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PROBIOTICS ENCAPSULATED FRUIT JUICE BUBBLES AS FUNCTIONAL FOOD PRODUCT

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ABSTRACT: This experimental research investigates encapsulation of *Pediococcus pentosaceus* ARG-MG12 in pure orange juice bubbles. In the study, four tropical fruit juices: guava, watermelon, pineapple, and orange, were first evaluated in terms of sensory characteristics: color, odor, flavor, texture, overall acceptance, using a 9-point hedonic scale; and orange juice whose sensory scores were statistically highest was selected for further development into probiotic fruit bubbles. The physicochemical properties of probiotic orange bubbles were examined and the sensory evaluation undertaken. Furthermore, the probiotic bubbles were subjected to gastric juice (pH 2.0) and bile salt (0.6% w/v) environment for 3 h to simulate human gastrointestinal tract and to variable storage durations (0, 3, 5, 7, 14 days) at 4 ^{o}C to assess cell viability. The findings showed that the probiotic orange bubbles had 390.71 g/sec of stiffness, DPPH radical-scavenging activity of 294 μg ascorbic acid eq./ml, and L-ascorbic acid content of 0.095 mg/ml. Meanwhile, the survival rates of bacterial cells in low pH environment and bile salt were 83.27% and 94.24%, respectively. At day 14, the remaining bacteria were above 8 log CFU/g, given that the minimum concentrations of probiotics are 10^6–10^7 CFU/ml at the end of product's shelf life. Moreover, sensory testing panelists were unable to differentiate between non-probiotic and probiotic orange bubbles.

Keywords: Probiotic, Encapsulation, Fruit bubble, Functional food

1. INTRODUCTION

First invented in Taiwan in the 1980s [1], bubble tea is a trendy drink popular among teenagers. The colorful beverage is concocted from green or black tea mixed with milk and fruit syrup. As a marketing gimmick, the drink contains small spherical tapioca starch balls of 10 -15 mm in diameter (also known as pearls, bubbles, or boba), and is served with a wide colorful straw through which the bubbles are sucked up [2].

Encapsulation involves the entrapment of active substance within another substance acting as coating material. The encapsulation technology is commonly used by the food industry for preservation and delivery of flavors, dyes, stabilizers, antioxidants, enzymes, probiotics, lipids, mineral salts, and vitamins [3]. For probiotics, encapsulation protects microorganisms against environmental stresses. Specifically, microcapsules creates an anaerobic environment conducive to probiotics growth and provide protection against harsh environment, including acidic fruit juice, freezing, and gastric conditions, thereby mitigating cell injury [4,5].

Of particular interest is the survival of probiotics in food products given that significant proportions of cells deteriorate during processing and storage. According to the International Dairy Federation, the minimum concentrations of probiotics should be 10^6–10^7 CFU/ml at the end of product's shelf life [6]. The health benefits of probiotics include, for example, bio-availability of macro- and micro-nutrients, antioxidant activity, and production of anti-microbial compounds [7],[8].

Despite widespread encapsulation of probiotics in a variety of food products, research on probiotics encapsulation for fruit juice bubbles is limited and rare. Thus, this research aims to develop fruit bubbles containing probiotics encapsulated with sodium alginate as functional food product. In the study, four tropical fruit juices: guava, watermelon, pineapple, and orange, were first evaluated in terms of sensory features (i.e., color, odor, flavor, texture, overall acceptance) using a 9-point hedonic scale and the fruit juice with statistically highest scores selected for further development into probiotic fruit bubbles. The physicochemical properties of the probiotic fruit bubbles were then characterized in terms of size, texture, antioxidant activity, and L-ascorbic acid content; and the sensory evaluation undertaken in comparison with commercially non-probiotic fruit bubbles. In addition, the probiotic fruit bubbles were subjected to simulated human gastrointestinal tract and variable storage durations at 4 ^{o}C to determine the cell viability.

2. MATERIALS AND METHODS

2.1 Preparation of Fruit Bubbles

Fresh tropical fruits were acquired from a local fresh market in Thailand's Pathum Thani province, consisting of 10 kg each of guava, watermelon,

pineapple, and orange. The fruits were washed, peeled, and ground by a blender for 5 min at maximum speed (Philips, HR-2118). The slurry was manually squeezed through a muslin cloth for juice, and pH values were measured by pH meter (Sartorius PB-20, Germany) and titratable acidity determined [9].

