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## Effect of Thai fruits on Sensory properties of Fruit Yogurt and Survival of Yogurt Starter Culture added with Probiotic Strains in Fruit Yogurt

Duangrutai Thumrongchote\*

Rajamangala University of Technology Krungthep, Faculty of Home-Economics Technology, Department of Food Technology and Nutrition 149 Chareon Krung Rd., Yannawa, Sathorn, Bangkok 10120, Thailand

### ABSTRACT

The effect of Thai fruits on the sensory properties of fruit yogurt, and the survival of yogurt starter culture, with added probiotic strains in fruit yogurt, were studied at a refrigerated temperature ( $5 \pm 2^{\circ}\text{C}$ ) during 3 weeks of storage. The purpose of this research was to study the influence of Thai fruits, including pineapple (*Ananas comosus* (L) Merr.), papaya (*Carica papaya* L.), and mango (*Manifera Indica* Linn.), on both the sensory properties and survival of fruit yogurt. The results revealed that the addition of Thai fruit purée was not significantly different ( $p > 0.05$ ) on the accepted mean score of fruit yogurt. The highest average mean score was fruit yogurt with pineapple (7.14), while fruit yogurt with mango and papaya had an average mean score 6.98 and 6.93. The decline of probiotic bacteria cells in pineapple fruit yogurt and mango fruit yogurt were significantly greater than papaya fruit yogurt. The morphology of fruit in yogurt after being stored for three weeks at a temperature of  $5 \pm 2^{\circ}\text{C}$ , after examination with an Electron microscope, revealed that papaya has a more porous structure than mangos and pineapples. Therefore, probiotic bacteria can be embedded and survive in papaya longer than in the other types of fruit purée.

**Keywords:** Probiotics; survival of probiotic bacteria; fruit yogurt; pineapple yogurt; papaya yogurt; mango yogurt.

\*Corresponding author

## INTRODUCTION

Yogurt is defined as a product resulting from milk by fermentation with the mixed starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* [1, 2]. It is normally produced using skimmed milk as a raw material. Yogurts differ according to their chemical composition, the method of production, the type of flavoring used, and the nature of the post incubation process. There are many types of yogurt; flavored yogurt or fruit yogurt, frozen yogurt, low-lactose yogurt, and heated yogurt.

In Thailand, yogurt containing beneficial microorganisms or probiotic cultures, such as lactobacilli and bifidobacteria, have become more popular. Probiotic bacteria in yogurt, which are living microorganisms that survive their passage through the digestive system, and help to restore microflora balance in the gut [3, 4]. There are many benefits of probiotic bacteria on the consumer health, such as control of number of bacteria in the colon, stimulation of the human immune system, the inhibition of some types of tumor cells, the reduction of cholesterol, and the symptoms of diarrhea [5, 6]. The Food and Drug Administration of Thailand declared that probiotic bacteria should be viable, active, and abundant (at least  $10^7$  CFU/mL) in fermented milk yogurt to the date of minimum duration [7]. The viability of probiotic bacteria in yogurt depends on strain, product acid, dissolved oxygen, fermentation time, storage temperature, incubation temperature, nutrients, the concentration of sugars, and food additives.

Lactobacilli are Gram positive and rod-shaped, while bifidobacteria are Gram positive rods of variable morphology that show branching and pleomorphism. Bifidobacteria were first isolated by Tissier at the Pasteur Institute, France, and dominate the gut flora of breast-fed infants. *Lactobacillus acidophilus* and *Bifidobacterium* spp. are difficult to propagate because of their specific nutritional requirements. Bifidobacteria are anaerobic, and therefore high oxygen levels may affect their growth and viability. However, *Lactobacillus acidophilus* is reported to have a high cytoplasmic buffering capacity (pH 3.72 to 7.74), which allows it to resist changes in cytoplasmic pH and to gain stability under acidic conditions. *Lactobacillus acidophilus* is more tolerant of acidic conditions than Bifidobacteria. The growth of *Bifidobacterium* spp. is significantly retarded below pH 4.0 [1, 8].

