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Comparison of Fermentation Process in Thai Fermented Pork Sausage (I-San Sausage) on Quality and Safety

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The comparison of different fermentation process for I-san sausage was demonstrated in 5 conditions as follow 1) Hang at room temperature for 3 day 2) incubation 37˚C for 3 day 3) vacuum 37˚C for 3 day 4) incubation 37˚C for 2 day with one day hanging at room temperature and 5) vacuum 37˚C for 2 day with one day hanging at room temperature. The pH value, total acidity percentage, weight loss percentage, lactic acid bacteria, coliform/*Escherichia coli* and yeast/mold were analyzed. The results showed that the pH value declined rapidly from 5.97-5.99 to 4.35-5.16 during fermentation for 3 day in all methods. The pH reduction corresponded to an increase in total acidity from 0.35-0.38% to 0.73-1.17%. The lowest pH value and the highest total acidity found in I-san sausage incubation 37˚C for 3 days. The weight loss of fermenting I-san sausage generally decrease as the fermentation time increased. As the fermentation process, I-san sausage hang at room temperature for 3 day had the greatest weight loss (14.93%) but I-san sausage with fermentation condition at incubation 37˚C for 3 day or vacuum 37˚C for 3 day displayed the lowest weight loss value as 3.94 and 3.22%, respectively. The lactic acid bacteria count at day 3 increased in all methods with value between 7.31-7.81 log cfu/g on day 3. The highest population of lactic acid bacteria was found in fermentation condition with 37˚C incubation for 3 days (7.81 log cfu /g). The count of yeast/mold in all methods had no differences (P>0.05) on day 1-3 of fermentation. The coliform decreased on day 1 and displayed low limit of detection. The detection of *Escherichia coli* on day 0-3 of fermentation in all methods was no differences (P>0.05) with undetectable level.

Keywords: Thai fermented pork sausage (I-san sausages), fermentation process

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Introduction

I-san sausage or Sai krok Prew (Traditional, Thai fermented meat-rice sausages) is a very popular meat product in Thailand (Vatanyoopaisarn *et al*., 2011; Sriphochanart and Skolpap, 2011). I-san sausage is usually made of pork 50%, pork lard 30%, cooked rice 20% and food additives, mixed well and stuffed tightly in edible casing. It is fermented in room temperature at normally 30˚C for 2-3 days and cooked before eating (Phromraksa *et al.*, 2004; Phalakornkule and Tanasupawat, 2006). In Thailand, most of the production is in the household itself (Phromraksa *et al.*, 2004; Sriphochanart *et al.*, 2011). Generally, the fermentation process of Thai fermented sausages with rice as food ingredient induce lactic acid bacteria which helps to control pathogen as it may come from raw materials (Azam *et al*., 2017). The most important microorganisms during the spontaneous fermentation are lactic acid bacteria such as *Lactobacillus plantarum* and *Pediococcus cerevisiae* with have been shown to become the dominant microorganisms (Malti and Amarouch, 2008; Rantsiou and Cocolin, 2008; Axelsson *et al.*, 2012; Vangpikul and Kansandee, 2014; Tamang *et al*., 2016). Unfortunately, the conventional process of Thai fermentation sausage production has difficulties with unstable product quality and health risks on pathogenic disease (Sriphochanart and Skolpap, 2011). The occurrence of pathogen such as *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli* O157:H7, *Clostridium botulinum* and *Listeria monocytogenes* were found in fermented pork sausage (Visessanguan *et al.*, 2006; Chokesajjawatee *et al.*, 2009; Yörük and Güner, 2017). The bacterium, *S. aureus*, a salt-and nitrite-tolerant microorganism, is also able to grow under anaerobic conditions and produce toxins (González-Fandos *et al.*, 1999; Hui *et al.*, 2004; Schelin *et al.*, 2011; Holck *et al.*, 2017). Therefore, a novel process of sausage fermentation has been developed in I-san sausage. There are several ways for I-san sausage production. Especially, in fermentation stage such as hanging at room temperature, vacuum and incubation process (Phalakornkule and Tanasupawat, 2006). However, some manufacturers were experiencing problems in producing of process, or quality in consistent taste, contamination and mold growth surface on I-san sausage during fermented (Holck *et al.*, 2017).

