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Safety control indices for *plaa-som*, a Thai fermented fish product

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This study was aimed to determine chemical and microbiological changes during *plaa-som* fermentation process. The results have been considered for use as safety control indices to obtain a higher quality and safer *plaa-som*. The fermentation process can be divided into an initiation stage (samples 1 to 3) and a maturation stage (samples 4 to 9) based on the changes obtained. At the initiation stage, pH remained stable at 6.3 and then rapidly declined during the maturation stage as from 6.3 to 4.5. Total acidity showed a continuous increasing trend from 0.12% (w/w) in sample 1 to 1.17% (w/w) in sample 9. Lactic and citric acids were detected as major acids during the initiation stage in a range of 0.22 - 0.29% (w/w). The main acids detected during the maturation stage, however, were lactic and acetic acids and these reached maximum levels at 2.82 and 0.16% (w/w) in samples 9 and 8, respectively. Substantial discrepancies between total viable counts (TVC) and lactic acid bacteria (LAB) counts were obtained during the initiation stage, particularly in sample1 where the TVC was 2.97 log CFU/g and LAB count was lower than 10 CFU/g. This indicated an existence of undesirable indigenous microorganisms other than LAB. At the maturation stage, the two counts concomitantly increased and no discrepancy was found. Maximum counts of TVC and LAB were 6.83 and 6.72 log CFU/g in samples 6 and 9, respectively. Good practices at particular steps and possible critical control points were noted and proposed for a more controllable *plaa-som* production process which will guide to a higher standard in both safety and quality consistency of the product.

Key words: chemical changes, good manufacturing practices, initiation stage, maturation stage, microbiological changes, organic acids profile, safety control indices, spontaneous fermentation.

INTRODUCTION

Plaa-som is described as a group of traditional Thai fermented fish products obtained from the fermentation of either whole fish or fish fillets with salt, steamed rice or sticky rice, and garlic until its taste becomes sour (TISI 2005). However, *plaa-som* recipes can be varied depending on local organoleptic preferences and ingredient availability in each region of Thailand (Valyasevi and Rolle 2002). The fermentation relies on a septic fermentation in which the process is performed in an open system without sterilization or selected starter cultures (Owen and Mendoza 1985; Lee 1997; Motarjemi 2002). The fermentation spontaneously occurs due to the presence of natural adventitious microorganisms, mainly lactic acid bacteria (LAB), that are found in the raw materials, on the processing utensils and in the local atmosphere as natural starters to initiate the fermentation process (Khieokhachee et al., 1997; Valyasevi and Rolle 2002; Visessanguan et al., 2004). Appropriate conditions are vital for accomplishment of fermentation by LAB such as the presence of particular carbohydrate and antimicrobial substances containing ingredients; salt and garlic

ABBREVIATIONS

Calcium Carbonate ($CaCO_3$); Critical Control Point (CCP); de Man, Rogosa and Sharpe MRS; Good Manufacturing Practices (GMPs); Lactic Acid Bacteria (LAB); Hazard Analysis and Critical Control Point (HACCP); Plate Count Agar, PCA; Potato Dextrose Agar, PDA; revolutions per minute (rpm); Total Viable Counts (TVC); weight by volume (w/v); weight by weight (w/w).

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Table 1. The *plaa-som* sample designations and descriptions at each step of sampling.

Sample designation	Description of sample
1	Instantaneously after mixing
2	6 h after mixing
3	After freezing at -18°C and packing
4	24 h of fermentation
5	48 h of fermentation
6	72 h of fermentation
7	96 h of fermentation
8	120 h of fermentation
9	144 h of fermentation

garlic; and microaerophilic conditions created by a wrap-up package design. These conditions are aimed to promote the growth of LAB instead of other indigenous microorganisms including spoilage and pathogenic groups which also co-contaminate the raw materials and ingredients. Moreover, LAB produces organic acids, especially lactic acid, aroma substances, ethanol, and bacteriocin; an antimicrobial agent produced by some members of LAB. These metabolic products are useful to enhance product quality and safety (Kandler 1983; Leroy and Vuyst 2004). *Plaa-som* production is usually conducted in small scale or cottage industries thus it entirely depends on spontaneous fermentation by LAB and is without implementation of either Good Manufacturing Practices (GMPs) or Hazard Analysis and Critical Control Point (HACCP) systems. This is considerably very risky (Nout and Motarjemi, 1997). This experiment aimed to determine chemical and microbiological changes during the spontaneous fermentation of *plaa-som* obtained from GMPs implemented by a local producer. The results can then be used to serve as safety control indices to determine additional practices for GMPs and to evaluate possible critical control points (CCPs) for small scale or cottage industries which will lead to better production process practice imparting higher quality and safety standards for *plaa-som* production.

