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Contributed Paper

Selection of Starter Cultures for the Production of Vegetarian Kapi, a Thai Fermented Condiment

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ABSTRACT

Kapi is a traditional fermented shrimp paste that serves as a flavoring in various Thai foods. 7 kapi samples from local markets in Chiang Mai, Trad, Rayong, Chon Buri and Nakhon Sawan, Thailand, were used as a source for the isolation of their bacterial strains. The total viable count of the 7 samples was in the range of 8.48 – 9.30 log cfu/g. A total of 23 isolates were obtained, 10 of which had the ability to produce both proteolytic and amylase activity and these were selected to produce vegetarian kapi using mung bean protein as a substrate. Among the 10 strains, isolate RY1 which was identified as *Bacillus subtilis* by 16s rDNA analysis, was selected to be the starter culture due to its ability to produce the highest total quantities of nitrogen (110.07 $\pm$ 2.76 g/kg), free amino acids (7.92 $\pm$ 1.34 mg/g) as well for its low ammoniacal nitrogen content (5.63 $\pm$ 0.20 g/kg). The quality of the vegetarian kapi produced by isolate *B. subtilis* RY1 was compared to that of the commercial vegetarian kapi samples. There were significant differences *($p<0.05$)* in moisture, protein, pH, free amino acid and ammoniacal nitrogen between the vegetarian kapi produced from *B. subtilis* RY1 and the commercial vegetarian kapi. In addition, organoleptic evaluation scores showed no significant differences between laboratory vegetarian kapi and commercial vegetarian kapi. With the use of the starter culture *B. subtilis* RY1, the vegetarian kapi could be produced in a shorter period compared with the commercial vegetarian kapi.

Keywords: *Bacillus subtilis*, vegetarian kapi, fermentation, mung bean, food condiments.

1. INTRODUCTION

Kapi is a Thai fermented shrimp paste that has been widely used as a seasoning for many decades. Traditional production of kapi is as follows: small-sized shrimp are washed, mixed with 20-25% (w/w) salt; the liquid is then removed by the process of filtering the shrimp through a cloth-sheet and the shrimp are sun dried before being ground and ultimately fermented in a jar for at least 4 months at ambient temperature [1]. The finished product has a paste-like consistency and a distinctive odor and flavor. Similar fermented shrimp paste and fish paste products from different countries in Asia have been referred to as bagoong in the Philippines [2], shiokara in Japan [3] and terasi in Indonesia [4]. Previously, several microbiological studies of shrimp paste have been conducted.

Moderate quantities of halophilic bacteria have been isolated from shrimp paste such as, *Lentibacillus kapialis, Salinicoccus siamensis* and *Oceanobacillus kapialis* [5-7]. Surano and Hosano [8] revealed the microflora of the terasi (Indonesian shrimp paste) to be the species of *Bacillus*, *Pseudomonas*, *Micrococcus*, *Kurthia* and *Sporolactobacillus*, and also reported that the starter of terasi, is composed of *Bacillus brevis*, *Bacillus pumilus*, *Bacillus megaterium*, *Bacillus coagulans*, *Bacillus subtilis* and *Micrococcus kristinae*. Moreover, *Tetragenococcus halophilus* and *T. muriaticus*, the lactic acid bacteria were also isolated from terasi by Kobayashi *et al.* [9]. Additionally, Chotwanawirach [10] has reported that some strains of lactic acid bacteria in kapi are *Staphylococcus*, *Micrococcus* and *Bacillus*.

To accommodate vegetarians, light soy sauce or salt-fermented soybean products are often used as substitutes. Traditional fermented soybean condiments, such as soybean paste and soy sauce, are commonly consumed. Typical examples of fermented soybean foods in Japan are Shoyu (soy sauce) and Miso (soy bean paste) that are produced with a traditional fermentation process of over 3 months [11]. In Thailand, a similar product is produced involving the fermentation of soybeans by *Aspergillus oryzae* for 6-8 months, and the final product is known as soy bean paste [12]. Moreover, some soybean products in vegetarian markets are produced from the traditional soybean fermentation by *Aspergillus* sp. for a month, and called "vegetarian kapi".

