

## Survival of probiotic bacteria in fruit juice jelly products

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### Abstract:

The aims of this work were to determine the sensory evaluation (in terms of color, odor, flavor, texture and overall liking) of three types of fruit juice jelly products, growth curves of *Lactobacillus acidophilus* TISTR 1338 and *L. casei* TISTR 390 and their survivals in fruit juice jelly products during storage at 5°C for 9 days. The sensory scores showed that there was no significant difference of overall liking scores among jelly products. According to their growth curves, both probiotic bacteria had entered the stationary phase with an average of 9 log cfu/ml after incubation for 26 h. Probiotic bacteria were cultured for 26 h for studying the survival of probiotic bacteria in fruit juice jelly products. It was found that the numbers of *L. acidophilus* TISTR 1338 and *L. casei* TISTR 390 decreased from the initial numbers of 9.23 log cfu/g and 8.97 log cfu/g to 6.80 log cfu/g and 7.73 log cfu/g, respectively, after 9 days of refrigerate storage. However, there was no significant difference ( $p > 0.05$ ) between viabilities of *L. acidophilus* TISTR 1338 and *L. casei* TISTR 390 on day 9. The survival of bacteria reduced during storage; however the final cell concentrations were still higher than the minimum therapeutic levels ( $\geq 5$  Log cfu/g) for probiotics in the products.

**Keywords:** Probiotic bacteria, Survivals, Fruit juice jelly

### Introduction

Probiotics are living microorganisms that provide a beneficial effect on human health, such as a decrease in serum cholesterol level, reduction of lactose intolerance, stimulation of immune system, improving the digestive system (Sanders et al., 2013), inhibiting the growth of pathogenic bacteria as well as synthesizing vitamins and antimicrobial agents. The efficiency of added probiotics depends on the dose level and their viability throughout storage, product

shelf life and their survival in the gut environment (Kailasapathy & Chin, 2000). Therefore, the concentration of probiotics in the product should be at least  $10^5$  cfu  $g^{-1}$  or  $mL^{-1}$  at the moment of consumption (FAO/WHO, 2001). Probiotic bacteria are usually added to dairy products, such as yogurt and milk because there is no need for great changes in the manufacturing processes. (Boza-Mendéz et al., 2012) but the use of dairy products may also be limited by lactose-intolerance, allergies and vegetarian (Perricone et al., 2014).

Recently, there has been an interest in the development of fruit and vegetable juices as functional beverages with probiotic (Mortazavian et al., 2006). Fruit juices may represent the alternative means of providing probiotic cultures to consumers because they are considered healthy products and are regularly consumed (Sheehan et al., 2007). Moreover, they are rich in sugars, minerals and vitamins, which can be used by probiotics and they contain no starter cultures to compete with the probiotic cultures for substrates (Ding & shah, 2008). However, juice supplementation with probiotics is more complicated than in dairy products, since the juices present insufficient quantities of peptides and free amino acid, required for the metabolism of probiotic cultures and the strains are usually sensitive to more acidic conditions (Sheehan et al., 2007). Moreover, probiotic cultures may change juice sensory characteristics (Granto et al., 2010).

Few studies have evaluated the effect of supplementation of fruit juice jelly products with probiotics. This can be seen as a further way to protect and preserve the probiotic cultures, since the product is classified as moderately moist and stable on storage, which is expected to reduce the growth rate of other microorganisms and also help stabilize the probiotic bacteria that are added to the product to achieve the required standard level of survival during storage.

Therefore, the objectives of this study were to evaluate the sensory characteristics of different fruit juice jelly products, growth curves of probiotic bacteria and their survivals in those products during storage at refrigeration temperature.

## **Materials and methods**

### ***Preparation of fruit juice jelly products***

Three different commercial fruit juices were purchased from a super market: orange juice (pH, 4.22; soluble solids, 17 °Bx), Lychee juice (pH, 4.05; soluble solids, 15 °Bx) and grape juice (pH, 3.49; soluble solids, 17 °Bx). Jelly preparation, drinking water (160 mL/L)

was heated to 80 °C, mixed with 80 g/L of sucrose and 6 g/L of carrageenan, then stirred for 3 min and mixed with 752 mL/L of juices. The prepared jelly was adjusted to pH 3.5-3.7 with 2 g/L citric acid. Samples of different juices were poured in a plastic cup and left at room temperature for gelling and stored at 5 °C.

### ***Activated probiotic cultures***

Freeze-dried cultures of *L. acidophilus* TISTR 1338 and *L. casei* TISTR 390 were obtained from Bangkok MIRCEN, TISTR, Thailand. The strain were inoculated into MRS (de Man, Rogosa, Sharpe) broth (HiMedia<sup>®</sup>) and incubated at 37 °C for 24 h, checked for purity and maintained on MRS Agar (HiMedia<sup>®</sup>). The strain was reactivated for three consecutive before use.