MATERIALS AND METHODS

Fermented fish sample preparation

Fermented fish (*plaa-som*) samples were prepared at a GMPs-implemented local producer in Khon Kaen province, Thailand. Regarding the selected local producer, GMPs including personal hygiene, basic process controls, sanitation operations, sanitary facilities, plant, and grounds are in place. The fish were prepared according to the producer's traditional techniques and recipe. A common Thai fresh water SILVER BARB (*Barbodes gonionotus*) fish, locally called *plaa-ta-pien*, weighing approximately 300 g each, was used as the main ingredient. Fish were prepared by scaling, gutting, slitting, thoroughly washing, and soaking in 25% (w/v) brine for approximately 2 - 3 h. They were finally rinsed and drained of all excessive brine before being mixed with the other ingredients according to the recipe. Ten kilograms of fish of fish were then subsequently well mixed with steamed jasmine rice and minced garlic at a ratio of 1:0.5 by weight. After mixing of all ingredients, the rest of the steamed jasmine rice and minced garlic were stuffed into the fish bellies. The mixed fish were placed in bowls which were covered with plastic sheets and left at room temperature for 6 h in order to initiate the fermentation process. The 6 h fermented fish were then kept in a -18°C freezing container for an overnight period. Immediately after the overnight period, they were packed at approximately 3 fish per plastic bag. Most of the air inside each bag was expelled by pressing before closing the bag tightly with a rubber band. Thereafter, all the packed fish from the same production batch were subjected to further fermentation at 30ºC.

Sample designation and their descriptions

Fish samples were taken for both microbiological and chemical investigations, in conditions according to each step of the production process from the selected local producer; instantaneously after mixing, designated as sample 1 (after stuffing all ingredients into the fish bellies), 6 h. after mixing, after freezing at -18°C and packing, and also at every 24 h. interval during fermentation at 30ºC for 144 h at sample 9. These designated samples and their descriptions (samples 1 to 9) are listed in Table 1.

Chemical analyses

Fish samples were analyzed for pH, total acidity, salt content, and organic acids. All determinations were done in duplicates. Total acidity and pH were analyzed according to the methods of AOAC (2000) . To conduct this, one whole fish was withdrawn from a plastic bag and homogenized in a homogenizer (model # 32BL80, Waring Blender, Connecticut, USA). For pH determination, 10 g of homogenized sample were mixed with 90 ml of carbon dioxide free distilled water. Direct pH measurement was measured using a standard pH meter (S20 SevenEasy™, Mettler-Toledo, Inc., Ohio, USA) while total acidity was determined in the form of titratable acidity. For total acidity, 10 g of homogenized sample were mixed with 40 ml of carbon dioxide free distilled water. The homogenate was centrifuged at 3000 rpm for 15 min. in an appropriate centrifuge (Avanti® J-25, Beckman coulter, Inc., Connecticut, USA). The supernatant was filtered through a Whatman filter paper No.1, (Whatman plc., Kent, UK). The filtrate was titrated against standard 0.1 M sodium hydroxide (BDH Laboratory Supplies, Poole, UK) with an addition of a few drops of 1% phenolphthalein (Fluka Chemie AG, Buchs, Switzerland) as an indicator. The total acidity was calculated as an equivalent to lactic acid and reported as % (w/w).

Salt content was determined as the amount of sodium chloride content using silver nitrate titration according to Mohr method, AOAC (2000). Five grams of homogenized sample were mixed with 25 ml distilled water and filtered through a filter cloth and a Whatman filter paper No. 1. The filtrate was titrated against 0.1 M silver nitrate (Sigma Chemical Co., Missouri, USA) with an addition of 1 ml of 0.25 M potassium chromate (E.Merck Co., Darmstadt, Germany) as an indicator. Salt content was calculated from the volume of 0.1 M silver nitrate used to reach the end point of titration and reported as %sodium chloride (w/w).

Organic acids were determined using a high performance liquid chromatography technique (HPLC) according to the method of Visessanguan et al. (2004) with some modifications. Five grams of homogenized sample were centrifuged at 3000 rpm for 15 min. Protein in the supernatant was removed by precipitation with 0.5 N perchloric acid (E.Merck Co., Darmstadt, Germany) at the ratio 1: 1 between supernatant and perchloric acid. The mixture was left at room temperature for 5 min for protein precipitation then was cen-

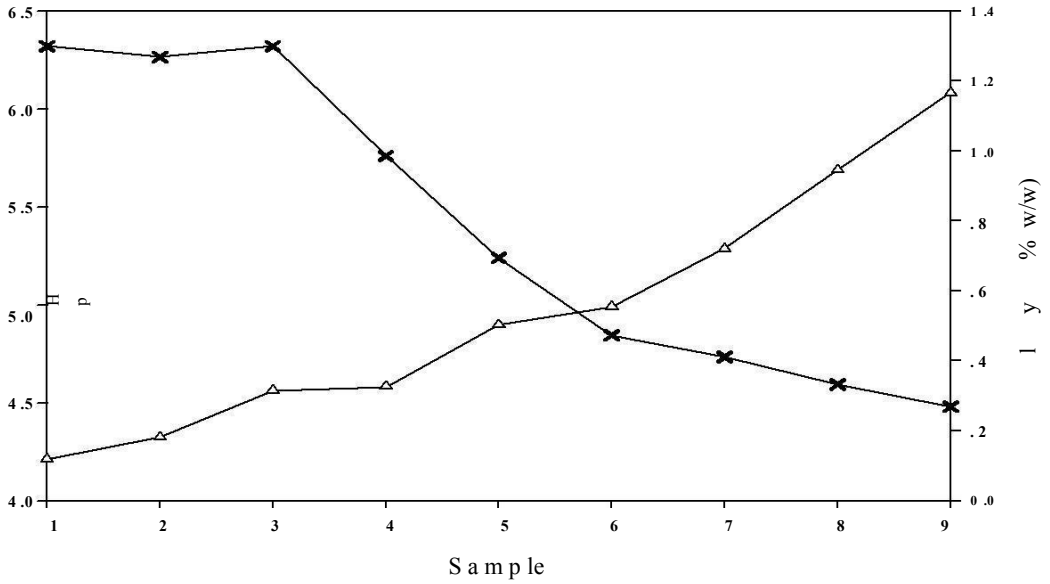

Figure 1. Changes in pH ( ) and total acidity ( ) during the spontaneous fermentation process of *plaa-som.*