In this research, the encapsulation followed Krasaekoopt and Kitsawad [11] whereby 1% sodium alginate (w/w) was mixed with 1000 ml fruit juice and heated at 70 °C for 10 min. The mixture was extruded dropwise through 8-mm needle into sterile 0.1 M $CaCl_2$ as hardening solution and left for 10 min. The fruit juice bubbles were cleaned by potable water and particle sizes measured using Vernier caliper.

The sensory attributes (color, odor, flavor, texture, overall acceptance) of bubbles of four fruit juices: guava, watermelon, pineapple, and orange, were evaluated by a team of 30 untrained panelists using a 9-point hedonic scale, where 1 denotes extremely dislike and 9 extremely like. Randomized complete block design was utilized to select the fruit juice with statistically highest sensory scores ($p \leq 0.05$) for subsequent experiment [10].

2.2 Preparation of Inoculum

Pediococcus pentosaceus ARG-MG12 was cultured in 10 ml de Man, Rogosa and Sharpe (MRS) broth (Merck, Darmstadt, Germany), followed by overnight incubation at 37 °C. The culture was transferred to 100 mL MRS broth and incubated overnight at 37 °C. Cultures were harvested by centrifugation at 3000 g at 25 °C for 10 min and washed with sterile saline, and the procedure was repeated prior to the collection of cultures.

2.3 Encapsulation of *Ped. pentosaceus*

In encapsulation, *Ped. pentosaceus* ARG-MG12 bacterial cultures (10^{11} CFU/ml) were aseptically suspended in the selected sodium alginate-mixed fruit juice (i.e., the fruit juice with statistically highest sensory scores). The suspensions were extruded dropwise through 8 mm needle into sterile 0.1 M $CaCl_2$ as hardening solution and left for 10 min. The probiotic fruit bubbles were then washed by potable water.

To determine the bacterial survival, 10 g of the probiotic fruit bubbles was mixed with 90 ml of sterile phosphate buffer in mixer bag and broken up by stomacher (Seward, UK) at room temperature for 1 min. The mixture was diluted and the number of viable cells determined by pour plate count (log CFU/ml) in MRS agar after incubation at 37 °C for 48 h. The survival rate (%) was then calculated using Eq. (1):

$$\text{Survival rate (\%)} = (N/N_0) \times 100 \quad (1)$$

where N is the number of viable cells in the fruit bubble (log CFU/ml) and N_0 is the number of free viable cells in suspension containing sodium alginate, fruit juice, and bacterial culture (log CFU/ml).

2.4 Texture Profile Analysis

Texture profile analysis of fruit bubbles was carried out using Texture Analyser (Model TA. XT. Plus, Make: Stable Micro Systems, UK) equipped with 10 kg load cell. The data from the texture profile (force-time) curve was used to determine chewiness (g), energy to chew (g.%), and stiffness (g/sec) [12].

2.5 Antioxidant Activity and L-Ascorbic Acid

The antioxidant activity of probiotic fruit bubbles was determined according to [13] using free radical 2,2-diphenyl-1-picrylhydrazil (DPPH), whereby 0.2 ml of the suspension (containing sodium alginate, fruit juice, and bacterial culture) and 3.8 ml of 0.1 mM DPPH in ethanol were briefly mixed in test tube, and retained in darkness for 30 min. The absorbance was measured at 517 nm by visible light spectrophotometer (772s, Precision and Scientific Instrument Co., Shanghai, China). L-ascorbic acid was used as standard and ethanol as blank. The antioxidant activity was expressed in µg of ascorbic acid eq./ml. Meanwhile, the Association of Official Agricultural Chemists (AOAC)'s titrimetric method was used to determine L-ascorbic acid content [9].

2.6 Tolerance of Probiotic Fruit Bubbles to Simulated Human Gastrointestinal Tract

In this research, the tolerance of probiotic fruit bubbles was characterized under simulated gastric juice (pH 2.0) and bile salt (0.6% w/v) conditions. The simulated gastric and intestinal juices were to mimic human gastrointestinal (GI) tract [14]. In the experiment, fruit bubbles were introduced into the simulated gastric juice and incubated at 37 °C for 3 h. Afterward, 10 g of fruit bubbles was mixed with 90 ml of sterile phosphate buffer and broken up by the stomacher for 1 min at room temperature. The mixture was diluted and the number of viable cells determined by pour plate count (log CFU/ml) in MRS agar after incubation at 37 °C for 48 h [15]. The survival rate (%) was then determined.

