CHAPTER V

DISCUSSION AND CONCLUSION

DISCUSSION

Proximate analysis

Moisture :

The moisture contents of unfermented soybean residue, soybean residue tempeh, soybean residue tooa-nao, soybean, soybean tempeh, and soybean tooa-nao were slightly different . In this study, we calculated moisture contents of all products as % weight on dry weight basis. Therefore the moisture contents of all products seem to be less than the results from the other investigations (ลาวัณย์ ไกรเดช, 2519 ; Sarkar and Tamang,1995) which the contents were calculated as % weight on fresh weight basis.

Crude protein :

Our data suggested that the protein contents of soybean were higher than that of soybean residue (Table1). In the production of soy milk, protein was extracted from soybean. Therefore the protein content in soybean residue was decreased. The findings that protein contents of soybean residue tempeh and soybean tempeh slightly increased after fermentation were similar to the results from previous investigations by Murata et al. (1967) from 45.7% to 50.6% (w/w of dry matter), by Wang, Ruttle, and Hesseltine (1968) from 47.8% to 48.1% (w/w of dry matter), by Zamora and Veum (1988) from 45.0% to 45.5% (w/w of dry matter). This result was also considerably consistent with the previous study of Mak (1986) in that the protein level of tempeh increased from that of the soybean milk waste. However, Steinkraus et al. (1960) found that during 72-hour

fermentation of tempeh, total soluble solids and soluble nitrogen increased from 13% to 28% and 0.5% to 2.5%, respectively but the total nitrogen remained fairly constant. Nout and Rombouts (1990) also reported that the effect of tempeh fermentation on total nitrogen content was negligible. Porntip Charoenthamawat (1991) showed that the protein contents of soybean residue tempeh remained unchanged after fermentation. In addition, van Buren, Hackler, and Steinkraus (1972) observed a slightly decrease in total protein from 43.2% to 41.6% (w/w of dry matter). A slightly increase in protein contents of soybean residue tempeh and soybean tempeh may attribute to the loss of nitrogen during fermentation. Hachmeister and Fung (1993) stated that fermentation of whole soybean accounted for 8.6% of the loss of nitrogen.

The most likely explanation of an apparent increase in total protein content is that there is a shift in the composition of the dry matter during fermentation (de Reu et al., 1995). There is a loss of 10% dry matter during fermentation (van der Riet et al., 1987). During fermentation carbohydrates and lipids were used to form biomass, carbondioxide, and water (de Reu et al., 1994). This would result in a relative increase in protein content. Increase in total protein content were small but seemed to be related to the growth rate of *Rhizopus oligosporus* . The optimum temperature for growth is about 37 to 42°C ; the lower the inoculation temperature, the smaller the observed increases in total protein content (de Reu et al., 1995).

In this study, we also found that protein contents of both soybean residue tooa-nao and soybean tooa-nao slightly increased after fermentation. This finding agrees with previous studies by Sarkar et al. (1994), that kinema contained only 1% higher protein content than its substrate. Sarkar and Tamang (1995) reported that the increase in total nitrogen content of kinema, over the raw soybean was significant. However, after the first 8 hour of fermentation, protein nitrogen content of raw soybean declined significantly at every 8 hour interval. Hayashi (1974) found a 4% increase in total nitrogen in natto over that of the raw soybean. This may be due to the ability of *Bacillus subtilis* to fix dinitrogen. Kim et al. (1995) conducted an experiment to investigate the changes of saccharides

and amino acids in spice added natto during fermentation. They found that amino-type nitrogen content of natto increased gradually during the 24 hour fermentation.

Crude fat :

The fat content of soybean residue and fermented soybean residue were lower than those of unfermented and fermented soybean because fat in soybean was extracted into soymilk during soymilk production. The decrease in the fat content of soybean residue tempeh and soybean tempeh were consistent with previous studies (Wang et al., 1968 ; Zamora and Veum, 1988 ; Pitch-on Wana-intarayude and Warawut Krusong, 1992) in that *Rhizopus oligosporus* was unable to use the carbohydrates present in soybeans (Sorenson and Hesseltine, 1966) ; instead the mold easily used the oil as its energy source (Wang et al., 1968). Lipolytic activity from the molds also breaks down fat in soybean (Lim, 1991).