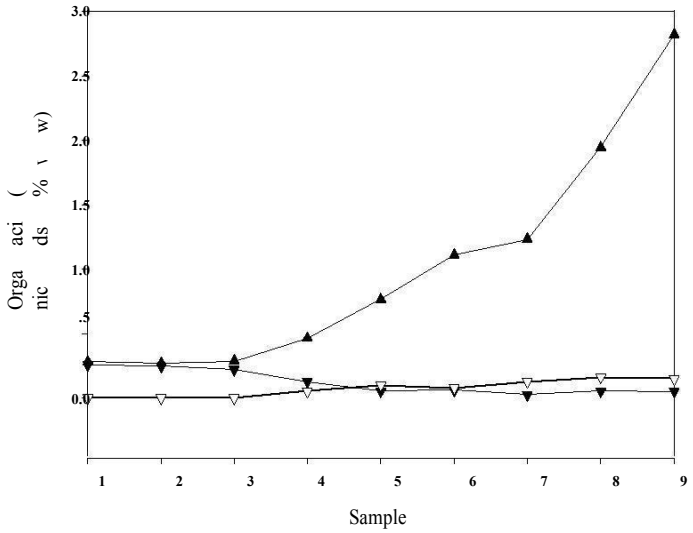

Figure 2. Organic acid profiles during the spontaneous fermentation process of *plaa-som*; lactic acid ( ), acetic acid ( ), and citric acid ( ).

trifuged at 12,000 rpm for 10 min (Avanti® J-25, Beckman coulter, Inc., Connecticut, USA) to remove all the protein precipitates. The obtained supernatant was then followed by an addition of petroleum ether at a 1:1 ratio between the petroleum ether and the supernatant for fat extraction. Afterward, the bottom supernatant was harvested and adjusted to 100 ml with 0.1% (v/v) phosphoric acid (Fluka Chemie AG, Buchs, Switzerland) . The mixture was then collected and filtered through Whatman filter paper No. 1. The filtrate was again filtered through a 0.45 µm membrane filter (Chrom tech. Inc., Minnesota, USA) prior to performing HPLC analysis. The filtrate was analyzed with a Waters HPLC system (Milford, USA) including a Waters™600 controller, membrane typed Waters™ inline degasser, auto sampler Waters™ 710, Waters™486 absorbance detector (210 nm absorbance), Supelcogel™ C- 610H (Sigma-Aldrich, Inc., Saint Louis, MO, USA) and a computer with a data analysis software program called Millennium 2000 package. The samples were analyzed in isocratic mode at 0.5 ml/min. The column temperature was steadily maintained at 30°C. Lactic acid, acetic acid, and citric acid (Fluka Chemie AG, Buchs, Switzerland) were used as external standards.

Microbiological analyses

Total viable counts (TVC) and LAB (in the form of acid producing bacteria) counts were performed according to standard total viable count methods described by Speck (1984). One whole fish was withdrawn from a plastic bag and was aseptically cut into small pieces prior to homogenization and dilution. The homogenates were aseptically prepared using a stomacher 400 Lab Blender (Seward Ltd., Worthington, UK) at high speed for 3 min. Appropriate 10 times serial dilutions were prepared with saline peptone water (0.1% (w/v) peptone (Criterion, California, USA) in 0.85% (w/v) sodium chloride (BDH supplies, Poole, England) and used for colony counting via a standard total viable count technique. Each appropriate dilution was then inoculated in triplicates for both TVC and LAB counts. The TVC counts were determined using plate count agar, PCA (Himedia, Mumbai, India) as a cultivation medium with aerobic incubation at 30°C for 2 - 3 days. LAB counts were determined using micro-aerobic incubation at 30°C for 3 - 5 days on de Man, Rogosa and Sharpe agar, MRS agar (Pronadisa ®, Madrid, Brazil) modified by the addition of 1.0% (w/v) calcium carbonate ($CaCO_3$-MRS), in order to facilitate the observation of clear zones exhibited by acid producing colonies. After an optimal incubation period, PCA and $CaCO_3$ - MRS plates with 30 - 300 discrete colonies were counted and the results were reported in log CFU/g of sample. After counting all acid producing colonies surrounded by clear zones on $CaCO_3$ -MRS agar plates, colonies were randomly picked from samples 1 to 9 for LAB confirmation using Gram staining, catalase test, glucose fermentation test according to Sneath et al. (1986). Additionally, potato dextrose agar, PDA (Himedia, Mumbai, India) was used for general mold detection in *plaa-som.*