Although there have been reports on the use of starter cultures in the food production process in Thailand [13-14], there has not been a systematic study of starter cultures to produce vegetarian kapi. Another bean type, the mung bean is of interest as a possible substrate for food condiment products due to its high protein content (20-27%) when compared to soy beans. It is generally used in supplementing cereal-based products because of its amino acid pattern [15]. Therefore, the main objective of this study was to screen for possible microorganisms isolated from traditional kapi samples that could be used as starter cultures for vegetarian kapi production. A new type of vegetarian kapi has been developed with a selected starter culture to produce a good quality vegetarian kapi that could be a new alternative for vegetarians. We have used mung bean protein from a nearby vermicelli plant that is inexpensive, readily available, and having high protein content as a suitable substrate for vegetarian kapi fermentation.

2. MATERIALS AND METHODS

2.1 Preparation of Fermentation Substrate

Mung bean protein, obtained from a vermicelli plant in Lampang Province, was centrifuged at 1500 rpm for 15 min. The mung bean protein precipitate (MPP) consisted of 74.03% moisture, 22.40% protein, 1.75% lipid and 0.42% carbohydrate, and was then separated and used as the substrate for vegetarian kapi fermentation.

2.2 Collection of Kapi Samples

Two kinds of commercial vegetarian kapi were collected from a local market in Chiang Mai (VCM) and Chiang Rai (VCR) Provinces in Thailand. Four kinds of shrimp paste kapi were collected from markets in Trad (STD), Rayong (SRY), Chon Buri (SCR) and Nakhon Sawan (SNW) Provinces, and one kind of fish paste kapi was collected from Trad (FTD) Province in Thailand.

2.3 Isolation of Microorganisms

Kapi samples (1 g) were taken aseptically and then were homogenized in 9 ml of NaCl solution (0.85% w/w). Serial dilutions of homogenate were made and total plate counts were determined using the pour plate method

on plate count agar following the standard AOAC methods [16]. All plates were incubated at 37°C for 48 h.

Several strains of bacteria were isolated from kapi samples. One gram of each sample was homogenized with 9 ml of sterilized nutrient broth (LAB-SCAN 409101) and shaken at room temperature for 24 hours. Serial dilutions were made and 100 µl of each of the samples was plated on Brain Heart Infusion agar (CRITERION 06027) containing 15% (w/v) NaCl and were incubated at 37°C for 2-3 days. The different colonies were preserved by refrigeration at 4°C for further study of their morphological characteristics and fermentation abilities.

Selected bacterial strains were maintained on Brain Heart Infusion agar (BHI agar) slants at 4°C and were subcultured monthly.

2.4 Screening of the Isolates for Proteolytic and Amylase Activity

2.4.1 Protease Activity

A total of 23 strains were screened for their proteolytic activity. The isolated strains were precultured in inoculum medium, which contained (per 100 ml of distilled H_2O): glucose 1 g, peptone 1 g, $CaCl_2.2H_2O$ 0.02 g, $MgSO_4$. $7H_2O$ 0.025 g and were incubated on the rotary shaker (180 rpm) for 18 h at 37°C. Five milliliters of inoculum were mixed with 50 ml of protease production medium and incubated on the rotary shaker (180 rpm) at 37°C. The composition of the protease production medium (per 100 ml of distilled H_2O) was: glucose 1 g, peptone 1 g, yeast extract 0.5 g, $CaCl_2.2H_2O$ 0.02 g, $MgSO_4.7H_2O$ 0.025 g. After 24 h, the cultures were centrifuged at 6000 rpm, 4°C for 15 min and the supernatant was tested for protease activity. Hagihara's Method [17] was used in protease activity assay using casein as the substrate. A reaction mixture of 0.5 ml of appropriate enzyme diluted solution and 0.5 ml of 1 % (w/v) casein was incubated in a water bath at 37°C for 30 min. The reaction was terminated with 4 ml of 10% (w/v) trichloroacetic acid and the reaction mixtures were then cooled to 4°C for 30 min. The mixture was centrifuged at 6000 rpm for 10 min and the absorbance of the supernatant was measure at 275 nm.

One unit of protease activity was defined as the amount of enzyme that catalyzes the formation of 1 µmol of tyrosine per min under assay conditions.

