ORIGINAL ARTICLE

The effect of kefir starter on Thai fermented sausage product

Marisa Jatupornpipat[1] and Payom Keatikumjorn[2]

Abstract

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The effect of kefir starter from Wilderness Family Naturals Company on the initial formulation of Thai fermented sausage were evaluated. The differences found among batches in the main microbial populations and pH were not significant. Only, the total acid of batch D (added the kefir starter 15 ml) was significantly higher (P<0.05) than those of batches C, A and B. (control : not added the kefir starter, added the kefir starter 1 and 7 ml. respectively). The odour, flavor and overall acceptability of the B batch was different from those of the other three (P<0.05), C , A and D, which were very similar to each other (P>0.05). It is concluded that the addition of kefir starter (7 ml) could be useful to improve the final quality of Thai fermented sausages. The addition of kefir starter that initiates rapid acidification of the raw meat and that leads to a desirable sensory quality of the end-product are used for the production of fermented sausages, and represents a way of improving and optimizing the sausage fermentation process and achieving tastier, safer, and healthier products.

Key words : Thai fermented sausage, Kefir starter

[1]Ph.D. (Biotechnology), Asst. Prof., [2]B.Sc. (Biology), Scientist, Department of Applied Biology, Faculty of Science , King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520 Thailand.
Corresponding e-mail : kjmarisa@kmitl.ac.th, kjmarisa@gmail.com
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บทคัดย่อ

มาริสา จาตุพรพิพัฒน์ และ พยอม เกียรติกำจร
ผลของการเติมหัวเชื้อคีเฟอร์ต่อผลิตภัณฑ์ไส้กรอกอีสาน
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การเติมหัวเชื้อคีเฟอร์จากบริษัท Wilderness Family Naturals ลงในสูตรเริ่มต้นของไส้กรอกอีสาน พบว่าความแตกต่างของปริมาณหัวเชื้อคีเฟอร์ในแต่ละตัวอย่างไม่มีผลต่อจุลินทรีย์และพีเอช มีเพียงค่าปริมาณกรดทั้งหมดของตัวอย่าง D (เติมหัวเชื้อคีเฟอร์ 15 มล.) ให้ค่าสูงกว่าตัวอย่าง C, A และ B (ชุดควบคุมไม่เติมหัวเชื้อคีเฟอร์ เติมหัวเชื้อคีเฟอร์ 1 และ 7 มล. ตามลำดับ) อย่างมีนัยสำคัญ ส่วนการยอมรับทางประสาทสัมผัส ได้แก่ กลิ่น รสชาติ และการยอมรับที่ทั้งหมดของตัวอย่าง B ให้ค่าแตกต่างอย่างมีนัยสำคัญเมื่อเทียบกับตัวอย่าง C, A และ D ดังนั้นการเติมหัวเชื้อคีเฟอร์ปริมาณ 7 มล. สามารถช่วยปรับปรุงคุณภาพสุดท้ายของผลิตภัณฑ์ไส้กรอกอีสาน การเติมหัวเชื้อคีเฟอร์มีผลต่อกระบวนการการสร้างกรดในเนื้อได้รวดเร็วในระยะเริ่มต้น ส่งผลต่อคุณภาพทางประสาทสัมผัสของผลิตภัณฑ์สุดท้าย ซึ่งจำเป็นสำหรับการผลิตไส้กรอกอีสาน ทำให้เกิดการปรับปรุงที่เหมาะสมของกระบวนการหมักไส้กรอกอีสานที่ให้ผลิตภัณฑ์อร่อยกว่า ปลอดภัยกว่า และมีประโยชน์ต่อสุขภาพ

ภาควิชาชีววิทยาประยุกต์ คณะวิทยาศาสตร์ สถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหารลาดกระบัง เขตลาดกระบัง กรุงเทพ 10520

