SURVIVAL OF FREE AND MICROENCAPSULATED BIFIDOBACTERIA IN ORANGE, MANGO AND PINEAPPLE JUICES

Journal

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ABSTRACT

The survival and effect of free and κ-carageenan microencapsulated probiotic *Bifidobacterium lactis* Bb-12 on pH and sensory quality of orange, mango and pineapple juices were studied over 4 weeks of refrigerated storage. Our results showed that the juices containing free cells of bifidobacteria had the lowest pH values at the end of storage compared to the control and the juices containing microencapsulated cells. The orange juice showed the lowest pH values. The viable numbers of free cells of bifidobacteria in all tested juices decreased significantly during storage period. The viable counts of free cells of bifidobacteria in orange, mango and pineapple juices were remained above the level of 10^6 cfu/ml for 2, 3 and 4 weeks of refrigerated storage, respectively. The viable counts of free bifidobacterial cells in orange, mango and pineapple juices decreased significantly at the end of storage period by 3.37, 3.37 and 0.87 log cycles, respectively, these values below the level of 10^6cfu/ml. A positive correlation between the pH values of tested juices and the viable counts of free cells of *B. lactis* Bb-12 was found. On the other hand, the microencapsulated cells in orange, mango and pineapple juices remained viable at levels of 10^8 cfu/ml during storage period up to 4 weeks. No significant difference changes was found in the viable counts of microencapsulated cells in all tested juices during storage period. The viable count of microencapsulated cells in orange, mango and pineapple juices decreased only by 0.05, 0.17 and 0.08 log cycle after 4 week of storage period. No significant changes in odor of

control orange juice and there containing microencapsulated cells was observed. The taste of orange and pineapple juices containing free or encapsulated cells changed significantly compared with its control juices. No significant differences in appearance and color, odor, taste and consistency of control mango juice and that containing either free or encapsulated cells were found. This study has shown the microencapsulation protected *Bifidobacterium lactis* Bb-12 from the effect of low pH of orange, mango and pineapple juices

INTRODUCTION

The consumption of food and beverages containing probiotic microorganisms is a growing, global consumer trend (Verbeke, 2005). Probiotics have been defined in several ways, depending on our understanding of the mechanisms of action of their effects on the health and well-being of humans. The most commonly used definition is that of Fuller (1989): probiotic are live microbial feed supplements that beneficially affect the host by improving its intestinal microbial balance. Recently, Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) define probiotic as live microorganisms (bacteria or yeasts), which when ingested or locally applied in sufficient numbers confer one or more specified demonstrated health benefits for the host (FAO/WHO, 2001). It is clear that these definitions have: (1). restricted the use of the word probiotic to products which contain live microorganisms (2). pointed out the need for providing an adequate dose of probiotic bacteria in order to exert the desirable effects. It is worthy to note that Kurmann et al., (1992), mentioned that, 10^6- 10^7 probiotic organisms per day would ensure transit of viable bacteria through the stomach. Thus, studying the viability of bifidobacteria took much attention. Several species of *Bifidobacterium* are considered to be the important among the probiotic organisms. Bifidobacteria and other probiotic bacteria have been added to yoghurt and other fermented dairy products, but lactose intolerance and the cholesterol content are two drawbacks related to their consumption. Nowadays, fruit juice is positioned as a healthy food product by a large percentage of the global consumer population. Furthermore, fruit juice are rich in functional food components such as minerals, vitamins, dietary fibers, antioxidants (phytochemicals), and do not contain any dairy allergens that might prevent usage by certain segments of the population (Luckow and Delahunty, 2004). Incorporation of bifidobacteria has been shown to enhance the therapeutic value of carrot, orange, pineapple, cranberry juices (Kun, et al., 2008 and Sheehan, et al., 2007). However, the low pH of fruit juices, typically between pH 2.5 and 3.7, and bacterial sensitivity to acidic conditions mean that is imperative that only strain which can remain viable for an acceptable shelf-life are chosen to ensure actual benefits to the consumer (Sheehan, et al., 2007). The viable count of *Bifidobacterium lactis* Bb-12 decreased in orange and pineapple juices by ~2 and 3.5 log cycles between weeks 6 and 10 and between 4 and 6, respectively, to levels below the critical value of 10^6 cfu/ml. Moreover no cells were recovered by week 12 and 10 in orange and pineapple juices, respectively (Sheehan, et al., 2007).

