https://doi.org/10.17221/39/2018-CJFS

Fruit Juices With Probiotics – New Type of Functional Foods

Šárka HORÁČKOVÁ[1], Kristýna ROKYTOVÁ[1], Kristina BIALASOVÁ[1], Iveta KLOJDOVÁ[1] and Marcela SLUKOVÁ[2]*

[1]Department of Dairy, Fat and Cosmetics and [2]Department of Carbohydrates and Cereals, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Prague, Czech Republic

**Corresponding author: sarka.horackova@vscht.cz*

Abstract

Horáčková Š., Rokytová K., Bialasová K., Klojdová I., Sluková M. (2018): Fruit juices with probiotics – new type of functional foods. Czech J. Food Sci., 36: 284–288.

The cells of commercial strain *Bifidobacterium animalis* subsp. *lactis* Bb12 were encapsulated using emulsion encapsulation in a milk protein matrix. The volume based median of the microcapsules was 52.1 ± 6.2 μm. The stability of free and encapsulated cells was compared during 28 day-storage in pineapple juice and in strawberry-apple juice at 8 ± 1°C and 22 ± 1°C. Encapsulation ensured a higher number of cells compared to the free cells only at 8 ± 1°C. Strawberry-apple juice was found to be not suitable as probiotic vehicle. Both free and encapsulated cells lost their viability after 14 days at 22 ± 1°C. The number of bifidobacteria cells, pH and lactic and acetic acid content did not change in pineapple and strawberry-apple juice after 24 h cultivation at 37°C.

Keywords: bifidobacteria; encapsulation; pineapple juice; strawberry juice

Currently, consumers are increasingly interested in so-called 'functional foods' containing bioactive compounds such as fibre, oligosaccharides or probiotic microorganisms whose consumption can lead to the prevention or reduction of the risk of food-borne illness. Probiotics together with prebiotics (non-digestible food ingredients that positively and selectively stimulate the growth and/or activity of beneficial bacteria in the colon (Gibson & Roberfroid 1995)), phytonutrients and unsaturated fatty acids complement the traditional functional food ingredients such as vitamins, minerals and micronutrients (Jankovic *et al*. 2010).

Many foodstuffs containing probiotics or prebiotics are of milk origin but the interest of both manufacturers and consumers is extended also to non-dairy products. Greater attention is now given to fruit juices that are considered to be healthy due to their high content of bioactive compounds and are consumed by all age groups of the population. Fruit juices with added probiotics can be a choice for people who cannot consume dairy products for health (lactose intolerance) or other reasons (not available in the market, lifestyle habits) (Antunes *et al*. 2013; Martins *et al*. 2013). Fruit juices containing probiotics are commercially available only in limited variants, mainly supplemented with *L. plantarum* 299v or *L. rhamnosus* GG (Perricone *et al*. 2015). They are not always suitable for a probiotics application because of their composition (minimum proteins and aminoacids content, presence of phenolic compounds inhibiting bacteria, flavonoids and organic acids lowering pH) (Nualkaekul *et al*. 2013). Paradoxically, fruit juices can contain ingredients which promote the viability of probiotics such as ascorbic acid, which reduces redox potential (Antunes *et al*. 2013), saccharides or organic acids usable as a carbon source (Nualkaekul & Charalampopoulos 2011)

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. 20-SVV/2017

https://doi.org/10.17221/39/2018-CJFS

or cellulose, which may help to protect probiotics during manufacturing and storage (Martins *et al*. 2013). Factors influencing the stability of probiotics in fruit matrices include the microorganism strain and its inoculum, the juice's composition, which determines pH, oxygen concentration, the presence of antimicrobial compound, artificial dyes and flavours, and last but not least the production processes and subsequent handling (pasteurization, storage temperature and packaging material used) (Perricone *et al.* 2015). Many studies demonstrating how to increase the stability of probiotics in fruit juices have been published. The most successful methods are fortification by prebiotics, cellulose, β-glucans (Saarela *et al*. 2006), storage at low temperature in atmosphere enriched by carbon dioxide (Corbo *et al.* 2014), antioxidants addition (Nag & Das 2013) or the probiotics encapsulation (Tsen *et al*. 2004; Ding & Shah 2008). The most commonly used process is an extrusion method using alginate, chitosan, gelatine or cellulose derivatives as an encapsulation material (Nualkaekul *et al*. 2013). Emulsion encapsulation in a protein matrix (Heidebach *et al*. 2009) has not been mentioned in literature regarding the application of probiotics in fruit juices so far.