The purpose of this study was to compare different methods during I-san sausage fermentation for better product acceptable both in quality and safety.

Materials and Methods

Formulations and processing in I-sausage

The preparation procedures used to make I-san sausage are described in Phromraksa *et al*., (2004) and Vatanyoopaisarn *et al*., (2011) with slight modification. Freshly-manufactured I-san sausages, prepared using conventional techniques by mixing (46.27%) (w/w) of minced lean pork, (32.39%) (w/w) of minced pork lard and (13.88%) (w/w) of steamed rice with 4.63% (w/w) of finely chopped garlic, 0.38% (w/w) of pepper powder, 0.46% (w/w) of sugar, 0.09% (w/w) of sodium erythrobate, 0.23% (w/w) of monosodium glutamate, 0.28% (w/w) of trisodium polyphosphate, 0.93% (w/w) of sodium nitrate and 0.46% (w/w) of salt. The mixture was then stuffed into 2.5 cm diameter natural casing and tied with thread. Each piece of fresh sausages was of 3 cm in length. Fresh sausages were fermented 1) hang at room temperature for 3 day 2) incubation 37˚C for 3 day 3) vacuum 37˚C for 3 day 4) incubation 37˚C for 2 day with one day hanging at room temperature and 5) vacuum 37˚C for 2 day with one day hanging at room temperature. Samples were taken at day 0, 1, 2 and 3 for chemical, physical and microbial analysis.

Physical and chemical analysis

pH

Direct pH measurement was taken using a standard pH meter Mettler Toledo 320 (Mettler Toledo, Greifensee, Switzerland)

Total acidity

Total acidity of I-san sausage was determined according to the method of (Friedrich, 2001). 2 g of samples were homogenized with 20 ml distilled water. The homogenate was centrifuged at 4000g for 5 min. The supernatant was filtered through filter paper (Whatman No. 1). The filtrate was titrated with standardised 0.1N Sodiumhydroxide (NaOH) (Sigma, Germany) with Phenolph- thalein (Sigma, Germany) as the indicator.

Weight loss

Weight loss was determined as described by Nakao *et al.*, (1991). I-san sausage with casing was accurately weighed before fermentation. During fermentation process, I-san sausage was taken and then reweighed. Difference in weight of I-san sausage before (A) and after (B) fermentation was referred to as weight loss.

$$\text{Weight loss (\%)} = (A-B) \times 100 / A$$

Microbial analysis

Twenty-five grams of each I-san sausage sample was aseptically transferred to a sterile plastic bag containing 225 ml of 0.85% sodium chloride (NaCl) (Merck, Germany) for 1 min and homogenized in a stomacher bag mixer (400 model VW, France) to get the 10^{-1} dilutions. Then, 1 ml of these 10^{-1} dilutions was pipetted into a test tube containing 9 ml of 0.85% sodium chloride to get a 10^{-2} dilution. This step was repeated to get 10^{-3}, 10^{-4} and 10^{-5} dilutions. Appropriate dilutions were used for microbial enumeration. The following media and incubation conditions were used: lactic acid bacteria was determined by the spread plate method 0.1 ml of each dilution was counted on MRS agar (Merck, Germany) containing 0.5% calcium carbonate ($CaCO_3$) (Merck, Germany) and then bacteria were anaerobically incubated at 30°C for 24-48 h. While coliform/*Escherichia coli* were counted on Chromocult agar (Merck, Germany) incubation at 37°C for 24-48 h. This was done according to the method presented by AOAC (2006). For determination of yeast/mold, method of AOAC (2005) was followed. 1 ml of dilution was poured plate which contained malt agar (Merck, Germany) pH 3.5 and incubation at 26°C for 3-5 days. Colonies froming units (CFU) between 30-300 colonies were selected from each plate. Select the plate with counts forming. Microbial colonies were counted and expressed as $\log_{10}$ cfu/g meat sample.