Microencapsulation was successfully used to increase the viability of bifidobacteria cells in yoghurt (Adhikari, et al., 2000 and 2003 respectively), cheese (Ozer, et al., 2009) and mayonnaise (Khalil and Mansour, 1998). Microencapsulation segregates the cells from adverse environment, thus potentially reducing cell injury.

The main objective of this study was to examine the effect of microencapsulation on the survival of bifidobacteria in orange, mango and pineapple juices during refrigerated storage. Finally we studied the sensory characteristics of these juices containing free and microencapsulated bifidobacteria to determine consumer acceptability of such products.

MATERIALS AND METHODS

Bacterial strain

Bifidobacterium lactis Bb-12, was supplied by Chr. Hansen Laboratories, Copenhagen, Denmark.

Preparation of culture for inculation into the juices.

B. lactis Bb-12 was propagated in MRS broth (Oxoid, CM0359B) supplemented with 0.05% (w/v) L.cysteine HCl (Sigma Chemical Co., St. Louis, Mo., USA) at 37°C under anaerobic conditions achieved using anaerobic gas jars containing gas generating packs (Oxoid, BR0038B, UK). *Bifidobacteria* was grown initially for 48 h followed by subculturing and incubating for a further 17 h. The culture was harvested by centrifugation (1700 xg, 10 min). The pellets were washed twice in one-quarter-strength Ringer's solution (Oxoid) and concentrated 10-fold in the same diluent. A 1% inoculum of culture was distributed into the various juices to obtain a final concentration of ~10^8 cfu /ml

Preparation of microencapsulated bifidobacteria for inculation into the juices.

Microencapsulation of bifidobacterial cells were prepared by the method of Adhikari et al., (2003) as following: Bifidobacterial cells were grown in MRS broth containing L-cystein- HCl, maintained at 37°C for 24 h. the cells were harvested by centrifugation at 5000 xg, washed twice in sterile normal saline under the same centrifugation conditions and resuspended in 10 mL of sterile normal saline. A 2% κ-carageenan solution containing 0.9% NaCl (to improve dispersability of the κ-carageenan) was prepared and heat treated at 96°C for 6 min. Sixty mL of κ-carageenan solution was thoroughly mixed with 20 ml of cell suspension, and temporarily kept in a water bath at 47±°C. Ten mL of soybean oil containing 0.1% Tween 80 was tempered by stirring to 40°C on a stirrer hotplate for 2 to 3 min. the mixture of cells and κ -carageenan was then quickly added with continuous stirring to the oil in the beaker and the resultant mixture was further stirred for about 10 min to allow for emulsification and encapsulation to occur. The emulsion was removed by the addition of 150 mL of sterile 0.3 M KCl. After that, the oil phase was removed from the top of the mixture with a sterilized separator funnel under the laminar flow, the capsules were harvested from the KCl solution by gentle centrifugation at 350 xg for 10 min, the capsules were washed twice with 0.3 M KCl for better stability under the same centrifugation condition and finally stored in refrigerator before use. The capsules was distributed into the various juices to obtain a final concentration of ~10^8 cfu /ml

Fruit juices

Three bottles from each juice of fresh orange, mango and pineapple were collected in the production day from Al-Ahsa local markets in Saudi Arabia. These juices were free from sugar and preservatives additives. The bottles divided as follows:

The first bottles for each remained without inculation of bifidobacteria (control), second bottles, inculated with free cells of bifidobacteria and the third bottles, inculated with microencapsulated bifidobacteria. The above treatments were stored in refrigerator for 4 weeks.

Enumeration of bifidobacteria

Ten grams of juice treatments containing microencapsulated bifidobacteria were mixed with 90 mL of 0.05 M EDTA solution made in 0.1 M sodium phosphate buffer (pH 7), to release the cells from the capsules and incubating at 42°C for 20 min according to Adhikari et al., (2003). The count of released bifidobacteria and in the other treatments (juice containing free bifidobacteria) was determined according to Dinakar and Mistry (1994) by using MRS agar medium (Oxoid, CM0361B). L.cysteine-HCl (Sigma chemical Co., St. Louis, Mo) was added at the rate of 0.5%, to decrease the redox potential of the medium. Plates were incubated anaerobically at 37^{o}C for 48h.

pH measurement

pH was measured for all juice samples using a pH meter (Model 955; Fisher Scientific).

