Chapter 4

Improvement of *Thua Nao* Production Using Protein-rich Soybean and *Bacillus subtilis* TN51 Starter Culture

4.1 Introduction

Thua Nao, a traditional fermented soybean of Northern Thailand is typically present in two forms: fresh *Thua Nao* (*Thua Nao Mer*) and dried form (*Thua Nao Kab*). Traditional fermentation of *Thua Nao* is as follows: soybeans are cooked by boiling and fermented spontaneously at ambient temperature for 3 - 4 days. The completion of fermentation is indicated by the formation of a dominant ammoniacal smell, a typical umami taste and a white viscous fluid on the beans. Like other traditional soy-fermented products, *Thua Nao* is currently produced basically on a traditional manner. This household practice often yields inconsistent quality products, strong ammonical smell, risk of foodborne disease and short shelf-life. A number of spoilage and foodborne pathogenic organisms have been identified in the traditional *Kinema* (Nout *et al*., 1998), soy-*Dawadawa* (Jideani and Okeke, 1991; Dike and Odunfa, 2003) and *Thua Nao* (Leejeerajumnean, 2003; Chukeatirote *et al*., 2006). In order to eliminate these problems, there is a need for scientific investigation to improve production methods. In recent years, many attempts are made by several researchers aiming to develop a starter culture(s). A series of successful scenarios have been described in various products such as *Natto* (Wei *et al*., 2001; Wei and Chang, 2004), *Kinema* (Tamang *et al*., 1993; Sarkar and Tamang, 1995; Tamang and Nikkuni, 1996) and soy-*Daddawa* (Omafuvbe *et al*., 2002; Omafuvbe, 2008). As reported in these studies, the use of potential pure starter culture and controlled fermentation process appears to be the gold standard toward improvement of fermented soybean products. In addition to starter organism, there are many factors affecting the quality of fermented soybean such as soybean cultivar and processing conditions i.e. soaking, cooking and fermentation (Wei *et al*., 2004). Small types of soybeans with high protein and low fat contents are suggested for producing the quality *Natto* products (Taira and Suzuki, 1983; Steinkraus, 1983; Ohta, 1986; Griffis

and Wiedermann, 1991; Hosoi and Kiuchi, 2003). Soybean with high carbohydrate content correlated with the lesser ammonia content in *Natto* products (Ohta, 1986). The positive correlation of ammonia production with the hardness of steamed soybeans has also been reported by Taira *et al*. (1987).

The attempts of using pure starter culture to improve the quality of *Thua Nao* production have also been investigated in Thailand. Changes in proteolytic activity and some biochemical qualities during fermentation of *Thua Nao* by using pure starter culture of *Bacillus subtilis* strain 38 (Chantawannakul *et al*., 2002) and BIOTEC7123 (Visessanguan *et al*., 2005) have been studied. Also, the finding of Tangjitjaroenkun *et al*. (2004), the mixture cultures of *B. amyloliquefaciens* and *Klebsiella* sp. KB2 which isolated from *Thua Nao* could enhance vitamin B12 contents in the fermented soybean. However, the nutritive value and organoleptical quality of the product are still requiring study. In our previous study, proteolytic activities of bacteria isolated from *Thua Nao* were screened and *Bacillus subtilis* TN51 is the highly active proteolytic activity strain (Dajanta *et al*., 2009). Therefore, the objective of this study was to propose *B. subtilis* TN51 as starter culture for the fermentation suitably soybean cultivar into a high nutritive *Thua Nao*. Changes in biochemical quality of soybean to form *Thua Nao* along with a natural fermentation were monitored during controlled fermentation including the final acceptability attributes of these products were also involved in this investigation.