This result is similar to those of Sarkar et al. (1994) and Sarkar and Tamang (1995) in that the fat contents of soybean residue tooa-nao decreased after fermentation compared to unfermented soybean residue. This may be due to the lipolytic activities of the micro-organisms during fermentation. Sarkar et al. (1994) found that during kinema production, strong lipolytic activities of the micro-organisms degraded a quarter of the fat content of soybean. On the other hand, our data showed that the fat content of soybean tooa-nao increased significantly after fermentation. This result agrees with the investigation of Taira and Suzuki (1983). The increase in fat content may due to the active assimilation of carbohydrates and limited consumption of lipids, resulting in an accumulation of crude fat at the end of fermentation (Sarkar et al., 1996).

Ash :

In this experiment, the ash contents of soybean residue and soybean were not significantly changed by tempeh or tooa-nao fermentation. This finding

is in agreement with previous studies by Porntip Charoenthamawat (1991) and Wang et al. (1968).

Crude fiber :

This finding agrees with previous study (ลาวัณย์ ไกรเดช, 2519) in that there was a slightly decrease in crude fiber contents of soybean residue and soybean after tempeh fermentation. The fermentation process didn't affect the fiber content of soybean residue tooa-nao. However, there was a slightly increase in fiber content of soybean tooa-nao.

Carbohydrate :

The carbohydrate content in soybean residue tempeh and soybean tempeh were increased after fermentation. This finding were similar to the results from Wang et al. (1968) and Pitch-on Wana-intarayude and Warawut Krusong (1992). The increase in carbohydrate and a decrease in fat contents of soybean residue tempeh and soybean tempeh after fermentation found in this study further substantiate previous finding. Sorenso and Hesseltine (1966) suggested that *Rhizopus oligosporus* was unable to use the carbohydrate present in soybean ; however, the mold easily used the oil as its energy source. Nout and Rombouts (1990) stated that *Rhizopus oligosporus* cannot utilize sucrose, stachyose, and raffinose and derives much of its energy from soya bean lipid, particularly oleic acid. While the carbohydrate content of soybean residue tooa-nao remained unchanged, the carbohydrate content of soybean tooa-nao decreased significantly. It is probably due to the active assimilation of carbohydrate and limited consumption of lipids in soybean tooa-nao (Sarkar et al., 1996). Sarkar and Tamang (1995) stated that it seems to be that sugars were initially used as substrates for metabolism and growth of the organisms.

Moisture (fresh weight basis)

The moisture contents of soybean residue tempeh and tooa-nao were lower than those of fermented soybean products. This may be due to the lower moisture content of raw material, soybean residue. Sarkar et al. (1996) suggested that the significant increase in moisture content during fermentation of soybean in the presence of kinema micro-organism was probably due to the volume of water added with the inocula. In addition, the increase in moisture content of soybean tooa-nao might be attribute to the combined effects of dry matter consumption and production of water during aerobic catabolism by the *Bacillus* (Sarkar and Tamang, 1995). The increase in moisture content of soybean tempeh may be explained by the same way as soybean tooa-nao.

Amino acids composition

In this study, the amino acids contents of the fermented soybean residue and soybean remained unchanged or decrease or increase by less than 5-10% as compared to their unfermented substances , which was in agreement with the results reported by Smith et al. (1964), Zamora and Veum (1979). However, almost all the amino acid contents in soybean residue tempeh increased after fermentation. The amino acid scores of all essential amino acids in soybean residue tempeh were also increase after fermentation. The results obtained from this study indicated that improvements the nutritive value of protein in soybean residue were accomplished by the tempeh fermentation. Murata et al. (1967) noted that some amino acids increased while others decreased as a result of mold activity. However, they observed an overall increase in the content of free amino acids as the fermentation progressed. Sano (1961) found the increase in essential amino acids in natto, prepared by fermenting soybean with *Bacillus natto (subtilis)*.

According to the amino acid scores, we found that the nutritive value of protein in soybean residue tempeh was better than that of soybean residue

tooa-nao. However, the nutritive values of protein in soybean tempeh and soybean tooa-nao were similar.