Sensory evaluation

Sensory evaluation of control juices and juices containing free and microencapsulated *B. lactis* Bb-12 were carried out by a regular score panel. A consumer panel consisting of 13 panelists was used to determine appearance and color, odor, taste and consistency of juice sample treatments.

Statistical analysis

Data are presented as means and standard deviation. The randomized complete block design, split-plot in time, was used for statistical analysis. All analyses were performed using procedures for the general linear model (PROC GLM) of SAS (1990) (SAS Institute, Inc., Cary, NC, USA). The significant differences among juice treatments were evaluated using a one-way ANOVA to analyze the points of sensory evaluation by Least Significant Different (LSD) at $P < 0.05$

RESULTS AND DISCUSSION

1. Changes in pH values of fruit juices containing free and microencapsulated *B. lactis* Bb-12

Table 1. showed that the pH values of juices containing free and microencapsulated *B. lactis* Bb-12 during refrigerated storage period. The pH values were decreased slightly in all tested sample during refrigerated storage. The decrease in pH were almost identical for control sample and samples containing microencapsulated bifidobacteria. The decrease in pH was significant (*P*>0.05) for the treatments containing free cells at the end of storage period. While, almost the same for the control and the treatments containing microencapsulated cells. This effect was much more evident for the orange juice containing free cells. Generally, the juices containing free cells of bifidobacteria had the lowest pH values at the end of storage compared to the control and the juices containing microencapsulated cells, while the orange juice showed the lowest pH values. This decreasing may be due to the metabolic activity of bifidobacteria, which resulting in the production of organic acid.

Table (1): Changes in pH values of orange, mango and pineapple juices containing free and microencapsulated *B. lactis* Bb-12 during refrigerated storage period.

Storage period (week)	Orange juice			Mango juice			Pineapple juice		
	Control*	Free	E.C	Control*	Free	E.C	Control*	Free	E.C
Zero time [Ψ]	4.5±0.1[a]	4.4±0.0[a]	4.6±0.1[a]	4.7±0.1[a]	4.7±0.0[a]	4.5±0.0[a]	4.4±0.0[a]	4.5±0.1[a]	4.3±0.1[a]
1	4.5±0.0[a]	4.4±0.1[a]	4.4±0.2[a]	4.7±0.1[a]	4.7±0.1[a]	4.5±0.1[a]	4.4±0.0[a]	4.5±0.1[a]	4.3±0.0[a]
2	4.5±0.1[a]	4.3±0.1[a]	4.4±0.2[a]	4.6±0.1[a]	4.6±0.1[b]	4.5±0.0[a]	4.4±0.0[a]	4.3±0.0[b]	4.4±0.0[a]
3	4.3±0.0[b]	4.0±0.1[b]	4.3±0.1[ab]	4.4±0.0[b]	4.3±0.0[b]	4.3±0.1[b]	4.2±0.1[b]	4.3±0.0[b]	4.1±0.0[b]
4	4.3±0.0[b]	3.8±0.1[c]	4.2±0.1[bc]	4.3±0.0[b]	4.0±0.1[c]	4.3±0.1[b]	4.2±0.1[b]	4.0±0.0[c]	4.1±0.1[b]

[a-c]Mean values (±SD; n =3) in column with the same letters are not significantly different from each other at P>0.05.

* Juice without adding *B. lactis* Bb-12

E.C: Encapsulated *B. lactis* Bb-12

Free: Free *B. lactis* Bb-12

[Ψ] After adding *B. lactis* Bb-12

2. Survival of free and microencapsulated of *B. lactis* Bb-12 in fruit juices

In case of orange juice, Data in Table 2. showing changes in count of free and microencapsulated of bifidobacteria during refrigerated storage period. The results showed that the number of free cells of bifidobacteria in orange juice decreased significantly during storage period. The count was remaining viable above the critical level of 10^6 cfu/ml for 2 weeks. The viable cell counts decreased by 3.37 log cycles to levels below the critical level 10^6 cfu/ml in the end of storage period. Although *B. lactis* Bb-12 is recognised as being more robust when confronted with hostile condition in comparison to other bifidobacteial strains (Crittenden, et al., 2001), continued exposure to oxygen under acidic conditions during refrigerated storage was most probably responsible for the reduction in the numbers of the strain over time (Sheehan, et al., 2007). On the other hand, the microencapsulated cells remained viable counts 10^8 cfu/ml during storage period up to 4 week. No significant difference changes in the viable counts of microencapsulated cells during storage period were found. The viable count of microencapsulated cells decreased only by 0.05 log cycle after 4 week of storage period. Sheehan, et al., (2007), reported that the number of *B. lactis* Bb-12 was decreased in orange juice by ~ 2 log cycles between weeks 6 and 10 to levels below 10^6 cfu/ml and no cells were recovered by week 12.