Furthermore, Sadaghar *et al.* [9] reported that the type of fruit effects the decline in cell count of probiotic bacteria in fruit fermented milk. They found that the decline in cell count of Lactobacilli strains in strawberry fermented milk was significantly greater than that of peach fermented milk. The twin objectives of this study were to assess the impact of adding Thai fruits to the sensory qualities of fruit yogurt, and to investigate the survival of probiotic cultures in fruit yogurt after being stored at a temperature of  $5 \pm 2^{\circ}\text{C}$  for three weeks.

## MATERIALS AND METHODS

### MATERIALS

#### *Strains*

Freeze-dried yogurt starter culture (Lyofast SAB442B) was obtained from Clerici-Sacco, Italy. The culture consisted of yogurt starter bacteria (*Streptococcus thermophilus*) and probiotic bacteria (*Lactobacillus acidophilus* and *Bifidobacterium animalis*).

#### *Starter culture*

The starter culture was prepared by mixing the yogurt starter culture in homogenized and sterilized milk at the ratio of milk: starter culture of 100:0.5 (w/w). The mixture was held overnight at a temperature of  $45 \pm 2$  °C.

#### *Thai fruit preparation*

The Thai fruits in this research consisted of three types; pineapple (*Ananas comosus* (L.) Merr.), papaya (*Carica papaya* L.), and mango (*Manifera Indica* Linn.). The fruit was peeled and cut into small pieces  $0.5 \times 0.5 \times 0.5$  cm<sup>3</sup>. The fruits were cooked in boiled 20 % (w/w) sucrose solution for 10 minutes, and then cooled to a temperature of  $37 \pm 2$  °C.

#### *Yogurt preparation*

Homogenized and sterilized milk was heated to a temperature of  $45 \pm 2$  °C and skim milk powder (SMP) was added with high-speed stirring, to make 180g/l total solids in yogurt. The mixture was heated to  $85 \pm 2$  °C and held at this temperature for 30 minutes, and then cooled to  $45 \pm 2$  °C. The yogurt starter culture (no. 2.2) was added to the yogurt mixture at ratio of 0.5 L starter culture: 2 L mixture and dispersed by mixing. The 100 mL yogurt mixture was poured in 4 oz plastic cup, which has 10 g Thai fruit (no. 2.3) in the bottom of cup, and incubated at  $45 \pm 2$  °C for 5 hours, after that the fruit yogurt was kept at  $5 \pm 2$  °C for 3 weeks.

### METHODS

#### *Determination of pH*

The pH of fruit yogurt samples was determined using a digital pH meter (Eutech, Cyberscan 1000, Singapore). The pH meter was calibrated using reference pH 4.0 and 7.0 buffer solution. An analysis to evaluate the pH level was made after the storage of fruit yogurt at a temperature of  $5 \pm 2$  °C for 0, 1, 2, and 3 week periods. All samples were analyzed in triplicate.

### **Sensory evaluation**

Fruit yogurt samples were coded with three digit random numbers and served at  $10 \pm 2$  °C to 30 untrained panelists, consisting of students from the Department of Food Technology and Nutrition, Rajamangala University of Technology Krungthep. A nine-point hedonic scale was used for appraising the sensory traits, viz. appearance, odor, texture, flavor and overall preference [10].

### **Microbiological analysis**

The viability of probiotic microorganisms in fruit yogurt samples were analyzed by viable plate count method under anaerobic conditions [11] after the storage of fruit yogurt at a temperature of  $5 \pm 2$  °C for 0, 1, 2, and 3 week periods. A one gram yogurt sample was weighed directly into a sterile 10 mL tube and mixed with 9 mL of peptone water (1.0g/L) and spread inoculated in duplicate onto plates of MRS agar (Merck, Germany). The inoculated plates were incubated at 37 °C for 72 hours under anaerobic conditions. All samples were analyzed in triplicate.

