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Conference title

Effect of Different Combinations of Soybean and Wheat Bran on Enzyme Production from *Aspergillus oryzae* S.

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Abstract

To investigate the enzyme production from *Aspergillus oryzae* S. NPUST-FS-206-A1, different combinations of soybean and wheat bran (40%:60%, 50%:50%, and 60%:40% w/w) in koji were used as substrates in soybean koji fermentation. During cultivation period, pH change of various samples was similar. However, moisture content of koji obviously decreased. Koji with high content of soybean conducted high protease activity. After 48 h of cultivation, koji containing 60% soybean showed the highest neutral protease activity of 84.38 U/g dry weight. Sample with high amount of wheat bran presented high amylase activity of 731.53 U/g dry weight. Reducing sugar content of koji was related to amylase activity. Moreover, different inoculum sizes of *A. oryzae* S. spore had no effect on the enzyme production.

Keywords: Aspergillus oryzae S., soybean koji, protease, amylase

1. Introduction

The solid-state fermentation (SSF) has been a common method in traditional fermentation. It is characterized as a fermentation process carried out on a solid medium with low moisture content (a_w), typically 0.4-0.9, in nonseptic and natural state. Various agricultural materials such as wheat straw, rice hulls, and corncobs have been used as substrates for SSF [1].

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Koji fermentation is also a kind of SSF system. Koji producing is the first step in making fermented foods such as soy sauce, miso, mirin and sake [2]. In traditional soy sauce fermentation, soybean has been used as the protein source for koji. The koji mold (*Aspergillus oryzae* S.) breaks down carbohydrates and proteins on soybean to synthesize enzymes. There are various extracellular proteins produced from the culture by *A. oryzae* AS 3.951 in soybean koji including leucine aminopeptidase, TAKA-amylase (α-amylase), oryzin (alkaline protease), glutaminase, alanyl dipeptidyl peptidase, X-Prolyl aminopeptidase, and methallopeptidase [3]. Moreover, wheat is the most suitable starchy material or carbon source in koji fermentation [4].

There are many factors affecting enzyme production during koji fermentation, such as substrates, inoculum size of starter culture, moisture content of substrate, and cultivation temperature [5, 6]. Even though soybean and wheat bran have been used as substrates for traditional soy sauce production, few reports illustrated enzyme production using the combination of these two substrates. In this study, the effects of various combinations of soybean and wheat bran as the substrate and the inoculum size of *A. oryzae* S. spore on biochemical change, especially enzyme production during koji fermentation period were investigated.

2. Materials and methods

2.1. Koji preparation

Soybean were soaked in water for 6-8 h then autoclaved at 121 °C for 40 min. Wheat bran was roasted to dark brown and cracked then 32% of total wheat bran was ground to powder and mixed with culture before spreading to raw material to ensure through mixing of raw material. Different combinations of soybean and wheat bran (40%:60%, 50%:50%, and 60%:40% w/w) inoculated with 0.1% of culture spore were used as substrates for koji fermentation and incubated at 30°C for 72 h. Spores of *A. oryzae* S. NPUST-FS-206-A1 in 2 inoculum sizes (0.1% and 0.3% w/w) were inoculated into the raw material.

2.2. Enzyme extraction

To extract the enzyme, the fermented matter was mixed with 0.1 M phosphate buffer (pH 6.9) (1:2 w/v) by a shaking incubator (150 rpm, 30 °C, 30 min). After centrifugation at 10,000 xg, 4 °C for 15 min, the supernatant was collected as crude enzyme extract.

2.3. Analytical methods

The moisture content of a fermented sample was determined by an infrared moisture determination balance (FD-720, Kett Electric Laboratory, Tokyo, Japan). The fermented matter was mixed with deionized water (1:2 w/v) and measured the pH value by a pH meter (DKK-TOA, HM-25G, Japan). Neutral and alkaline protease activity were determined using 0.65% casein solution in 0.05 M phosphate buffer (pH 6.9) and 0.05 M carbonate buffer (pH 10) as substrate, respectively. One unit of enzyme activity was defined as the amount of enzyme that liberated 1μg of tyrosine per minute under assay conditions [7].

Amylase activity was measured with 1.0% soluble starch in 0.1 M sodium acetate buffer (pH 5.0). One unit of enzyme activity was defined as the amount of enzyme that released 1 mM of reducing sugar as maltose per minute under the assay conditions [5]. Reducing sugar content was determined by 3, 5-dinitrosalicylic acid method [8]. All samples were analyzed in triplicate.