When survival of *B. lactis* Bb-12 was monitored in mango juice the viable count of free cells of bifidobacteria were significant reduced gradually during storage period (Table 3). The log reduction was 1.9, 2.37, 2.38 and 3.37 log cycles after 1, 2, 3, 4 weeks, respectively. The count was remaining viable above the critical level of 10^6 cfu/ml for 3 weeks. After 4 week the viable counts decreased to levels below the critical level 10^6 cfu/ml. In the other side, the viable counts of microencapsulated cells were still 10^8 cfu/ml during storage period up to 4 weeks. No significant difference changes in the viable counts during storage period. The viable count of microencapsulated cells los only 0.17 log cycle after 4 week of storage period.

Viable count of bifidobacteria in mango juice was higher than the count in orange juice, this findings are due to the orange juice had lower pH values than that in mango juice (Table 1)

Table (2): Survival of free and microencapsulated of *B. lactis* Bb-12 in orange juice during refrigerated storage period.

Storage period (week)	Orange juice			
	Free cells		Microencapsulated cells	
	Count*	Log reduction	Count*	Log reduction
Zero time [Ψ]	8.41±0.01[a]	0	8.25±0.05[a]	0
1	6.58±0.04[b]	1.83	8.24±0.02[a]	0.01
2	6.26±0.07[c]	2.15	8.22±0.01[a]	0.03
3	5.95±0.06[d]	2.46	8.20±0.06[a]	0.05
4	5.04±0.06[e]	3.37	8.20±0.03[a]	0.05

[a-d]Mean values (±SD; n =3) in column with the same letters are not significantly different from each other at *P>0.05*.
* log 10 CFU/ml
[Ψ] After adding *B. lactis* Bb-12

Table (3): Survival of free and microencapsulated of *B. lactis* Bb-12 in mango juice during refrigerated storage period. Robust confronted

Storage period (week)	Mango juice			
	Free cells		Microencapsulated cells	
	Count*	Log reduction	Count*	Log reduction
Zero time [Ψ]	8.53±0.02[a]	0	8.32±0.02[a]	0
1	6.63±0.05[b]	1.9	8.27±0.06[a]	0.05
2	6.16±0.21[c]	2.37	8.25±0.01[a]	0.07
3	6.15±0.05[c]	2.38	8.20±0.01[a]	0.12
4	5.16±0.04[d]	3.37	8.15±0.07[a]	0.17

[a-d]Mean values (±SD; n =3) in column with the same letters are not significantly different from each other at *P>0.05*.
* log 10 CFU/ml
[Ψ] After adding *B. lactis* Bb-12

In case of pineapple juice, although, the viable counts of free cells of bifidobacteria were reduced significantly, but the count was remaining viable above the critical level of 10^6 cfu/ml and still above 10^7 cfu/ml after 4 weeks of storage period (Table 4). The log reduction of free cells was very low compared with that in orange and mango juices during all time of storage period. A positive correlation between the pH values of tested juices and the viable counts of free cells of *B. lactis* Bb-12 was found. Sheehan, et al., (2007), found the cell counts of *B. lactis* Bb-12 in pineapple juice were recovered above 10^6 cfu/ml and also found no viable cell were recovered after 10 week. The microencapsulated of bifidobacteria in pineapple juice takes the same trend of the viable count in orange and mango juices during storage period. The microencapsulation protected *B. lactis* Bb-12 from the low pH of orange, mange and pineapple juices and remained viable during refrigerated storage period.

