# Impact of Selected Cultures of Probiotics on Quality of Vanilla Low Fat Ice Cream during Storage

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**บทคัดย่อ**

เชื้อโพรไบโอติกสายพันธุ์ที่แตกต่างกันมีความสามารถในการผลิตสารประกอบอินทรีย์ที่มีความหลากหลายทั้งชนิดและปริมาณ ส่งผลให้ผลิตภัณฑ์อาหารหมักที่ได้มีลักษณะทางประสาทสัมผัสที่เป็นเอกลักษณ์ ในงานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของเชื้อโพรไบโอติก 3 สายพันธุ์ต่อคุณภาพของไอศกรีมในระหว่างการเก็บรักษา โดยผลิตไอศกรีมวนิลาไขมันต่ำจำนวน 4 ทรีทเมนต์ ได้แก่ ไอศกรีมวนิลาไขมันต่ำผสม *Lactobacillus acidophilus* BCC51147, ไอศกรีมวนิลาไขมันต่ำผสม *Lactobacillus rhamnosus* DSM20021, ไอศกรีมวนิลาไขมันต่ำผสม *Lactobacillus casei* 01 และไอศกรีมวนิลาไขมันต่ำสูตรควบคุม (ไม่เติมเชื้อโพรไบโอติก) แล้วเก็บตัวอย่างไว้ที่อุณหภูมิ -20 องศาเซลเซียส เป็นเวลา 40 สัปดาห์ วัดการรอดชีวิตของเชื้อโพรไบโอติกในไอศกรีม พีเอช ปริมาณกรด สมบัติการละลาย และการยอมรับทางประสาทสัมผัสของไอศกรีม ในสัปดาห์ที่ 0, 20 และ 40 หลังการเก็บรักษาเป็นเวลา 40 สัปดาห์พบว่า เชื้อโพรไบโอติกทุกสายพันธุ์มีอัตราการรอดชีวิตอยู่ที่ 96-98% จากปริมาณเชื้อตั้งต้นซึ่งคิดเป็นจำนวนมากกว่า 7 log cfu/g ไอศกรีมที่เติมโพรไบโอติกมีค่าพีเอชต่ำกว่าไอศกรีมสูตรควบคุมอย่างมีนัยสำคัญ ($p< 0.05$) โดยไอศกรีมที่เติม *L. acidophilus* มีค่าพีเอชต่ำที่สุด ไอศกรีมสูตรควบคุมและไอศกรีมที่เติม *L. rhamnosus* มีปริมาณกรดต่ำกว่าสูตรอื่นอย่างมีนัยสำคัญ ($p< 0.05$) ไอศกรีมที่เติม *L. acidophilus* และไอศกรีมที่เติม *L. rhamnosus* มีอัตราการละลายสูงที่สุดอย่างมีนัยสำคัญ ($p< 0.05$) การทดสอบทางประสาทสัมผัสพบว่าไม่มีความแตกต่างกันอย่างมีนัยสำคัญ ($p>0.05$) ระหว่างไอศกรีมที่เติมโพรไบโอติกและไอศกรีมสูตรควบคุมในด้านลักษณะปรากฏ สี และความชอบโดยรวม และเมื่อเก็บไอศกรีมที่เติมโพรไบโอติกไว้เป็นระยะเวลานานขึ้นพบว่า การยอมรับทางด้านกลิ่นรส รสชาติ และเนื้อสัมผัส มีค่าสูงกว่าไอศกรีมสูตรควบคุม ผู้บริโภคไม่พบความแตกต่างทางด้านลักษณะปรากฏ สี กลิ่นรส รสชาติ และความชอบโดยรวมของไอศกรีมที่เติมโพรไบโอติก ในขณะที่ไอศกรีมที่เติม *L. acidophilus* และ ไอศกรีมที่เติม *L. rhamnosus* มีคะแนนการยอมรับในด้านเนื้อสัมผัสสูงที่สุด

