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Koji fermentation using toasted defatted soybean grit

(Fermentasi koji dengan menggunakan kersik kacang soya tanpa lemak)

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Key words: soy sauce koji, defatted soybean grit

Abstract

Defatted soy bean grit have good potential for use as a substrate in koji production. The koji is the source of enzymes and other biochemical changes during soy sauce fermentation. The changes in total reducing sugar, total soluble nitrogen, amino and ammonia nitrogen are described. The activities of protease and α-amylase produced are also discussed. Overall changes showed that 2 days was the optimum fermentation time for the koji production using toasted defatted soy bean grit incubated at ambient temperature.

Abstrak

Kersik kacang soya tanpa lemak berpotensi untuk digunakan sebagai substrat dalam penghasilan koji. Koji merupakan sumber enzim dan sumber perubahan biokimia semasa fermentasi kicap. Perubahan dalam jumlah gula penurun, jumlah nitrogen terlarut, nitrogen amino dan nitrogen ammonia telah dihuraikan. Aktiviti enzim protease dan α-amilase yang dihasilkan juga dibincangkan.Perubahan keseluruhannya menunjukkan bahawa 2 hari ialah tempoh fermentasi yang terbaik bagi penghasilan koji daripada kersik kacang soya tanpa lemak yang dieram pada suhu bilik.

Introduction

Soy sauce is man's oldest prepared seasoning and is known as 'shoyu' in Japan, 'chiang-yiu' in China, 'kanjang' in Korea, 'toyo' in the Philippines, 'see-ieu' in Thailand and 'kecap' or 'kicap' in Indonesia and Malaysia. It is mainly used as an all-purpose seasoning.

The processing of soy sauce generally involves two stages of fermentation, namely the 'koji' and 'moromi' stages. Koji is a dark green fungal-coated mass produced by solid substrate fermentation, with a pleasant aroma and has high protease and α-amylase activities. Other important enzymes that have also been found to be involved in the koji fermentation include sucrase, amylase, protease, lipase, cellulase, amyloglucosidase and maltase. The activities of these enzymes in hydrolysing the polysaccharides, protein and fat in the soy bean-wheat mixture have also been mentioned (Yong and Wood 1977; Aidoo et al. 1981, 1984). In conventional koji-making as practised in Malaysia, koji mould is grown on cooked soy beans coated with wheat flour and allowed to ferment for 72 h under ambient temperature.

This study monitors the activities of protease and α-amylase as well as other principal biochemical changes during koji fermentation using toasted defatted soy bean grit as the main substrate.

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Materials and methods

Inoculum

Koji inoculum was obtained from the microbiology laboratory of the Food Technology Division, MARDI. It is a powdered pure culture of *Aspergillus oryzae* spores containing about 2 x 10^6conidia/g.

Toasted defatted soybean grit

The grit was obtained from Soon Soon Oil Mills Sdn Bhd., Prai Industrial Estate, Province Wellesley, Malaysia. The grit contained at least 45.8% protein, 13.0% moisture, 1.5% oil, with a granule size of 1.0–2.0 mm.

Koji preparation

A volume of 500 mL of sterilized distilled water was added to 1 kg of sterilized toasted defatted soy bean grit and mixed thoroughly. Two-hundred grams of roasted wheat flour containing 1 g koji inoculum (0.1% of the grit) was mixed with the moist grit and then spread on sterilized bamboo tray to a thickness of 2–2.5 cm. The tray was covered with muslin cloth and incubated at room temperature (30 °C ± 5 °C). Temperature at the centre of the koji was measured every day. Samples were taken every day during the 4 days of fermentation.

Preparation of koji extracts for chemical and enzymic analysis

The koji extracts were prepared according to the modified methods of Yong and Wood (1977). Ten grams of koji was weighed into a 100 mL Erlenmeyer flask and 20 mL of distilled water (for chemical analysis) or 10 mL of 0.02 M phosphate buffer at pH 7 was added. The sample was then crushed with a glass rod and an additional 20mL of extractant was added. The flask was stoppered and stirred using a magnetic rod in a cool water bath at 10 °C for 20 min.