The aim of this study was to verify the viability of a commercial strain *Bifidobacterium animalis* subsp. *lactis* Bb12 (Bb12) in the form of free and encapsulated cells in pineapple and strawberry-apple juices during long-term storage at 8 ± 1°C and 22 ± 1°C, as well as to test the effect of encapsulation on maintaining the original sensory properties of juices.

MATERIAL AND METHODS

Used microorganisms. Commercial probiotic lyophilized DVS culture *Bifidobacterium animalis* subsp. *lactis* Bb12 (Ch. Hansen, Denmark) was cultured in MRS broth (Merck, Germany) at pH 6.5 with the addition of L-cysteine hydrochloride monohydrate (0.5 g/l) (Sigma, USA) anaerobically for 24 h at 37°C.

Fruit juices. Relax® 100% pineapple juice made from pineapple concentrate (pineapple juice from concentrate, pineapple pulp, pasteurized, proteins 0 g/100 ml, carbohydrates 12 g/100 ml, of which sugar 12 g/100 ml) and Relax® Strawberry-apple juice, partly made from concentrates and fruit puree (water, juice from concentrates and puree (33%), of which strawberries 25%, apples 8%, citric acid, concentrate of black carrots, flavour, proteins 0 g/100 ml, carbohydrates 9.3 g/100 ml, of which sugars 9.3 g/100 ml, fats 0 g/100 ml) were purchased on the market.

Encapsulation process. Encapsulation of *B. animalis* subsp. *lactis* Bb12 was performed according to Heidebach *et al.* (2009) in a modification according to Lisova *et al.* (2013). Briefly, 30 g of 35% (w/w) reconstituted skimmed milk (skimmed-milk powder Laktino; PROMIL, Czech Republic) was kept for 2 h at 5°C with agitation (500 rpm) in a closed 50 ml vessel. Afterwards, about 2 g of fresh culture and 400 µl of diluted rennet (1 : 4) Naturen™ PREMIUM 145 (Christian Hansen, Germany) were added. The mixture was stirred (500 rpm) at 5°C for 1 h and subsequently 180 µl of 10% (w/w) $CaCl_2$ was added. Fifteen grams of this mixture was immediately transferred to 150 ml of canola oil with 0.5% (w/w) soya lecithin (Solae Europe S.A., Switzerland), purity of 96%, and stirred for 5 min to emulsify the mixture into the oil. Then, still stirring, the mixture was heated up to 40°C with 15 min duration. The capsules were separated by gentle centrifugation (500 rpm, 2 min and 4°C), washed 3 times with distilled water and stored in wet form at 4°C before further use.

Particle size determination and capsules optical control. The mean size distribution of microcapsules was measured using Mastersizer 2000 (Malvern, UK) with dispersion unit Hydro G (Malvern, UK) at 25°C. A refractive index 1.45 and absorption coefficient 0.001 were selected for the measurement. The results are the means from two independent encapsulation processes; the sample analyses were repeated seven times. The mean of volume based median ($d_{0.5}$) (i.e. 50% of total volume is composed of microcapsules with diameters equal or lower than $d_{0.5}$) and 90% fractiles ($d_{0.9}$) were calculated. Microscopic observations of the microcapsules were performed on a Leica DM LF (Leica Microsystems, Germany) after methylene blue staining.

Analytical determination. To determine the number of cells in the microcapsules, 9 ml of citrate buffer supplemented with Tween 80 (0.5 g/l), pH 7.5 was added to 1 g of wet microcapsules, followed by dilution with physiological solution and using the cultivation plate method at MRS agar (Merck, Germany), pH 6.8 with the addition of L-cysteine hydrochloride monohydrate (0.5 g/l) (anaerobic cultivation, 72 h and 37°C). The same plate method was used to count the number of free cells in samples. The active acidity was measured with a pH meter (Jenway, UK) provided with a combined electrode.