### ***Growth curves determination of probiotic bacteria***

The cultures were grown in MRS (de Man, Rogosa, Sharpe) (HiMedia<sup>®</sup>) medium 10% (v/v) inoculum and incubated at 37 °C for 48 h. A 3 mL sample of the culture was taken every 2 h to determine the absorbance at OD<sub>600</sub> via spectrophotometer and viable cell count using pour plate method.

### ***Sensory evaluation***

The sensory properties such as color, odor, flavor, texture and overall liking of three fruit juice jelly (orange, lychee and grape) were evaluated by 30 untrained panelists (11 women and 9 men) through 9 point hedonic scale test (9 = like extremely, 1 = dislike). Data from each panelist for each attribute were analyzed by two-way ANOVA.

### ***Survival of probiotic bacteria in grape juice jelly***

*L. acidophilus* TISTR 1338 and *L. casei* TISTR 390 were inoculated in MRS (de Man, Rogosa, Sharpe) (HiMedia<sup>®</sup>) medium and incubated at 37 °C for 26 h. Cultures were harvested by centrifugation at 2000xg at 25 °C for 15 min and washed twice with 0.85% sterile saline solution. The washed bacterial cells were re-suspended in 0.85% sterile saline solution and then added to the grape juices jelly solution. The grape juices jelly were then stored at 5 °C for 9 days.

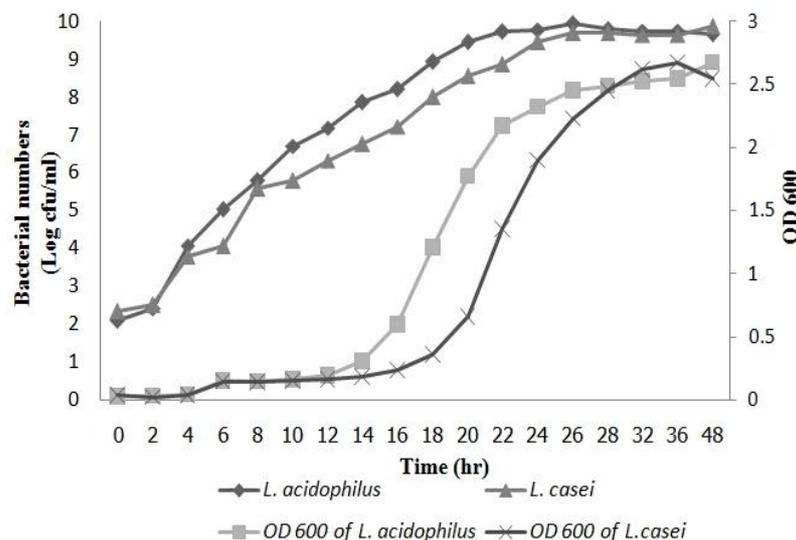
### Statistical analysis

The data were submitted to ANOVA by using SPSS version 17 for windows. The determination of significant differences among treatment means  $\pm$  standard deviation was done by Duncan's new multiple range tests.

### Results and discussion

#### Growth curves

The bacterial growth curves were presented in Fig. 1 for *L. acidophilus* TISTR 1338 and *L. casei* TISTR 390. The relation between OD<sub>600</sub> and log cfu/mL were used to estimate the concentration of viable cells in MRS medium under the same growth conditions. As seen in Fig. 1 and Fig. 2, it was found that both strains were in lag phase at 0-2 h, with the numbers of viable cells about 2- 2.5 log cfu/mL and then entered the log phase at 3-24 h, with an increase in numbers. After that, they entered the stationary phase after 26 h, with an average number of 9 log cfu/mL.



**Figure 1** OD 600 and viable count (log cfu/mL) of *L. acidophilus* TISTR 1338 and *L. casei* TISTR 390 over time in MRS medium incubated at 37 °C for 48 hrs.

### Sensory evaluation

The liking scores in term of color, odor, flavor, texture and overall liking of the jelly products are shown in Table 1. The results revealed that all three types of fruit juice jelly products had the liking scores in the range of 6-7 on a 9–point hedonic scale, indicating that the panelists had evaluated the products liking scores from “slightly” to “moderately”. In addition, attributes of color, odor, texture and overall liking of all products were not significantly different ( $p > 0.05$ ). However, the flavor scores of lychee and grape jelly products were significantly higher than orange jelly product ( $p \leq 0.05$ ). Eventually, grape jelly product was selected for further experiment.