3. Results and discussions

3.1. Physical properties

The initial pHs of different treatments were all around pH 6.32, then the pH decreased followed by an increasing after 36 h of fermentation (Fig. 1a). The results showed that the koji sample containing 60% soybean presented the highest increasing of pH to 6.97 after 72 h of cultivation. The increasing of pH was due to the production of various extracellular proteins from *A. oryzae* S. during soybean koji fermentation [3].

On the contrary, moisture content decreased with the fermentation time. It changed from 35-40% to 19-22% during 72 h of fermentation in 3 different combinations of substrates (Fig. 1b). The sample contained higher content of soybean showed higher initial moisture content. The decreasing of moisture content was caused by the mycelia growth of *A. oryzae* S. during fermentation. Moreover, moistening brings a suitable a_w for mold growth because of the easy penetration of mycelia into the substrates [9]. It was opposite to the results of Chutmanop et al. [5] that the moisture content of fermented wheat and rice bran increased during fermentation. It might be due to the different substrates. Soybean particle is bigger than rice and wheat bran. It supports air circulation and affect oxygen and heat transfer in the substrate.

3.2. Effect of different combinations of substrates on enzymes production

The protease activities in various combinations of substrates were similar at the beginning of fermentation. After 24 h, neutral protease activity of 60% soybean sample increased rapidly and reached the highest activity of 84.38 U/g dry weight at 48 h of fermentation, then it declined (Fig. 1c).

The highest alkaline protease activity was 41.35 U/g dry weight at 72 h of fermentation in 60% soybean sample (Fig. 1d). Whereas both neutral and alkaline protease activities were lower in the 40% and 50% soybean sample. The previous study reported that production of enzymes required amino acids by digesting proteins in substrate. *A. oryzae* can utilize a pool of nitrogen sources in soybean to produce amino acids, it decreases the total protein content of soybean koji during cultivation [3, 5].

Soybean is a good substrate containing high nitrogen content to produce proteolytic enzymes. The result indicated that substrate contained high content of soybean (60%) was the most optimal condition for protease production in soybean koji fermentation.

However, higher content of wheat bran in the substrate showed higher amylase activity (Fig. 1e). The highest amylase activity of 731.53 U/g dry weight was obtained in the 40% soybean sample. It is clear that the high carbon source contained substrate encourages the amylase production. The digestion of carbohydrates in substrates is required for the metabolism of *A. oryzae* S. Therefore, amylase production was accompanied with the growth of fungi [5].

3.3. Effect of different combinations of substrates on reducing sugar content

The profile of reducing sugar production was similar to the amylase activity. The reducing sugar content increased during the cultivation period. The highest reducing sugar content was 341.01 mM/g dry weight presented in 40% soybean sample (Fig. 1f). The increasing of reducing sugar content is caused by the enzymatic hydrolysis of starch in the substrates during fermentation [9].

