

Sensory and Acceptance Assessment of Yogurt Containing Probiotic Beads in Thailand

Wunwisa Krasaekoott* and Atittaya Tandhanskul

ABSTRACT

Although the encapsulation technique has been proved to increase the survival of probiotics in yogurt, the sensory and acceptance assessment of yogurt containing probiotic beads has not been reported. In this research, the acceptance assessment of this product was performed by consumers in Thailand and the sensory of products was evaluated by using descriptive analysis. Four hundreds yogurt consumers from Bangkok and the suburbs of Bangkok were recruited. Plain and strawberry yogurts containing probiotics beads were served with the questionnaire to determine the consumer demographic, buying behavior and consumer acceptance. The consumers bought yogurt because of its taste (8.8) and nutrients (8.5). Although the addition of probiotic beads affected the texture characteristics by increasing viscosity and difficulty of swallowing, most of consumers accepted plain (82.3%) and strawberry (94.9%) yogurts with probiotic beads, giving scores for texture and overall acceptability of 6.7 and 6.8 for plain yogurt; and 7.5 and 7.6 for strawberry flavored yogurt. They were also willing to try (92.5%) and buy (89.8%) the products, indicating a potential market exists for yogurts containing probiotic beads in market.

Key words: probiotics, microencapsulation, yogurt, chitosan, consumer acceptance, alginate

INTRODUCTION

Probiotics are known as beneficial microorganisms by improving intestinal microbial balance (Fuller, 1997). Their benefits include reducing intestinal infection, reducing serum cholesterol levels, beneficially influencing the immune system, improving lactose utilization in persons who are classified as lactose maldigestors, and having anticarcinogenic activity (Gilliland, 1991). The traditional cultures, such as *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*, used in many dairy products also may provide benefits, but not through their ability to survive and grow in the

gastrointestinal tract. Probiotic effects are influenced very strongly by the ability of the organism to survive in the product and also survive and multiply within the host. To achieve the benefits, probiotics should be metabolically stable and active both in the product and host.

The viability of probiotic bacteria in yogurt over long periods at refrigerated temperature is unsatisfactory to provide the health benefits (Shah *et al.*, 1995; Dave and Shah, 1997; Vinderola *et al.*, 2000). Protection of probiotics by encapsulation in alginate beads is one method of improving their viability in food products. Encapsulation is a process in which a material or a mixture of materials, such as bacterial cells, is

Department of Food Technology, Faculty of Biotechnology, Assumption University Hau Mak Campus, Hau Mak, Bangkok 10240, Thailand.

* Correspondence author, e-mail: wunwisaKrs@au.edu

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coated with or entrapped within another material or system. The entrapped material is most often in liquid form and is referred to as the core material or internal phase. The material forming the coating is referred to as wall material, carrier or membrane coating. The benefits of encapsulation of bacterial cells include protection of cells from bacteriophages (Steenenson *et al.*, 1987; Tanaka *et al.*, 1989), increased survival during freeze-drying and freezing (Sung, 1997; Kebary *et al.*, 1998; Sha and Ravula, 2000), greater stability during the storage (Woo *et al.*, 1999; Koo *et al.*, 2001) and increased survival after exposure to gastric solution (Lee and Heo, 2000; Sun and Griffiths, 2000; Lee *et al.*, 2001).

Although alginate is the most suitable supporting material, it has some limitations, such as low stability and high porosity (Smidsrod and Skjak-Braek, 1990); coating of the beads is required to improve the properties of encapsulated beads. Coating not only prevents cell release but also increases mechanical and chemical stability. Encapsulation of probiotic in alginate beads coated with chitosan has been reported to improve the survival of probiotics in both yogurt and severe conditions, such as in simulated gastric and intestinal juices and bile salt solution (Krasaekoopt *et al.*, 2004). Therefore, these probiotics will remain in yogurt at the level above therapeutic minimum level (Krasaekoopt *et al.*, 2006).