### **Scanning Electron Microscope (SEM)**

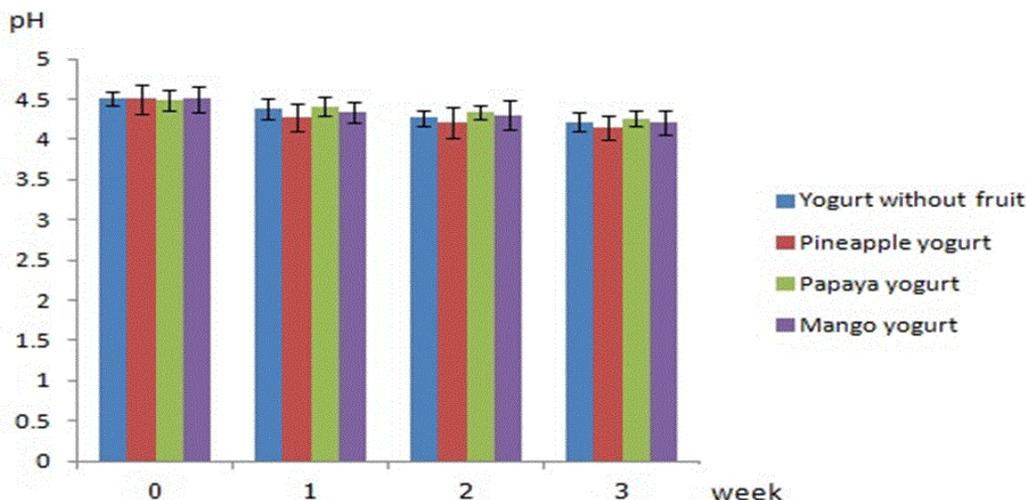
The samples of fruit set in yogurt, after being stored at a temperature of  $5 \pm 2$  °C for a three week period were analyzed with a SEM microscope (JEOL model JSM-6510, Tokyo, Japan) at an accelerating voltage of 15kV and a magnification rate of 15000 X.

## **RESULTS AND DISCUSSION**

### ***pH changes during fruit yogurt storage***

The pH changes in fruit yogurt samples during storage at  $5 \pm 2$  °C for a period of 3 weeks were not significantly different in all samples (in Fig. 1). The decline in pH of all the stored fruit yogurts occurred during the shelf life study. The decrease of pH levels in stored fruit yogurt had a tendency to decrease more than in yogurt without fruit. The initial pH value of the yogurt samples was around 4.50, while the final pH value of yogurt without fruit, pineapple yogurt, papaya yogurt, and mango yogurt were 4.22, 4.14, 4.26, and 4.20 respectively. This result was consistent with the findings of Sadaghdar et al. [9]; Kailasapathy, K. [12] and Saxelin et al. [13]. There was a significant difference ( $p < 0.05$ ) between the pH at day 0 and day 21 across all types of stored yogurt samples. The decrease in the pH value of yogurt, throughout its shelf life, may be due to the fact that the pH value of yogurt is dependent on factors including nutrient composition, and continued fermentation by yogurt bacteria in yogurt samples [14, 15]. In the initial phase of yogurt fermentation, *Streptococcus thermophilus* grows rapidly, reducing the pH value to approximately 5.4, which then stimulates the growth of *Lactobacillus* spp., which is both acid-tolerant and produces lactic acid, therefore reducing the pH value. *Sc. thermophilus* uses oxygen during its growth, which makes its oxidation-reduction potential more favorable for *Lactobacillus* spp., and it also produces formic acid, which stimulates the growth of

*Lactobacillus*, until the pH decreases to a level of 4.5. During storage at low temperatures, *Lactobacillus* and *Bifidobacterium* in fruit yogurt grow more slowly [16].