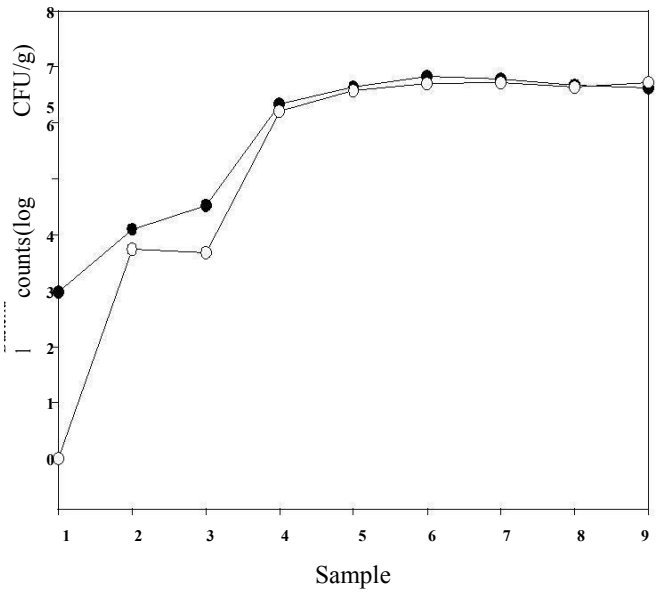

Figure 3. Total viable counts ( ), and lactic acid bacteria counts ( ) during the spontaneous fermentation process of *plaa-som*.

RESULTS

Chemical changes during the fermentation

Salt content in the *plaa-som* was found to be 1.43% (w/w). The pH and total acidity changes during the fermentation are illustrated in Figure 1. The pH was initially found at 6.3 and remained stable throughout the time periods for samples 2 and 3. Thereafter, pH was found to decrease rapidly from 6.3. in sample 3 to 4.8 in sample 6. From this point onward, pH of *plaa-som* had a tendency to drop slowly from 4.7 in sample 7 to 4.5 in sample 9. Total acidity, calculated as titratable acid equivalent to lactic acid, generally was revealed to have an increasing trend throughout the fermentation, as from 0.12% (w/w) in sample 1 to a final maximum level at 1.17% (w/w) in sample 9. From these results, pH and total acidity exhibited an inverse relationship to each other from samples 3 to 9. Thus, the lowest pH at 4.5 and highest total acidity at 1.17% (w/w) were found in sample 9. However, from samples 1 to 3, while the total acidity was found to maintain an increasing trend, from 0.12 to 0.32% (w/w), the pH was found to be stable around 6.3.

Lactic and citric acids were found in significant amounts in sample 1 at 0.28 and 0.26% (w/w), respectively (Figure 2). From samples 1 to 3, lactic acid showed a stable amount between 0.27 - 0.29% (w/w) whereas citric acid started to decrease, but not greatly, from 0.26% (w/w) in sample 1 to 0.22% (w/w) in sample 3. Lactic acid increased rapidly from samples 3 to 6 at 0.29 to 1.11% (w/w). The period between samples 6 to 7, lactic acid slightly increased from 1.11 to 1.23% (w/w) . The amount of lactic acid then continually increased in a greater ex tent from sample 7 at 1.23% (w/w) peaking at 2.82% (w/w) in sample 9. In contrast, citric acid showed a decreasing trend as from sample 3 and was found to be lowest at 0.03% (w/w) in sample 7. Citric acid minutely increased to 0.06 and 0.05% (w/w) in sample 8 and sample 9, respectively. Acetic acid was first detected in sample 4 at 0.06% (w/w) and gradually increased to 0.17% (w/w) in sample 8. Acetic acid finally decreased to 0.15% (w/w) in sample 9.

Minced garlic, the other ingredient used for *plaa-som* production was found to contain lactic acid, citric acid, and acetic acid at 0.54, 0.63, and 0.31% (w/w), respectively

Microbiological changes during the fermentation

Randomly picked acid producing colonies on $CaCO_3$-MRS agar plates from samples 1 to 9 were all confirmed to be LAB as they exhibited Gram-positive, catalase-negative, and sugar-fermentative characteristics (data not shown). TVC and LAB counts during the fermentation are shown in Figure 3. The TVC count was 2.97 log CFU/g whereas the LAB count was estimated to be lower than 10 CFU/g in sample 1. At sample 2, the TVC count was found to have slightly increased to 4.10 log CFU/g and the LAB count was found to have dramatically increased to 3.74 log CFU/g. In sample 3, both TVC and LAB counts were found to be retarded at 4.52 log CFU/g and 3.68 log CFU/g, respectively. Thereafter, both TVC and LAB counts dramatically increased and reached their maximum levels at 6.83 log CFU/g in sample 6 and 6.72 log CFU/g in sample 9, respectively. The results show the microbial counts, and the main organic acid, lactic acid, together with total acidity and pH exhibit coinciding trends from samples 3 to 9, as shown in Figure 4. In general, as fermentation proceeded, the resulted organic acids had accumulated, as a consequence, the higher in total acidity and the lower in pH were obtained. The amount of lactic acid was found to be increased from 0.47% (w/w) in sample 4 until reached its highest value at 2.82% (w/w) in sample 9. LAB count and total acidity were also increased from 6.21 log CFU/g, 0.33%(w/w) in sample 4 and reached their highest values at 6.72 log CFU/g, 1.17% (w/w), in sample 9, respectively. The pH was also exhibited a decreasing trend from 5.8 in sample 4 to its minimum at 4.5 in sample 9**.**

DISCUSSION

Based on the chemical and microbiological changes observed in this study, the *plaa-som* fermentation profile can be divided into 2 stages: an initiation stage, and a maturation stage (Figure 4). The initiation stage extends from the mixing step through to the packing step (encompassing samples 1 to 3), and the maturation stage involves the period of fermentation at 30°C (encompassing samples 4 to 9) where development of product characteristics takes place. This 2 stage fermentation