Thai fermented sausage is a typical local meat-product, which is composed of a mixture of pork meat, lard, cooked rice, salt, pepper, anise, cinnamon additives (nitrate, nitrite, antioxidants) and spices. It is known that the typical flavor of a Thai fermented sausage is a result of the micro-organisms and the metabolism of carbohydrates, proteins and lipids in combination with spices. Microbiological changes during this ferment sausage have been observed in previous report (e.g., Bruna *et al.*, 2000, Montel *et al.*, 1998 and Ockerman *et al.*, 2001). The acceptability of these products by the consumer is strongly influenced by the final flavor. The flavor of Thai fermented sausage depends on many factors such as raw meat quality and rice, processing conditions and additives, spices. The enzymatic and chemical phenomena involved in flavor generation include carbohydrate fermentation, lapillus, proteolysis, lipid oxidation and amino acid catabolism. The biochemical changes can be accelerated by the exogenous addition of different microorganisms and/or enzymes to the initial formulation (Lucke, 2000, Talon *et al.*, 2002 and Toldra *et al.*, 2001). Nowadays, producers of Thai fermented sausages are interested in extending the shelf-life of this product in order to increase the potential market and satisfy consumer demands. It is known that the addition of enzymes (proteases or lipases) is useful to accelerate the production of free amino acids and free fatty acids but these do not always lead to improvements in sensory quality (Ansorena *et al.*, 2002 and Ordonez *et al.*, 1999). This is because the final flavor also depends on the subsequent generation of volatile compounds through lipid oxidation and amino acid catabolism. Kefir is a fermented milk beverage that originated in Eastern Europe. The starter culture used to produce this beverage is an irregularly shaped, gelatinous white/yellow grain (Guzel *et al.*, 2000). These Kefir grains have a varying and complex microbial composition that includes species of yeasts, lactic acid bacteria (LAB), acetic acid bacteria (AAB), and mycelia fungi. Some LAB that have been isolated from Kefir include: *Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus helveticus, Lactobacillus kefiri, Lactobacillus parakefiri, Lactococcus lactis* and *Leuconostoc mesenteroides* (Assadi *et al.*, 2000; Cogan *et al.*, 1997; Fujisawa *et al.*, 1988; Kandler and Kunath, 1983; Micheli *et al.*, 1999; Pintado *et* *al.*, 1996; Takizawa *et al.*, 1998; Witthuhn *et al.*, 2004). Yeasts isolated from Kefir grains include *Kluyveromyces marxianus, Torula kefir, Saccharomyces exiguus* and *Candida lambica* (Assadi *et al.*, 2000; Garrote *et al.* 1997; Kwak *et al.*, 1996; Pintado *et al.*, 1996; Witthuhn *et al.*, 2004). *Acetobacter aceti* and *A. rasens*. The mycelial fungus, *Geotrichum candidum* has also been isolated from Kefir grains (Pintado *et al.*, 1996). The "normal flora" in kefir is made of very strong strains of microorganisms which inhibit the growth of undesirable and pathogenic microbes. Kefir will repopulate the digestive tract with good organisms. Thus, current knowledge of the amino acid catabolic pathways provides new prospects for controlling flavor development in fermented foods. The starter culture can be decisive for the sensory properties of the final product, since the ability of microorganisms to degrade amino acids to aroma compounds is highly strain dependent (Berdague *et al.*, 1993). The present paper reports the results obtained on the addition of the kefir starter at difference initial inoculum to a Thai fermented sausage for the microbiological and sensory quality of the final product. In the future, probiotic strains (the kefir starter) may be used in functional, probiotic meat products.

Materials and Methods

Materials

The kefir starter used in this study were provided by the Wilderness Family Naturals its contains the following probiotic organisms: *Lactococus lactis* subsp. lactis, *Lactococus lactis* subsp. cremoris, *Lactococus lactis* subsp. *diacetylactis, Leuconostoc mesenteroides* subsp. *cremoris, Lactobacillus kefyr, Kliyveromyces marxianus var. marxainus, Saccharomyces unisporus*. Activating the starter and transfering followed the Wildness Family Naturals instructions.

Samples and processing of Thai fermented sausages

The Thai fermented sausage composition was as follows: pork meat, lard and cooked rice (1.8, 1.5 and 3.5 kg. respectively), pork skin, garlic, salt, sugar and pepper (300, 350, 80 and 70 g., respectively) and fermented garlic solution 70 ml. Four batches, including batch C which was the control, and batch A , B and D with 1, 7 and 15 ml of kefir starter added were prepared, respectively. The mixture of each batch was manually stuffed in casings of regenerated collagen 27 mm in diameter and hand-linked to form approximately 2.5 cm long links. All four samples were fermented at 30°C for 48 h. The Thai fermented sausages from each batch were taken for microbial counts, determined by pour plate method every 8 h for 2 days. The initial dilution was made by aseptically blending in a stomacher for 60 s, 10 g of sample with 90 ml 1 g/l peptone solution. Lactic acid bacteria counts were estimated on plates of MRS Agar (Scharlau chemie S.A., Barcelona, Spain). The viable yeast counts were estimated on plates of YM agar (Scharlau chemie S.A., Barcelona, Spain). Two plates were inoculated from each dilution for each sampling point and the plates were incubated at 30°C for 48 h (lactic acid bacteria) and 28°C for 2-5 days (yeast). After incubation, plates with 30-300 colonies were counted and the results were expressed as log colony forming units (CFU)/g sample. The pH and total acid with lactic acid were determined (A.O.A.C., 2002). At the end of the curing process (2 days) each batch was vacuum packaged and stored at 4°C for sensory analysis and microbiological analysis