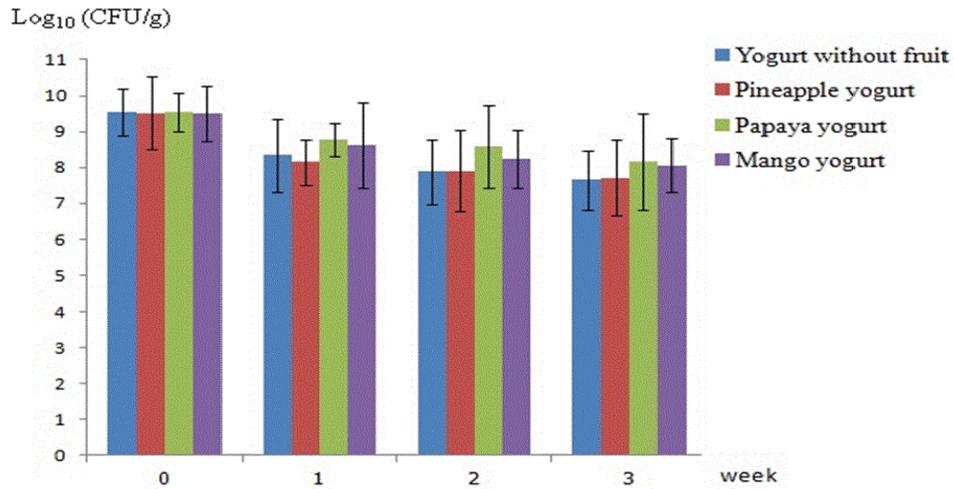
**Fig. 1** Changes in pH throughout the shelf life of fruit yogurt samples.

### **Effect of Thai fruit on probiotic viability**

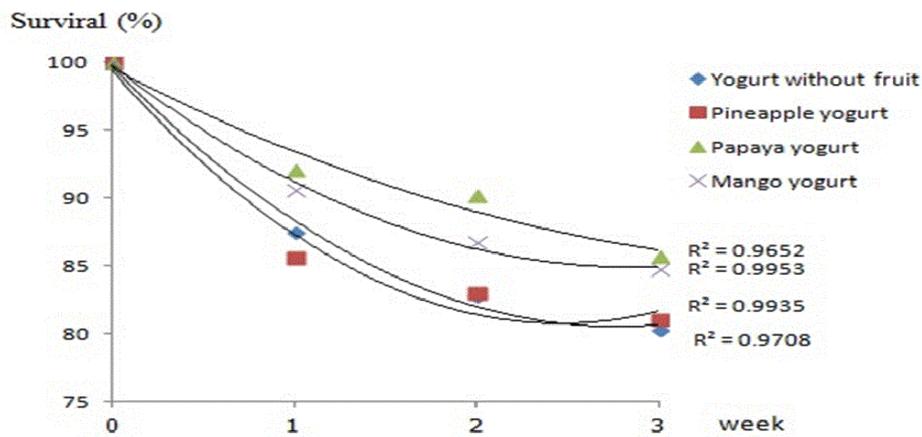
Fig. 2 shows the effect of Thai fruit on probiotic bacteria viability (*Lactobacillus acidophilus* and *Bifidobacterium animalis*) in yogurt samples after storage at a temperature of  $5 \pm 2$  °C for a period of three weeks. The average viability of probiotic bacteria decreased from  $3.37 \times 10^9$  cfu/g on day 0 to  $8.17 \times 10^7$  cfu/g on day 21, and all stored yogurt contained a higher amount of probiotic bacteria than the recommended level ( $10^6$ -  $10^7$  cfu/g) [7, 17]. The viability of probiotic bacteria in papaya yogurt was  $1.47 \times 10^8$  cfu/g on day 21, which was the highest count. The decline in bacterial counts may be due to the decline in the pH value of yogurt.

The results of the study showed that the survival of probiotic bacteria depends on the type of fruit used in yogurt. The numbers of viable cells in yogurt without fruit and pineapple yogurt decreased more than in papaya yogurt and mango yogurt during storage (Fig. 3). Thus, the type of fruit has an effect on the survival of probiotic bacteria in yogurt samples, especially papaya and mango. This result was consistent with the findings of Kailasapathy et al. [18].