Protein digestibility

The results indicated that protein digestibility of soybean residue tempeh was the highest of all products in this study. The protein digestibility of soybean tempeh was significantly higher than that of unfermented soybean. This finding are consistent with the previous study (van Veen and Shaefer, 1950) in that the mold made the soybean more digestible. Ko and Hesseltine (1979) stated that tempeh was easily digested and was tolerated by patients suffer from dysentery and nutritional edema (Ko and Hesseltine, 1979). In 1979, Zamora and Veum reported that fermentation of cooked soybean inoculated with *Rhizopus oligosporus* improved its digestibility in rats. The high digestibility of tempeh may be ascribed to the predigestion of soybean nutrients and their breakdown into soluble solids and nitrogen by the mold during fermentation. The beans, therefore, become soft and tender. The proteases, produced by the mold, breakdown the proteins into amino acids and other water soluble products which are readily assimilated by the body. The protein digestibility of soybean tooa-nao was also improved after fermentation. This may attribute to the hydrolysis of soybean proteins to peptides and amino acids by proteolytic enzymes of *Bacillus subtilis* (Ohta, 1986). However, the protein digestibility of soybean residue tooa-nao was fairly constant after fermentation. It is possible that the fermentation condition was unsuitable for the micro-organism.

Vitamins

The results of our investigation demonstrated that all vitamins, except thiamin and pantothenic acid, in soybean residue increased after fermentation. Various researchers have reported 2-fold increase in riboflavin, 7-fold increase in niacin, and 33-fold increase in cobalamine (Mital and Garg, 1990). Other investigations reported the higher levels of niacin, riboflavin, pantothenic acid, and pyridoxine than those in raw soybean (Roelofsen and Talens, 1964 ; Murata et al., 1967). Liem et al. (1977) found that pure tempeh mold obtained from

different source did not produce cobalamine. However, a bacteria that accompanied the mold during fermentation was found to produce cobalamine in commercial tempeh purchased in Canada. An increase of pantothenic acid during tempeh fermentation was not observed by Steinkraus et al. (1961). Our finding agrees with other studies in that the pantothenic acid content of soybean residue tempeh was fairly constant after fermentation. However, we also found that the thiamin content of soybean residue tempeh was not change after fermentation. The increase in riboflavin, pyridoxine, and cobalamine during the fermentation of soybean residue to make tooa-nao were less than those in making tempeh. Thiamin and pantothenic acid contents were relatively constant in soybean residue tooa-nao. However, we found a decrease in niacin content of soybean residue tooa-nao.

CONCLUSION

This study investigated the production of tempeh and tooa-nao from soybean milk waste and soybeans. The nutritive value of protein in soybean residue was found to improve by the fermentation process. The crude protein contents in soybean residue tempeh, soybean residue tooa-nao, soybean tempeh, and soybean tooa-nao were increase 2.40%, 1.65%, 2.55%, and 1.85%, respectively. Valine, tyrosine, and phenylalanine contents were found to increase 9% to 16% after soybean residue tempeh fermentation. The contents of alanine and phenylalanine in soybean tempeh increase 31% and 15%, respectively. In soybean residue tooa-nao, cystine content increased 11.2% but valine content decreased 13%. In soybean tooa-nao, the contents of valine and lysine increased 26% and 11%, respectively but arginine content decreased 15%. Amino acid scores of some essential amino acids in fermented products were increase. The protein digestibility of soybean residue tempeh was increase from 67.68% to 74.95% whereas that of soybean residue tooa-nao remained constant. We also found that protein digestibility of soybean tempeh and soybean tooa-nao increased from 59.65% to 68.18% and 66.92%, respectively. Undoubtedly, vitamins contents were also affected by the fermentation process and contributed to the overall changes in nutritional value. The contents of riboflavin, niacin, pyridoxine, and cobalamine increased during soybean residue tempeh fermentation, while those of thiamin and pantothenic acid remained unaltered. Tooa-nao fermentation also improved the vitamins contents in soybean residue , except for niacin which decreased after fermentation.

Although most indigenous fermented foods used soybean as substrate, soybean milk waste has undergone in this study as possible substrate. Advantages of fermented soybean residue over the raw, unfermented material included improved organoleptic properties, enhance nutritional value, and improved digestibility.