**คำสำคัญ :** *Lactobacillus acidophilus*; *Lactobacillus rhamnosus*; *Lactobacillus casei* การรอดชีวิต, ไอศกรีม

**Abstract**

Different strains of bacteria can produce different types and ratios of organic acids and volatile compounds, resulting in unique overall sensorial characteristics of fermented food products. The objective of this study was to investigate the effect of probiotic strains on ice cream quality during frozen storage. Three strains of probiotic, *Lactobacillus acidophilus* BCC51147, *Lactobacillus rhamnosus* DSM20021, and *Lactobacillus casei* 01, were incorporated into low fat vanilla ice cream. The samples were kept at -20ºC for 40 weeks. The viability of the probiotics at 0, 20, and 40 weeks was measured by viability count. The pH, acidity, melting property, and consumer acceptance of the ice cream were also determined at 0, 20, and 40 weeks. After 40 weeks of storage, all probiotic strains showed survival rates of 96-98% with viability of more than 7 log cfu/g. Probiotic ice cream had significantly lower pH than the control (no probiotic added) ($p< 0.05$). Ice cream with added *L. acidophilus* showed the lowest pH. Acidity of the control and probiotic ice cream with added *L. rhamnosus* had the lowest acidity ($p< 0.05$). Probiotic ice cream samples with added *L. acidophilus* and *L. rhamnosus* had the highest melting rate ($p< 0.05$). Consumer acceptance of probiotic ice cream was not significantly different from that of the control in terms of appearance, color, and overall acceptance ($p> 0.05$). At longer storage times, higher acceptance scores in terms of flavor, taste, and texture were observed for probiotic ice cream ($p< 0.05$) compared to those of the control. No significant difference in sensory scores for appearance, color, flavor, taste, or overall acceptance were found among probiotic ice cream samples, whereas probiotic ice cream containing *L. acidophilus* and *L. rhamnosus* was found to have the highest consumer acceptance in terms of texture.

**Keywords:** *Lactobacillus acidophilus*; *Lactobacillus rhamnosus*; *Lactobacillus casei*; Viability; Ice Cream

**Introduction**

Probiotics are defined as “live microorganisms which when administered in adequate amounts confer health benefits on the host” [1]. Food products containing probiotics have been commercialized and gained popularity worldwide [2], [3]. To exhibit health benefits, probiotics have to survive in the product. It is recommended that food used as a probiotic carrier should contain at least 6 log colony-forming units of viable probiotics per gram of food [4], [5], [6]. However, panelists differentiated a product with an added probiotic from the control [7]. Bacteria cultures of different species and strains have different abilities to produce various metabolites contributing to food product characteristics including physicochemical and sensory properties. The ratio of lactic acid, acetaldehyde, acetoin, and ethanol in milk fermented by *Lactobacillus johnsonii*, *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, *Lactobacillus acidophilus*, and *Bifidobacterium animalis* were different [8]. Ice cream containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum* or both had different product characteristics including viscosity and pH [9]. Addition of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* changed the pH and acidity of the ice cream [7]. However, there is little information concerning changes during storage of low fat ice cream added with *Lactobacillus rhamnosus*, *Lactobacillus casei,* and *Lactobacillus acidophilus*. This study investigated the influence of *L. acidophilus*, *L. rhamnosus* and *L. casei* on physicochemical properties and consumer acceptance of the vanilla low fat ice cream during frozen storage.

**Materials and Methods**

**Preparation of probiotic mixture**

Probiotic mixture was prepared by adding *Lactobacillus casei* 01 (Chr. Hansen, Hørsholm, Denmark), *Lactobacillus rhamnosus* DSM20021 (Institute of Food Science, BOKU, Austria), or *Lactobacillus acidophilus* BCC51147 (BIOTEC, NSTDA, Thailand) to sterilized skim milk. The sterilized skim milk was prepared by heating ten percentage (w/w) of skim milk (CP - Meji Co., Ltd., Thailand) in the formula supplemented with 3% (w/w) inulin (Jebsen and Jessen Technology Co., Thailand) at 121°C for 15 minutes. The mixture was incubated at 37ºC until the final probiotic quantity of 9 log cfu/g was obtained.