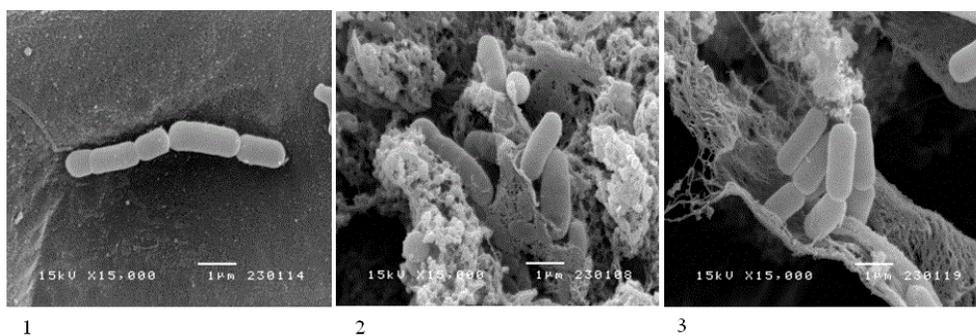
In this study, the morphology of fruit in yogurt samples indicated that fruit tissue promoted the survival of probiotic bacteria in yogurt stored at a temperature of  $5 \pm 2$  °C. Papaya tissue was determined to be more porous than that of mango or pineapple tissue, so probiotic bacteria could be inserted into the more porous tissue of papaya in order to survive longer (Fig. 4).



**Fig. 2** Viability of probiotic bacteria in fruit yogurt during storage at  $5 \pm 2$  °C



**Fig. 3** Survival rate (percentage) of probiotic bacteria in fruit yogurts during storage at  $5 \pm 2$  °C



**Fig. 4** Scanning electron micrographs of pineapple in pineapple yogurt (1), papaya in papaya yogurt (2), and mango in mango yogurt (3) after kept at  $5 \pm 2$  °C for 3 weeks with magnification of 15000 X.

**Sensory evaluation**

The sensory scores of the yogurt samples are provided in Table 1. The results showed that the addition of pineapple or mango significantly ( $p \leq 0.05$ ) affected odor, flavor, and overall preference more than yogurt with papaya or without fruit. However, it had no effect on the appearance, color and texture of yogurt. This may be due to the fact that the odor and the flavor of pineapple and mango improved the odor and the flavor of the yogurt more than the papaya did.

**Table 1** Sensory properties of fruit yogurt and various kinds of fruit

Characteristics	Yogurt without fruit	Pineapple yogurt	Papaya yogurt	Mango yogurt
Appearance <sup>ns</sup>	7.14±1.52	6.96±1.66	7.12±1.27	6.90±1.49
Color <sup>ns</sup>	6.62±1.80	6.81±1.02	6.75±1.48	6.57±1.22
Odor	6.48±1.40 <sup>b</sup>	7.57±1.19 <sup>a</sup>	6.52±1.05 <sup>b</sup>	7.06±1.13 <sup>ab</sup>
Texture <sup>ns</sup>	7.56±1.21	7.21±1.30	7.54±1.37	7.38±1.15
Flavor	6.40±0.54 <sup>bc</sup>	7.44±1.05 <sup>a</sup>	6.26±0.92 <sup>c</sup>	7.26±1.33 <sup>ab</sup>
Overall preference	6.13±1.07 <sup>b</sup>	7.34±0.92 <sup>a</sup>	5.76±1.45 <sup>b</sup>	7.05±1.10 <sup>a</sup>

**CONCLUSION**

The types of Thai fruits used in the yogurt affected some sensory properties, as well as the survival rate of the probiotic bacteria in the yogurt. The sensory scores, in terms of order and flavor, ranked pineapple yogurt and mango yogurt higher than papaya yogurt and yogurt without fruit ( $p \leq 0.05$ ). In addition, the type of fruit can significantly improve the survival rate of probiotic bacteria in fruit yogurt stored at a temperature of at  $5 \pm 2$  °C for three weeks. The decrease in percentage of the survival rate of probiotic bacteria in yogurt without fruit was greater than that of both papaya yogurt and mango yogurt. Furthermore, this study also revealed that the viability of probiotic bacteria was influenced by the type of fruit tissue.

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