4.2 Materials and methods

4.2.1 Soybean cultivars

Five soybean cultivars namely CM60, Pabong7, Kunpae, TG55 and TG145 were obtained from Field Crop Research Center, Institute of Agriculture, Chiang Mai, Thailand. These soybeans were initially characterised based on their production yield and size. The production yield was calculated as weight per area (kg/rai) in which the soybean weight used was 12% dry matter of moisture and these data were obtained from 4 experimental replications in dry season (December 2007 to March 2008) at Field Crops Research Center. For soybean size, 100 seeds were randomly selected and

used for the analysis. In addition, protein and fat contents were analysed based on the AOAC's protocol (AOAC, 2000).

4.2.2 Bacterial strain

Bacillus subtilis TN51 previously isolated from commercial *Thua Nao* was used as pure starter culture due to its high proteolytic activity (Dajanta *et al*., 2009). The bacteria were routinely cultured on nutrient agar and, for stock culture, the 20% glycerol bacterial culture was prepared and kept at -20°C. For inoculum preparation, the bacteria were grown in nutrient broth at 37°C for 24 h. The cells were then harvested, resuspended in sterile distilled water and adjusted to 10^6 – 10^7 CFU/ml. This cell suspension was ready to serve as inoculum for soybean fermentation.

4.2.3 Preparation of *Thua Nao*

Protein-rich soybeans variety TG145 obtained from the Field Crop Research Center, Institute of Agriculture, Chiang Mai, Thailand, were washed and soaked in tap water for 16 h at ambient temperature (~25°C). Before fermentation, different methods were used to prepare cooked non-fermented soybeans (CNF). Soaked soybeans were cooked by boiling for 4 h (CNF1) and left to ferment by naturally occurring microbes (TNMX). In contrast, for *Bacillus*-inoculum fermentation, soaked soybeans were sterilised at 121°C for 40 min (CNF2), and inoculated with 10^4 CFU (per g of sterilised soybeans) of *B. subtilis* TN51 starter culture (TNB51). Fermentation process was then allowed to proceed at 42°C for 72 h. Changes in microbiological and physicochemical qualities of soybean during the fermentation were then determined every 24 h for 3 days.

4.2.4 Analysis of physicochemical and microbiological qualities

Changes in proximate composition including protein, ammonia, fat ash, moisture, pH, reducing and total sugars as well as soybean surface colour were carried out as described in Section 3.2.3. Ammonia concentration was also determined by titrating 1 g sample with 0.1 M HCl after steamed distillation with Kjeltec Distillation

Unit. For changes in microbiological quality, total viable count, spore count and total yeasts and moulds were also determined as described in Section 3.2.2.

4.2.5 Sensory evaluation

Cooked *Thua Nao* products (TNMX and TNB51) and commercial product purchased from Mae Hia market (MH), Chiang Mai were evaluated for consumer's acceptability by 50 untrained local panelists using a 7-point hedonic scale test (see Appendix B).

4.2.6 Statistical analysis

All data except those of sensory evaluation were expressed as means ± standard deviation of triplicate observations. The results from sensory evaluation were calculated from the mean values of the 50 panelists. The data were also subjected to analysis of variance (ANOVA), *t*-test, and Duncan's multiple range tests. The significant differences between means were defined at $P \leq 0.05$.

4.3 Results and discussion

4.3.1 Quality of soybeans

Five soybean cultivars namely CM60, Pabong7, Kunpae, TG55 and TG145 were selected for this study. In general, their seeds are oval and round with yellow seed colour and dark brown hila (Figure 4.1). In this study, some nutritive qualities such as protein and fat contents, and the production yield and seed-size were used as criteria to select the potential soybean cultivar as raw material for producing high nutritive value of *Thua Nao*. As shown in Table 4.1, the protein contents of the five soybean cultivars were between 39.38 - 45.85% which were similar to those of soybean cultivars used to produce *Natto* (39.30 - 45.31%) as reported by Taira and Suzuki (1983). The effect of peptides and amino acids derived from the activities of fermenting organisms on the flavour appeals of *Natto*, *Douchiba*, and soy source have been reported, thus protein-rich soybeans were preferred to produce a quality of these products (Hosoi and Kiuchi, 2003; Qin and Ding, 2007; Yanfang and Wenyi, 2009).

