VIABILITY OF BIFIDOBACTERIUM IN FERMENTED CARROT FLAKES

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(Manuscript received 14 January 2008)

Abstract

Probiotic fermented carrot flakes were manufactured using single Bifidobacterium lactis Bb-12, Bifidobacterium longum Bb-46 or mixed, salt brine 5%, and with or without 0.5% orange peel powder, incubated at 35 ± 2ºC under anaerobic condition till the pH value reached ~ 4.5, then stored at 5ºC. The results indicated that Bifidobacterium counts after fermentation of carrot flakes ranged from 8.21 – 10.02 (10^8 cfu/g). Also, addition of orange peel powder to carrot flakes along with Bifidobacterium was highly increased of the Bifidobacterium growth and resulted in good sensory characteristics.

Results indicated also, that Bifidobacterium gradually decreased during storage at 5ºC. However, there still in the range of the recommended level (10^7cfu/g). Besides, the sensory evaluation indicated that no significant differences caused by storage in quality attributes were detected between fermented samples especially those prepared by adding both Bfidobacterium and orange peel powder.

Key words : Bifidobacterium, Carrot, Fermentation, Storage

INTRODUCTION

The preservation of vegetables and fruits by lactic acid fermentation is considered one of the most ancient methods of food preservation. Fermented carrots were increasing popularly for the consumer due to high quality of sensory evaluations (Niketic-Aleksic *et al.,* 1973).

Probiotic bacteria are frequently used as the active ingredients in functional foods in dairy products such as bio-yoghurt and cheese. Also, they include cookies, frozen desserts and fermented beverages (Oliveira *et al.,* 2002, Saleh *et al.,* 2004 and Gouda, 2006).

The claimed beneficial effects and therapeutic application of probiotic bacteria in human can be summarized as balancing of colonic microbial, vaccine adjuvant effect, reduction of faecal enzymes implicated in cancerintiation, enhancement of the immune systems, reduction of serum cholesterol and reduction of lactase intolerance (Hattingh and Viljoen 2001).

Probiotic food must contain at least 10^7 cfu/g probiotic bacteria and should be consumed at levels higher that 100g/day to have positive effects of health (Ishibashi and Shimanura, 1993).

Orange peels are sources of dietary fiber being 35.8% of crude fiber and 14.3% pectin on dry weight (Abou-El-Maati, 1999).

Ibrahim *et al.,* (2003) mentioned that dietary fibers are non-starch polysaccharides enhanced growth of lactic acid bacteria and probiotic bacteria. Also, it increased the consistency value of products as well as being anticarcinogenic activities.

Therefore, this study was carried out to produce a probiotic fermented carrot flakes using different strains of probiotic bacteria as single or mixed, with or without addition of orange peel powder and its effect on the quality of the product during storage.

MATERIALS AND METHODS

Materials

Carrot vegetables (*Daucus carrota*) Chenteny variety and Baladi orange fruits (*Citrus sinensis*) were obtained from the Horticultural Research Institute, Giza, Egypt.

Salt (Sodium chloride) was obtained from Sigma Chemical Co. USA.

Bifidobacterium lactis (Bb-12) and *Bifidobacterium longum* (Bb-46) were obtained from Chr. Hansen Lab., Copenhagen, Denmark.

All microbiological media used were obtained from Oxoid Division of Oxiod Ltd., London.

Methods

Processing methods

Preparation of dried Baladi orange peels powder

Baladi orange peels were washed, cut into halves, then dried at 65ºC until constant weight. After drying the dried peels were ground separately in a mill, then screened by passing through U.S standard No. 100 sieve, then packed in polypropylene bags.

Manufacture of fermented carrot flaks

Carrots were washed, flaked before blanching with steam for 5 min, then cooled in tap water. The blanching water used to make brine 5%, which was added to carrot flakes at ratio 1:2 (w/w) brine: carrot flakes.

The mixture was divided into 8 portions, as follows:

1- Treatment (1) without addition (control).
2- Treatment (2) containing 0.5% (w/w) of orange powder peels.
3- Treatment (3) containing 1% (w/w) of activated *Bifidobacterium lactis* Bb-12.
4- Treatment (4) containing 1% (w/w) of activated *Bifidobacterium longum* Bb-46.
5- Treatment (5) containing 1% (w/w) of activated *Bifidobacterium* Bb-12 and Bb-46 of (1:1).
6- Treatment (6) containing 1% (w/w) of activated *Bifidobacterium* Bb-12 and 0.5% (w/w) of orange powder peels.
7- Treatment (7) containing 1% (w/w) of activated *Bifidobacterium* Bb-46 and 0.5% (w/w) of orange powder peels.
8- Treatment (8) containing 1% (w/w) of activated *Bifidobacterium* Bb-12 and Bb-46 of (1:1) and 0.5% of orange powder peels.