In Thailand, with the growing number of health-conscious consumers, especially the young generation, typically age between 14 and 25 years, the annual growth rate of yogurt were 20% (Mercer, 2006). The average sale value is approximately THB10.5bn, which is equivalent to one-third of all dairy products sale in Thailand. Although the annual consumption of yogurt products is still fairly low – at one or two liters per person – when compared with Thailand's milk consumption, which stands at around 15.9 liters per person, addition of probiotic beads in yogurt for health benefit may increase the amount of

consumption of health-conscious consumers (Mercer, 2006).

Although the protection of probiotics using encapsulation technique has been studied, the consumer assessment of yogurt containing encapsulated probiotics has not been reported anywhere else. The addition of probiotics encapsulated in alginate beads coated with chitosan may affect the consumer preference and sensory attributes of the product due to the size of the beads.

Therefore, the aim of this research was to investigate the assessment of plain and strawberry yogurts containing probiotic beads by consumers in Thailand, simultaneously with the study of the effect of probiotic beads on sensory attributes using descriptive sensory analysis.

MATERIALS AND METHODS

Preparation of probiotics

A slant of *Lactobacillus casei* (TISTR 390) (Microbiological Resource Center, MIRCEN) was inoculated in 10 mL MRS broth (Oxiod) and incubated at 37°C for 24 h under aerobic conditions and then transferred into 95 mL MRS broth. The cells were harvested by centrifuging at 1500 g for 15 min at 25°C and washed twice with sterile water.

Encapsulation of probiotics

The extrusion technique of encapsulation was derived from Krasaekoopt *et al.* (2004) using alginate as the supporting matrix. After washing, the cells were suspended in 5 mL of sterile distilled water and mixed with 20 mL of 2% (w/v) sodium alginate solution (D 3247 AJAX Chemicals Ltd.) sterilized at 121°C for 15 min. The cell suspension was placed in a sterile syringe and injected through a 0.11 mm needle into sterile 0.05 M CaCl₂. The 0.11 mm needle was used to produce beads with a diameter of 1-2 mm. Moreover, stirring with a magnetic stirrer was applied during dropping to

produce spherical beads, and the distance between the needle and the surface of the solution was controlled to approximately 1 to 2 cm to avoid formation of flat beads. After 30 min gelification in CaCl_2 , the beads were rinsed with, and then kept in, sterile water at 4°C.

Coating alginate beads with chitosan

Alginate beads were coated with chitosan by using a two-stage method, derived from Krasaekoopt *et al.* (2004). Low molecular weight chitosan (0.4 g; low viscosity 14 mPas in 1% w/v solution; Fluka) was dissolved in 90 mL distilled water acidified with 0.4 mL of glacial acetic acid to achieve a final concentration of 0.4% (w/v). The pH was then adjusted to between 5.7 and 6 by adding 1 M NaOH. The mixture was filtered through Whatman #4 filter paper and the volume was adjusted to 100 mL before autoclaving at 121°C for 15 min. Then 15 g of washed beads were immersed in 100 mL of chitosan solution with gentle shaking at 100 rpm for 40 min on an orbital shaker for coating. The chitosan-coated beads were washed and kept in sterile water at 4°C. The diameters of 120 randomly selected beads were measured with an objective micrometer on an optical microscope at a magnification of 100x.

Application of encapsulated probiotics in yogurt

Ten grams of microencapsulated beads of probiotic bacteria were added aseptically into 100 g of commercial stirred yogurts, which were sponsored by Foremost Dairies Co. (Bangkok) Ltd. The yogurts were then stored overnight at 4°C. The same treatments were applied in both plain and strawberry yogurts.

Sensory evaluation

Descriptive sensory analysis

Eight trained panelists were recruited from Faculty of Biotechnology, Assumption University, Bangkok, Thailand. They were assisted

in developing a consensus vocabulary for aroma, appearance, texture and flavor attributes for plain and strawberry yogurts with and without probiotic beads. This procedure for attribute development is similar to that used in some other recent sensory studies (Vara-Ubol *et al.*, 2004; Matta *et al.*, 2005; Chambers *et al.*, 2006). The sensory lexicon used for the plain yogurt in this study included 9 terms: 1 appearance, 1 odor, 2 texture and 5 flavor attributes and for the strawberry yogurt also included 9 terms: 1 appearance, 2 odor, 1 texture, and 5 flavor attributes (Table 1). After the vocabulary was established, the panelists were trained 8-10 sessions by rating the intensity of each attribute in 15-cm unstructured line scale that was anchored on both ends with extremes of each descriptive term until the standard deviation of each attribute was less than 1.5. Then the panelist rated the intensity of the products. Plain and strawberry yogurts were served in white polystyrene cups (40 mL in a 120 mL cup), topped with transparent and vented lids, respectively. They were maintained and served at 4°C. Yogurts were evaluated in triplicate.