**Production of vanilla low fat ice cream**

Ice cream containing 3% milk fat, 13% milk solid non fat, 11% sucrose, 5.5% glucose syrup, 0.1% emulsifier, and 0.32% stabilizer was formulated. Skim milk and cream (38% fat) (Foremost Friesland (Thailand) PCL.) were mixed and heated to 40ºC prior to adding skim milk powder (FA groups Co., Ltd., Thailand), sugar, glucose syrup, di-mono glycerol (Berli Jucker Public Co., Ltd., Thailand), 0.3% xanthan gum, and 0.02% carrageenan (NutritionSc Co., Ltd., Thailand). The ice cream mix was pasteurized at 75ºC for 15 minutes and homogenized (Ystral GmbH, Germany). Then the mix was cooled to 4ºC and refrigerated for 16 hours. After adding 0.05% vanilla flavor, the mix was frozen (Homemate® freezer, China). The soft ice cream was hardened at -20ºC for 24 hours. The ice cream was stored at -20ºC until analysis. For production of ice cream added with probiotic, the probiotic mixture was added to the ice cream mix prior to freezing.

**Overrun**

Overrun of the ice cream was determined by using weight of the mix before freezing and weight of equal volume of the ice cream after freezing according to the method described by Marshall *et al* [10] and calculated as shown in the following equation.

$$\text{Overrun}(\%) = \frac{\text{Weight of mix} - \text{Weight of ice cream}}{\text{Weight of mix}} \times 100$$

**Probiotic viability**

Pour plate technique on MRS agar (Becton, Dickinson and Company, USA.) was used to measure the viability of the probiotics. Plates were incubated at 37ºC for 48 hours in an anaerobic condition. Viability was recorded as logarithm of colony forming units (cfu)/g sample. The survival rate of the probiotic was calculated as shown in this equation.

$$\text{Survival rate}(\%) = \frac{\text{log cfu/g}}{\text{log cfu/g on Day 1}} \times 100$$

**Acidity**

Acidity was determined by titration of ten grams of melted ice cream with 0.1 N sodium hydroxide and expressed as %acidity (lactic acid equivalent) [11] as shown in the following equation.

$$\text{Acidity}(\%) = \frac{\text{Normality of NaOH} \times \text{Volume of NaOH} \times 0.009 \times 1000}{\text{Weight of melted ice cream}}$$

**Melting property**

Forty-two grams of ice cream was placed on a 20-mesh screen above a beaker and was left to melt. After first drip, ice cream was weighed every 5 minutes. The melting rate of the sample was reported as a linear regression coefficient of weight of the melted ice cream on the melting time.

**Sensory acceptance**

One hundred consumers aged between 19-23 years old were recruited to evaluate the ice cream with respect to their degree of liking the color, appearance, flavor, taste, texture, and overall liking using a 9-point hedonic scale (1=dislike extremely and 9=like extremely).

**Statistical analysis**

Completely Randomized Design (CRD) was applied for all experiments. Analysis of variance (ANOVA) was applied for the determination of the main effect. Ducan's new multiple range test was used to separate the mean of the main effect when significant differences ($p < 0.05$) were observed.

**Results and Discussion**

The number of *L. acidophilus* in the probiotic mixture was the lowest and the number of *L. casei* was the highest (Table 1). Probiotic mixture prepared from *L. acidophilus* had the lowest pH and the highest acidity, whereas probiotic mixture prepared from *L. rhamnosus* had the highest pH and the lowest acidity (Table 1). Although *L. acidophilus* presented in low quantity, it produced the highest amount of acid, showing the greatest acid producing ability among cultures used in this study, while *L. rhamnosus* produced the least amount of acid. This is in accordance with a previous report [8].

**Table 1** Probiotic content, pH, and acidity of probiotic mixture before added to ice cream mix

| Probiotic mixture | Probiotic (log cfu/g) | pH | %Acidity (lactic acid equivalent) |
|---|---|---|---|
| added *L. acidophilus* | $8.88 \pm 0.06^{c}$ | $4.11 \pm 0.04^{c}$ | $1.56 \pm 0.06^{a}$ |
| added *L. rhamnosus* | $9.13 \pm 0.05^{b}$ | $5.06 \pm 0.02^{a}$ | $0.72 \pm 0.04^{c}$ |
| added *L. casei* | $9.40 \pm 0.09^{a}$ | $4.50 \pm 0.04^{b}$ | $1.12 \pm 0.06^{b}$ |

a, b, … Different letters in the same column indicates statistically different ($P < 0.05$).