All treatments were poured into jars, tightly sealed and incubated at 35±2°C and maintained at anaerobic condition, till pH~ 4.5 then stored at 5°C. Samples were analyzed periodically for chemical, microbiological and sensory evaluations

Analytical methods

Moisture content, Total soluble solids (T.S.S.), Total titratable acidity, pH value, total reducing and non reducing sugars, ash, crude fiber and total carotenoids were determined according to the methods described in the A.O.A.C. (2000).

Microbiological analysis

Total bacterial counts as well as yeasts and moulds and coliforms bacteria were determined according to Marshall (1992).

Bifidobacterium were determined according to Dinakar and Mistry (1994) using the MRS agar supplemented with 0.05% L. cysteine-HCL. The antibiotic mixture (2g of neomycin sulphate, 0.3 g of nalidixi acid and 60 g of lithium chloride) as a selective agent was prepared in 1L of distilled water and sterilized by filtration through 0.2 µm Millipore filter (Gelman Sci., England), then added to the medium at a rate of 50 ml/L medium just before poring the plates. The plates were anaerobically incubated at 73°C for 48 hrs.

Sensory evaluation

Taste, flavor, color and texture were evaluated using the methods described by Larmond (1977).

Statistical analysis

The results of sensory evaluation were statistically analyzed using ANOVA procedure of the SPSS statistical package (SPSS, 1990).

RESULTS AND DISCUSSION

Chemical composition of fresh carrots

The chemical composition of fresh carrots Chenteny variety presented in Table (1). The moisture content of fresh carrots was 88.94%. Also, in the same table indicated that total, reducing and non-reducing sugars of fresh carrots were 71.52, 49.19 and 22.33%) on dry weigh basis), respectively. Carrots are rich in carotenoids being 73.33 mg/100g dry weight as compared to other fruits. Data in Table (1) showed that crude fiber and ash of carrots were 6.87 and 4.07% (on dry weight basis), respectively

Table 1. Chemical composition of fresh carrots

Properties		Carrots (Chenty variety)	
		Fresh weight	Dry weight
Moisture content	(%)	88.94	
T.S.S.	(%)	9.8	
pH		6.1	
Total acidity(as lacticacid)	(%)	0.20	1.81
Total sugars	(%)	7.91	71.52
Non-reducing sugars	(%)	2.47	22.33
Reducing sugars	(%)	5.44	49.19
Total carotenoids	(mg/100g)	8.11	73.33
Ash	(%)	0.45	4.07
Crude fiber	(%)	0.76	6.87

Effect of fermentation and storage on acidity and pH value of carrot flakes

The combination of Bifidobacterium Bb-12, Bb-46 and orange peel powder caused an increase in acidity as lactic with a simultaneous decrease in pH value. The obtained results indicated that the metabolization of different fermentative microbial and/or the presence of orange peel powder (Andersson *et al.,* 1990). Also, Fleming *et al.,* (1983) found that acidity increased in carrots during storage indicating bacterial

growth. Results in Table (2) and Fig. (1) showed that the acidity gradually decreased till the end of storage at 5ºC. Also, the acidity of fermented carrot flakes with Bifidobacterium was higher than that of the control sample during storage. This may be due to metabolic activities of Bifidobacteria as reported by Saleh *et al.*, (2004). Also, from the same table the addition of orange peel powder to carrot flakes enhanced increasing of acidity during storage.