2.4.2 Amylase Activity

A total of 23 strains were screened for their amylase activity. The isolated strains were precultured in nutrient broth, and incubated on the rotary shaker (180 rpm) for 18 h at 37°C. Five milliliters of inoculum were mixed with 50 ml of amylase production medium (nutrient broth with 1 %(w/v) starch) and incubated on the rotary shaker (180 rpm) at 37°C. After 24 h, the cultures were centrifuged at 6000 rpm, 4°C for 15 min and the supernatant was tested for amylase activity. The assay procedure described by Bernfeld's method [18] was adopted with 0.05 M Tris-HCl buffer (pH 7.5) in order to extract buffer and reaction time. The assay mixture comprised 0.5 ml 1 % (w/v) starch solution and 0.5 ml enzyme solution incubated at 37°C for 30 min. The enzyme reaction was stopped by adding 1 ml of DNS reagent. The tube was heated for 5 min in boiling water and then cooled in running tap-water. After the addition of 10 ml of distilled water and being mixed well, the absorbance of brown solution was read at 540 nm.

One unit of α-amylase activity was defined as the amount of enzyme releasing 1 µmole of maltose per min under assay condition.

2.5 Selection of Starter Culture for Vegetarian Kapi Fermentation

Ten bacterial strains which had proteolytic and amylase activity were screened for their feasibility in vegetarian kapi fermentation. Thirty grams of mung bean protein precipitate (MPP) were sterilized. The isolated strains in nutrient broth were incubated on a rotary shaker (180 rpm) for 16 hours at 37°C. Cells were suspended in 0.85% (w/v) NaCl solution at a density of 10^3 cells/ml. Each strain was inoculated separately into sterile MPP and incubated at 37°C. The samples were then collected after 8 days of fermentation and their total nitrogen (TN), free amino acid (FA) and ammoniacal nitrogen (AN) contents were assessed. The RY1 strain of bacteria produced vegetarian kapi with the highest content of TN, FA as well as having low AN, was selected for further study. According to the Thai Industrial Standard for Thai Kapi, ammoniacal nitrogen of not more than 7 g/kg AN is acceptable [19]. The selected strain, RY1 was also identified using 16S rDNA sequencing method by BIOTEC Culture Collection (BCC), Thailand.

2.6 Production of Vegetarian Kapi Using the Starter Culture

Thirty grams of MPP was added into an Erlenmeyer flask, stopped with cotton wool and aluminium foil, and was sterilized at 121°C for 20 min. The selected strain, RY1 was precultured for 16 h at 37°C in nutrient broth medium and cells were suspended in 0.85% (w/v) NaCl solution at density of 10^3 cells/ml. Three milliliters of cell suspensions were inoculated into sterilized MPP. The contents of the flask were thoroughly mixed using a sterile glass rod, and incubated at 37°C for 10 days. Each batch of fermenting MPP was sampled at 0, 2, 4, 6, 8 and 10 day intervals for microbiological (viable count), chemical (pH, moisture content, free amino acid and ammoniacal nitrogen) analysis. Fermented samples were refrigerated at -20°C until being analyzed.

The chemical composition and sensory evaluation of laboratory vegetarian kapi and commercial vegetarian kapi were compared.

2.7 Chemical analysis

The samples were analyzed for pH, fat, moisture, protein, total nitrogen, free amino acid and ammoniacal nitrogen content following the standard AOAC methods [16]. All experiments were carried out in at least triplicate, and the results have been presented as the mean value with standard deviation.

2.8 Sensory analysis

The laboratory vegetarian kapi sample produced by starter cultures of RY1 was evaluated for acceptability alongside two types of commercial vegetarian kapi samples based on 4 attributes: color, aroma, texture and general acceptability. The samples were assessed by a panel of 12 regular consumers of kapi using a 5-point hedonic scale [20] with 1 (dislike extremely) to 5 (like extremely). The data obtained were subjected to analysis of variance (ANOVA) and Randomized Complete Block Design was used to compare the means where statistical significance was defined at p value < 0.05.

2.9 Statistical Analysis

All experiments were determined in triplicate and expressed as mean standard deviations. The significance was verified by performing Duncan's multiple range tests using the SPSS (SPSS 13.0 for window, SPSS Inc., Chicago, Ill., U.S.A.) software package. Statistical significance was accepted at p value < 0.05.