Statistical analyses

All experiments in this study was carried out by Randomized Complete Block Design. Mean values were compared by the Duncan's multiple range test. Statistical analysis was performed using SAS software (SAS Institute Inc., Cary, N.C.).

Results and Discussion

pH and total acidity (%)

Changes in pH and total acidity of I-san sausage during the fermentation by comparison five fermentation processes 1) Hang at room temperature for 3 day 2) incubation 37˚C for 3 day 3) vacuum 37˚C for 3 day 4) incubation 37˚C for 2 day with one day hanging at room temperature and 5) vacuum 37˚C for 2 day with one day hanging at room temperature were determined. The initial pH of I-san sausage was 5.97, 5.98, 5.96, 5.99 and 5.98, respectively at day 0 ($P>0.05$). The decrease in pH could be mainly due to the production of acid such as lactic acid of the lactic acid bacteria present in the I-san sausage. A

rapid decrease in pH was observed in fermentation method 2, 3 and 4, while pH value of I-san sausage fermentation process by hanging at room temperature was not much decline after hanging for 3 day. The lowest pH value of I-san sausage at day 3 found in fermentation by incubation 37˚C for 3 day (4.35). However, It was not different (P>0.05) with fermentation process 3, 4 and 5 which showed pH value 4.47, 4.48 and 4.56, respectively. However, It was observed that pH value in I-san sausage of fermentation process 2, 3, 4 and 5 were significent lower than pH value in sausage which fermented by process 1 (hang at room temperature for 3 day). The data show in table 1.

For total acidity value of I-san sausage, after fermentation for 2-3 day, the lowest pH value of I-san sausage was corresponding to the highest total acidity in I-san sausage. The lowest pH value and the highest of total acidity was displayed in I-san sausage fermentation by incubation 37˚C for 3 day 3. The total acidity in I-san sausage at the first day of all methods, was not significant different (P>0.05) as shown 0.35, 0.36, 0.35, 0.36 and 0.38%, respectively. The total acidity of all process increased after 2-3 day fermentation. The highest total acidity at 3 day fermentation was observed in fermentation process 2 (1.17%) different form after fermentation process (P>0.05) which showed value at 0.73, 1.00, 0.96 and 0.91% for process 1, 3, 4 and 5, respectively. The data show in table 2.

The results were in agreement with the literature that pH values of fermented sausages decreased sharply at the first 3 days of fermentation (Bozkurt and Bayram, 2006; Baka *et al.*, 2011). The decrease in pH values of the fermented sausages correspond to the production of organic acids such as lactic acid and acetic acid by lactic acid bacteria (Komperda *et al.*, 2004; Bozkurt and Bayram, 2006; Saithong *et al.*, 2010). The pH fall could be related to an accumulation of organic acids, mainly lactic, present in this type of sausages as a result of carbohydrate breakdown during fermentation (Baka *et al.*, 2011; Zaho *et al.*, 2011; Esmaeilzaden *et al.*, 2013). *Lactobacilli* are the major producers of lactic acid responsible for the decrease in pH and the increase in acidity during the fermentation (Valyasevi *et al.*, 2002; Thongruck *et al.*, 2017). The pH of the sausage should be lowered to 4.6 after fermentation, there by preventing the growth of other microbes, particularly foodborne bacterial pathogens such as *Staphylococcus aureus* (Chokesajjawatee *et al.*, 2009; Holck *et al.*, 2017; Loypimai *et al*., 2017). A pH change pattern, which consisted of a rapid decrease at first, followed by a steady or slow decrease, and then finally a rise during the processing and storage time, was observed in a Spanish dry-cured sausage (González-Fernández *et al.*, 2006)