Sensory analysis

Sensory analyses were conducted by 25 panelists (laboratory panel) from students and faculty members in our institute who were experienced in sensory evaluation of foods, but received no specific training relevant to these products. Panelists were asked to indicate how much they liked or disliked each product on a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely) according to colour, odour, flavor, texture, and overall acceptability characteristics.

Bacteriological analysis

AOAC (1998) methods were used to determine total aerobic bacterial, *Escherichia coli, Staphylococcus aureus, Salmonella, Clostridium perfringens* and molds-yeasts counts. Total aerobic counts were determined on Plate Count Agar (PCA), after incubation at 35°C for 48 h. The presence of *E. coli* was confirmed by VRBA-MUG and the plates were incubated at 37°C for 48 h. Those giving positive fermentation and gas production in lactose broth were further characterized as *E. coli* using indole, methyl red, Voges-Proskauer and citrate (IMVC) identification tests. The presence of *Staphylococcus aureus* was tested by surface plating on prepoured and dried Baird Parker Agar with egg yolk tellurite enrichment. The plates were incubated at 37°C for 48 h. The presence of *C.perfringens* was tested by Trypticase Sulfide Neomycin and the plates were incubated at 37°C for 48 h. For the presence of *Salmonella*, samples enriched in Selenite Cysitein Broth were tested by Bismuth Sulfide Agar. The plates were incubated at 35°C for 24 h. Confirmation of *Salmonella* was by Triple Sugar Iron.

Statistical analysis

A one-way ANOVA was performed for pH, total acid and overall sensory quality values to determine significant differences ($P<0.05$) by using the Duncan's multiple range test using the SPSS version 10.0.

Results and Discussion

pH and total acid analysis

Results of pH and total acid are shown in Figure 1. The pH showed a sharp reduction during the fermentation stage (16-32 h.), the pH values decreased (from ~pH 5.5- 5.7 to ~pH 5.1-4.8) which could be due to production of organic acids by bacteria (Lucke, 1994), and then remained constant in the four batches up to the end of the process.(~pH 4). No significant differences ($p<0.05$) are detected between the pH and total acid