For bile salt tolerance, the probiotic bubbles were submerged in the bile salt (Sigma, USA) and incubated at 37 °C for 3 h. Afterward, 10 g of the sample was mixed with 90 ml of sterile phosphate

buffer and disintegrated for 1 min at room temperature using the stomacher. The mixture was diluted and the number of viable cells determined by pour plate count (log CFU/ml) in MRS agar after incubation at 37 °C for 48 h [15]. The survival rate (%) was then determined.

2.7 Sensory Evaluation

Color, odor, flavor, texture, and overall acceptance of probiotic fruit bubbles were evaluated by a team of 30 untrained panelists. Two sets of fruit bubbles, i.e. commercially non-probiotic (Ding Fong Foods, Thailand) and probiotic fruit bubbles, were served to the panelists who subsequently scored on the sensory features (color, odor, flavor, texture, and overall acceptance) using a 9-point hedonic scale, where 1 denotes extremely dislike and 9 extremely like. Randomized complete block design was used to assess the statistical difference between the non-probiotic and probiotic fruit bubbles ($p \leq 0.05$) [10].

2.8 Statistical Analysis

All the experiments were carried out in triplicate and the results expressed as mean ± standard deviation and average percentage for the survival rate. Analysis of variance (ANOVA) was used to analyze the data and Duncan's multiple-range test to compare means. Statistical significance was based on the 5% significance level ($p \leq 0.05$).

Use at most three levels of headings that correspond to chapters, sections and subsections. The first level headings for chapter titles should be in 10pt, bold, justified, and uppercase font. Leave one blank line before and after the first level headings, respectively.

3. RESULTS AND DISCUSSION

3.1 Comparison of Fruit Bubbles

Table 1 tabulates the pH and total acidity of four fruit juices (guava, watermelon, pineapple, and orange) mixed with sodium alginate.

According to Gandomi, Abbaszadeh, Misaghi, Bokaie and Noori [16], formulating probiotic fruit juices presents greater challenges than dairy products because fruit juices normally have low pH (3.0-4.5), which affects the stability of probiotics. In Table 1, pH of fruit juices, except watermelon, were below 4.5, which is unconducive to bacterial viability.

Sensory features of fruit bubbles are also important in formulating probiotic food products. Fig.1 presents the sensory evaluation of the four fruit bubbles on color, odor, flavor, texture, and overall acceptance.

Table 1 pH and total acidity of fruit juices

Fruit bubbles	pH	Titratable acidity (%)
Guava	4.31 ± 0.02^{b}	0.70 ± 0.07^{c}
Watermelon	5.73 ± 0.04^{a}	0.62 ± 0.15^{c}
Pineapple	4.12 ± 0.02^{d}	1.65 ± 0.80^{a}
Orange	4.23 ± 0.03^{c}	1.23 ± 0.10^{b}

The experiments were carried out in triplicate, and the results are expressed as mean ± standard deviation. Different superscripts in the same column indicate significant difference ($p \leq 0.05$).

The results indicated that the evaluation scores of orange bubbles were statistically highest in nearly all sensory characteristics ($p \leq 0.05$). Orange fruit juice (i.e., 100% genuine orange juice) was thus selected for subsequent development into probiotic fruit bubbles.

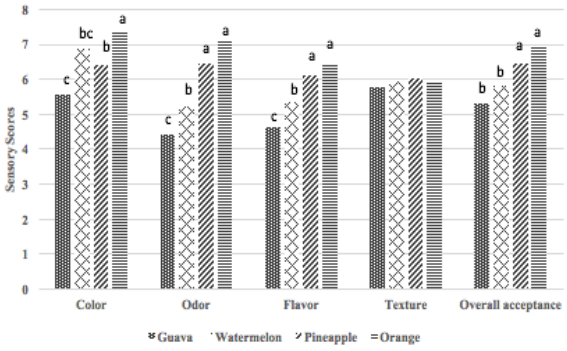

Fig.1 Sensory evaluation of four experimental fruit bubbles. Different superscripts in the same column indicate significant difference ($p \leq 0.05$).