Figure 4.1 Appearances of soybean cultivars used in this study.

Table 4.1 Some qualitative data of soybean cultivars

Soybean variety	Protein[1] (%dbs)	Fat[1] (%dbs)	Production yield[2] (kg/rai)	Weight of 100 seeds[2] (g)
TG145	45.85 ± 0.75[a]	15.59 ± 0.02[d]	358	11.1
TG55	40.70 ± 0.24[c]	18.06 ± 0.43[b]	347	14.5
Kunpae	39.38 ± 0.63[c]	17.07 ± 0.37[c]	366	12.9
Pabong7	43.01 ± 1.37[b]	17.00 ± 0.05[c]	360	13.6
CM60	39.48 ± 0.16[c]	19.91 ± 0.43[a]	371	15.9

[1]Data are mean ± standard deviation (n = 3). Means within a column with different letter are significantly different ($P \leq 0.05$).

[2]Data calculated from experimental 4 replications in dry season (December 2007 to March 2008) were obtained from Field Crop Research Center, Institute of Agriculture, Chiang Mai, Thailand.

This study also found that the fat content of five soybean cultivars ranged from 15.59 – 19.91% which were consistent with the fat contents of suitable soybean cultivars for *Natto* processing (18.71 - 20.51%) as the reported of Taira and Suzuki (1983). Besides these, small soybean seed types having the weights of 100 seeds

between 8.1 to 18.2 g at 11.08-13.02% of moisture content were suggested to use in quality *Natto* production (Taira *et al.*, 1987). In this study, seed-size of five tested soybean cultivars based on the weight of 100 seeds (moisture contents around 12%), displayed in the range 11.1 to 15.9 g with the smallest one in TG145 and the biggest one in CM60 (Table 4.1 and Figure 4.1). The suitability characteristics of soybean seed for making quality *Natto* have been studied and suggested as small size of round seed, light yellow hilum, brilliant light yellow or yellow coloured and smooth seed coat (Steinkraus, 1983; Ohta, 1986; Hosoi and Kiuchi, 2003). The smaller soybean produced the better product because it has a high water-absorbing capacity, easier to cook and provide a better aerobic fermenting environment (Teng *et al*., 2004). It has been reported that, soybeans with a protein level of 43% or greater and low fat content could improve nutritional quality of *Natto* (Griffis and Wiedermann, 1991).

Of five soybean cultivars tested, the TG145 cultivar displayed highest protein content and lowest fat content. For production yield, there was no significant difference among these cultivars ($P > 0.05$). By the criteria of high protein, low fat content, high production yield and smaller seed-size, TG145 cultivar is highly regarded for using in high nutritional *Thua Nao* production.

4.3.2 Microbial profiles of *Thua Nao* during fermentation process

Based on previous screening and isolation of protease-producing bacilli from *Thua Nao*, *Bacillus subtilis* strain TN51 exhibited highest proteolytic activity (Dajantan *et al*., 2009). This strain was therefore selected as a potential starter culture to improve nutritive *Thua Nao*. Bacterial cell and colony morphology are illustrated in Figure 4.2.