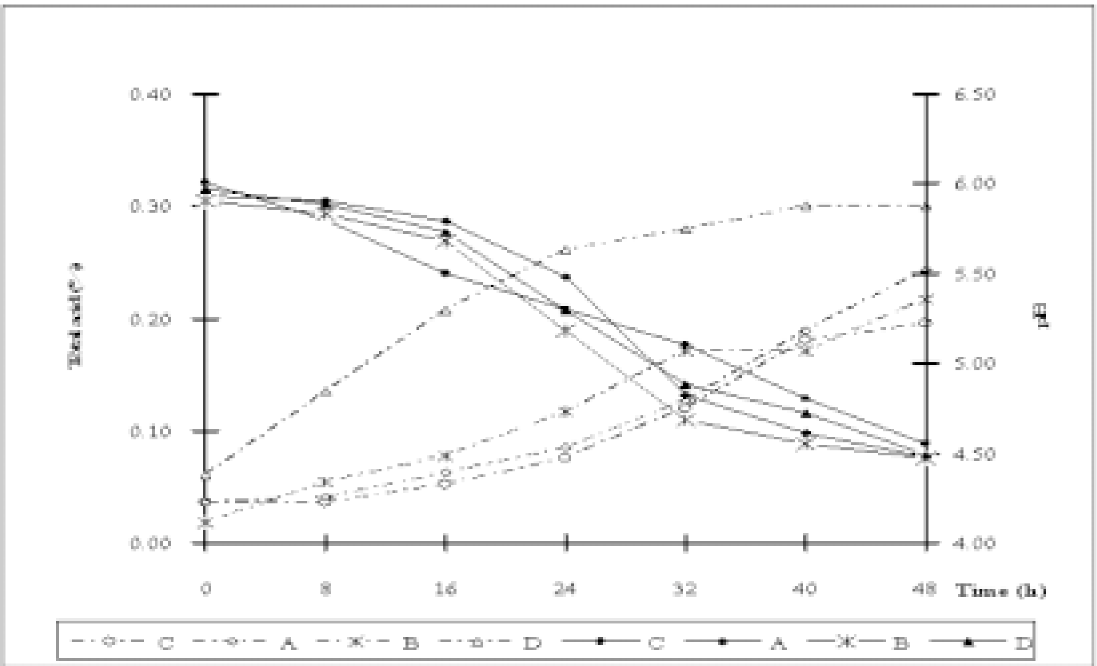

Figure 1. Evolution of pH (solid lines) and total acid (short dash lines) in different batches of Thai fermented sausages. batch C, which was the control, batches A , B and D had 1, 7 and 15 ml of kefir starter added respectively.

of the different batches (~0.05 %). These results were in agreement with literature that pH values decreased sharply during the first day of ripening due to the production of acid (Bozkurt and Erkmen, 2004). The pH was not significantly affected (*P*> 0.05) by the addition of kefir starter. The decline in the pH value during the first days of ripening (fermentation) is very important due to the inhibition of undesirable bacteria, rate of conversion of color, and formation of desired flavor in dry-fermented sausages (Lucke,1994). Only the total acidity of batch D containing kefir starter 15 ml was significantly higher ($p<0.05$) than those of the batches C, A and B. This is because the high amount inoculum gives a faster of end product rate. This could be explained by the fact that these bacteria are continuously in an acid environment, while the bacteria in a normal inoculum get refreshed. This was in agreement with Peter (1999) who considered a higher amount of inoculum (*Lactobacillus plantarum*) gave a quicker lowering of the pH from lactic acid production from whole grain wheat and bagerivetemjol. Another reason concerns the role in promoting symbiosis among the (LAB, yeasts); the lactic acid bacteria acidify the milk and the yeasts perform the alcoholic fermentation (Sorensen and Samuelsen, 1996 and Sorensen,1997). Lucke (1996) considered a number of LAB involved in the fermentation of a range of foods and beverages, such as dairy fermentations, meat and fish fermentations, vegetable fermentations, sourdough fermentation, wine and cider fermentations. However, in batch A, B, lipolysis was not promoted, showing the smallest total acid ($p<0.05$). This could be due to the addition of a smaller amount of kefir starter.

Microbial analysis

Figure 2 shows the evolution of lactic acid bacteria (LAB) and yeast counts through the