**Table 1** Sensory scores of three types of fruit juice jelly products

| Attributes     | Types                    |                          |                          |
|----------------|--------------------------|--------------------------|--------------------------|
|                | Orange                   | Lychee                   | Grape                    |
| Color          | 7.36 ± 1.24 <sup>a</sup> | 6.96 ± 1.56 <sup>a</sup> | 7.46 ± 1.38 <sup>a</sup> |
| Odor           | 6.86 ± 1.43 <sup>a</sup> | 7.13 ± 1.43 <sup>a</sup> | 7.16 ± 1.57 <sup>a</sup> |
| Flavor         | 6.80 ± 1.47 <sup>b</sup> | 7.63 ± 1.12 <sup>a</sup> | 7.66 ± 1.18 <sup>a</sup> |
| Texture        | 7.56 ± 1.22 <sup>a</sup> | 7.66 ± 1.24 <sup>a</sup> | 7.60 ± 1.37 <sup>a</sup> |
| Overall Liking | 7.20 ± 1.12 <sup>a</sup> | 7.50 ± 0.97 <sup>a</sup> | 7.70 ± 1.17 <sup>a</sup> |

Means ± standard deviation in the same row followed by the same superscripts are not significantly different ( $p > 0.05$ ).

### Survival of probiotic bacteria in grape juice jelly.

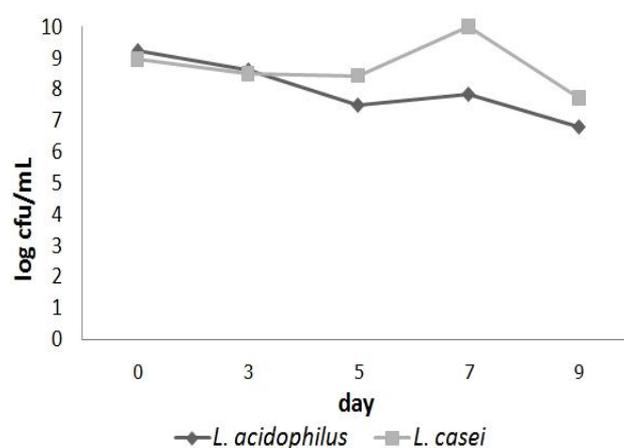
The survivals of *L. acidophilus* TISTR 1338 and *L. casei* TISTR 390 in the grape juice jelly during refrigeration storage (5 °C) for 9 days were determined. The numbers of surviving bacterial cells on day 0, 3, 5, 7 and 9 are presented in Fig. 3. The numbers of *L. acidophilus* TISTR 1338 and *L. casei* TISTR 390 decreased from the initial ones of 9.23 log cfu/g and 8.97 log cfu/g to 6.80 log cfu/g and 7.73 log cfu/g respectively, after 9 days of refrigeration storage. However, there was no significant difference ( $p > 0.05$ ) between viabilities of *L. acidophilus* TISTR 1338 and *L. casei* TISTR 390 on day 9. The results were similar to the studies of Mousavi et al., (2011), who reported that the microbial population of *L. paracasei* and *L. acidophilus* in pomegranate juice decreased approximately three

logarithmic during the first week of cold storage (4 °C) and lost their viability after 2 weeks. The reason could be related to the lack of their ability to survive in the low pH and high acidity of the pomegranate juice. Also Sheehan et al., (2007) reported that low pH fruit juices, with a range of pH typically between 2.5 and 3.7, caused the bacterial sensitivity to stressful conditions to increase.

A loss of probiotic viability could be related to acidity and the presence of oxygen in the product. When probiotic cells are present in low pH environments (<4.5), increased energy is required to maintain the intracellular pH, causing a lack of ATP for other critical functions and thereby causing cell death (Nualkaekul et al., 2011). In addition, the presence of oxygen can cause formation and accumulation of toxic metabolites in cells, which can lead to cell death by oxidative damage (Boza-Mendéz et al., 2012).

In order to enhance the survival of probiotic bacteria in low pH products, Sohail et al., (2012) improved the viability of *L. rhamnosus* and *L. acidophilus* using microencapsulation method, which reduced the acidification and assured the survival of probiotics at 25 °C for at least 9 days in orange juice. Ding and shah (2008) also reported that fruit juices containing microencapsulated probiotic bacteria were more stable than those containing free probiotic. Moreover, encapsulated probiotics (*L. rhamnosus*, *L. salivarius*, *L. plantarum*, *L. acidophilus*, *L. paracasei*, *B. longum*, *B. lactis* type Bi-o4 and Bi-07) were protected from the acidic environment of orange juice.

Although in the present study, the survival of probiotic bacteria reduced during storage, the final cell count was still higher than the minimum therapeutic dose (FAO/WHO, 2001).



**Figure 3.** Survival of *L. acidophilus* TISTR 1338 and *L. casei* TISTR 390 in the grape juice jelly during refrigeration storage (5 °C) for 9 days.

## Conclusion

Grape juice could successfully be used to deliver probiotic bacteria to consumers since the probiotic numbers were higher than the minimum therapeutic dose after refrigeration storage. Thus, this product could be a challenging one for alternative choice of functional foods in the market.

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