Consumer acceptance test

Consumer testing was performed at public places in Bangkok (four places) and the suburb of Bangkok (four places). Based on the formula developed by Cochran (1963), four hundreds yogurt consumers, previously screened by oral screening either they like yogurt or not (150 males, 250 females, age range from 14 to 55 years), participated in this study. The participation of the consumers was voluntary and no monetary compensation was given. The consumers answered demographic questions (gender, age, education, occupation and income); buying behavior (frequency and amount of buying, quality of yogurt); and their attitudes of consumers for yogurt containing probiotics.

After answering, the consumers were served with the plain and strawberry yogurts,

Table 1 Product attributes: definitions and corresponding reference standards used in the trained panel evaluation.

Term		Definition	Character references (Highest intensity)
Plain	Strawberry		
Yellow color		Degree to which the sample is visually pale yellow	Foremost pasteurized milk
	Pink color	Degree to which the sample is visually pale pink	Foremost pasteurized strawberry flavored milk
Yogurt odor	Yogurt odor	Odor of fermented milk	4-week old yogurt
	Strawberry odor	Odor of strawberry	Foremost pasteurized strawberry flavored milk
Viscosity	Viscosity	Flow rate of sample	Mayonnaise
Swallow ability		Degree of easiness to swallow the sample	Kanom-Kho (Chinese white cake)
Sourness	Sourness	Fundamental taste	Lime
Sweetness	Sweetness	Fundamental taste	Sugar
Yogurt flavor		Flavor of fermented milk	4-week old yogurt
Milky flavor	Milky flavor	Flavor of fresh milk	Foremost pasteurized milk
	Strawberry flavor	Flavor of strawberry	Foremost pasteurized strawberry flavored milk
Oiliness	Oiliness	Oily residue left in the mouth after swallow	Whipped cream

respectively, both with and without probiotic beads, in white polystyrene cups (40 mL cup) topped with transparent, vented lids, at 4°C. The consumers were given the instruction not to chew the beads in the yogurt because the protection of entrapped cells from the gastric and intestinal juices decreases. They were asked to rate their liking by using 9-point hedonic scale for texture and overall acceptance of the yogurts. The consumers also were asked to rinse their mouth with water before and after tasting the samples.

Statistical analysis

ANOVA and least significant differences (LSD) ($p < 0.05$) were executed with SPSS software (version 11.5) on descriptive analysis of the yogurts containing probiotic beads, while the hedonic ratings of the yogurt quality was used for consumer preference on texture and overall characteristic and purchasing decision.

RESULTS AND DISCUSSION

Consumer acceptance test

Four hundreds yogurt consumers participated in this research were 250 female and 150 male with 79.7% were in the age range of 16-25 yrs. This test paralleled to the report of Mercer (2006), who found that the number of health-conscious consumer was the young generation of 14-25 years. The educational background of most consumers was bachelor degree (69.0%), which were students (56.2%). Moreover, the income of yogurt consumers was in the range of 5,001 to 10,000 Baht (38.5%).

Yogurt quality is the most important factor affecting the buying behavior of consumers. The quality attributes included appearance, taste, texture, odor, brand, nutritional value, convenience, price and innovation (Figure 1). Consumers rated taste (8.8) as the most important

criteria ($p<0.05$) followed by nutrition (8.3), texture (7.7) and odor (7.4) for yogurt without beads. This implied that most of consumers buy yogurt due to its taste rather than the others.