Table 2. Changes of total acidity and pH value of carrot flakes during fermentation and storage

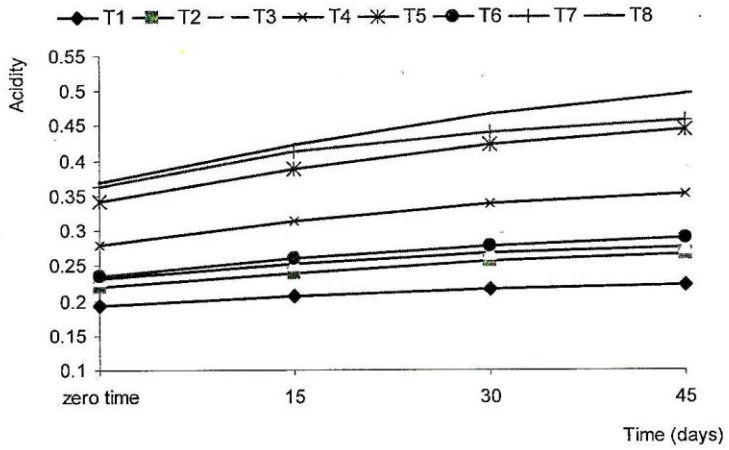
Storage period (days) / Treatments	Total acidity (as lactic acid) (%) (%)(%)(%)(%)				pH value			
	zero time	15	30	45	zero time	15	30	45
T1	0.193	0.207	0.217	0.222	4.65	4.60	4.56	4.53
T2	0.220	0.240	0.256	0.265	4.57	4.52	4.47	4.45
T3	0.231	0.252	0.267	0.276	4.52	4.47	4.42	4.38
T4	0.280	0.314	0.339	0.351	4.48	4.42	4.37	4.32
T5	0.343	0.388	0.423	0.443	4.46	4.39	4.34	4.29
T6	0.236	0.260	0.278	0.288	4.50	4.43	4.38	4.33
T7	0.364	0.413	0.439	0.456	4.45	4.38	4.33	4.28
T8	0.368	0.423	0.466	0.495	4.44	4.36	4.29	4.24

T1 : Carrot flakes (control)
T2 : Carrot flakes + orange peel
T3 : Carrot flakes + *Bif.lactis* Bb-
T4 : Carrot flakes + *Bif. longum* Bb-46
T5 : Carrot flakes + *Bif. lactis* Bb-12+ *Bif. longum* Bb-
T6 : Carrot flakes + orange peel powder + *Bif. lactis*
T7 : Carrot flakes +orange peel powder + *Bif. longum*
T8 : Carrot flakes +orange peel powder+*Bif.lactis* Bb-12+ *Bif. longum* Bb-46

Effect of fermentation and storage on microbial counts of carrot flakes

Total bacterial counts

Data in Table (3) and Fig. (2) illustrated that the total bacterial counts of control treatment was lower than those of the other treatments.



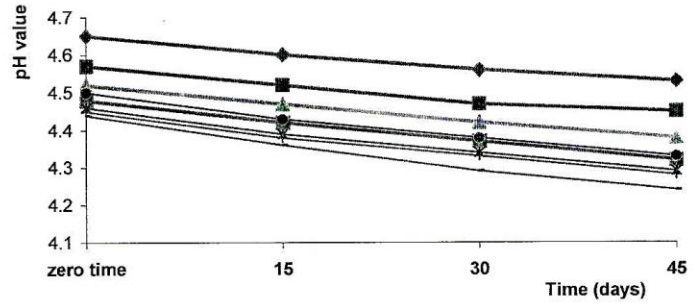


Fig1. Effect of fermentation and storage period on total acidity and pH value .

T1	:	Carrot flakes (control)	T5	:	Carrot flakes + *Bif. lactis* Bb-12+ *Bif. longum* Bb-
T2	:	Carrot flakes + orange peel	T6	:	Carrot flakes + orange peel powder + *Bif. lactis*
T3	:	Carrot flakes + *Bif.lactis*Bb-	T7	:	Carrot flakes +orange peel powder + *Bif. longum*
T4	:	Carrot flakes + *Bif. longum* Bb-46	T8	:	Carrot flakes +orange peel powder+*Bif.lactis*Bb-12+ *Bif. longum* Bb-46

This may be due to the addition of probiotic bacteria and orange peel powder as dietary fiber. These results are in agreement with those obtained by El-Nagar and

Berennan (2001) who found that the addition of fiber to stirred yoghurt enhanced the growth of bacteria. Also, from the same table, the total bacterial count were increased from zero time to 15 days of storage, then decreased at storage period of 30 and 45 days.This may be attributed to the increase of the acidity in products.