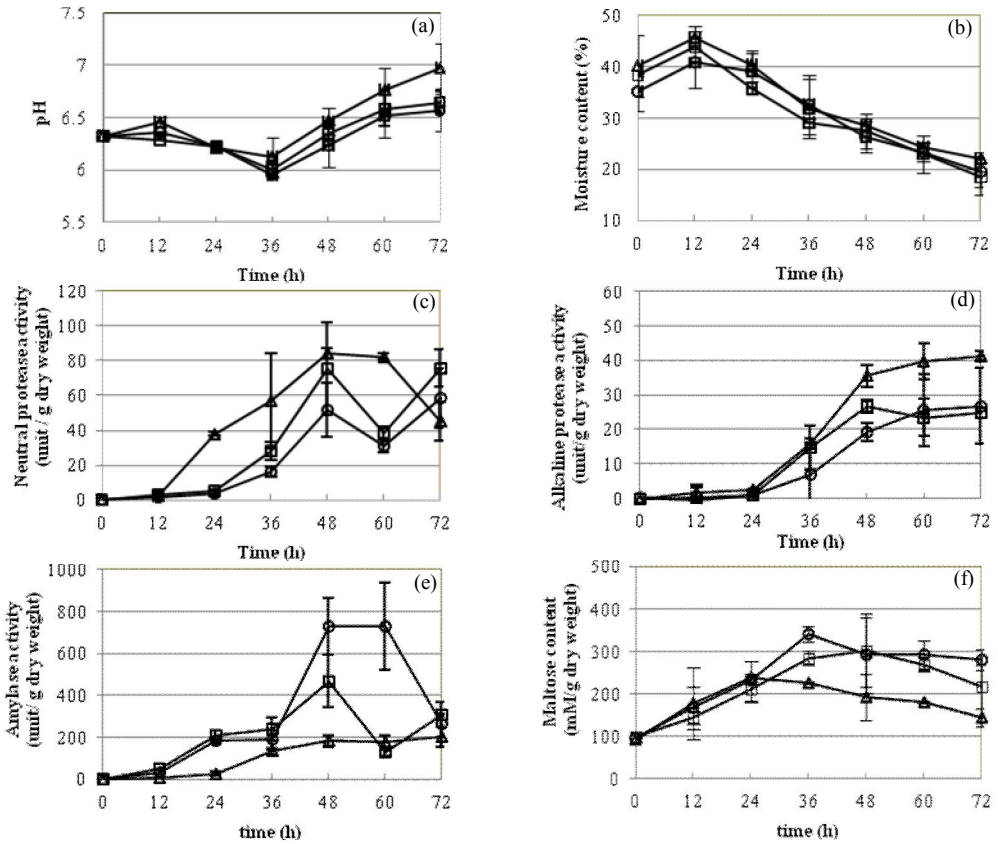

Fig. 1. Effect of different combinations of soybean and wheat bran on pH (a), moisture content (b), activity of neutral protease (c), alkaline protease (d), amylase (e), and reducing sugar content (f) during koji fermentation. The substrate combinations were 40% soybean (○), 50% soybean (□), 60% soybean (△).

3.4. Effect of inoculum sizes on enzyme production

The 60% soybean and 40% soybean samples were used to investigate the effect of inoculum size on protease and amylase production, respectively. The alkaline protease activity was no difference after 72 h of fermentation. The results indicated that higher neutral protease activity was shown from 0.1% inoculation. Furthermore, 0.3% inoculum reduced the neutral protease production (Fig. 2a).

The increase of inoculum size did not increase amylase activity or reducing sugar content (Fig. 2b). The results summarized that the maximal activities of various enzymes were obtained from 0.1% inoculation of koji. Even the inoculum size is an important biological factor, the large inoculum size decreases enzyme yield due to the shortage of nutrient available for the large culture and faster growth of the culture [6].

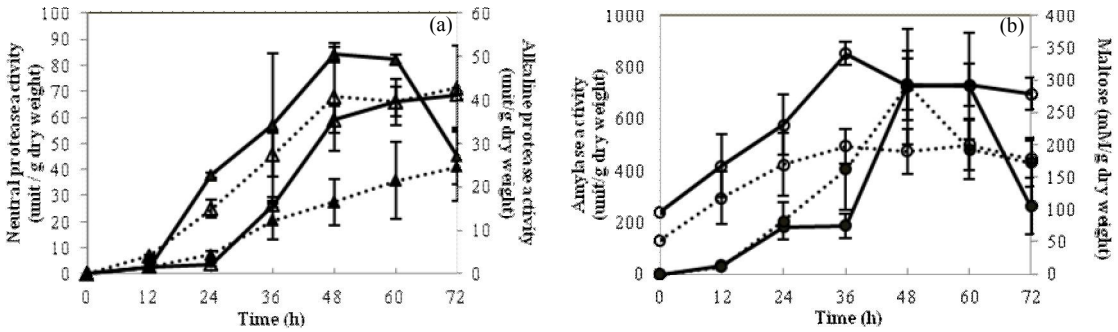

Fig. 2. Effect of inoculum sizes on neutral (▲) and alkaline protease activity (△) (a), amylase activity (●) and reducing sugar content (○) (b) during koji fermentation. Solid line and dotted line are represented for 0.1% and 0.3% inoculation, respectively.

4. Conclusion

The results indicated that different combinations of soybean and wheat bran affected enzyme production in koji fermentation. The high content of nitrogen source in the substrate like soybean encouraged protease activity, whereas high wheat content in the substrate promoted amylase activity. Increasing of inoculation did not increase the enzyme production. High nitrogen source material is required to elevate the protease production in koji fermentation.

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