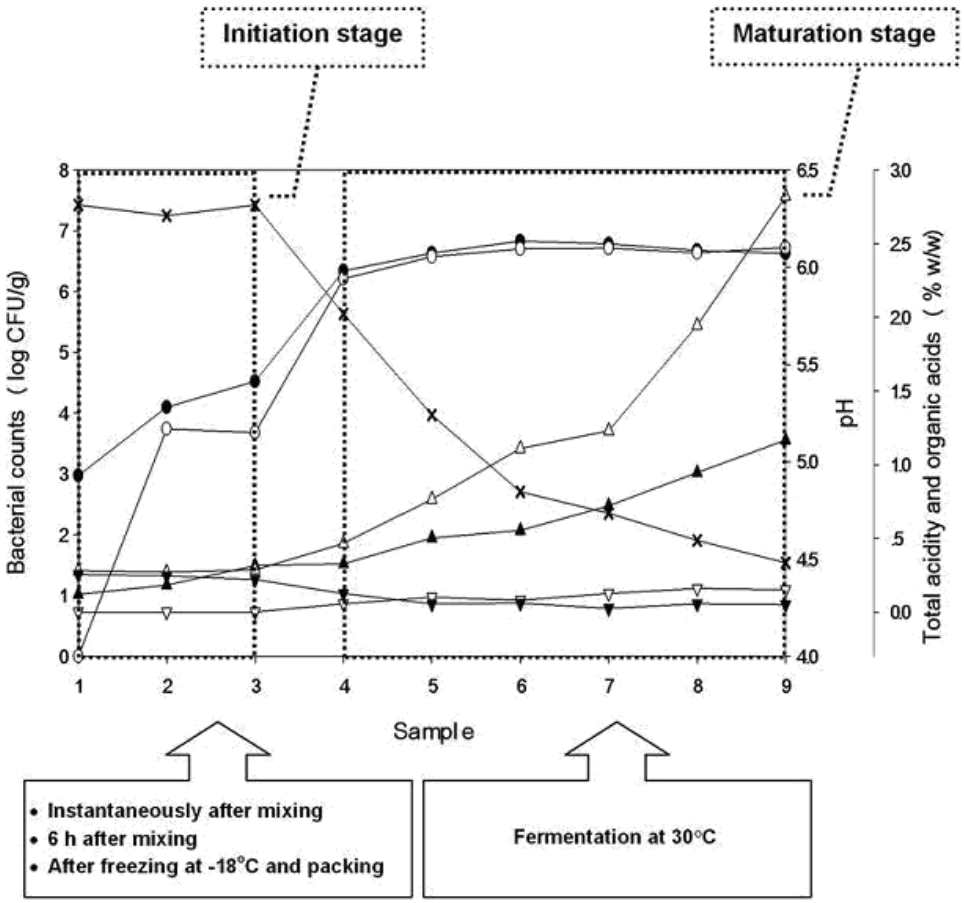

Figure 4. Elucidation of the initiation and maturation stages during the spontaneous fermentation process of *plaa-som*: pH ( ); total acidity ( ); lactic acid ( ); acetic acid ( ); citric acid ( ); total viable counts ( ); and lactic acid bacteria counts ( ).

profile is also in accordance to realistic practices from both producer and consumer. By this, the initiation stage is conducted via production steps employed by the selected local producer and the maturation stage is conducted principally by the consumer who maintains the *plaa-som* at ambient temperature allowing development of the preferred sour taste prior to consumption. The length of the maturation stage, therefore, largely depends on consumer preference and ambient temperature which is influenced by seasonal changes in the region (Adams et al., 1985; Saisithi, 1987). The length of the maturation stage in this study was assigned 144 h at 30°C, equating to 6 days, at which time the fermented fish started to develop unacceptable appearances by exude and fishy odor and were no longer fit for consumption. Thus the fermentation at 30^{o}C was brought to an end at sample 9. At extended times, the depletion of fermentable carbohydrates may adversely impact the LAB population, and the preservation effect becomes inoperative.

In this experiment, the salt content in *plaa-som* was found to be 1.43% (w/w) which corresponds to its catego - rization as being a low salt fermented fish containing less than 8% (w/w) salt (Tanasupawat and Komagata 1995). However at this level it is considered relatively low in salt content compared to other reports of fermented fish products. For example, *burong dalug* from the Philippines contained 3.04% (w/w); and *som-fak* from Thailand contained 3.04 and 2.9% (w/w) (Orillo and Pederson 1968; Paludan-Müller 2002b). This may be due to a variant recipe in accordance to regional organoleptic preferences. Higher salt may mask real delicate aroma and flavor of fermented food products (Pederson 1979). However, at low levels of salt there is less inhibition influence as a hurdle factor on undesirable micro-organisms in these food products (Ingram and Kitchell 1967; Lee 2004).