Bifidobacterium counts

Results in Table (3) and Fig. (2) indicated that Bifidobacterium Bb-12 and Bb-46 highly increased after fermentation. These results are in accordance with those obtained by Zaki *et al.,* (2004). Also, the results show that the counts of Bifidobacterium slightly decreased after 15 day of storage, then sharply decline after 30day of storage

The decline in Bifidobacterium may be due to the effect of increasing the acidity during storage. The orange peels powder as dietary fiber in fermented carrot flakes were enhanced the viability of Bifidobacterium. These results are in agreement with those obtained by Gouda (2006) who reported that Bifidobacterium had higher growth rate at 0.5% cellulose than control.

Although, the counts decreased during storage of the Bifidobacterium they were still in the range of the recommended counts at least 10^7 cfu/g.

Coliform, Yeasts and Moulds

Coliforms were not detected in all treatments and during storage. These results reflect the good hygienic condition during manufacture and storage. Results from Table (3) showed that moulds and yeasts were detected only after 30 days of storage and slightly increased parallel with storage but still lower than 10^2 cfu/g of all products at the end of storage period.

Sensory evaluation

The results in Table (4) show the sensory characteristics of fermented carrot flakes after fermentation and during storage period at 5ºC for 45 days. These results indicated that taste, flavor, color and texture of fermented carrot flakes with orange peels powder or bifidobacteria had higher scores than control. These results are in agreement with those recorded by El-Nagar and Berennan (2001). Also, from the same table (4) data showed that extending time of storage up to 45 days had no significant effect on taste and flavor for all treatments, but the scores decreased after 30 days. Results in Table (4) are confirmed by those appeared in table (2) concerning the increasing acidity of fermented carrot flakes during storage.

Results in table (4) showed that color and texture did not differ significantly in all treatments during storage till 45 days. Niketic-Aleksic

et al., (1973) showed that carrots can be successfully subjected to the lactic acid fermentation giving good retention of the color and texture and the salt sour flavor which is typical of fermented vegetables.

From the aforementioned results, it could be concluded that the fermented carrot flakes with Bifidobacterium and orange peel powder gave high quality of sensory evaluation and beneficial microorganisms till 45 days of storage at 5°C.

Table 3. Survival of microbial counts of carrot flakes after fermentation and during

Microbial group / Treatments	Total bacterial (10^8 cfu/g)				*Bif. lactis* Bb-12 (10^8 cfu/g)				*Bif. Longum* Bb-46 (10^8 cfu/g)				Yeasts and moulds (10 cfu/g)			
	zero time	15	30	45	zero time	15	30	45	zero time	15	30	45	zero time	15	30	45
T1	12.5	15.75	9.9	5.83	----	----	----	----	----	----	----	----	ND*	ND	5.21	6.8
T2	15.5	20.15	13.1	8.25	----	----	----	----	----	----	----	----	ND	ND	5.75	7.8
T3	37	43.6	29.3	21.5	8.4	6.8	3.46	1.11	----	----	----	----	ND	ND	5.75	7.8
T4	42.5	51.00	35.7	27.13	----	----	----	----	9.1	7.74	4.73	1.5	ND	ND	6.7	7.8
T5	72.1	83.1	62.3	49.5	8.21	7.18	2.93	0.85	8.84	7.78	4.18	1.24	ND	ND	7.6	9.5
T6	47.5	57.1	39.9	30.1	8.79	7.52	4.08	1.58	----	----	----	----	ND	ND	6.9	8.21
T7	61.8	76.1	54.6	42.8	----	----	----	----	9.95	8.86	4.86	1.69	ND	ND	7.11	9.72
T8	98.4	116.6	90.3	72.4	8.97	7.71	3.2	0.93	10.02	9.02	5.16	1.81	ND	ND	6.2	8.13

storage at 5°C.ND: Not detected

T1 : Carrot flakes (control)

T2 : Carrot flakes + orange peel

T3 : Carrot flakes + *Bif.lactis*Bb-

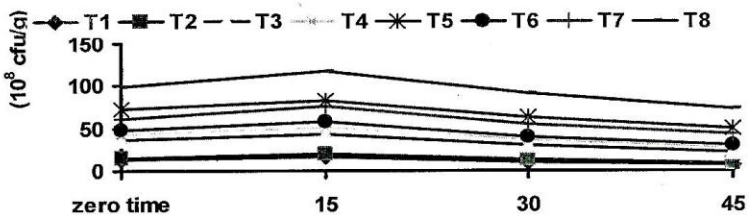
T4 : Carrot flakes + *Bif. longum* Bb-46