Enzyme extracts were then transferred to centrifuge tubes and centrifuged at 10 000 rpm at 5 °C for 30 min (RC-5 Superspeed Refrigerated Centrifuge, Sorvall). The supernatant was decanted, the residue resuspended in about 15 mL of extracting buffer and recentrifuged. The supernatants were combined and made up to 50mL with buffer. Extracts for chemical analysis were filtered through Whatman No. 4 filter paper, the residue washed with distilled water and the combined volume made up to 100 mL.

Analytical procedures

Dry weight Two grams of koji sample was weighed in a metal dish and dried overnight in an oven at 105 °C to a constant weight.

Total reducing sugar It was determined according to the method suggested by Sumner (1925).

Total soluble nitrogen It was obtained according to the standard Kjeldahl method (AOAC 1984).

Amino (formal) nitrogen It was determined according to the method of Cowan (1974).

Ammonia nitrogen It was determined according to the standard AOAC method (AOAC 1984).

Protease assay It was done according to the method used by Aidoo et al. (1984). One unit of protease activity was defined as the amount of enzyme which hydrolysed 1 mg of casein per min under the assay conditions.

α-Amylase assay It was done according to the method suggested by Obi and Odibo (1984). One unit of α-amylase activity was defined as the amount of enzyme which produced 1 mg of glucose per min under the assay conditions.

Results

The results presented are based on the means of the two experiments, each with triplicates.

The changes in temperature, total moisture content and total reducing sugar levels during koji fermentation are shown in *Figure 1*. There were not much temperature changes throughout the process i.e., between 27.5 °C and 30.5 °C. However, a rapid decrease in the moisture content was observed during the first 2 days of fermentation, by about 40% of the starting materials. The decrease in moisture content was more gradual as time of fermentation progressed.

There was a rapid increase in total reducing sugar concentration after 24 h of koji fermentation, giving a maximum value of 19.00 mg/g. This was followed by a lesser decrease towards the end of the fermentation period.

The changes in total soluble nitrogen, amino and ammonia nitrogen during koji fermentation are shown in *Figure 2*. A tremendous increase in total soluble nitrogen content was observed after 2 days of fermentation, reaching a maximum value of 12.04 mg/g. This was followed by a rapid decrease as fermentation continued.

Amino nitrogen content showed a more gradual increase, reaching a maximum value

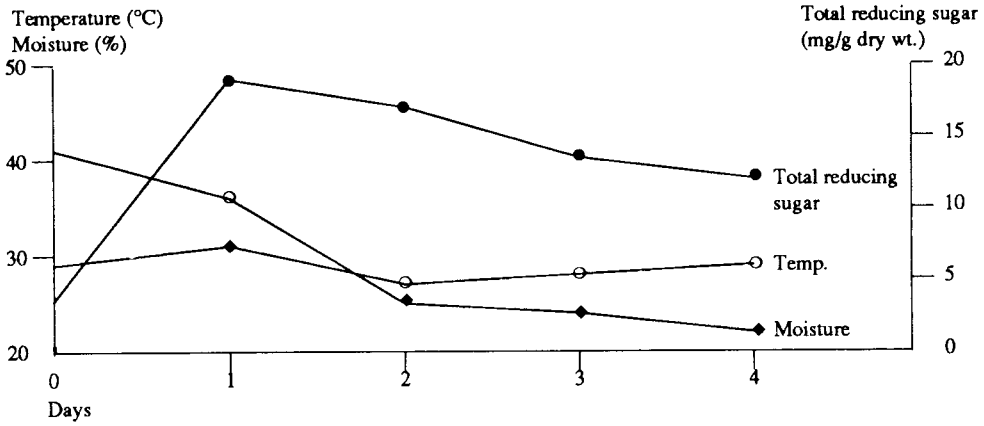

Figure 1. Changes in temperature, moisture and total reducing sugar during koji fermentation