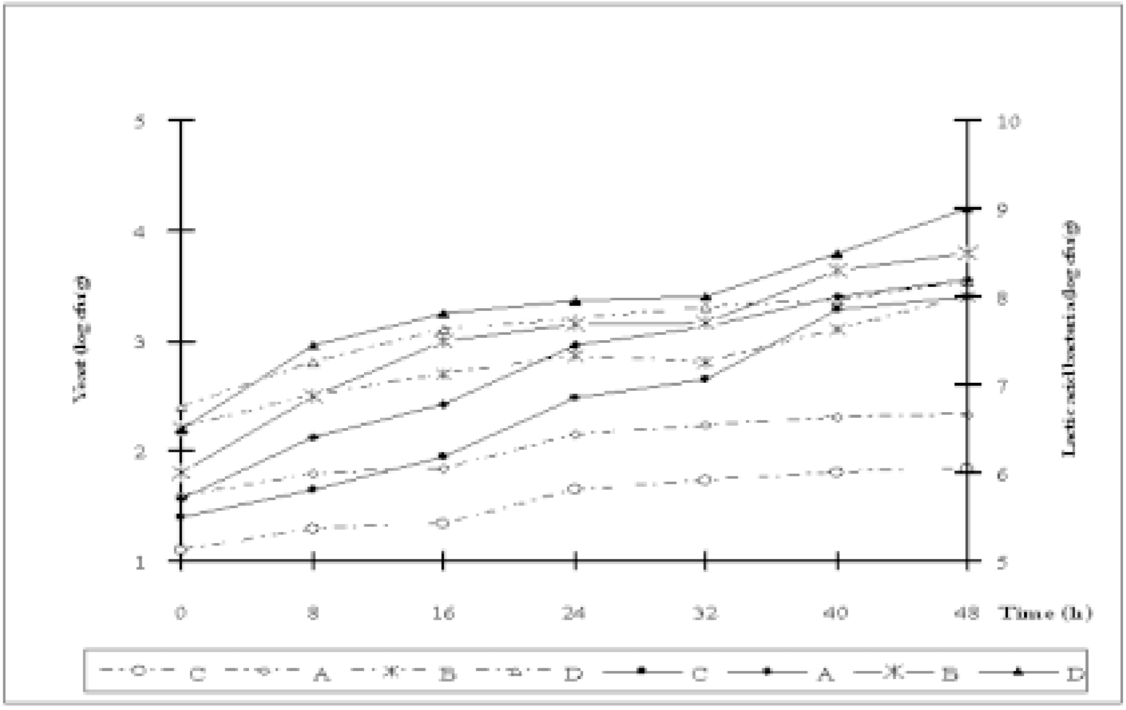

Figure 2. Evolution of lactic acid bacteria (solid lines) on MRS agar and yeast (short dash lines) YM agar in different batches of Thai fermented sausages. batch C, which was the control, batches A, B and D had 1, 7 and 15 ml of kefir starter added respectively.

fermentation of different batches of Thai fermented sausages. In general, the number of LAB and yeast were within the expected range for fermented sausages (Dura *et al.*, 2004 and Santos *et al.*, 2001), and the differences were not significant (p<0.05) among the four batches. LAB counts increased during the fermentation stage reaching the highest value (p<0.05) at 48 h. in the D batch. A good correlation was found between lactic acid bacteria count and pH, as also shown by Bloukas *et al.* (1997). The microbiological changes of fermented sausage during fermentation as a result of the combined effects of lowering the pH, increasing the brine content and decreasing the water activity due to drying, resulting in high populations of lactic acid bacteria (Flores and Bermel, 1996).

Sensory evaluation

In relation to the sensory properties no significant differences were observed in colour and texture between the batches (Table 1). The odour, flavor and overall acceptability of the B batch were different from those of the other three (P<0.05), C, A and D, which were very similar to each other (P>0.05). This is in agreement with the reports of Berdague *et al.*, 1993 and Witthuhn *et al.*, (2004) who showed that the starter culture can be decisive for the sensory properties of the final product, since the ability of microorganisms to degrade amino acids to aroma compounds is highly strain dependent. The enzymatic and chemical phenomena involved in flavor generation include carbohydrate fermentation, lipolylis, proteolysis, lipid oxidation and amino acid catabolism. The biochemical changes can be accelerated by the exogenous addition of different microorganisms and/or enzymes to the initial formulation (Lucke, 2000, Talon *et al.*, 2002 and Toldra *et al.*, 2001). Yeasts play an important role in promoting symbiosis among the microorganisms present, CO_2 formation and development of the characteristic taste and aroma (Koroleva, 1988). Also, the addition of *Lactococcus lactis* subsp. cremoris could result in a higher amount of free amino acids (Herranz *et al.*, 2004). The combination of LAB and yeast from kefir starter promoted the generation of volatile compounds derived from lipid oxidation and carbohydrate fermentation.

Bacteriological analysis

Total count of microorganism was found 10^2 cfu/g. *E. coli, S. aureus, Salmonella* spp., *C. perfringens* and yeasts-molds were not detected suggesting good hygienic quality in processing (data not show). This was in agreement with Geisen (1992), who considered high populations of lactic acid bacteria in fermented sausages inhibited the growth of spoilage and pathogenic bacteria, especially *Staphylococcus aureus*

Table 1. Sensory colour, odour, flavor, texture, and overall acceptability scores on a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely) of Thai fermented sausage with different kefir starter. batch C, which was the control, batches A , B and D had 1, 7 and 15 ml of kefir starter added respectively. At the end of the curing process (2†days)

Kefir starter	Sensory properties				
	Colour	Odour	Flavor	Texture	Overall acceptability
C	5.63 [a]	6.18 [b]	5.54 [b]	5.28 [a]	5.37 [b]
A	5.84 [a]	6.60 [b]	6.00 [b]	5.40 [a]	5.46 [b]
B	6.52 [a]	7.28 [a]	7.68 [a]	5.68 [a]	7.72 [a]
D	6.32 [a]	6.00 [b]	6.12 [b]	5.88 [a]	5.88 [b]

Note: Means in the same column with common letters (añb) are not different (p>0.05)

Conclusion

The pH values and main microbial counts did not show remarkable differences as a result of the incorporation of kefir starter in comparison to normal process. However, the added kefir starter improved the sensory quality of Thai fermented sausages. The addition of kefir starter (7 ml) could be useful to improve the final quality and the hygienic quality of Thai fermented sausages.

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