetic) during the preparation phase of miso are described by Abiose et al. (1).

6) *Organoleptic evaluation* (Table 2): Most test persons found in all three misos an aromatic or sour odor; additionally, pea miso had a sweet-like smell. The flavor of the miso specimen was predominantly characterized as sour-aromatic. Most test persons found that pea miso had a sweet-like flavor. A legume-like taste was never recorded.

7) *Comparison of the three types of miso.* Besides the traditional soybean, peas *(Pisum sativum)* and beans *(Phaseolus vulgaris)* are suited as substrates for the preparation of miso. The biochemical processes during fermentation are very similar yielding products of similar quality. There are only slight differences in odor and taste. All miso types were well accepted by the test persons as seasoning agents.

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Table 2. Sensory evaluation by 40 judges of three types of legume miso (expressed in % of all test persons)

	Soybean miso	Pea miso	Bean miso
Odor			
– aromatic	44	37	48
– sour	45	23	41
– bread-like	11	–	10
– sweet-like	–	40	–
Flavor			
– aromatic	49	40	55
– sour	34	12	24
– sweet-like	–	48	–
– fresh	17	–	7
– musty	–	–	14

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