At the initiation stage, the pH of *plaa- som* was found to be 6.3 and remained stable throughout this stage. In the same period total acidity showed an increasing trend from 0.12 to 0.32% (w/w). The pH stability may due to the buffering capacity of fish muscle protein (Adams et al., 1987). The fermentation process of the maturation stage was accompanied by a dramatic decrease in pH with increasing fermentation time after the fermentation had

initiated as from sample 3 to 72 h at sample 6, and then a gradual further decrease from pH 4.7 when fermentation time reached 96 h in sample 7 to pH 4.5 when fermentation time reached 144 h in sample 9. The rapid pH drop from samples 3 to 6 was probably due to increasing amounts of organic acids, mainly lactic acid produced by LAB during the fermentation process. The pH drop correlated to a rapid increase in total acidity from the time when fermentation had initiated right after sample 3 to 72 h at sample 6. While pH can be used as a simple scientific indicator of sourness of the product, the evaluation for total acidity can only be used as an indicator to quantify the concentration of hydrogen ions able to be dissociated from any titratable acid. The pH levels are consistent with earlier reports on other fermented fish products, such as *plaa-som* from Thailand; pH 6 - 4.5, *som-fak* from Thailand; pH 6.5 - 4.5, *burong dalag* from the Philippines; pH 6.7 - 4.0, and *sikhae* from Korea; pH 6.6 - 4.4 (Orillo and Pederson 1968; Lee 1997; Paludan-Müller et al., 2002a,b). In general, pH of the fermented fish products should be below 5 – 4.5 in order to effectively inhibit undesirable bacteria, including pathogenic and spoilage bacteria (Owens and Mendoza 1985; Østergaard et al., 1998). Therefore, the pH of *plaa-som* in this study was found to be in an acceptable range for safety aspects. The pH was below 5 from samples 6 to 9 corresponding to fermentation times of 72 - 144 h. Another reference indicates that to ascertain the safety for consumption of a fermented meat product, such as *nham*, pH has to be lower than 4.6 (Visessanguan et al., 2006). Thus basic chemical characteristics, pH and total acidity, can be both used as safety control indices for *plaa-som* production.

Organic acid analysis, as determined by HPLC, was conducted in order to both quantify and identify the organic acids present initially and resulting from the fermentation process, led by LAB. Organic acid analysis at the initiation stage revealed that lactic and citric acids were the major organic acids present. Sample 1 was collected immediately after the mixing step and before the metabolic biological process was allowed to take place. This suggests that lactic acid and citric acid might already exist in some of the ingredients used in the process. Organic acid analysis of the minced garlic used in this study was additionally performed to determine the exact source of lactic and citric acids found in the initiation stage of fermentation. The HPLC result showed that lactic acid, citric acid, and acetic acid peaks were detected in the minced garlic at approximately 0.54, 0.63, and 0.31% (w/w) respectively. These organic acids detected in the minced garlic may be introduced by garlic bulbs contaminated with mold rot**.** Since some of the common molds such as *Fusarium* spp., *Botrytis* spp., and *Penicillium* spp. have been reported to infect garlic bulbs (Pitt and Hocking 1997). Generally, filamentous molds are able to produce organic acids as their metabolites, such as citric, lactic, and acetic acids (Wolschek et al., 1998; Magnuson and Lasure 2004). This is consistent with the finding of a relatively low number of mold colonies on PDA plates for sample 1 (data not shown) which occasionally can be found in the open fermentation process routinely employed in the production of fermented food (Khieokhachee et al., 1997; Visessanguan et al*,.* 2006). To reduce the mold load in *plaa-som*, some additional practices can be applied to existing GMPs including selection of fresh intact garlic bulbs for *plaa-som* production and using a well ventilated, low-humidity room for garlic storage*.* In addition, ensuring micro-aerophilic conditions in the *plaa-som* wrap-up package is considered a possible way to inhibit growth of molds during the fermentation.

Organic acid profiles revealed an increasing trend of lactic acid and a decreasing trend of citric acid in the maturation stage. The decrease of citric acid may due to citric acid metabolism by microorganisms in the fermentation system, including LAB, in which some metabolites (e.g. acetoin and diacetyl) are formed (Drainan et al.,1976; Caplice and Fitzgerald 1999; Mugula et al., 2002). These metabolites are important contributors to the flavor, aroma, and texture developments of fermented food products (Drainan et al., 1976; Fox et al., 2000; Visessanguan et al., 2006). In addition, some of these metabolites, such as diacetyl, can serve as antimicrobial agent (Visessanguan et al., 2006). Acetic acid was first detected in sample 4 when fermentation was proceeded for 24 h at 0.06% (w/w) and slightly increased to 0.15% (w/w) in sample 9 when fermentation has progressed up to 144 h. Both lactic and acetic acids found in this study indicate the presence of LAB populations in the mixed microbial communities inhabiting the samples and could possible be used as another safety indices for *plaa-som* production. LAB performs the vital role of converting carbohydrates, contained in the *plaa-som* ingredients, to organic acids, mainly lactic acid (Orillo and Pederson 1968; Kandler 1983; Lee 1997; Østergaard et al., 1998). LAB is a group of bacteria comprising both homofermentative LAB, producing 70 - 90% lactic acid, and heterofermentative LAB, producing at least 50% lactic acid and acetic acid, carbon dioxide and ethanol (Tanasupawat and Komagata 1995). Thus, detection of both lactic and acetic acids also indicates that fermentation found in *plaa-som* is principally lactic acid fermentation by both groups of LAB as described. These results closely conform to other studies on fermented meat products where lactic acid is found in a significant amount together with acetic acid (Visessanguan et al., 2006).