https://doi.org/10.17221/39/2018-CJFS

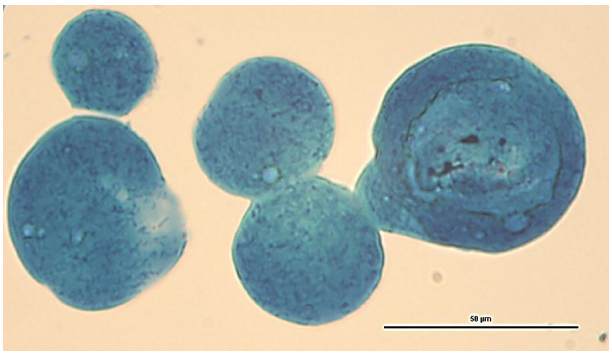

Figure 1. Microcapsules with *B. animalis* subsp. *lactis* Bb12 in milk protein matrix after methylene-blue staining

The metabolic activity of free and encapsulated cells in both juice was tested based on cell count, pH, lactic and acetic acids contents determined by capillary isotachophoresis (ITP analyser IONOSEP 2003; Recman, Czech Republic) after 24 h cultivation at 37°C. The anionic lead electrolyte was composed of 10 mmol/l HCl, 1% hydroxypropyl methylcellulose and 22 mmol/l ε-aminocaproic acid (pH 4.5). The end electrolyte contained 5 mmol/l of capric acid. Lithium lactate and ammonium acetate were used as standards for calibration curves measurements. The results are the means from two parallel assays, both were analysed twice (n = 4).

Storage. The stability of free (cultivated in MRS broth and washed in physiological solution) and encapsulated cells were monitored during storage in pineapple and strawberry juice for 28 days at 8 and 20 ± 1°C. Initial cells concentration was approx. 10^8 CFU/ml of juice. The number of viable cells was determined at weekly intervals.

RESULTS AND DISCUSSION

The Figure 1 illustrates the example of microcapsules obtained by the encapsulation process. The mean of volume based median $d_{0.5}$ was 52.1 ± 8.2 µm and $d_{0.9}$ value was 193.6 ± 24.7 µm. The size of microcapsules can significantly influence the sensory characteristics. The size reported to have no potential sensory defect was approx. 250 µm (Heidebach *et al*. 2009) and is usually achieved by emulsion technique rather than by the extrusion method of encapsulation (Burgain *et al*. 2011). The changes in the shape and size of the microcapsules were monitored microscopically during the storage period. Microcapsules were compact even after 28 days storage at both temperatures in pineapple juice and at 8 ± 1°C in strawberry-apple juice, while total disruption in strawberry-apple juice stored for 21 days at room temperature was observed.

The main goal of this experiment was to access bacteria stability when applied in different fruit juices. Figures 2 shows changes in the number of viable cells (free and encapsulated) during storage in both media tested at 8 ± 1°C (Figure 2A) and 22 ± 1°C (Figure 2B). During the first 3 weeks of storage, no effect of encapsulation on cell viability in pineapple juice at 8 ± 1°C was observed. But a more significant difference in a decrease of cells number of free cells (1.7 log cycles) compared to encapsulated cells (only 0.7 log cycle) was detected after 28 days storage. The same trend was noted at room temperature storage. Acidity of pineapple juice (pH 3.8 ± 0.1) did not change during the whole period at either temperature. Some previously published studies also confirmed pineapple juice as a suitable carrier of probiotics due to its high fibre content, low content of antimicrobial phenolic compounds and higher pH (Espinosa & Navarro 2010; Nualkaekul & Charalampopoulos 2011).

The level of cell count reached when applying free and encapsulated *B. animalis* subsp. *lactis* Bb12

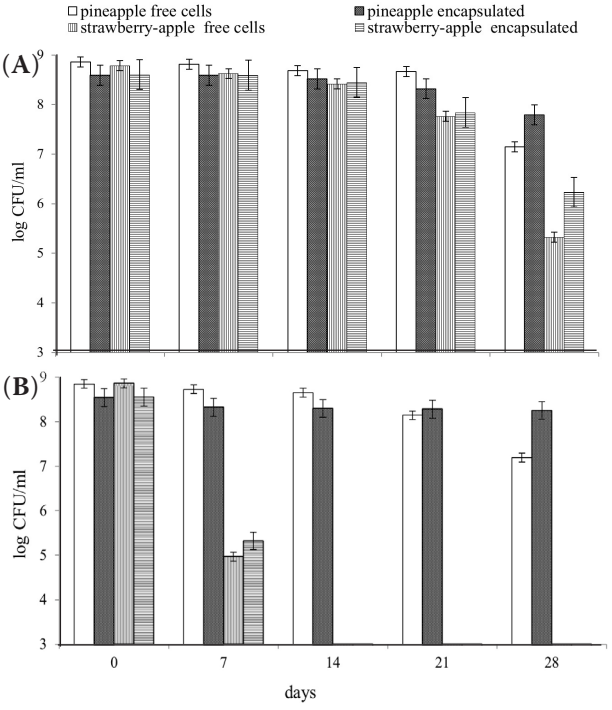

Figure 2. The number of free and encapsulated cells in milk protein matrix during storage in pineapple and strawberry-apple juices at 8 ±1°C (**A**) and 22 ± 1°C (**B**)

https://doi.org/10.17221/39/2018-CJFS

Table 1. Viable count and metabolic activity of free and encapsulated cells in juices after 24 h cultivation at 37°C in aerobic condition