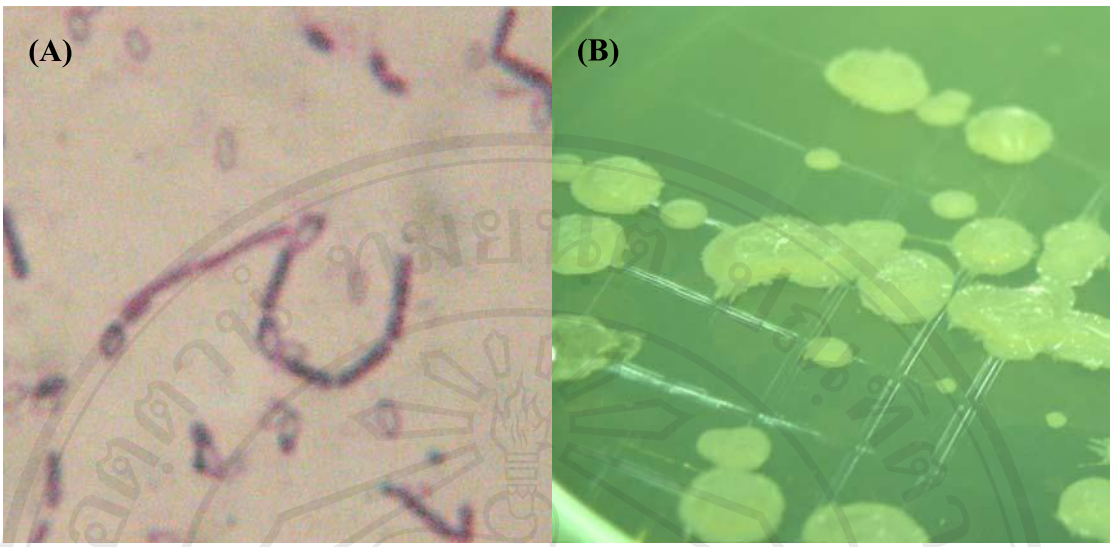

Figure 4.2 Cell (A) and colony (B) morphology of *Bacillus subtilis* strain TN51

As shown in Table 4.2, *B. sutilis* TN51 seems to only have an effect at the start of the fermentation process of soy because the significant higher contents of initial organisms were recorded. After the fermentation process, total viable count (TVC) and spore count (SPC) were greatly increased in the first 24 h then the growth of microorganisms seem to be a stationary phase as evidence of the stabilities values of microorganisms were recorded till 72 h of fermentation time. Spore-forming bacteria were suggested as the mainly role in *Thua Nao* productions, since greater 92% of SPC were identified throughout fermentation processes. This result is in agreement with the findings by other researchers for predominant spore-forming bacteria in *Thua Nao* productions (Sundhagul *et al*., 1972; Visessanguan *et al*., 2005; Chukeatirote *et al.*, 2006). In general, natural *Thua Nao* production (TNMX) showed the higher amount of microorganisms with respect to pure starter fermented process (TNB51), suggesting that more than one species of *Bacillus* involved in the naturally fermented *Thua Nao*. Another *Bacillus* species such as *B. licheniformis*, *B. pumilus*, *B. megaterium and B. cereus* including other organisms (i.e. *Lactobacillus* sp. and Gram-positive cocci) have been identified in spontaneously fermenting *Thua Nao* (Leejeerajumnean, 2003; Chukeatirote *et al*., 2006). This study also presented small amount of yeast and mould (< 2 log CFU/g) throughout fermentation processes. This observation is inconsistent with the finding of Chukeatirote *et al.* (2006), who found a

large number (13 log CFU/g) of fungi in 72 h fermentation of natural *Thua Nao*, it may be indicative of a safety risk of the product.

Table 4.2 Changes in microbial population during soybean fermentation

Fermentation time (h)	Total Viable Count		Spore Count	
	TNB51	TNMX	TNB51	TNMX
0	4.09 ± 0.09^{cA}	2.80 ± 0.14^{cB}	4.08 ± 0.07^{cA}	1.23 ± 0.21^{cB}
24	8.89 ± 0.04^{aA}	8.89 ± 0.11^{bA}	8.36 ± 0.13^{bA}	8.17 ± 0.03^{bA}
48	8.91 ± 0.03^{aB}	9.57 ± 0.13^{aA}	8.66 ± 0.14^{aB}	9.58 ± 0.02^{aA}
72	8.62 ± 0.17^{bB}	9.67 ± 0.02^{aA}	8.59 ± 0.03^{aB}	9.62 ± 0.07^{aA}

Data are mean ± standard deviation (n =3) and expressed as log CFU/g wet weight. Means within a column with different small letter are significantly different within interval samples ($P \leq 0.05$) and means within a row with different capital letter are significantly different between types of *Thua Nao*, analysed by *t*-test method ($P \leq 0.05$). TNB51, *Thua Nao* was prepared by fermentation of autoclaved soybeans with pure starter culture of *B. subtilis* TN51; TNMX, *Thua Nao* was prepared by fermentation of naturally occurring microbes of boiled soybeans.