These accumulated organic acids detected during fermentation are not only considered to be causative agents for decreasing pH and increasing total acidity of *plaa-som* but also participate in the product's taste, flavor and texture, such as its unique sourness, aromas, and sponginess (Valyasevi and Rolle 2002; Visessanguan et al., 2006). Lactic and acetic acids are expected to play essential roles in imparting the tangy sour characteristic

on the product (Visessanguan et al., 2004). These organic acids can serve as indicators of successful batch fermentation (Motarjemi 2002; Valyasevi and Rolle 2002).

Both microbiological counts were also served as another safety index for spontaneous fermentation process of *plaa- som*. Substantial discrepancies between TVC and LAB counts were observed during the initiation stage of fermentation. This suggested a co-existence of a non-LAB group as a majority and the LAB group as a minority. The non-LAB group was considered to comprise undesirable microorganisms introduced through co-contamination with raw materials and ingredients used in the recipe. In addition to basic GMPs used by the local producer, an appropriate concentration of chlorine dioxide wash water applied to wash raw materials, such as fish and garlic bulbs, with an adequate contact time would reduce an initial load of undesirable micro-organisms (Andrews et al., 2002).

After the freezing step (sample 3), the TVC count was retarded and the LAB count was markedly reduced by a freeze-shock effect. This shows that the freeze-shock effect not only affected the LAB population but also other adventitious bacteria present in the ingredients. This freezing step is a unique step employed at the selected local production site. The step is claimed to assist in maintaining a firmness of fish texture as well as allowing long term storage for large production batch. At ambient temperature, fish muscle proteins are naturally degraded by enzymatic reactions, from enzymes native in fish itself and those that are produced from microorganisms, resulting in softening of fish texture. These reactions can be retarded by various methods such as drying, salting, and also freezing. (Huss 1995; Johnston et al., 1994; Valyasevi and Rolle 2002). With the local production site the freezing step effectively retarded the enzymatic reactions in *plaa-som*.

Both TVC and LAB counts increased from 4.52 and 3.68 log CFU/g in sample 3, to maximum counts at 6.83 log CFU/g in sample 6 and 6.72 log CFU/g in sample 9, respectively. During the maturation stage, the TVC counts more or less equaled to the LAB counts. This assures that LAB is the predominant microbial group dominating throughout the fermentation of *plaa-som* in this study. In addition, the total acidity and the amount of lactic acid produced from the fermentation process were found to be correlated to LAB counts, particularly when entering the maturation stage. In this stage, as the LAB count increased the amount of lactic acid and total acidity also increased until maximums were reached at 6.72 log CFU/g, 2.82 and 1.17% (w/w), respectively. This also suggests that most of the bacteria comprising the TVC were LAB, indicating good hygiene for this selected local production site. The predominant presence of LAB and accompanying acidity thus contributes to the taste and food preservation against undesirable microorganisms (Tanasupawat and Komagata 1995; Østergaard et al., 1998). This implies that the LAB, a particular group of bacteria generally recognized as safe, was a major contribute to *plaa-som* fermentation process and insured product safety from the selected local production site.

Furthermore, some practices are necessarily added in the initiation stage in order to reduce the number of undesirable microorganisms cross-contaminated from the raw materials. However, an excessive cleaning practice on raw materials may reduce the initial number of LAB hence adversely affecting the fermentation process. The use of LAB starter may then become advantageous for *plaa-som* production. Therefore, an adequate concentration of a LAB starter may be used as a critical control point to ensure a rapid pH decrease to the safety range within the restricted time when product characteristics and consumer acceptability are being considered. The chemical and microbiological changes during the maturation stage assure the accomplishment of the fermentation. This implies that the length of the maturation stage should be considered as the other possible CCP to assign keeping conditions and shelf-life of the product.

In conclusion, this study demonstrated that an evaluation of salt content, pH, acidity, main organic acid types and microbiological changes during spontaneous fermentation of *plaa-som* is necessary to ensure the success and safety of the production batch. These chemical and microbiological changes can possibly be used to underline important aspects existing in the current GMPs; to add some practices of improvement for GMPs; and to propose possible critical control points (CCPs) for small scale or cottage industries such as LAB counts, pH, and the length of maturation stage. This indicates the sustainable benefits of applying GMPs as a basic program in the fermented food processing industry which will lead to better production practices leading to higher safety standards for *plaa-som* production.

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REFERENCES

Adams MR, Cooke RD, Rattagool P (1985). Fermented fish products of South East Asia. Trop. Sci. 25: 61-73.

Adams MR, Cooke RD, Twiddy DR (1987). Fermentation parameters involved in the production of lactic acid preserved fish-glucose substrates. Int. J. Food Sci. Tech. 22:105-114.

Andrews LS, Key AM, Martin RL, Grodner R, Park DL (2002). Chlorine dioxide wash of shrimp and crawfish an alternative to aqueous chlorine. Food Microbiol 19:261-267.

AOAC International (2000). Official Methods of Analysis of AOAC International. AOAC International, Gaithersburg, MD, USA.

Caplice E, Fitzgerald FG (1999). Food fermentation: role of microorganisms in food production and preservation. Int. J. Food Microbiol. 50:131-149.

de Man JC, Rogosa M, Sharpe ME (1960). A medium for the cultivation of Lactobacilli. J. Appl. Bacteriol. 23:130-135.

Drainan DF, Tobin S, Cogan TM (1976). Citric acid metabolism in hetero- and homofermentative lactic acid bacteria. Appl. Environ. Microbiol. 31:481-486.