Cells	Pineapple juice				Strawberry-apple juice			
	free		encapsulated		free		encapsulated	
	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
Number (log CFU/ml)	8.72 ± 0.05	7.89 ± 0.08	8.56 ± 0.12	7.99 ± 0.16	8.57 ± 0.08	6.91 ± 0.10	9.20 ± 0.17	9.23 ± 0.12
pH	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	3.2 ± 0.1
Lactic acid (g/l)	0.12 ± 0.03	0.13 ± 0.01	0.21 ± 0.06	0.23 ± 0.05	0.19 ± 0.02	0.15 ± 0.02	0.23 ± 0.03	0.18 ± 0.04
Acetic acid (g/l)	nd	0.01 ± 0.0	nd	0.02 ± 0.0	nd	nd	nd	nd

nd – not detected

in strawberry-apple juice showed that bifidobacteria were significantly less stable in this environment. The acidity of strawberry-apple juice was stable during storage at both temperatures and reached pH 3.2 ± 0.1. The cells stability was influenced both by storage temperature and encapsulation. The difference in the cell count decrease between free and encapsulated cells was 1.1 log cycle (3.5 and 2.4, respectively) at 8 ± 1°C after 28 days. Juice with encapsulated cells still contained more than 10^6 CFU ml, which corresponds with the requirements for functional foods (Boylston *et al.* 2004; Anal & Singh 2007). At the same time the encapsulation did not affect cells viability when strawberry-apple juice was stored at 22 ± 1°C. Already during the first week there was a decrease by 3.9 (free cells) and 3.2 (encapsulated) log cycles. After 14 day-storage no live cells were detected in either of the samples. Juices obtained from small-berry fruits contain a high amount of phenolic compounds and have lower pH and are therefore not suitable for probiotics application (Espinoza & Navarro 2010). This was observed in the study by Perricone *et al.* (2015). The positive effect on the probiotic lactobacilli viability was demonstrated only for peach, orange and tomato juices and for carrot juice in case of bifidobacteria.

Further, the metabolic activity of free and encapsulated cells in both tested juices was compared after 24 h cultivation at optimal temperature 37°C in order to determine whether bifidobacteria can cause any undesirable changes in sensory properties because acetic acid production. The results are summarized in Table 1. It is evident that both type of cells were neither able to grow nor increase the concentration of lactic and acetic acids in the samples. The tested parameters did not significantly change. Some authors have demonstrated that probiotics do not affect the acceptability of juices for consumers (Perricone *et al.* 2014 for *Lactobacillus reuteri* in pineapple juice; Rodríguez *et al.* 2009 for *L. casei* in apple juice), but in other studies the addition of probiotics degraded their sensory properties (Luckow & Delahunty 2004; Krasaekoopt & Kitsawad 2010). In this case tentative sensory valuation confirmed that encapsulated cells did not influence either the taste of pineapple juice by acid off-flavour or by the negative effect of capsules on consistency. Juice with free bifidobacteria cells were not positively accepted by evaluators.

CONCLUSIONS

It was confirmed that juice type selection is a key factor for the stability of added probiotics. In this case, pineapple juice was proved to be suitable for *B. animalis* subsp. *lactis* Bb12 cells viability. Encapsulation increased the cells stability after 28 days of storage. Microcapsules were not stable in strawberry-apple juice at 22 ± 1°C and both free and encapsulated cells died after 14 days. Encapsulation was important for maintaining the sensory properties of pineapple juice. Based on these results, pineapple juice with a commercial probiotic Bb12 strain encapsulated in a milk protein matrix seems to be a promising area for probiotic fruit juice development.