4.3.3 Physicochemical changes of fermented soybeans

As shown in Figure 4.3, there is a distinct difference between non-fermented and fermented soybeans. The colour of autoclaved soybean (Fig.4.3B) was stronger compared to boiled soybean (Fig.4.3A) due to high temperature treatment. After 72 h fermentation, it was found that *Thua Nao* prepared by *B. subtilis* TN51 was sticky covered white-coloured viscous substance (Fig.4.3D). The distinct odour of *Natto*-like was also observed. In contrast, traditionally fermented soybean did not have such characteristics (Fig. 4.3C). Similar results have been reported in Korean *Chungkukjang* produced by pure starter *B. subtilis* for higher production of sticky substance and desirable odour (Lee *et al*., 2005a).

Figure 4.3 Appearance of cooked non-fermented and fermented soybeans. A, boiled non-fermented soybeans (CNF1); B, autoclaved non-fermented soybeans (CNF2); C, naturally fermented *Thua Nao* (TNMX); D, *B. subtilis* TN51 fermented *Thua Nao* (TNB51).

According to the report of Chunhachart *et al*. (2006), the degradation of γ-glutamyl hydrolase which produced from some *Bacillus* strains contaminant in traditional fermentation of *Thua Nao* with resulted a lesser viscous materials in final product. The sticky substance comprises of polyglutamic acid and levan (β-2,6-fructan) is produced by certain *Bacillus* strains as a capsular or an extracellular during fermentation process of soybeans (Kunioka and Goto, 1994; Ogawa *et al*., 1997). In *Natto*, the mucous substance is an important criterion to consider the high-quality product. The benefits of this material has been reported as dietary fiber to reduce the cholesterol level in serum (Tsuji and Tsuji, 1986) and also acts as antibiotic agents against food spoilage and pathogenic bacteria such as *Staphylococcus aureus, Bacillus cereus, Escherichia coli* O157, and *Salmonella* (Youn *et al.,* 2001).

The changes in soybean chemical composition during starter *B. subtilis* fermentation have been discussed by several researchers (Sarkar and Tamang, 1995; Omafuvbe *et al.*, 2002; Wei and Chang, 2004; Visessanguan *et al*., 2005; Terlabie *et al*., 2006). In this study, changes in physicochemical quality of *Thua Nao* during fermentation of soybeans at different incubation periods (0, 24, 48 and 72 h) are shown in Table 4.3. The moisture contents in fermented *Thua Nao* showed a little change throughout fermentation (57.6 to 56.74 for TNB51 and 66.51 to 68.19% for TNMX). However, boiled soybean production *Thua Nao* (TNMX) showed higher moisture contents significantly throughout fermentation process ($P \leq 0.05$) when compared with autoclaving the fermented product.

The pH values and ammonia contents of soybeans increased with fermentation time in both *Thua Nao* productions. However, the final ammonia content of TNMX was two times higher than TNB51 product. This is likely due to the effect of cooking process. The nutrient utilisation (i.e., protein degradation) by microorganisms could therefore occur easily and rapidly (Taira *et al*., 1990; Wei *et al*., 2001; Hosoi and Kiuchi, 2003). According to Table 4.3, the patterns of change in pH values generally coincides raise trends of TVC and SPC (Table 4.2) every 24 h interval products. This observation might be due to the activity of *Bacillus* species utilising soy proteins leading to release of amines and ammonia which causes a rise of pH value in final product (Terlabie *et al.*, 2006; Omafuvbe, 2008). The strong proteolytic activity of *B. subtilis* TN51 has been investigated previously by Dajanta *et al.* (2009) and their report also presented that 99% isolated bacterial strains from traditional *Thua Nao* were capable of producing protease enzymes and around 76% of these were identified as endospore-forming bacilli. Volatile ammonia is presented at the pH value reach about 8.0 - 8.3 and results in a strong unpleasant ammoniacal smell of the *Thua Nao* product as observed by Campbell-Platt (1980) and Sarkar *et al*. (1993).