3. RESULTS AND DISCUSSION

3.1 Strain isolation and screening for selection of the starter culture

The kapi samples were used as sources for the isolation of microorganisms obtained from many parts of Thailand. Table 1 shows the microbial load of kapi samples. The shrimp paste kapi from Rayong (SRY) sample had the highest of total viable count with values 9.30 log cfu/g, while the vegetarian kapi from Chiang Mai (VCM) sample recorded the lowest count of 8.48 log cfu/g. The BHI agar containing 15 % (w/w) NaCl was used for isolation to obtain the salt tolerant bacteria that are present in the majority of organisms found in various salted and fermented fish products [21]. Twenty-three bacterial isolates were obtained and further studied for their macroscopic and microscopic morphological characterization. Following the results of the investigation (data not shown), the strains were characterized as: 11 isolates predominant were rod-Gram positive, 6 isolates were cocci-Gram-positive, 5 isolates were rod-Gram-negative and 1 isolate was short rod-Gram-negative. Previous studies of shrimp paste fermentation [1] reported that the predominant bacteria were cocci-Gram-positive.

Table 1. Microbial Load of Market Kapi Samples.

Samples	Total viable count (log cfu/g)
VCM	8.48 ± 0.02^{a}
VCR	8.85 ± 0.03^{b}
STD	9.06 ± 0.04^{c}
SRY	9.30 ± 0.03^{d}
SCR	8.70 ± 0.03^{e}
SNW	8.64 ± 0.02^{f}
FTD	9.08 ± 0.04^{c}

Values represent average scores of triplicate determinations
Means having the same superscripts within the same column do not differ significantly ($p<0.05$)

VCM = Vegetarian kapi from Chiang Mai Province
VCR = Vegetarian kapi from Chiang Rai Province
STD = Shrimp paste kapi from Trad Province
SRY = Shrimp paste kapi from Rayong Province
SCR = Shrimp paste kapi from Chon Buri Province
SNW = Shrimp paste kapi from Nakhorn Sawan Province
FTD = Fish paste kapi from Trad Province

The 23 isolates were then investigated for their protease and amylase production. From Table 2, it was found that 13 of 23 isolate-strains demonstrated proteolytic activity while 16 of 23 isolates demonstrated amylase activity. However, 10 of 23 isolate-strains exhibited both proteolytic and amylase activity. These 10 strains were screened for their suitability for vegetarian kapi production. Mung bean protein obtained from vermicelli

production was sterilized before being inoculated by each of the 10 strains to produce vegetarian kapi. The data of the strains that demonstrated high total nitrogen, high free amino acid content and low ammoniacal nitrogen content are present in Table 3 as probable candidates that could be employed as starter cultures for vegetarian kapi fermentation. Based on the values obtained, isolate RY1 was chosen as the starter organism for the laboratory preparation of vegetarian kapi. The selected strain, RY1 was subjected to 16S rDNA sequence analysis. Homology search was performed by using the standard nucleotide BLAST from NCBI web server against previously reported sequences at the GenBank/EMBL/DDBJ database for determination of the nearest sequences. The 16S rDNA sequence analysis indicated that RY1 isolate clearly belonged to *Bacillus subtilis*.

Table 2. Protease and Amylase Activity of Bacterial Strains Isolated from Market Kapi Samples.

Source	Bacterial isolates	Protease activity* (mU/ml)	Amylase activity* (mU/ml)
VCM	CM1	4.23 ± 0.99	23.00 ± 1.22
	CM2	nd.	12.65 ± 0.98
	CM3	nd.	6.55 ± 1.23
VCR	CR1	2.33 ± 0.67	7.88 ± 1.23
	CR2	nd.	nd.
STD	TD1	10.05 ± 2.12	46.56 ± 3.00
	TD2	30.09 ± 2.00	68.76 ± 4.13
	TD3	8.76 ± 1.35	35.67 ± 2.45
	TD4	nd.	14.32 ± 2.01
	TD5	nd.	nd.
SRY	RY1	6.25 ± 2.21	15.71 ± 2.39
	RY2	6.00 ± 1.98	20.02 ± 3.00
	RY3	nd.	25.64 ± 2.34
	RY4	nd.	nd.
SCR	SC1	13.45 ± 1.22	30.00 ± 3.45
	SC2	9.87 ± 2.33	nd.
	SC3	nd.	nd.
SNW	NW1	4.33 ± 0.78	15.47 ± 0.89
	NW2	3.45 ± 0.91	nd.
	NW3	nd.	7.81 ± 0.45
FTD	FT1	3.21 ± 1.00	nd.
	FT2	4.44 ± 2.01	20.09 ± 2.34
	FT3	nd.	14.23 ± 1.89