References

Anal A.K., Singh H. (2007): Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends in Food Science and Technology, 18: 240–251.

https://doi.org/10.17221/39/2018-CJFS

Antunes A.E.C., Liserre A.M., Coelho A.L.A., Menezes C.R., Moreno I., Yotsuyanagi K., Azambuja N.C. (2013): Acerola nectar with added microencapsulated probiotic. Food Science and Technology, 54: 125–131.

Boylston T.D., Vinderola C.G., Ghoddusi H.B., Reinheimer J.A. (2004): Incorporation of bifidobacteria into cheeses: challenges and rewards. International Dairy Journal, 14: 375–387.

Corbo M.R., Bevilacqua A., Petruzzi L., Casanova F.P., Sinigaglia M. (2014): Functional beverages: The emerging side of functional foods: commercial trends, research and health implications. Comprehensive Revue in Food Science and Food Safety, 13: 1192–1206.

Ding W.K., Shah N.P. (2008): Survival of free and microencapsulated probiotic bacteria in orange and apple juices. International Food Research Journal, 15: 219–232.

Espinoza Y.R., Navarro Y.G. (2010): Non-dairy probiotic products. Food Microbiology, 27: 1–11.

Gibson G.R., Roberfroid M.B. (1995): Dietary modulation of the human colonic microbiota – introducing the concept of prebiotics. Journal of Nutrition, 125: 1401–1412.

Heidebach T., Först P., Kulozik U. (2009): Microencapsulation of probiotic cells by means of rennet-gelation of milk proteins. Food Hydrocolloids, 23: 1670–1677.

Jankovic I., Sybesma W., Phothirath P., Ananta E., Mercenier A. (2010): Application of probiotics in food products-challenges and new approaches. Current Opinion in Biotechnology, 21: 175–181.

Krasaekoopt W., Kitsawad K. (2010): Sensory characteristics and consumer acceptance of fruit juice containing probiotics beads in Thailand. AU Journal of Technology, 14: 33–38.

Lisová I., Horáčková Š., Kováčová R., Rada V., Plocková M. (2013): Emulsion encapsulation of *Bifidobacterium animalis* subsp. *lactis* Bb 12 with the addition of lecithin. Czech Journal of Food Sciences, 31: 270–274.

Luckow T., Delahunty C. (2004): Which juice is 'healthier'? A consumer study of probiotic non-dairy juice drinks. Food Quality and Preferences, 15: 751–759.

Martins E.M.F., Ramos A.M., Vanzela E.S.L., Stringheta P.C., de Oliveira Pinto C.L., Martins J.M. (2013): Products of vegetable origin: A new alternative for the consumption of probiotic bacteria. Food Research International, 51: 764–770.

Nag A., Das S. (2013): Improving ambient temperature stability of probiotics with stress adaptation and fluidized bed drying. Journal of Functional Foods, 5: 170–177.

Nualkaekul S., Charalampopoulos D. (2011): Survival of *Lactobacillus plantarum* in model solutions and fruit juices. International Journal of Food Microbiology, 146: 111–117.

Nualkaekul S., Cook M.T., Khutoryanskiy V.V., Charalampopoulos D. (2013): Influence of encapsulation and coating materials on the survival of *Lactobacillus plantarum* and *Bifidobacterium longum* in fruit juices. Food Research International, 53: 304–311.

Perricone M., Bevilacqua A., Altieri C., Sinigaglia M., Corbo M.R. (2015): Challenges for the production of probiotic fruit juices. Beverages, 1: 95–103.

Perricone M., Corbo M.R., Sinigaglia M., Speranza B., Bevilacqua A. (2014): Viability of *Lactobacillus reuteri* in fruit juices. Journal of Functional Foods, 10: 421–426.

Rodríguez H., Curiel J.A., Landete J.M., de las Rivas B., de Felipe F.L., Goméz-Cordovés C., Mancheño J.M., Muñoz R. (2009): Food phenolics and lactic acid bacteria. International Journal of Food Microbiology, 132: 79–90.

Saarela M., Virkajarvi I., Nohynek L., Vaari A., Matto J. (2006): Fibres as carries for *Lactobacillus rhamnosus* during freeze-drying and storage in apple juice and chocolate-coated breakfast cereals. International Journal of Food Microbiology, 112: 171–178.

Tsen J.H., Lin Y.P., King V.A.E. (2004): Fermentation of banana media by using κ-carrageenan immobilized *Lactobacillus acidophilus.* International Journal of Food Microbiology, 91: 215–220.

Received: 2018–02–12
Accepted after corrections: 2018–06–19