Protein, ash and fat contents of *Thua Nao* were mostly constant throughout fermentation, except ash and fat contents of TNMX that increased significantly in the first 24 h of fermentation and significantly dropped at the end of fermentation ($P \leq 0.05$). These different patterns may be due to a difference of capabilities to utilise lipid or carbohydrate of starter microbial organisms during *Thua Nao* fermentation. In

literatures, the variable trends of fat contents have also been recorded in production of *Natto* (Taira and Suzuki, 1983; Wei and Chang, 2004)

Table 4.3 Physicochemical qualities of soybean during fermentation

Quality	Fermentation time (h)	TNB51	TNMX
Moisture content (%dbs)	0	57.60 ± 0.60^{aB}	66.51 ± 0.22^{aA}
	24	57.18 ± 1.18^{aB}	67.10 ± 0.64^{aA}
	48	56.53 ± 0.04aB	66.77 ± 1.68^{aA}
	72	56.74 ± 0.84^{aB}	68.19 ± 0.49^{aA}
pH	0	6.39 ± 0.01^{cB}	6.58 ± 0.01^{dA}
	24	7.66 ± 0.03^{bA}	7.30 ± 0.10^{cB}
	48	8.01 ± 0.08^{aA}	7.97 ± 0.06^{bA}
	72	8.00 ± 0.02^{aB}	8.33 ± 0.11^{aA}
Ammonia (mg/ml)	0	0.03 ± 0.00^{cA}	0.04 ± 0.01^{cA}
	24	0.04 ± 0.00^{cB}	0.21 ± 0.02^{bA}
	48	0.18 ± 0.02^{bB}	0.42 ± 0.03^{aA}
	72	0.29 ± 0.01^{aB}	0.42 ± 0.01^{aA}
Ash (%dbs)	0	6.29 ± 0.49^{aA}	4.93 ± 0.08^{cA}
	24	6.45 ± 0.47^{aB}	8.40 ± 0.23^{aA}
	48	6.17 ± 0.25^{aB}	8.27 ± 0.10^{aA}
	72	6.43 ± 0.19^{aB}	7.58 ± 0.07^{bA}
Fat (%dbs)	0	18.47 ± 0.55^{aA}	18.54 ± 0.26^{bA}
	24	16.93 ± 0.56^{aA}	22.08 ± 0.70^{aA}
	48	16.53 ± 0.69^{aA}	21.72 ± 0.73^{aA}
	72	16.40 ± 0.13^{aA}	19.06 ± 0.72^{bA}
Protein (%dbs)	0	45.74 ± 0.31^{aA}	45.27 ± 0.77^{aA}
	24	46.19 ± 1.06^{aA}	45.74 ± 0.35^{aA}
	48	46.00 ± 0.17^{aA}	46.73 ± 1.00^{aA}
	72	45.35 ± 0.28^{aA}	44.91 ± 0.12^{aA}
Total sugar (%dbs)	0	11.61 ± 0.15^{aA}	4.32 ± 0.08^{aB}
	24	5.90 ± 0.12^{bA}	2.73 ± 0.15^{bB}
	48	5.53 ± 0.16^{cA}	2.78 ± 0.14^{bB}
	72	6.02 ± 0.07^{bA}	2.64 ± 0.06^{bB}
Reducing sugar (%dbs)	0	3.27 ± 0.37bA	1.54 ± 0.01cB
	24	3.97 ± 0.12aA	2.53 ± 0.06abB
	48	4.04 ± 0.02aA	2.78 ± 0.14aB
	72	3.59 ± 0.14abA	2.47 ± 0.14bB

Table 4.3 (*continued*)