*Values represent average scores of triplicate determinations
nd. = not detected

Several *Bacillus* species have been reported to be associated with the production of traditional fermented food, especially *Bacillus subtilis*. The *B. subtilis* is often found as the dominant microorganism that plays the important role due to its ability to grow over a wide pH range, produce several extracellular enzymes and other useful biological compounds [14]. The previous studies revealed that *B. subtilis* were found responsible for the fermentation of soybeans into many fermented foods, for example thau-nao, natto, chungkukjang, kinema and soy-daddawa [14, 22-25]. In addition, *B. subtilis* have also been reported to be the main microorganisms involved with African oil bean fermentation to ugba and African locust bean into soumbala [26-27]. In general, *B subtilis* is considered an opportunistic microorganism with no pathogenic potential to humans. Boer and Diderichsem [28] reported that there were no cases demonstrating invasive properties and no evidence of any pathogenic potential of *B. subtilis*. Thus, they concluded that *B. subtilis* is a harmless host for the production of safe, food products.

Table 3. Total Nitrogen, Free Amino Acid and Ammoiacal Nitrogen Content of Mung Bean Kapi after Inoculation by Isolated Strains for 10 Days.

Source	Bacterial isolates	Total Nitrogen (g/kg)	Free amino acid (mg/g)	Ammoniacal Nitrogen (g/kg)
VCM	CM1	93.90 ± 3.23^{abc}	6.23 ± 1.32^{ac}	3.09 ± 0.34^{a}
VCR	CR1	90.09 ± 2.11^{a}	6.55 ± 1.32^{ac}	6.98 ± 0.55^{b}
STD	TD1	95.87 ± 1.98^{c}	7.00 ± 1.54^{ac}	2.55 ± 0.10^{ac}
	TD2	91.54 ± 1.33^{ab}	7.60 ± 1.20^{c}	3.00 ± 0.50^{a}
	TD3	96.45 ± 1.76^{c}	4.58 ± 0.51^{ab}	4.56 ± 0.23^{d}
SRY*	**RY1**	**110.07 ± 2.76^{d}**	**7.92 ± 1.34^{c}**	**5.63 ± 0.20^{e}**
	RY2	96.12 ± 2. 89^{c}	3.56 ± 0.85^{b}	6.00 ± 0.30^{e}
SCR	SC1	90.86 ± 2. 33^{ab}	5.87 ± 1.60^{abc}	1.23 ± 0.14^{f}
SNW	NW1	94.57 ± 2.00^{bc}	6.84 ± 1.76^{ac}	2.08 ± 0.19^{c}
FTD	FT2	93.21 ± 1.23^{bc}	5.78 ± 0.88^{abc}	7.65 ± 0.19^{g}

Values represent average scores of triplicate determinations
Means having the same superscripts within the same column do not differ significantly ($p<0.05$)

* The selected strains for further studies.

3.2 Production of vegetarian kapi using the starter culture

Chemical characteristics and viable counts of fermented samples during vegetarian kapi fermentation by the selected strain, *B subtilis* RY1 are shown in Figure 1. Fermentation time had an effect on pH, moisture content, free amino acid, ammoniacal nitrogen and viability count. During 10 days of fermentation, the pH values slightly increased from 5.54 ± 0.21 to 6.00 ± 0.12 and ammoniacal nitrogen increased significantly from 0.91 ± 0.30 g/kg to 5.64 ± 0.28 g/kg . The level of free amino acids in samples continuously increased

during the fermentation, ranging from 3.00 ± 0.09 mg/g to 7.01 ± 0.13 mg/g at the end of fermentation. Ammonia concentration which significantly increased during fermentation may be a general reflection of the proteolysis activities. It is presumed that the deamination of amino acid induced by *B. subtilis* RY1 resulted in ammoniacal production and led to an increase of the pH in the fermented products. However, the slight increased of pH values during vegetarian mungbean kapi fermentation may be due to the accumulation of volatile acids, such as acetic acid, in the fermented products (data not shown). The formation of ammonia also makes the substrate unsastisfactory that might spoil the products [29]. In this study, the ammoniacal nitrogen in the final product was 5.64 ± 0.28 g/kg, which is considered acceptable according to the Thai Industrial Standard (ammoniacal nitrogen content must not be more than 7 g/kg). During the fermentation of soybean into daddawa, Omafuvbe [25] observed a rise in pH and ammonia which often develops a strong ammonia-like odor. Moreover, the

