Effects of Fermenting and Dehydrating techniques on enzymatic activities

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ABSTRACT

The thesis was to study the effects of fermenting and dehydrating techniques on enzymatic activity and functional compounds of Natto and Tempeh. Six experiments were conducted to achieve the above objectives. Experiment I (Chapter 3) was conducted to evaluate the effect of fermentation time on the proteins in steamed soybean inoculated with Bacillus subtilis, natto. The results indicated that the contents of trichloroacetic acid soluble nitrogen (TCA-N) and the degree of hydrolysis (DH) of the protein increased as the fermentation time increased. Protein solubility initially decreased with increasing fermentation time, and then increased. Sodium dodecyl sulfate polyacrylamide gel electrophoretogram (SDS-PAGE) showed that soy protein components with molecular weight above 20 kDa disappeared from the electrophoretograms for the samples fermented for 24-48 h. Though most essential amino acids declined after 36 h of fermentation, except histidine all increased after 48 h of fermentation. Experiment II (Chapter 4), was conducted to investigate the effect of drying process on enzyme activities of natto. The dries fermented steamed soybeans (natto) were dried by different methods which are conventional oven-drying at 50°C, vacuum-drying at 40°C, and freeze-drying at -50°C, and then ground them into powder form for analysis after drying for two days. Enzymatic activities including milk-clotting enzyme, protease, lipase, nattokinase and superoxide anion scavenging ability were determined. All the tested enzymatic activities, except that of superoxide anion scavenging ability, were maintained at higher level or almost the same as the fresh natto after drying by current drying methods. We suggest that the drying methods utilized in this study are feasible for processing producing dry natto powder. Experiment III (Chapter 5) was conducted to investigate the effects of drying methods on angiotensin-converting enzyme (ACE) inhibitory activity, gamma-polyglutamic acid (γ -PGA), and isoflavone contents of steamed soybeans fermented by Bacillus subtilis (natto). Results indicated that vacuum drying, freeze-drying, or conventional oven-drying did not affect the stability of γ -PGA and isoflavone contents, but did affect ACE inhibitory activity in all dried natto samples. Experiment IV（Chapter 6）was to investigate effect of the fermentation conditions on bioactive properties of the steamed soybean inoculated with B. subtilis. The steamed soybean was inoculated with the starter culture of B. subtitles and incubated at different conditions (pH 5, 7 and 9; temperature 37, 40 and 43°C; time 24, 36 and 48 h, respectively). The milk-clotting, proteolytic activity, ACE-I and amino acid composition of the products were determined. The result indicated that most of amino acids deceased in the product from fermentation at pH 9, 40°C and 36 h when compared with the control, suggesting that the selection of fermentation conditions should depend on what purpose and substances need. Experiment V（Chapter 7）was to investigate effect of the steamed soybeans inoculated with R. oligosporus in advance then B. subtilis on the biological activity. Changes of soy proteins, enzymatic activities were determined. The results indicated that the soluble nitrogen content and degree of hydrolysis of soy protein increased with increasing fermenting time both of the two-step fermentation and single starter culture. The amino acid composition of the two-step culture fermented product was fluctuant during fermentation. The milk-clotting activity of the mixed culture fermented product was higher than those of single starter cultures. No certainty trend of enzymatic activity changes caused by fermentation time and drying method. However, the most of components with molecular weight above 20 kDa on electrophoretogram of the soy proteins of the product fermented with the two-step fermentation disappeared after 24 h of fermentation. Experiment VI（Chapter 8）was to investigate purification and milk-clotting activity of the enzymes produced by Bacillus subtilis var, natto and Rhizopus oligosporus compared with that of the commercial rennet. The clotting time, viscosity, curd tension and microstructure of the curd and electrophoretic patterns of milk proteins were determined. The milk-clotting activity, specific activity, purification ratio and recovery of the purified enzymes produced by both the tested organisms with ion exchange chromatography and gel filtration were also determined. The results revealed that the curd formed by the commercial rennet had the highest in viscosity and curd tension and the shortest clotting time among the three enzymes. However, those of the enzyme produced by Rhizopus was ranked as second. From the results of milk-clotting activity/proteolytic activity ratios（MCA/PA ratio ）and electrophoretogram analyses, it could be noted that the enzyme produced by B. subtilis had the highest in proteolytic activity, while the commercial rennet had the highest in milk-clotting activity. Key words: ACE inhibitory activity, Bacillus subtilis, bioactive property, drying method, enzymatic activity, fermentation condition, isoflavone, microbial rennet, milk clotting, natto, purification, Rhizopus oligosporus, γ -PGA, SDS-PAGE, soy protein, tempeh

Keywords : ACE inhibitory activity、Bacillus subtilis、bioactive property、drying method、enzymatic activity、fermentation condition、isoflavone、microbial rennet、milk clotting、natto、purification、Rhizopus oligosporus、γ -PGA、SDS-PAGE、soy

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