Quality	Fermentation time (h)	TNB51	TNMX
Colour L	0	45.96 ± 1.89^{aB}	59.37 ± 0.89^{aA}
	24	41.49 ± 1.81^{bB}	55.79 ± 1.77^{abA}
	48	38.70 ± 1.46^{bcB}	55.21 ± 2.43^{abA}
	72	36.42 ± 2.98^{cB}	53.74 ± 3.25^{bA}
Colour a*	0	8.76 ± 0.45^{bA}	5.08 ± 0.30^{bB}
	24	7.56 ± 0.24^{cA}	5.42 ± 0.08^{bB}
	48	8.90 ± 0.58^{bA}	5.54 ± 0.61^{bB}
	72	10.11 ± 0.85^{aA}	6.62 ± 0.63^{aB}
Colour b*	0	20.10 ± 0.78^{aA}	22.75 ± 1.78^{aA}
	24	14.55 ± 1.69^{bB}	20.91 ± 1.14^{abA}
	48	12.55 ± 0.10^{bB}	19.12 ± 0.72^{bA}
	72	14.57 ± 3.99^{bA}	20.06 ± 2.12^{abA}

Data are mean ± standard deviation (n =3). Means within a column with different small letter are significantly different within interval samples ($P \leq 0.05$) and means within a row with different capital letter are significantly different between types of *Thua Nao*, analysed by *t*-test method ($P \leq 0.05$). TNB51, *Thua Nao* was prepared by fermentation of autoclaved soybeans with pure starter culture of *B. subtilis* TN51; TNMX, *Thua Nao* was prepared by fermentation of naturally occurring microbes of boiled soybeans.

The contents of total and reducing sugars in *Thua Nao* presented similar pattern of change in both production processes. The total sugar decreased significantly in contrast to the increase of reducing sugar in the first 24 h of *Thua Nao* fermentation ($P \leq 0.05$) and dropped slightly at the end of the process. It is significant to note that the period of rapid increase in the levels of reducing sugars in contrast to the marked decrease of total sugars coincides with the period of increase in pH values (Table 4.3) and the great rise of TVC and SPC (Table 4.2). These observations may indicate that the total sugars of soybeans were used by fermenting organisms resulted in increase of reducing sugars, and the last stage of fermentation, free sugars were utilised by the great microbial population causing their decrease in the final products. Similar trends of these values have been reported in soy-*Daddawa* production (Omafuvbe, 2008). Comparing with starter fermented *Thua Nao*, significantly lower contents of total and reducing sugars were found throughout fermentation of TNMX, because at initial stage some of these components may be lost during boiling soybean in water further leading to their lower levels through the process. The faster fermentation rate based

on rise of pH values was found in *Bacillus* starter product. It may be related with a larger content of reducing sugar involved in the product. Taira (1990) has been reported the closely associated the composition of free sugars with the taste of *Natto* and influence the speed of fermentation. Hence, a higher fermentation rate based on increasing pH value of TNB51 and a greater acceptability in taste attribute (Figure 4.3) than those in TNMX may be related to the larger content of reducing sugar of the product.

Colour L (lightness) and b* (blue to yellow colour) values of soybeans were continuously decreased depending on the time of fermentation (Table 4.3). In contrast, a* value (green to red colour) showed a significant increase after fermenting for 72 h. It means that during fermentation process, the lightness and yellowness were lowered conversely to increasing of redness significantly responsible to the increasing of visible darker brown colour from cooked non-fermented soybeans to fermented products (Figure 4.2). Compared to TNMX, the lower values of lightness and yellowness were observed in TNB51 product throughout the fermentation period. This is due to the higher temperature treatment of the autoclaving practice that was used to cook soybeans in the fermentation process. According the effect of heat treatment, dark brown nitrogen-containing pigments melanoidins are formed from the Maillard reaction and Strecker degradation. There are several factors that influence the higher rate of Maillard reaction, such as high temperature of heat treatment, low moisture levels, alkaline pH condition and large levels of reducing sugars (Hurrell, 1982). For these reasons, autoclaved non-fermented soybean and its fermented product, TNB51, showed significantly darker brown colour throughout the process of fermentation ($P \leq 0.05$).