Table 1. Change in pH value of I-san sausage during fermentation

Fermentation process*	pH value			
	Day 0	Day 1	Day 2	Day 3
1	$5.97 \pm 0.05^{a,A,\dagger,\S,\ddagger}$	$5.83 \pm 0.18^{a,A}$	$5.55 \pm 0.18^{b,A}$	$5.16 \pm 0.29^{c,A}$
2	$5.98 \pm 0.04^{a,A}$	$5.66 \pm 0.16^{b,A}$	$4.68 \pm 0.03^{c,B}$	$4.35 \pm 0.12^{d,B}$
3	$5.96 \pm 0.05^{a,A}$	$5.74 \pm 0.22^{b,A}$	$4.77 \pm 0.10^{c,B}$	$4.47 \pm 0.11^{d,B}$
4	$5.99 \pm 0.05^{a,A}$	$5.73 \pm 0.12^{b,A}$	$4.73 \pm 0.07^{c,B}$	$4.48 \pm 0.09^{d,B}$
5	$5.98 \pm 0.04^{a,A}$	$5.78 \pm 0.15^{b,A}$	$4.79 \pm 0.04^{c,B}$	$4.56 \pm 0.05^{d,B}$

* 1) Hang at room temperature for 3 day 2) incubation 37˚C for 3 day 3) vacuum 37˚C for 3 day 4) incubation 37˚C for 2 day with one day hanging at room temperature and 5) vacuum 37˚C for 2 day with one day hanging at room temperature.
‡ Mean values and standard deviations obtained from three independent experiments.
§ Different letters in the same row are significantly different ($P<0.05$).
† Different letters within a column significant difference ($P<0.05$).

Table 2. Change in total acidity of I-san sausage during fermentation

Fermentation process*	Total acidity (% as lactic acid)			
	Day 0	Day 1	Day 2	Day 3
1	$0.35 \pm 0.06^{c,A,\dagger,\S,\ddagger}$	$0.51 \pm 0.04^{b,B}$	$0.62 \pm 0.08^{ab,B}$	$0.73 \pm 0.02^{a,C}$
2	$0.36 \pm 0.05^{d,A}$	$0.59 \pm 0.07^{c,A}$	$0.87 \pm 0.09^{b,A}$	$1.17 \pm 0.12^{a,A}$
3	$0.35 \pm 0.04^{d,A}$	$0.64 \pm 0.07^{c,A}$	$0.82 \pm 0.02^{b,A}$	$1.00 \pm 0.11^{a,B}$
4	$0.36 \pm 0.02^{c,A}$	$0.60 \pm 0.10^{b,A}$	$0.84 \pm 0.05^{a,A}$	$0.96 \pm 0.05^{a,B}$
5	$0.38 \pm 0.11^{c,A}$	$0.60 \pm 0.07^{b,A}$	$0.81 \pm 0.06^{a,A}$	$0.91 \pm 0.04^{a,B}$

* 1) Hang at room temperature for 3 day 2) incubation 37˚C for 3 day 3) vacuum 37˚C for 3 day 4) incubation 37˚C for 2 day with one day hanging at room temperature and 5) vacuum 37˚C for 2 day with one day hanging at room temperature.
‡ Mean values and standard deviations obtained from three independent experiments.
§ Different letters in the same row are significantly different ($P<0.05$).
† Different letters within a column significant difference ($P<0.05$).

Weight loss (%)

The results in table 3 showed that the average weight loss (%) in I-san sausage by comparison five fermentation processes 1) Hang at room temperature for 3 day 2) incubation 37˚C for 3 day 3) vacuum 37˚C for 3 day 4) incubation 37˚C for 2 day with one day hanging at room temperature and 5) vacuum 37˚C for 2 day with one day hanging at room temperature were determined. Weight of fermented sausage generally decreases as the fermentation time increased. The highest % weight loss in fermentation process 1 for 8.20, 11.28 and 14.53 after hanging day 1, 2 and 3, respectively. The lowest % weight loss was fermented in process 2 and 3 (3.94 and 3.22) which was not different significant ($P>0.05$), when compared to process 1, 4 and 5 (14.93, 8.38 and 11.90%).