These results were in agreement with Khalil and Mansour (1998) they found that the viability of bifidobacteria encapsulated was increased in calcium alginate were incorporated in mayonnaise, and storage studies were done for 16 week. Also, Saarela et al., (2006) studied whether different substrated affect the culturable stability of freeze dried *Bifidobacteium animal* subsp. *lactis* preparation during storage in milk and fruit juice. It was reported that cells produced in different ways had comparable stability in milk, whereas in juice, sucrose-protected cells survived better than reconstituted skim milk-protected cells. Dinaker and Mistry (1994), reported that the microencapsulaion has been shown to be effective method for maintaining the viability of probiotic cultures in yoghurt. On the other hand, Lee and Heo (2000), found that the death rate of *Bifidobacterium longum* in the calcium alginate beads decreased proportionally with an increase in both the alginate gel concentration and bead size. Adhikari et al., (2003), reported that the decline in population of *Bifidobactrium longum*, irrespective of strain, was significant from 5th day, there was a reduction of 89.3% (0.97 log cycles) and 91.8% (1.08 log cycles)in the population of *B. longum* B6 and *B. longum* ATCC 15708, respectively, in yoghurt containing nonencapsulated cells over the storage period. In a parallel line, Godward and Kailasapathy (2003) recorded that encapsulation followed by freeze-drying of the probiotic bacterial calls ensured the smallest reduction in the viability of cells in yoghurt.

Table (4): Survival of free and microencapsulated of *B. lactis* Bb-12 in pineapple juice during refrigerated storage period.

Storage period (week)	Pineapple juice			
	Free cells		Microencapsulated cells	
	Count*	Log reduction	Count*	Log reduction
Zero time Ψ	8.10±0.01[a]	0	8.43±0.04[a]	0
1	7.63±0.04[b]	0.47	8.36±0.01[a]	0.07
2	7.63±0.01[b]	0.47	8.36±0.01[a]	0.07
3	7.37±0.03[c]	0.73	8.27±0.01[a]	0.16
4	7.23±0.02[d]	0.87	8.35±0.04[a]	0.08

[a-d]Mean values (±SD; n =3) in column with the same letters are not significantly different from each other at $P>0.05$.
* log 10 CFU/ml
Ψ After adding *B. lactis* Bb-12

3. Sensory evaluation of fruit juices containing free and microencapsulated *B. lactis* Bb-12.

Table (5) showed the results of statistical analyses of sensory evaluation of orange, mango and pineapple juices containing free and microencapsulated *B. lactis* Bb-12. There was a significant differences in appearance and color and taste between orange juice control and that containing either free or encapsulated cells. No significant changes in odor of control orange juice and there containing microencapsulated cells were observed. The taste of orange juice containing free or encapsulated cells changed significantly compared with control juice. No significant differences were detected in consistency of control orange juice and that containing free or microencapsulated cells. The pineapple juice has a similar trend of orange juice for all sensory evaluation characteristics. No significant differences were found in appearance and color, odor, taste and consistency of control mango juice and that containing either free or encapsulated cells. These results were an agreement with Luckow and Delahunty, (2004), they reported that the appearance of the probiotic juice was significantly different than the appearance of the seven conventional juices. They also found, the older females (over 40) displayed a preference for the appearance, aroma and flavor characteristics of probiotic juices. However, research has shown that perceptible off-flavors are associated with probiotic orange juices (Luckow, et al., 2004). A tropical juice concentrate (e.g., containing pineapple, mango and passionfruit juices) was added to a portion of the pasteurized probiotic orange juice at a concentration of 10% to mask the off-favours associated with probiotic cultures in orange juice (Luckow, et al., 2006).

Table (5): Sensory evaluation of orange, mango and pineapple juices containing free and microencapsulated *B. lactis* Bb-12.

Products	Orange			
	Appearance and Color (10)	Odor (10)	Taste (10)	Consistency (10)
Control*	9.92±0.36[a]	9.85±0.61[a]	9.77±0.611[a]	9.62±0.76[a]
Free cells Ψ	8.00±1.08[b]	7.69±1.11[b]	7.85±1.34[b]	9.08±0.76[a]
Encapsulated cells Ω	6.85±1.12[c]	9.75±1.34[a]	6.08±0.76[c]	9.65±0.80[a]
	Mango			
	Appearance and Color	Odor	Taste	Consistency
Control*	9.46±0.76[a]	9.31±0.91[a]	9.77±0.43[a]	9.77±0.47[a]
Free cells Ψ	8.92±1.26[a]	8.38±1.74[a]	9.57±1.42[a]	9.66±1.13[a]
Encapsulated cells Ω	8.77±1.36[a]	8.23±1.50[a]	9.77±1.41[a]	9.68±1.50[a]
	Pineapple			
	Appearance and Color	Odor	Taste	Consistency
Control*	9.77±0.63[a]	9.92±0.36[a]	9.77±0.47[a]	9.84±0.43[a]
Free cells Ψ	8.92±0.86[b]	8.69±1.18[b]	8.77±1.01[b]	9.85±1.07[a]
Encapsulated cells Ω	6.38±1.56[c]	9.62±1.50[a]	6.54±0.97[c]	9.85±1.13[a]