3.2 Characteristics of Probiotic Orange Bubbles

Table 3 tabulates the average size, texture profile, L-ascorbic acid, and antioxidant activity of probiotic orange bubbles. The average size of probiotic bubbles was 11.4±0.05 mm, as opposed to 0.5-3 mm for conventionally probiotics beads using extrusion method [18]. In addition, the beads were spherical in shape given that sphericity plays a crucial role in preventing cell overgrowth in encapsulated beads [18].

Based on the bead/bubble size, encapsulation is classified into two categories: macroencapsulation (mm to cm) and microencapsulation (1-1000 µm) [8],[17]. The probiotic fruit bubbles of this experimental research belong to the macro-encapsulation group. Fig.2 illustrates the experimental probiotic orange fruit bubbles.

Table 3 also presents the texture of probiotic orange bubbles, including chewiness, energy to

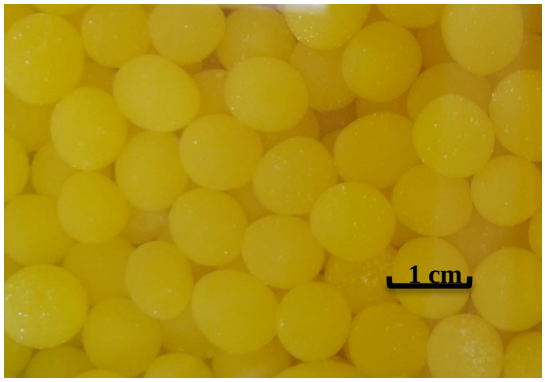

Fig.2 The experimental probiotic orange bubbles

Table 3 Characteristics of probiotic orange bubbles

Characteristics	Value
Size (mm)	11.4±0.05
Texture	
Chewiness (g)	220.03±26.02
Energy to chew (g.%)	3,400.57±421.92
Stiffness (g/sec)	390.71±25.66
L-ascorbic acid (mg/ml)	0.095±0.01
DPPH radical-scavenging activity (μg ascorbic acid eq./ml)	294±22.13

The experiments were carried out in triplicate, and the results are expressed as mean ± standard deviation.

chew, and stiffness. The texture of fruit bubbles was positively correlated with sodium alginate concentrations. Elevated sodium alginate concentrations induced a higher degree of cross-linkage between gel networks, resulting in denser and more rigid gel structure. Specifically, sodium alginate gelation developed as Ca^{2+} gelling ions crosslinked with sodium alginate whose optimal concentrations for probiotics encapsulation were 0.5 - 4% (w/v). Sodium alginate is preferable for probiotics encapsulation due to ease of use, biocompatibility, economy, non-toxicity, rapid gel matrix formation around bacterial cells, and mild processing [18]. In Table 3, the DPPH antioxidant activity and L-ascorbic acid content indicated adequate antioxidant capacity of the probiotic orange bubbles.

3.3 Viability of Probiotics in Fruit Bubbles under Simulated Gastrointestinal Condition

For health benefits, probiotics must be able to survive during gastrointestinal transition and exist in high proportions in the intestines (10^6 - 10^8 CFU/g of intestinal content) [19]. In this research, the viability of *Ped. pentosaceus* ARG-MG12 encapsulated in orange bubbles was investigated in the simulated gastric juice (pH 2.0) and bile salt (0.6% w/v) environments for 3 h each. Table 4 tabulates the assay results, where control denotes the initial condition at 0 h.

Encapsulation of probiotics in hydrocolloid beads protects the bacterial cells against damage caused by external environment and during gastrointestinal transition [18]. In Table 4, the bacterial stability decreased in both stimulated gastric juice (8.96±0.06 log CFU/g) and bile salt (10.14±0.04 log CFU/g), vis- à-vis the control (10.76±0.12 log CFU/g), with the corresponding survival rates of 83.27% and 94.24%. In comparison, unencapsulated *Ped. pentosaceus* strains in the simulated gastric juice suffered a reduction in the bacterial stability by two logarithmic cycles after 1h incubation [21].

Table 4 Viability and survival rate of probiotics under simulated gastrointestinal conditions

Conditions	Viability (logCFU/g)	Survival rate (%)
Control	$10.76±0.12^a$	100.00^a
Stimulated gastric juice (pH 2.0)	$8.96±0.06^c$	83.27^c
Bile salt (0.6%)	$10.14±0.04^b$	94.24^b

The experiments were carried out in triplicate, and the results are expressed as mean ± standard deviation. Different superscripts in the same column indicate significant difference ($p \leq 0.05$).