Similar results had been reported by Visessanguan *et al.*, (2006). The result showed that the lowest % weight loss were fermentation process by incubation at 30˚C for 84 h (3.14%) of Thai fermented pork sausage (Nham). Weight loss in meat products is mainly associated with loss in water and water-holding capacity (WHC) of meat. Increasing amounts of released and expressible water are possibly responsible for an increase in weight loss (Visessanguan *et al.*, 2004). Denaturation of sarcoplasmic proteins contributes to the decrease water-binding capacity of pork myofibrils (Wilson and Laack, 1999; De Luca *et al.*, 2016). Released water is generally refer to as the water retained in the casing and at the surface (Visessanguan *et al*., 2015). In contrast, expressible water is the water remaining in the sample which can be released when pressure is applied (Funami *et al.*, 1998; Visessanguan *et al*., 2015). An increase in expressible and released water presumably caused by denaturation of proteins during fermentation (Visessanguan *et al.*, 2004; Sİrİken *et al.*, 2009).

Table 3. Change in weight loss of I-san sausage during fermentation

Fermentation process*	**Weight loss (%)**		
	Day 1	Day 2	Day 3
1	$8.20 \pm 0.97^{a,A,\dagger,\S,\ddagger}$	$11.28 \pm 0.41^{b,A}$	$14.93 \pm 0.51^{c,A}$
2	$0.50 \pm 0.13^{b,B}$	$1.81 \pm 1.00^{b,B}$	$3.94 \pm 0.14^{a,D}$
3	$1.09 \pm 0.53^{b,B}$	$3.15 \pm 0.84^{a,B}$	$3.22 \pm 0.23^{a,D}$
4	$0.71 \pm 0.16^{b,B}$	$1.15 \pm 0.26^{b,B}$	$8.38 \pm 1.61^{a,C}$
5	$1.52 \pm 0.13^{b,B}$	$2.50 \pm 0.62^{b,B}$	$11.90 \pm 0.28^{a,B}$

* 1) Hang at room temperature for 3 day 2) incubation 37˚C for 3 day 3) vacuum 37˚C for 3 day 4) incubation 37˚C for 2 day with one day hanging at room temperature and 5) vacuum 37˚C for 2 day with one day hanging at room temperature.

‡ Mean values and standard deviations obtained from three independent experiments.

§ Different letters in the same row are significantly different ($P<0.05$).

† Different letters within a column significant difference ($P<0.05$).

Microbiological analysis

The microbiological analysis in I-san sausage during the fermentation by comparison five fermentation processes 1) Hang at room temperature for 3 day 2) incubation 37˚C for 3 day 3) vacuum 37˚C for 3 day 4) incubation 37˚C for 2 day with one day hanging at room temperature and 5) vacuum 37˚C for 2 day with one day hanging at room temperature were determined.

Lactic acid bacteria

Number of lactic acid bacteria increased during fermentation process 1-3 day. The initial number of lactic acid bacteria in I-san sausage of all fermentation process 1-5 were not significant different (P>0.05) as 4.22, 4.28, 4.17, 4.28 and 4.60 log cfu/g, respectively. The shorter fermentation period, the highest of number of lactic acid bacteria was observed. It was found that the lowest number of lactic acid bacteria displayed in I-san sausage with fermentation process 1 with significant difference when compare to process 2-5. The highest number of lactic acid bacteria after 3 day fermentation period demonstrated in I-san sausage with fermentation process 2 (7.81 log cfu/g). However, it was not significant different (P>0.05) when compared to process 3, 4 and 5 (7.66, 7.65 and 7.59 log cfu/g).