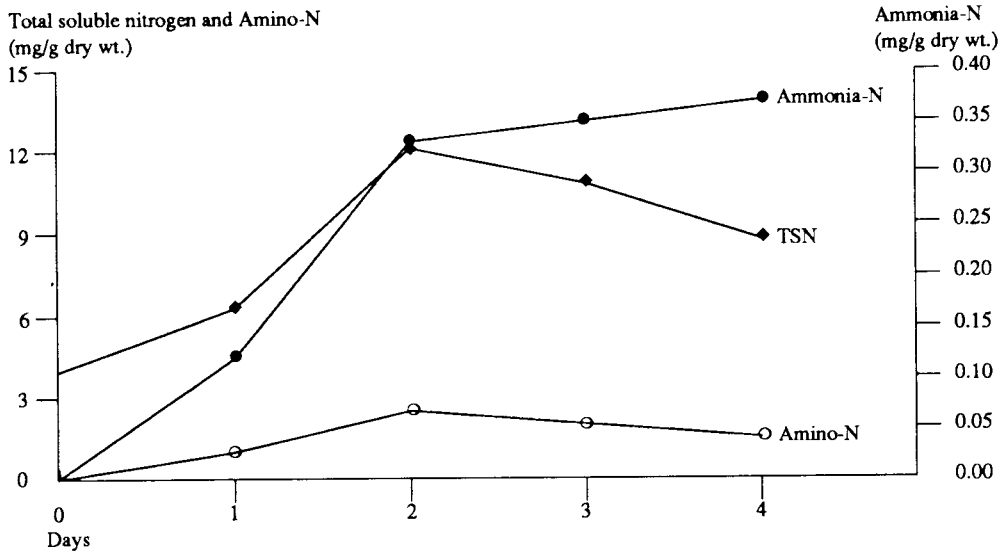

Figure 2. Changes in total soluble nitrogen, amino and ammonia nitrogen during koji fermentation

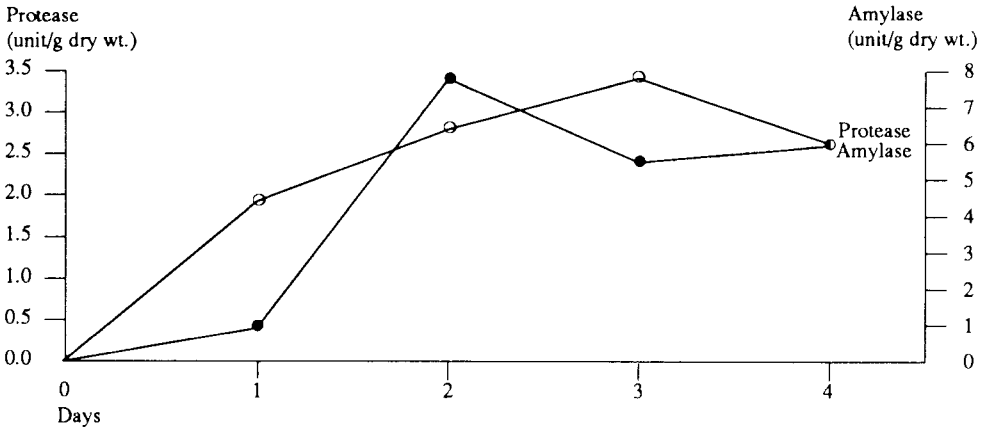

Figure 3. Protease and α-amylase activities during koji fermentation

after 2 days. It then decreased as fermentation time progressed. Ammonia nitrogen also increased rapidly during the first 2 days, followed by a slow increase towards the end of the process.