[a-c]Mean values (±SD; n =13) in column with the same letters are not significantly different from each other at $P>0.05$.

* Juice without adding *B. lactis* Bb-12

Ψ Juice containing free cells of *B. lactis* Bb-12

Ω Juice containing encapsulated *B. lactis* Bb-12

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الأعداد الحية لبكتيريا البيفيدو الحرة والمكبسلة في عصير البرتقال والمانجو والأناناس

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تم دراسة الأعداد الحية وتأثير بكتيريا البيفيدو (*Bifidobacterium lactis* Bb-12) الموجودة في صورة حرة أو المكبسلة بكبسولة الكابا كارجينان علي الأس الهيدروجيني (رقم الـ pH) والجودة الحسية لعصير البرتقال والمانجو والأناناس خلال التخزين لمدة أربعة أسابيع. وتبين من النتائج المتحصل عليها أن العصائر قيد الدراسة المحتوية على بكتيريا البيفيدو الحرة سجلت أقل قيم للأس الهيدوجيني في نهاية مدة التخزين بالمقارنة بالعينة القياسية (عصير بدون اضافة أي نوع من أنواع بكتيريا البيفيدو) والعينة المحتوية على بكتيريا البيفيدو المكبسلة، وقد سجل عصير البرتقال أقل قيم للأس الهيدروجيني. انخفضت الأعداد الحية لبكتيريا البيفيدو الحرة انخفاضا معنويا في كل عينات العصير المختبرة خلال مدة التخزين. ظلت أعداد بكتيريا البيفيدو الحية أعلى من 10^6 خلية/جرام في عصائر البرتقال والمانجو والأناناس لمدة اسبوعين وثلاث وأربع أسابيع علي التوالي خلال التخزين في الثلاجة. انخفضت أعداد بكتيريا البيفيدو معنويا إلي حد أقل من 10^6 خلية/جرام بواقع 3.37، 3.37 و 0.87 دورة لوغارتمية في عصائر البرتقال والمانجو والاناناس، على الترتيب وذلك في نهاية مدة التخزين. ظهرت علاقة طردية بين قيم الأس الهيدروجيني للعصائر المختبرة والأعداد الحية لبكتيريا البيفيدو الحرة . وعلى الجانب الاخر ظلت أعداد بكتيريا البيفيدو المكبسلة أعلى من 10^8 خلية/ جرام أثناء التخزين لمدة أربع اسابيع. لم يلاحظ أى اختلاف معنوى لأعداد بكتيريا البيفيدو المكبسلة فى كل عينات العصير قيد الدراسة أثناء فترة التخزين. انخفضت الأعداد الحية لبكتيريا البيفيدو المكبسلة فى عصير البرتقال والمانجو والأناناس بمقدار 0.05، 0.17 و 0.08 دورة لوغاريتمية فقط بعد التخزين لمدة 4 أسابيع. وعند عمل التقييم الحسى، وجد أنه لا توجد اختلافات معنوية فى رائحة عصير البرتقال القياسى والمحتوى على بكتيريا البيفيدو المكبسلة. أظهر مذاق عصير البرتقال والأناناس المحتوى على بكتيريا البيفيدو سواء الحرة أو المكبسلة اختلافا معنويا عن العينة القياسية. لم يلاحظ اختلافا معنويا فى مظهر ولون ورائحة ومذاق وقوام العينة القياسية لعصير المانجو وتلك المحتوية على بكتيريا البيفيدو الحرة أو المكبسلة. هذه الدراسة أظهرت أن وضع بكتيريا البيفيدو داخل كبسولة يقيها من تأثير الأس الهيدروجينى المنخفض فى عصائر البرتقال والمانجو والأناناس.