The microbiological changes during fermentation as a result of the combined effects of lowering the pH, resulting in high populations of lactic acid bacteria (Jatupornpipat and Keatikumjorn, 2007; Simion *et al.*, 2014; Yim *et al.*, 2015). The lactic acid bacteria constituted the major microflora of the sausages (7.7-8.5 log cfu/g). Because of the good adaptation to the meat condition and their faster growth rates which displayed during fermentation (Zdolec *et al.*, 2008; Zaho *et al.*, 2011). During this spontaneous fermentation, lactic acid bacteria such as *lactobacilli* and *pediococci* have been shown to become the dominant microorganisms (Malti and Amarouch, 2008).

Table 4. Number of lactic acid bacterial in I-san sausages during fermentation

Fermentation process*	**Lactic acid bacterial (log cfu/g)**			
	Day 0	Day 1	Day 2	Day 3
1	$4.22 \pm 0.59^{c,A,\dagger,\S,\ddagger}$	$5.70 \pm 0.58^{b,A}$	$6.70 \pm 0.31^{a,B}$	$7.31 \pm 0.35^{a,B}$
2	$4.28 \pm 0.63^{c,A}$	$6.91 \pm 0.12^{b,A}$	$7.53 \pm 0.19^{a,A}$	$7.81 \pm 0.38^{a,A}$
3	$4.17 \pm 0.50^{c,A}$	$6.36 \pm 0.96^{b,A}$	$7.12 \pm 0.47^{a,AB}$	$7.66 \pm 0.51^{a,AB}$
4	$4.18 \pm 0.51^{c,A}$	$6.83 \pm 0.59^{b,A}$	$7.22 \pm 0.39^{ab,A}$	$7.65 \pm 0.53^{a,AB}$
5	$4.16 \pm 0.48^{c,A}$	$6.47 \pm 1.10^{b,A}$	$7.40 \pm 0.69^{a,A}$	$7.59 \pm 0.45^{a,AB}$

*1) Hang at room temperature for 3 day 2) incubation 37˚C for 3 day 3) vacuum 37˚C for 3 day 4) incubation 37˚C for 2 day with one day hanging at room temperature and 5) vacuum 37˚C for 2 day with one day hanging at room temperature.

‡ Mean values and standard deviations obtained from three independent experiments.

§ Different letters in the same row are significantly different (P<0.05).

† Different letters within a column significant difference (P<0.05).

Yeast/mold, Coliform and *Escherichia coli*

Number of yeast/mold and coliform in I-san sausage were not significant differences (P>0.05) in all methods. However, the coliform was not found in all methods after 2 day fermentation process. In addition, in this study *Escherichia*

coli displayed lower limit of detection in I-san sausage. The data show in table 5, 6 and 7.

This indicated that vacuum packaging decreased mold growth but did not completely inhibit during I-san sausage storage. Mold increase was probably due to the fact that they consumed residual oxygen in vacuum packaging (Phromraksa *et al.*, 2004). The fermentation process by vacuum 37˚C for 3 day show the reduction of yeast/mold growth to 2.85 log cfu/g at day 3. Similar results have been reported by other researchers Casaburi *et al.*, (2007) who considered acidification to be the main cause of yeast/mold inhibition in dry fermented sausages. The pH drop below 4.5, which was well above the tolerance level of many yeast/mold (Malti and Amarouch, 2008).

The domination of lactic acid bacteria and the inhibition of gram negative bacteria in fermented sausages during fermentation are necessary for successful production of fermented sausages (Baka *et al.*, 2011; Esmaeilzadeh *et al.*, 2013). Enterobacteriaceae and gram negative bacteria, in general, are considered as undesirable microflora in fermented sausages (Esmaeilzadeh *et al.*, 2013). These reduction of Enterobacteriaceae are probably due to the rapid reduction of pH, acid production. The results are consistent with Roig-Sagués *et al.*, (1999) who reported that the enterobacteria counts in Spanish sausages (fuet) decreased steadily during ripening and were undetectable after ripening for 12 days. Although growth of pathogenic *Escherichia coli* during initial phases of fermented sausage production can occur, combinations of low pH and high total acidity an inhibit growth of *Escherichia coli* at the end of fermentation (Holck *et al.*, 2017).