T5 : Carrot flakes + *Bif. lactis* Bb-12+ *Bif. longum* Bb-

T6 : Carrot flakes + orange peel powder + *Bif. lactis*

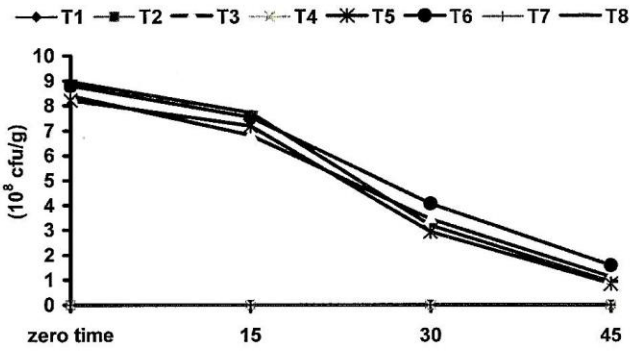
T7 : Carrot flakes +orange peel powder + *Bif. longum*

T8 : Carrot flakes +orange peel powder+*Bif.lactis*Bb-12+ *Bif. longum* Bb-46



Total bacterial counts ofcarrot flakes after fermentation and during storage at 5°C.

Survival of *Bif. longum* Bb-46 of carrot flakes after fermentation and during storage at 5°C.



Survival of *Bif. lactis* Bb-12 of carrot flakes after fermentation and during storage at 5°C.

Fig. 2. Effect of fermentation and storage on microbial counts.

T1 : Carrot flakes (control)
T2 : Carrot flakes + orange peel
T3 : Carrot flakes + *Bif.lactis*Bb-
T4 : Carrot flakes + *Bif. longum* Bb-46
T5 : rrot flakes + *Bif. lactis* Bb-12+ *Bif. longum* Bb-
T6 : rrot flakes + orange peel powder + *Bif. lactis*
T7 : Carrot flakes +orange peel powder + *Bif. longum*
T8 : Carrot flakes +orange peel powder+*Bif.lactis*Bb-12+ *Bif. longum* Bb-46

Table 4. Effect of fermentation and storage on sensory evaluation of carrot flakes

Treatments \ Storage period (days)	Taste				Flavor				Color				Texture			
	zero time	15	30	45	zero time	15	30	45	zero time	15	30	45	zero time	15	30	45
T1	1 ±0.64 ab	7.6 ± 0.83 ab	8.4 ± 0.62 a	8.1 ± 0.89 ab	7.0 ± 0.51 b	7.5 ±0.56 ab	7.9 ±0.85 ab	7.8 ±0.83 ab	9.5 ±0.86 a	9.3 ±0.97 a	9.0 ±1.01 ab	8.3 ±0.91 ab	8.5 ±0.75 a	8.5 ±0.67 a	8.4 ±0.80 ab	8.0 ±0.93 ab
T2	7.3 ±0.62 ab	7.8 ± 0.83 ab	8.5 ± 0.68 a	8.1 ± 0.54 a	7.5 ± 0.56 ab	8.0 ±0.79 ab	8.2 ±0.82 ab	8.5 ±0.71 ab	9.6 ±0.89 a	9.3 ±0.67 a	8.9 ±1.02 ab	8.4 ±0.62 ab	8.6 ±0.57 a	8.5 ±0.71 a	8.4 ±0.62 ab	8.1 ±0.57 ab
T3	7.45 ±0.92 ab	8.0 ± 0.5 a	8.4 ± 0.7 a	8.3 ± 0.53 a	7.75 ±0.62 ab	8.25 ±0.73 ab	8.6 ±0.83 a	8.5 ±0.61 a	9.4 ±0.89 a	9.1 ±0.81 a	8.7 ±0.71 ab	8.2 ±0.83 ab	8.7 ±0.85 a	8.5 ±0.93 a	8.3 ±0.85 ab	8.0 ±0.79 ab
T4	7.55 ±0.51 ab	8.2 ± 0.5 ab	8.5 ± 0.62 a	8.4 ± 0.81 a	8.0 ± 0.79 ab	8.5 ±0.7 ab	9.0 ±0.62 ab	8.9 ±0.67 ab	9.3 ±0.83 a	9.1 ±0.54 a	8.5 ±0.5 ab	8.0 ±0.71 ab	8.8 ±0.65 a	8.6 ±0.54 a	8.4 ±0.57 ab	8.0 ±0.72 ab
T5	7.65 ±0.75 ab	7.9 ± 0.83 a	8.6 ± 0.65 a	8.3 ± 0.67 a	7.5 ±0.56 ab	8.25 ±0.62 ab	8.7 ±0.65 ab	8.5 ±0.71 ab	9.4 ±0.86 a	9.0 ±0.93 a	8.4 ±0.151 ab	8.0 ±0.77 ab	8.8 ±0.55 a	8.5 ±0.61 a	8.3 ±0.74 ab	8.1 ±0.83 ab
T6	7.9 ±0.68 ab	8.3 ± 0.73 a	8.6 ± 0.96 a	8.4 ± 0.59 a	7.8 ±0.74 ab	8.5 ±0.5 ab	8.7 ±0.61 a	8.5 ±0.65 ab	9.5 ±0.53 a	9.2 ±0.91 a	8.7 ±0.67 ab	8.6 ±1.01 ab	8.8 ±0.65 a	8.6 ±0.25 a	8.4 ±0.25 ab	8.2 ±0.82 ab
T7	8.1 ±0.65 ab	8.5 ± 0.6 a	8.6 ± 0.97 a	8.4 ± 0.71 a	8.1 ±0.67 ab	8.6 ±0.67 ab	8.8 ±0.86 ab	8.7 ±0.86 ab	9.4 ±0.89 a	9.2 ±1.09 a	8.4 ±1.1 ab	8.0 ±0.84 ab	8.9 ±0.67 a	8.7 ±0.25 a	8.4 ±0.75 ab	8.1 ±0.62 ab
T8	8.1± 0.83	8.2 ±0.67	8.5 ± 1.02	8.3 ± 0.77	7.7 ±0.6	8.4 ±0.67	8.7 ±0.83	8.6 ±0.83	9.4 ±0.54	9.1 ±0.73	7.9 ±0.83	7.8 ±0.97	9.0 ±0.7	8.8 ±0.92	8.4 ±1.01	8.2 ±0.89

