The effect of kefir starter on Thai fermented sausage product

Marisa Jatupornpipat¹ and Payom Keatikumjorn²

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

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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 microorganisms 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 Leucanostoc mesenteroides (Assadi et al., 2000; Cogan et al., 1997; Fujisawa et al., 1988; Kandler and Kunath, 1983; Micheli et al., 1999; Pintado et
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 different 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: Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis, Leuconostoc mesenteroides subsp. cremoris, Lactobacillus kefyr, Kluyveromyces marxianus var. marxainus, Saccharomyces unisporus. Activating the starter and transferring followed the Wilderness 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, odor, 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 Cysitene 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.](image_url)
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](image_url)

**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, lipolysis, 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, CO₂ 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 10² 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>
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<th>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)</th>
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Note: Means in the same column with common letters (a–b) 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.

References


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