The attitude of the participants on yogurt containing probiotics is shown in Table 2. Although nutritional addition (97.5%) to yogurt was the consumers' opinion, they had less knowledge about the benefits of probiotic bacteria (24.2%). Nevertheless, they were willing to try (92.5%) and buy (89.8%) the yogurt containing probiotic beads after tasting the products. This implied that a potential market for the yogurts containing probiotic beads may exist in Thai market.

Most of the consumers accepted the yogurts containing probiotic beads about 82.3 and

94.9% with the overall score as 6.8 and 7.6 for the plain and strawberry yogurts, respectively (Table 3). Although the size of the beads were quite large, approximately 1-2 mm the texture score of the products were moderately as high as 6.7 and 7.5 for the plain and strawberry yogurts, respectively. Moreover, the scores for strawberry yogurt were higher than plain yogurt. Addition of fruit can reduce any off-flavors in yogurt (Drake *et al.*, 2001).

Descriptive sensory analysis

The presence of probiotic beads affected both color intensity and the textural characteristic of the plain yogurt (Figure 2a). Addition of probiotic beads significantly ($p<0.05$) increased the intensity of yellow color. The probiotics affect

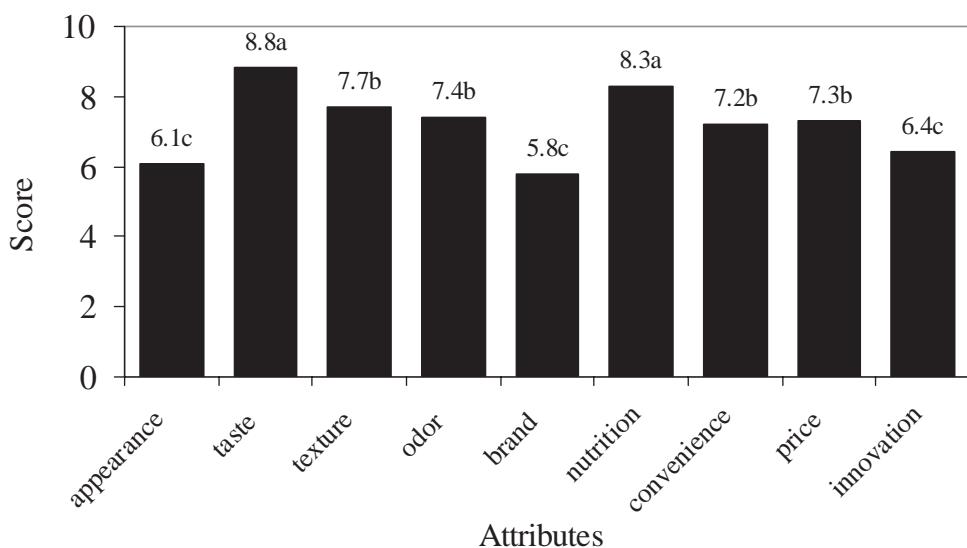


Figure 1 Means of the consumers' hedonic rating on qualities of yogurt used for purchasing the product ($n=400$).

Table 2 Percentages of consumer opinion on yogurt containing probiotic beads ($n=400$).

Topic	Percentages	
	Yes	No
Nutritional addition of yogurt	97.5	2.5
Knowledge about probiotics	24.2	75.8
Willingness to try the product	92.5	7.5
Willingness to buy the product	89.8	10.2

to the opacity of the beads resulting in darker color than normal when they were added into the yogurt. Thus, the color intensity of the yogurt containing probiotic beads (6.0) was higher than that without beads (4.3). In addition, the presence of probiotic beads affected the texture characteristics of the plain yogurt, such as viscosity and difficulty of swallowing. The plain yogurt containing probiotic beads demonstrated a significantly ($p<0.05$) higher viscosity (7.4) than the yogurt without beads (control) (5.3). Increasing viscosity paralleled to the difficulty of swallowing for the yogurt. The yogurt containing probiotic beads significantly ($p<0.05$) presented the higher difficulty of swallow (7.3) than that without beads (5.3). This probably is not only a direct increase in viscosity, but also by the size and spherical shape of the beads. Furthermore, other attributes found in plain yogurt with and without probiotics beads showed similar trends.