Table 5. Number of yeast/mold in I-san sausages during fermentation.

Fermentation process*	**Yeast/Mold (log cfu/g)**			
	Day 0	Day 1	Day 2	Day 3
1	$3.05 \pm 0.41^{b,A,\dagger,\S,\ddagger}$	$3.45 \pm 0.61^{ab,A}$	$3.88 \pm 0.80^{a,A}$	$3.93 \pm 0.81^{a,A}$
2	$3.03 \pm 0.49^{a,A}$	$3.84 \pm 1.10^{a,A}$	$4.05 \pm 1.22^{a,A}$	$4.15 \pm 0.96^{a,A}$
3	$3.01 \pm 0.51^{a,A}$	$3.28 \pm 1.82^{a,A}$	$3.31 \pm 1.55^{a,A}$	$2.85 \pm 1.48^{a,A}$
4	$3.04 \pm 0.49^{a,A}$	$3.40 \pm 1.31^{a,A}$	$3.74 \pm 0.60^{a,A}$	$3.43 \pm 1.29^{a,A}$
5	$2.95 \pm 0.57^{a,A}$	$3.14 \pm 0.68^{a,A}$	$3.51 \pm 1.00^{a,A}$	$2.98 \pm 0.67^{a,A}$

*1) Hang at room temperature for 3 day 2) incubation 37˚C for 3 day 3) vacuum 37˚C for 3 day 4) incubation 37˚C for 2 day with one day hanging at room temperature and 5) vacuum 37˚C for 2 day with one day hanging at room temperature.

‡ Mean values and standard deviations obtained from three independent experiments.

§ Different letters in the same row are significantly different ($P<0.05$).

† Different letters within a column significant difference ($P<0.05$).

Table 6. Number of coliform in I-san sausages during fermentation

Fermentation process*	Coliform			
	Day 0 (log cfu/g)	Day 1 (log cfu/g)	Day 2 (cfu/g)	Day 3 (cfu/g)
1	3.40±0.77[NS, ‡,†]	2.55±0.09	<1[¥]	<1
2	3.33±0.66	2.90±0.94	<1	<1
3	3.31±0.63	2.25±0.66	<1	<1
4	3.34±0.72	3.24±1.14	<1	<1
5	3.42±0.84	2.38±0.56	<1	<1

*1) Hang at room temperature for 3 day 2) incubation 37˚C for 3 day 3) vacuum 37˚C for 3 day 4) incubation 37˚C for 2 day with one day hanging at room temperature and 5) vacuum 37˚C for 2 day with one day hanging at room temperature.
‡ Mean values and standard deviations obtained from three independent experiments.
† Different letters in the same column and row are significantly different.
¥ <10 cfu/g.

Table 7. Number of *Escherichia coli* in I-san sausages during fermentation.

Fermentation process*	*Escherichia coli* (cfu/g)			
	Day 0	Day 1	Day 2	Day 3
1	<1[¥]	<1	<1	<1
2	<1	<1	<1	<1
3	<1	<1	<1	<1
4	<1	<1	<1	<1
5	<1	<1	<1	<1

*1) Hang at room temperature for 3 day 2) incubation 37˚C for 3 day 3) vacuum 37˚C for 3 day 4) incubation 37˚C for 2 day with one day hanging at room temperature and 5) vacuum 37˚C for 2 day with one day hanging at room temperature.
¥ <10 cfu/g.

Conclusion

Present study was to compare different methods during I-san sausage fermentation for better product acceptable both in quality and safety. There method were analysed including the pH value, total acidity percentage, weight loss percentage, lactic acid bacteria, coliform/*Escherichia coli* and yeast/mold. It was found that the fermentation process by incubation 37˚C for 3 day and vacuum 37˚C for 3 day gave the good quality I-san sausage, because of its shorter fermentation period. With these preliminary results, it is possible to apply both optimal condition fermentation process of I-san sausage for small entrepreneurs and industrials.

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