Fox PF, Guinee TP, Cogan TM, McSweeney PLH (2000). Fundamentals of cheese science. Aspen Publishers, Inc., USA.

Huss HH (1995). Fresh fish. Quality and quality changes. *FAO Fisheries Series No. 29.* Available at: http://www.fao.org/docrep/V7180E/V7180E00.HTM. Cited 11 September 2007.

Ingram M, Kitchell AG (1967). Salt as a preservative for foods. J. Food Technol. 2:1-15.

Johnston WA, Nicholson FJ, Roger A, Stroud GD (1994). Freezing and refrigerated storage in fisheries. *FAO Tech. Fish. Paper.* Available at: http://www.fao.org/DOCREP/003/V3630E/V3630E00.HTM. Cited 11 September 2007.

Kandler O (1983) Carbohydrate metabolism in lactic acid bacteria. Antonie Leeuwenhoek . 49:209-224.

Khieokhachee T, Praphailong W, Chowvalitnitithum C, Kunawasen S, Kumphati S, Chavasith V, Bhumiratana S, Valyasevi R (1997). Microbial interaction in the fermentation of Thai pork sausage. In: Proceedings of the 6th ASEAN food conference, 24-27 November 1997, Singapore. pp. 312-318.

Lee CH (1997). Lactic acid fermented foods and their benefits in Asia. Food Control 8:259-269.

Lee S-Y (2004). Microbial safety of pickled fruits and vegetables and hurdle technology. Int. J. Food Saf. 4:21-32.

Leroy F, Vuyst LD (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci. Tech. 15:67-78.

Magnuson JK, Lasure LL (2004). Organic acid production by filamentous fungi. In: Tkacz JS, Lange L (eds) Advances in Fungal Biotechnology for Industry, Agriculture, and Medicine, Kluwer Academic/Plenum Publishers, London, UK.

Motarjemi Y (2002). Impact of small scale fermentation technology on food safety in developing countries. Int. J. Food Microbiol. 75:213–229.

Mugula JK, Nnko SAM, Narvhus JA, Sorhaug T (2002). Microbiological and fermentation characteristics of *togwa*, a Tanzanian food. Int. J. Food Microbiol. 80:187-199.

Nout MJR, Motarjemi Y (1997). Assessment of fermentation as a household technology for improving food safety: a joint FAO/WHO workshop. Food Control. 8:221-226.

Orillo, CA, Pederson, CS (1968) Lactic acid bacterial fermentation of *burong dalag*. J. Appl. Microbiol. 16:1669-1671.

Østergaard A, Ben Embare PK, Yamprayoon J, Wedell -Neergaard C, Juss HH, Gram L (1998). Fermentation and spoilage of *som-fak*, a Thai low-salt fish product. Trop. Sci. 38:105-112.

Owens JD, Mendoza LS (1985). Enzymatically hydrolysed and bacterially fermented fishery products. J. Food Technol. 20:273-293.

Paludan-Müller C, Madsen M, Sophanodora P, Gram L, Moller RL (2002a). Fermentation and microflora of *plaa-som*, a Thai fermented fish product prepared with different salt concentrations. Int. J. Food Microbiol. 73:61-70.

Paludan-Müller C, Valyasevi R, Huss HH, Gram L (2002b). Genotypic and phenotypic characterization of garlic-fermenting lactic acid bacteria isolated from *som-fak*, a Thai low-salt fermented fish product. J. Appl. Microbiol. 92:307-314.

Pederson CS (1979). Microbiology of Food Fermentations. The AVI Publishing Company, Inc., Westport, Connecticut.

Pitt JI, Hocking AD (1997). Fungi and food spoilage. Blackie Academic and Professional, London, UK.

Saisithi P (1987). Traditional fermented fish products with special reference to Thai products. Asean Food J. 3:3-10.

Sneath PHA, Mair NS, Sharpe ME, Holt JG (1986). Bergey's Manual of Systematic Bacteriology. Williams and Wilkins, Baltimore.

Speck ML (1984). Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington DC.

Tanasupawat S, Komagata K (1995). Lactic acid bacteria in fermented foods in Thailand. World J. Microbiol. Biotechnol. 1:253-256.

TISI (2005). Thai Community Products Standard 26 / 2546. In *Thai Community Product Standard.* Thai Industrial Standards Institute, Ministry of Industry, Bangkok, Thailand.

Valyasevi R, Rolle RJ (2002). An overview of small-scale food fermentation technologies in developing countries with special reference to Thailand: Scope for their improvement. Int. J. Food Microbiol. 75:231-239.

Visessanguan W, Benjakul S, Riebroy S, Thepkasikul P (2004). Changes in composition and functional properties of proteins and their contributions to *Nham* characteristics. Meat Sci. 66:579-588.

Visessanguan W, Benjakul S, Smitinont T, Kittikun C, Thepkasikul P, Panya A (2006). Changes in microbiological, biochemical and physico-chemical properties of Nham inoculated with different inoculum levels of *Lactobacillus curvatus*. LWT-Food Sci. Technol. 3:814-826.

Wolschek MF, Narendja F, Karlseder J, Kubicek CP, Scazzocchio C, Strauss J (1998). *In situ* detection of protein-DNA interactions in filamentous fungi by *in vivo* footprinting. Nucleic Acids Res. 26: 3862-3864.