Overrun of the ice creams added with *L. rhamnosus*, *L. casei*, and the control were not significantly different and were higher than that of the ice cream added with *L. acidophilus* (Table 2). Overrun of the ice cream indicates air incorporated into ice cream. This result showed that the amount of air incorporated into the ice cream added with *L. acidophilus* was less than that in the ice cream added with *L. rhamnosus* and *L. casei*.

**Table 2** Overrun of vanilla low fat ice creams

| Ice cream sample | %overrun |
|---|---|
| Control | $48.34 \pm 1.56^{a}$ |
| added *L. acidophilus* | $37.11 \pm 2.39^{b}$ |
| added *L. rhamnosus* | $47.72 \pm 0.27^{a}$ |
| added *L. casei* | $48.54 \pm 0.86^{a}$ |

a, b, … Different letters in the same column indicates statistically different ($P < 0.05$).

The quantity of probiotic cultures in the ice cream was more than 7 log cfu/g (Table 3) which was higher than the recommended therapeutic dose [4], showing a survival rate of more than 96% throughout the storage time of 40 weeks. The survival rate and amount of probiotics in this study was the highest reported in any low fat ice cream added probiotics using the same culture under the same storage conditions. Viability of *L. casei* was the highest and did not reduce significantly during storage, whereas the viability of *L. acidophilus* was the lowest and significantly decreased with increasing storage time. Since the overrun of the ice cream added with *L. acidophilus* was the lowest, the reduction of *L. acidophilus* in ice cream may be due to low oxygen tolerance of the culture. Oxygen present in its habitat is toxic to *L. acidophilus* [12], leading to death of the bacteria. This is in agreement with Ferraz *et al* [13] who demonstrated that *L. acidophilus* was sensitive to oxygen present in ice cream.

Soon after the ice cream was manufactured, the ice cream added with *L. acidophilus* and *L. casei* had the lowest pH and highest acidity (Table 4). This low pH and high acidity may be due to the property of the probiotic mixture added during ice cream processing. Although the pH of the probiotic mixture prepared from *L. rhamnosus* was lower than that of normal milk, the pH and acidity of ice cream added with *L. rhamnosus* and the control were not significantly different (Table 4). This may be due to the high buffering capacity of milk proteins and milk salts [14], resulting in very low pH change in the ice cream added with *L. rhamnosus*. After 20 weeks of storage, only the ice cream added with *L. acidophilus* had the lowest pH and highest acidity, whereas *L. casei* had the highest viability, the ice cream added with *L. casei* did not have the lowest pH value. Since *L. acidophilus* survived in the ice cream with the lowest quantity (Table 3), it showed the ability of the culture to produce a high amount of acid even in frozen storage conditions. This high acid content may contribute to the complicated structure of the ice cream.

**Table 3** Viability of probiotics in vanilla low fat ice cream during 40 weeks of storage

| Ice cream sample | Day 1 | Week 20 | | Week 40 | |
|---|---|---|---|---|---|
| | log cfu/g | log cfu/g | Survival rate (%) | log cfu/g | Survival rate (%) |
| added *L. acidophilus* | 7.51±0.06$^{cA}$ | 7.46±0.01$^{cA}$ | 99 | 7.24±0.02$^{bB}$ | 96 |
| added *L. rhamnosus* | 7.69±0.08$^{b}$ | 7.68±0.05$^{b}$ | 100 | 7.51±0.14$^{ab}$ | 98 |
| added *L. casei* | 7.89±0.09$^{a}$ | 7.95±0.03$^{a}$ | 101 | 7.66±0.23$^{a}$ | 97 |

A, B, … Different letters in the same row indicates statistically different ($P < 0.05$).

a, b, … Different letters in the same column indicates statistically different ($P < 0.05$).