The activities of amylolytic and proteolytic enzymes during fermentation of koji are shown in *Figure 3*. It was observed that amylase activity increased rapidly reaching a maximum at day 3, followed by a rapid decline. On the other hand, protease activity showed a more fluctuating pattern, with a rather slow increase during the first 24 h, followed by a rapid increase to a maximum activity at about 48 h. As fermentation progressed, the activity dropped and then remained fairly constant at the later stage.

Discussion

Defatted soy bean grit (DSG) is prepared by extracting dehulled and crushed whole soy beans with a solvent, usually hexane of low boiling point. The DSG has low economic value as they are considered a by-product of soy bean oil production and is mainly used as animal feed.

In the production of soy sauce, the main purpose of koji fermentation is the production and utilization of its amylolytic and proteolytic enzymes for the conversion of starch and breakdown of the polypeptide of protein into amino acids (Fukushima 1985). In producing good koji, the moisture content of the starting materials should be 40–50% to avoid bacterial contamination. As fermentation of koji progresses, the moisture content decreases resulting in sporulation of the fungus. The koji is then transformed from the wet mouldiness of mycelial koji to a dry bitter sweet mouldiness of sporulated koji (Bull et al. 1985).

Another parameter observed during the koji process is total reducing sugar which showed rapid increase during the early stages of fermentation. Similar observations on koji from whole soy beans were obtained by Yong and Wood (1977). As fermentation continued, the total reducing sugar level showed a tendency to decline while α-amylase activity continued to increase. This increase in activity may be due to active sporulation that takes place during the later stages of the fermentation process. According to Yokotsuka (1986), the conversion of starch in the main substrate was about 20% and was dependent on the moisture content of the starting materials.

The extent of protein hydrolysis is regarded as the most important factor governing the quality of soy sauce and it is usually determined as soluble, amino and ammonia nitrogen. Among these

components, free amino acids have been of most interest because of their characteristic taste and contribution to quality.

During koji fermentation, free amino nitrogen obtained is in the range of 16–20% of total soluble nitrogen and as fermentation proceeds into mash fermentation (moromi), the levels increase to about 40–50% in "koikuchi" shoyu (Oka and Nagata 1974) and 35–42% in Chinese or "tamari" soy sauce (Chang et al. 1987). The presence of these amino acids not only contributes to the flavour of soy sauce but also to the colour of the final raw sauce which is due in part to browning reactions of sugars and amino acids (Nunomura and Sasaki 1986). However, the production of ammonia is of considerable importance since a high level is unacceptable to manufacturers and consumers of soy sauce (Yong and Wood 1977).

To ensure a high rate of substrate hydrolysis during koji fermentation, selection of koji mould is done based on its ability to produce good enzymic activity, especially high proteolytic activity as well as macerating power to decompose the tissue of soy bean and wheat flour (Yokotsuka 1986). The increase in proteolytic activity in koji of DSG was observed to follow a pattern similar to that of whole soy beans obtained in a laboratory scale study by Yong and Wood (1977). They indicated that most of the important enzymes reached their maximum level of activity after 50 h of fermentation. After 72 h incubation, profuse sporulation developed and the mild bitter sweet smell of koji was replaced by a harsh mouldy smell which will affect flavour of final soy sauce.

Conclusion

The role of koji in soy sauce is to produce the necessary enzymes for the conversion of the substrates to simpler compounds. In the Malaysian conventional method, koji is prepared from whole soy beans. The changes in the principal biochemical and enzymes activities during the fermentation are comparable with the koji produced using whole soy beans as the substrate. The maximum levels of total soluble nitrogen and amino nitrogen in the koji are reached during the second day of incubation. The major advantages in the use of defatted soybean grits are that the substrate contains high protein and are less expensive as soy bean grit is considered a by-product. However, the use of defatted soy bean grit in soy sauce making may need some modifications, especially in the extraction of the raw sauce due to the difference in the particle size of the substrate.

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