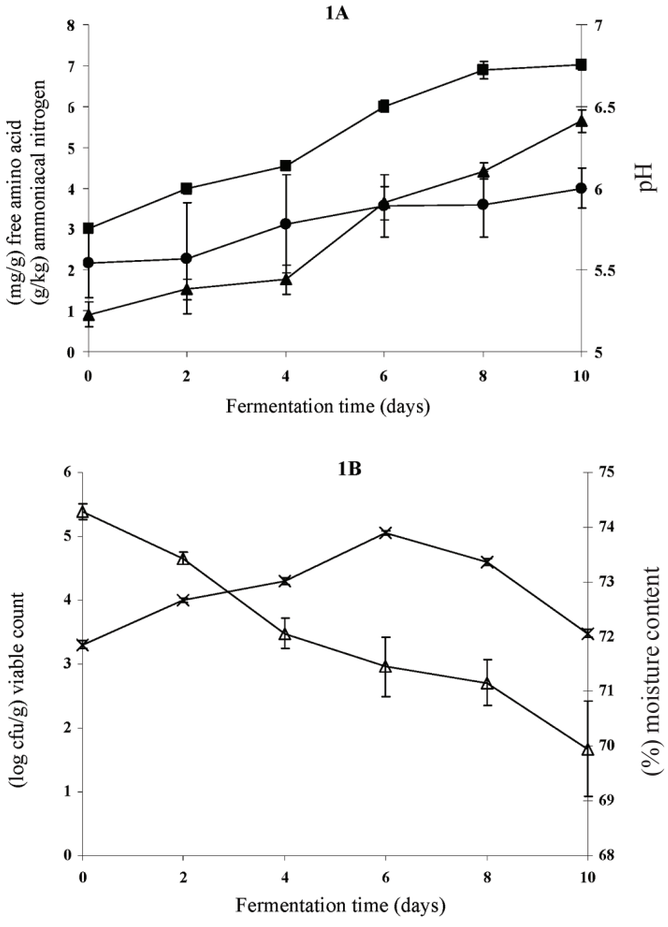

Figure 1. Change in pH (●), free amino acid (■) and ammoniacal nitrogen content (▲) in 1A and viable count (Δ) and moisture content (×) in 1B during the vegetarian kapi fermentation using selected starter culture (*B. subtilis* RY1). Bars represent standard deviation from triplicate determinations.

fermentation of the other protein rich-materials displayed a similar change in pH values, as had been reported [30-33].

The moisture content of the samples decreased from 74.29 ± 0.14% to 69.95 ± 0.87% when the fermentation time was extended up until 10 days of fermentation. The total microorganism content in vegetarian kapi was determined on plate count agar (PCA). The counts of total microorganisms increased from 3.30 log cfu/g to 5.05 log cfu/g in 6 days of fermentation, and then declined to 3.43 log cfu/g at 10 days of fermentation. This indicates that the starter culture of *B. subtilis* RY1 had reached the steady state. This trend is a similar pattern as had been reported in the fermentation of daddawa[25] and Natto[34]. The decrease in total microorganisms during fermentation may be due to the formation of toxic components such as carbon dioxide, hydrogen peroxide, diacetyl, ethanol and bacteriocins [35].

Table 4 shows the chemical composition of the laboratory vegetarian kapi and the commercial product. There were significant differences in the moisture, protein, pH, free amino acid and ammoiacal nitrogen contents. However, the difference in chemical composition may be due to the differences in the raw materials and/or the fermentation conditions. Sanni [26] reported the significant differences in pH and aroma between ugba fermented by starter culture and ugba obtained from the retail market. It is postulated that traditional ugba quality is usually unpredictable due to the presence of different associated microorganisms found in different batches of the same product caused by the uncontrolled fermentation process and which then results in an inconsistency in product quality.