The mean value is significant at P<0.05

T1 : Carrot flakes (control)

T2 : Carrot flakes + orange peel powder

T3 : Carrot flakes + *Bif.lactis*Bb-12

T4 : Carrot flakes + *Bif. longum* Bb-46

T5 : Carrot flakes + *Bif. lactis* Bb-12+ *Bif. longum* Bb-46

T6 : Carrot flakes + orange peel powder + *Bif. lactis* Bb-12

T7 : Carrot flakes +orange peel powder + *Bif. longum* Bb-46

T8 : Carrot flakes +orange peel powder+*Bif.lactis*Bb-12+ *Bif. longum* Bb-46

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القدرة على حياة بكتريا البيفيدو فى رقائق الجزر المتخّمر

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مركز البحوث الزراعية – معهد بحوث تكنولوجيا الأغذية – الجيزة

تم تصنيع مبشور (رقائق) الجزر المتخمر الداعم للحيوية باستعمال سلالتين من بكتريا البيفيدو *Bifidobacterium lactis* Bb-12 و *Bifidobacterium longum* Bb-46 سواء بصورة فردية أو في صورة مختلطة و ٠,٥% مسحوق قشور البرتقال و ٥% محلول ملحي والتحضين على ٣٥م° ±٢م° في ظروف غير هوائيةحتى الوصول الى pH حوالى ٤,٥ ثم التخزين على ٥°م.

أشارت النتائج أن بكتريا البيفيدو تراوحت من ٨,٢١ – ١٠,٠٢ (١٠ ^٨ خلية/جم) بعد عملية التخمر لرقائق الجزر. ايضاً إضافة مسحوق رقائق البرتقال إلى مبشور الجزر مع بكتريا البيفيدو زادت من نمو بكتريا البيفيدو وأدت إلى خصائص حسية جيدة.

كما أشارت النتائج أن بكتريا البيفيدو تقل تدريجيا أثناء التخزين على ٥°م ومع ذلك ظلت أعداد البكتريا في الحدود الموصى بها (١٠^٧ خلية/جم) بالإضافة إلى انة لا توجد فروق معنوية في الخصائص الحسية أثناء التخزين حتى ٤٥ يوم خصوصاً في رقائق الجزر المتخمر المضاف اليه بكتريا البيفيدو ومسحوق قشر البرتقال.