4.3.4 Sensory evaluation

Figure 4.3 illustrates the sensory evaluation of the TNMX, TNB51 and MH products. The overall acceptability, colour, texture, flavour and aroma attributes of *Thua Nao* products were assessed by 50 untrained panelists using 7-point hedonic scale test. High acceptability of consumers was observed as shown by highest scores ($P \leq 0.05$) in all attributes of the TNB51 product. Of these sensory attributes tested, the soft texture and mild aroma of TNB51 was most acceptable, whilst strong

ammoniacal smell of TNMX and MH were regarded as key factor affecting consumer's acceptability of these products. Previous investigations including this study have also presented that the organoleptically qualities of *Kinema*, soy-*Daddawa* and *Thua Nao* are successful to improve by inoculated sterilised soybean with controlled starter of *B. subtilis* production (Sarkar and Tamang, 1995; Omafuvbe *et al*., 2002).

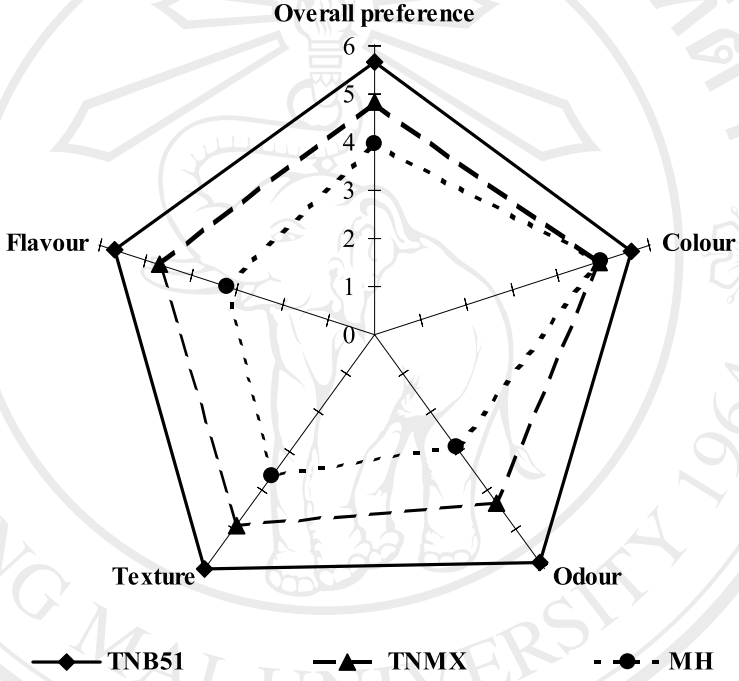

Figure 4.4 Sensory evaluation of *Thua Nao* produced by pure starter *B. subtilis* TN51 (TNB51), natural fermentation (TNMX), and commercial product (MH).

4.4 Conclusion

It is clear from this present study that the ability of pure culture of *B. subtilis* TN51 to bring about normal fermentation suggested that controlled fermentation could be accomplished in the production of *Thua Nao*. Furthermore, *B. subtilis* TN51 also could yield improved microbiological and chemical qualities, especially a smaller amount of ammonia that led to more organoleptically acceptability than the naturally fermented products. It should therefore be possible, through controlled fermentation, to produce a safer and more acceptable *Thua Nao*. Soy-fermented food is claimed to

be a functional food because of possessed several human health benefits with the mainly effect of pharmacological and antioxidant properties of soy isoflavone (Gotoh *et al*., 1998; Potter *et al*., 1998; Park *et al*., 2003; Jung *et al*., 2006). Therefore, further studies are needed to evaluate the qualities of soy isoflavones and antioxidant activity of *Thua Nao* prepared by pure starter *B. subtilis* TN51.