In this research, 83.27% of *Ped. pentosaceus* ARG-MG12 survived after 3 h in low pH environment, a time period sufficient for the probiotics to reach their action site in the intestine [20]. In addition, 94.24% of the bacteria were capable of surviving high bile salt concentration (0.6% w/v). In [21], un-encapsulated *Ped. pentosaceus* strains exhibited abundant growth at 0.5% concentration of bile salt but poor growth rates at 1-2% concentrations, suggesting that encapsulation contributes to the enhanced viability of probiotics in gastrointestinal tract.

3.4 In Storage Cell Survival and Sensory Evaluation

Table 5 tabulates the viability and survival rates of *Ped. pentosaceus* stored at 4 °C for 0, 3, 5, 7, and 14 days. The cell viability decreased with extended storage time ($p \leq 0.05$). According to Ding and Shah [22], encapsulated *Ped. pentosaceus* could survive in orange and apple juices for six weeks in

refrigerated storage (4 °C), whereas free cells (un-encapsulated) lost their viability within five weeks.

Table 5 Viability and survival rate of probiotics during storage at 4°C

Storage (days)	Viability (logCFU/g)	Survival rate (%)
0	11.38±0.05[a]	100.00[a]
3	9.02±0.04[b]	79.26[b]
5	9.12±0.02[b]	80.14[b]
7	9.11±0.02[b]	80.05[b]
14	8.58±0.20[c]	75.40[c]

The experiments were carried out in triplicate, and the results are expressed as mean ± standard deviation. Different superscripts in the same column indicate significant difference ($p \leq 0.05$).

In Table 5, the viability of probiotics in the orange bubbles steadily declined from day 0 through to termination (day 14). At termination, the remaining bacteria in the probiotic orange bubbles were still above 8 log CFU/g, given that the minimum concentrations of probiotics are 10^6–10^7 CFU/ml at the end of product's shelf life [6].

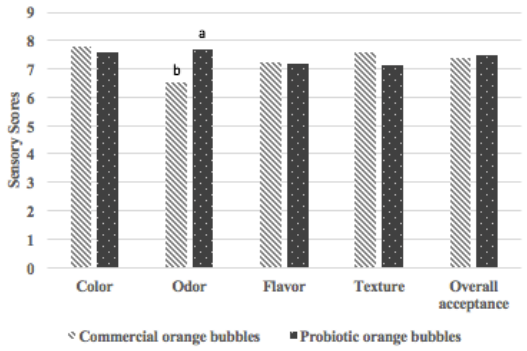

Fig.3 Sensory evaluation of commercial (non-probiotic) and probiotic orange bubbles. Different superscripts in the same column indicate significant difference ($p \leq 0.05$).

Fig.3 presents the sensory evaluation of commercially non-probiotic (15% orange juice) and probiotic orange (100% orange juice) bubbles by 30 untrained panelists. The results revealed that, except for odor where the probiotic orange bubbles received higher scores, the participants were unable to differentiate between the non-probiotic and probiotic orange bubbles ($p>0.05$). Essentially, the orange bubbles containing *Ped. pentosaceus* ARG-MG12 possess health and economic potential as a functional food product.

4. CONCLUSION

This research investigated the encapsulation of *Ped. pentosaceus* ARG-MG12 in pure orange juice bubbles as a functional food product. The probiotic bubbles also underwent gastric juice (pH 2.0) and bile salt (0.6% w/v) conditions for 3 h each to simulate human gastrointestinal tract; and variable storage times at 4 °C for 14 days to assess cell viability. The experimental results showed that the probiotic orange bubbles containing *Ped. pentosaceus* ARG-MG12 had 390.71 g/sec of stiffness, DPPH radical-scavenging activity of 294 µg ascorbic acid eq./ml, and L-ascorbic acid content of 0.095 mg/ml. The remaining bacteria at termination were above 8 log CFU/g, given that the minimum concentrations of probiotics should be 10^6–10^7 CFU/ml at the end of product's shelf life. Moreover, the sensory evaluation revealed that the participants were unable to differentiate between the non-probiotic and probiotic orange bubbles ($p>0.05$).

5. ACKNOWLEDGMENTS

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