Strawberry yogurt containing probiotic

beads was evaluated by the trained panelists. Nine attributes (2 odors, 1 appearance, 1 texture, and 5 flavors) were established (Figure 2b). Addition of probiotic beads in strawberry yogurt significantly ($p<0.05$) influenced the viscosity of the product, with score of 7.6 compared to without beads (5.3). While color, flavor, taste and oiliness showed similar trends.

One issue that was not measured in this study, but may be important outside Thailand is the perception of the beads in the yogurt. Most plain yogurts are smooth products even in Thailand while many flavored yogurts contain fruit pieces or seeds where the probiotic beads might be less noticeable in those cases. In this study the beads did result in a more viscous product, but consumers apparently did not care since they rated the products highly. This may be because particles or bead-like textures are common in Thai dairy products and fillings. Such products often include pearl tapioca or taro pieces and one of the most

Table 3 Mean (\pm SD) of texture, overall scores and consumer acceptance for yogurts with probiotic beads by consumer test (n=400).

Product	Texture score	Overall score	Acceptance (%)
Plain yogurt	$6.7^b \pm 1.4$	$6.8^b \pm 1.5$	82.3 ^b
Strawberry yogurt	$7.5^a \pm 1.3$	$7.6^a \pm 1.2$	94.9 ^a

* The same letter means no significant different at 95% confidential level.

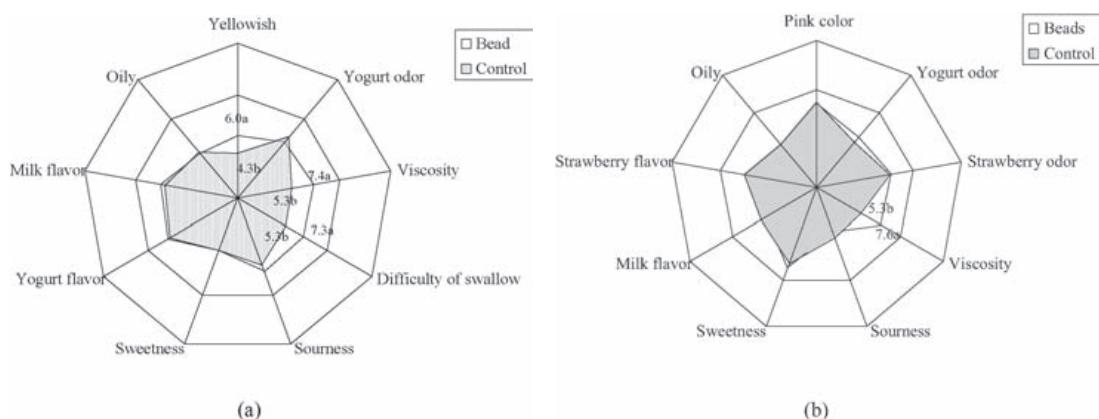


Figure 2 Typical spider plot for intensity of attributes in yogurt containing microencapsulated probiotics; (a) Plain yogurt and (b) Strawberry yogurt.

popular types of ice cream includes whole kernel corn. Thus, while Thai consumers were accepting of this texture, this may not be universally accepted in some other cultures where particles or bead-like textures might be objectionable.

Yogurts containing probiotic beads are classified as one of the premium product, so the price of the product is higher than the normal yogurts due to the addition of the probiotic beads; but the health benefits consumers obtained are more than that of normal yogurt or yogurt containing probiotics in the form of free cells. Yogurt cultures cannot colonize in the host (Gilliland and Kim, 1984) and probiotics in the form of free cells also cannot survive well in the products during refrigerated storage and also when they pass through the digestive system, with the number lower than the therapeutic level (10^7 cfu/g), compared to the encapsulated cells (Krasaekoont *et al.*, 2003; 2004). Therefore, the consumers may be willing to pay more for better quality of products.

CONCLUSION

A potential market for yogurts containing probiotic beads appears to strongly exist in market. Consumers bought yogurt due to its taste and nutrients. They also wanted yogurt with high health benefits such as yogurt containing probiotic beads. Although the addition of probiotic beads affected the texture characteristics by increasing viscosity and difficulty of swallowing, most of consumers accepted both plain and strawberry flavored yogurts.

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