**Table 4** pH and acidity of vanilla low fat ice cream during 40 weeks of storage

| **Ice cream sample** | **pH** | | | **%Acidity (lactic acid equivalent)** | | |
|---|---|---|---|---|---|---|
| | **Day 1** | **Week 20** | **Week 40** | **Day 1** | **Week 20** | **Week 40** |
| Control | $6.43±0.03^{aB}$ | $6.53±0.03^{aA}$ | $6.48±0.03^{aAB}$ | $0.27±0.01^{bB}$ | $0.29±0.01^{bA}$ | $0.28±0.01^{cA}$ |
| added *L. acidophilus* | $6.22±0.06^{bAB}$ | $6.26±0.02^{dA}$ | $6.16±0.02^{dB}$ | $0.33±0.02^{a}$ | $0.35±0.01^{a}$ | $0.32±0.01^{a}$ |
| added *L. rhamnosus* | $6.41±0.06^{a}$ | $6.45±0.01^{b}$ | $6.42±0.02^{b}$ | $0.29±0.01^{b}$ | $0.30±0.01^{b}$ | $0.29±0.02^{bc}$ |
| added *L. casei* | $6.24±0.02^{bB}$ | $6.33±0.02^{cA}$ | $6.31±0.03^{cA}$ | $0.31±0.02^{a}$ | $0.33±0.01^{a}$ | $0.31±0.02^{ab}$ |

A, B, … Different letters in the same row indicates statistically different ($P < 0.05$).

a, b, … Different letters in the same column indicates statistically different ($P < 0.05$).

The melting rate of the control was significantly lower than that of the ice cream added with probiotic and increased with increasing storage time (Table 5). The increasing melting rate may be due to the loss of ice cream air cells. During storage, air cells in close proximity coalesced, resulting in larger air cell sizes [15], which ultimately moved upwards and left the ice cream surface [16]. Since air cells retarded ice cream melting [15] due to their heat-proofing effect [10], ice creams stored for longer time melted faster. No significant change in the melting rate of ice cream added with *L. acidophilus* was found during storage. Ice cream added with *L. acidophilus* had a more complicated structure compared to other ice creams due to protein-protein interaction induced by high acid content. This complex structure may inhibit the movement of air cells out of the ice cream.

The consumer test (Table 6 – 8) revealed no significant differences between the control and the ice creams added with probiotic in appearance, color, and overall liking ($p>0.05$). Interestingly, acceptance scores of ice creams added with probiotic in flavor, taste, and texture increased with increasing storage time and ultimately were higher than those of the control ($p<0.05$). Among ice creams added with probiotics, ice creams added with *L. acidophilus* and *L. rhamnosus* had higher texture acceptance than the ice cream added with *L. casei* ($p<0.05$). The greater texture acceptability of the ice cream added with *L. acidophilus* may be due to the high acid content of the ice cream, leading to additional protein-protein interaction, and causing a smoother texture of the ice cream. Although *L. rhamnosus* had the lowest ability to generate acid, it was capable of producing exopolysaccharide [17], which in turn affected the consumer liking of the ice cream.

## Conclusion

Probiotics of different cultures can influence ice cream characteristics including pH, acidity, melting rate, and sensory properties. Ice creams added with probiotic, especially *L. acidophilus*, had lower pH, higher acidity, and higher melting rates, and received higher consumer acceptance compared to the control. Not only benefits and viability of probiotics to reach the recommended dose should be considered for producing probiotic products, but changes of product characteristics due to probiotic metabolites have to be taking into account. Compatibility of products and metabolites of probiotic cultures needs to be evaluated.

**Table 5** Melting rate of vanilla low fat ice cream during 40 weeks of storage

| Ice cream sample | Day 1 | Week 20 | Week 40 |
|---|---|---|---|
| Control | $4.62 \pm 0.36^{cB}$ | $4.70 \pm 0.30^{bB}$ | $5.60 \pm 0.06^{aA}$ |
| added *L. acidophilus* | $5.09 \pm 0.07^{ab}$ | $5.11 \pm 0.26^{ab}$ | $5.35 \pm 0.28^{a}$ |
| added *L. rhamnosus* | $5.20 \pm 0.09^{aA}$ | $4.79 \pm 0.17^{abB}$ | $5.23 \pm 0.17^{abA}$ |
| added *L. casei* | $4.78 \pm 0.11^{bcB}$ | $5.17 \pm 0.11^{aA}$ | $4.87 \pm 0.22^{bAB}$ |

A, B, … Different letters in the same row indicates statistically different ($P < 0.05$).

a, b, … Different letters in the same column indicates statistically different ($P < 0.05$).