Table 4. Chemical Composition of Starter Culture Produced (*B. subtilis* RY1) and Vegetarian Kapi Samples (VCM and VCR).

Samples	Moisture (%)	Protein (%)	Fat (%)	pH	Free amino acid (mg/g)	Ammoiacal Nitrogen (g/kg)
RY1	69.00 ± 0.20^{a}	18.63 ± 0.13^{a}	0.02 ± 0.01^{a}	6.00 ± 0.34^{a}	7.00 ± 0.13^{a}	5.41 ± 0.31^{a}
VCM	42.60 ± 0.34^{b}	24.59 ± 0.20^{b}	0.03 ± 0.01^{a}	4.40 ± 0.30^{b}	8.12 ± 0.25^{b}	6.85 ± 0.45^{b}
VCR	38.27 ± 0.40^{c}	23.46 ± 0.31^{c}	0.02 ± 0.01^{a}	6.07 ± 0.28^{c}	9.32 ± 0.31^{c}	5.93 ± 0.50^{a}

Values represent average scores of triplicate determinations

Means having the same superscripts within the same column do not differ significantly ($p<0.05$)

RY1 = laboratory vegetarian kapi by *B. subtilis* RY1

VCM = commercial vegetarian kapi from Chiang Mai Province

VCR = commercial vegetarian kapi from Chiang Rai Province

3.3 Sensory evaluation

The laboratory vegetarian kapi samples fermented by isolate *B. subtilis* RY1, including the commercial vegetarian kapi, were assessed by 12 regular consumers of kapi. There was no significant difference ($P<0.05$) in all the attributes scored for laboratory vegetarian kapi and commercial vegetarian kapi (Table 5). This implies that the isolate *B. subtilis* RY1 can produce acceptable vegetarian kapi. However, commercial vegetarian kapi VCM had the higher scores in terms of color while commercial vegetarian kapi VCM and VCR had the higher scores in terms of aroma. On

the other hand, the laboratory vegetarian kapi received the higher scores in terms of texture and general acceptability compared to commercial vegetarian kapi. It can be inferred that although the starter culture of isolate *B. subtilis* RY1 was able to produce acceptable vegetarian kapi, it did yield slightly different characteristics compared with commercial vegetarian kapi.

Table 5. Sensory Evaluation of the Vegetarian Kapi samples.

Samples	Sensory evaluation			
	Color	Aroma	Texture	General acceptability
RY1	3.42 ± 1.08[a]	3.67 ± 1.15[a]	4.00 ± 0.74[a]	3.92 ± 0.79[a]
VCM	3.75 ± 1.14[a]	3.75 ± 1.06[a]	3.67 ± 0.62[a]	3.75 ± 0.97[a]
VCR	3.67 ± 1.23[a]	3.75 ± 1.14[a]	3.75 ± 1.14[a]	3.67 ± 0.89[a]

Values represent the mean scores ± SD. (n=12)
Means having the same superscripts within the same column do not differ significantly ($p<0.05$)
Acceptance score: 5 = like extremely, 3 = neither like nor dislike, 1 = dislike extremely
RY1 = laboratory vegetarian kapi by *B. subtilis* RY1
VCM = commercial vegetarian kapi from Chiang Mai Province
VCR = commercial vegetarian kapi from Chiang Rai Province

4. Conclusions

This study describes the isolation of bacterial strains from Thai fermented shrimp paste and Thai vegetarian kapi and the screening of the isolated strains for the production of vegetarian kapi from mung bean protein precipitate (MPP) in terms of its proteolytic and amylase activity and ability to produce an acceptable vegetarian kapi product. Strains *B. subtilis* RY1 showed the ability to ferment MPP into vegetarian kapi. Moreover, no significant differences in color, aroma, texture and general acceptability were detected between vegetarian kapi by *B. subtilis* RY1 and commercial vegetarian kapi. This report is significant because it is the first to describe the use of the by-products of vermicelli production to prepare a suitable vegetarian kapi by starter culture substitute for shrimp paste kapi. This leads to fermentation within a more desirable and much shorter time period, compared with the traditional fermentation process.

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