**Table 6** Consumer acceptance of vanilla low fat ice cream on first day of storage

| Ice cream sample | Appearance $^{ns}$ | Color $^{ns}$ | Flavor | Taste | Texture | Overall liking |
|---|---|---|---|---|---|---|
| Control | 6.52±1.51 | 6.76±1.30 | $6.64 \pm 1.59^{b}$ | $7.16 \pm 1.34^{b}$ | $6.97 \pm 1.50^{b}$ | $7.07 \pm 1.17^{b}$ |
| added *L. acidophilus* | 6.76±1.17 | 6.78±1.28 | $6.54 \pm 1.65^{ab}$ | $6.86 \pm 1.52^{ab}$ | $6.91 \pm 1.40^{b}$ | $6.97 \pm 1.20^{ab}$ |
| added *L. rhamnosus* | 6.65±1.36 | 6.85±1.25 | $6.57 \pm 1.57^{ab}$ | $6.70 \pm 1.78^{a}$ | $6.90 \pm 1.62^{b}$ | $7.04 \pm 1.32^{b}$ |
| added *L. casei* | 6.54±1.42 | 6.741.19 | $6.27 \pm 1.37^{a}$ | $6.64 \pm 1.44^{a}$ | $6.53 \pm 1.44^{a}$ | $6.73 \pm 1.22^{a}$ |

a, b, … Different letters in the same column indicates statistically different ($P < 0.05$).

ns Not significantly different.

**Table 7** Consumer acceptance of vanilla low fat ice cream after 20 weeks of storage

| Ice cream sample | Appearance | Color $^{ns}$ | Flavor$^{ns}$ | Taste | Texture | Overall liking$^{ns}$ |
|---|---|---|---|---|---|---|
| Control | $6.20 \pm 1.37^{a}$ | 6.33±1.34 | 6.41±1.29 | $6.59 \pm 1.36^{a}$ | $6.64 \pm 1.57^{a}$ | 6.69±1.35 |
| added *L. acidophilus* | $6.38 \pm 1.32^{ab}$ | 6.54±1.26 | 6.56±1.35 | $6.80 \pm 1.40^{b}$ | $6.62 \pm 1.35^{a}$ | 6.82±1.23 |
| added *L. rhamnosus* | $6.48 \pm 1.30^{b}$ | 6.49±1.46 | 6.51±1.69 | $6.81 \pm 1.63^{b}$ | $6.53 \pm 1.57^{a}$ | 6.74±1.44 |
| added *L. casei* | $6.36 \pm 1.27^{ab}$ | 6.56±1.24 | 6.49±1.26 | $6.97 \pm 1.12^{c}$ | $7.05 \pm 1.15^{b}$ | 6.98±1.15 |

a, b, … Different letters in the same column indicates statistically different ($P < 0.05$).

ns Not significantly different.

**Table 8** Consumer acceptance of vanilla low fat ice cream after 40 weeks of storage

| Ice cream sample | Appearance [ns] | Color [ns] | Flavor | Taste | Texture | Overall liking |
|---|---|---|---|---|---|---|
| Control | 6.80±1.19 | 6.93±1.27 | 6.09±1.66[b] | 6.53±1.45[b] | 6.52±1.37[c] | 6.75±1.20[b] |
| added *L. acidophilus* | 6.94±1.32 | 7.05±1.25 | 6.66±1.51[a] | 6.89±1.48[a] | 6.94±1.41[a] | 7.11±1.30[a] |
| added *L. rhamnosus* | 6.89±1.41 | 7.10±1.31 | 6.61±1.63[a] | 6.90±1.72[a] | 6.85±1.55[ab] | 6.94±1.52[b] |
| added *L. casei* | 6.84±1.35 | 6.99±1.29 | 6.31±1.71[ab] | 6.57±1.90[ab] | 6.62±1.61[bc] | 6.79±1.55[b] |

a, b, … Different letters in the same column indicates statistically different ($P < 0.05$).

ns Not significantly different.

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