Anthocyanin-Colored Microencapsulation Effects on Survival Rate of *Lactobacillus rhamnosus* GG, Color Stability, and Sensory Parameters in Strawberry Nectar Model

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Abstract

Probiotic microencapsulation is a promising way to produce functional food, while their stability and sensory acceptability still a challenge. This study aims to enhance the functional properties of strawberry (*Fragaria × ananassa*, cultivar Camarosa) nectar and sensory acceptance using novel anthocyanin-colored microencapsulation of *Lactobacillus rhamnosus*. Four formulations (F1–F4) of coated materials (alginate, whey protein, and pullulan) integrated with anthocyanin pigment were used for encapsulation. The physical properties of microencapsulated probiotics (size, color, efficiency, stability, and survival rate) and quality parameters of nectar (pH, anthocyanin, and sensory acceptability) during 4 weeks of storage at 4 and 25 °C were evaluated. All formulations exhibited high encapsulation efficiency (> 89%), medium bead size (406–504 µm), and proper color (red color). The microencapsulated cells were stable in simulated gastrointestinal and processing conditions (up to 7 log₁₀ CFU mL⁻¹) compared to free cells. F4 (alginate 2% + anthocyanin 0.1% + whey protein 2% + pullulan 2% + cocoa butter 1% + *L. rhamnosus* GG) showed the greatest viability in nectar during storage (6.72 log₁₀ CFU mL⁻¹/4 °C/4 weeks), while a significant decrease in pH (< 2) and anthocyanin (< 60 mg 100 g⁻¹) was observed in nectar-containing free cells. The sensory scores with a difference-preference test as exploratory and preliminary responses revealed that colored probiotic microcapsules enhanced the sensory characters (up to 4 weeks) and commercially accepted (> 80% agreed) of strawberry nectar. Results demonstrated that anthocyanin-colored alginate-whey protein-pullulan matrix had the potential to enhance probiotic viability in functional nectar without negative impact.

Keywords Microencapsulation · *Lactobacillus rhamnosus* GG · Strawberry nectar · Quality parameters · Anthocyanin

Introduction

Strawberry (*Fragaria × ananassa*) nectar is considered one of the most important beverages, highly consumed in Egypt and worldwide (Yildiz & Aadil, 2020). It is a source of vitamins, minerals, carbohydrates, dietary fibers, and antioxidants, moreover tasty, attractive to all ages, and refreshing (Salehi, 2020; Xu et al., 2021). However, they are lower in health benefits and functional properties than fruit juice (Pereda et al., 2020). Therefore, there is a growing interest in functional nectar by incorporation of healthy ingredients, such as probiotics and/or bioactive compounds (de Oliveira Vieira et al., 2020). According to Tripathi and Giri (2014), the probiotic foods represent approximately 60–70% of functional foods. Recently, the demand for probiotic products has gradually increased, the global market was about US\$ 377 million in 2016 and anticipated to expand US\$ 719 million by 2025 (Anand et al., 2018), while probiotic products

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estimated US\$ 61.1 billion in 2021 and anticipated to expand US\$ 91.1 billion by 2026 (MarketsandMarkets, 2018).

Probiotic such as *Lactobacillus rhamnosus* GG is one of the most important legalized clinical bacteria that is widely used as a probiotic culture in food products (Sohail et al., 2013). *L. rhamnosus* has many health benefits as it reduces the severity of diarrhea (Morales et al., 2016), improves the immunity (Costabile et al., 2017), and inhibits the adhesion of *Salmonella enterica* and *Clostridium histolyticum* (Medveďová et al., 2008). However, there are several factors affecting the viability of probiotics such as temperature (Hao et al., 2021; Østlie et al., 2005), pH (Sohail et al., 2012), bile salt (Guerin et al., 2003), gastric juice (Praepanitchai et al., 2019), and food additives.

One of the ways to protect and improve the viability of probiotics in food products is the application of coating system using alginate (Mokarram et al., 2009). But, the maximum loading cells in the entrapped beads are restricted to 25% per volume due to weak mechanical strength (Liu et al., 2017). Another effective approach to overcome these limitations is using a multilayer technique encapsulation (Khorasani et al., 2017).

Encapsulation is a process in which the cells are held within a membrane to minimize cell injury and death (Celli et al., 2015). Recently, microencapsulation is receiving considerable interest as an approach for providing probiotic cells with a barrier against ingestion, storage, and food (Kailasapathy, 2009). Therefore, enhancing the viability of probiotic cells in gastrointestinal juice and during food processing is important for the microorganisms to reach sufficient numbers (10^6 CFU g^{-1} or mL^{-1}) of intestinal contents (Moumita et al., 2017) and have a positive impact on the host (Karimi et al., 2011). The encapsulation of probiotic cells has been investigated in various food products such as sausage (Bilenler et al., 2017), yogurt (El Kadri et al., 2018), soft cheese (Vasile et al., 2020), bread (Zhang et al., 2018), cereal bars (Bampi et al., 2016), chocolate (Malmo et al., 2013), and juice (Mokhtari et al., 2017). One study showed that chitosan-alginate encapsulation improved the survival of *Lactobacillus rhamnosus* in apple juice (Praepanitchai et al., 2019). Also, Krasaekoopt and Watcharapoka (2014) demonstrated that galacto-oligosaccharide-alginate microencapsulation kept the viability of *Lactobacillus acidophilus* and *Lactobacillus casei* at 10^7 CFU mL^{-1} in fruit juice. Also, Rodrigues et al. (2012) found that microencapsulation in alginate with double coating protected *Lactobacillus paracasei* L26 over 50 days in orange juice.

Anthocyanins are water-soluble pigments, responsible for the red color in most fruits and vegetables (Alvarez-Suarez et al., 2021; Giuffrè et al., 2017), as well as a good source of polyphenols and flavonoids as antioxidants (Gralléc et al., 2019; Suriano et al., 2021). Anthocyanins have many components such as delphinidin-3-*o*-glucoside,

cyanidin-3-*o*-glucoside, cyanidin-3-*o*-acetylglucoside, malvidin-3-*o*-glucoside, delphinidin-3-*o*-acetylglucoside, malvidin-3-*o*-*p*-coumarylglucoside, malvidin-3-*o*-acetylglucoside, petunidin-3-*o*-*p*-coumarylglucoside, peonin-3-*o*-*p*-coumarylglucoside, petunidin-3-*o*-glucoside, peonidin-3-*o*-glucoside, petunidin-3-*o*-acetylglucoside, and peonidin-3-*o*-acetylglucoside (Giuffrè, 2013; Fan-Chiang & Wrolstad, 2005). They are a permitted colorant in food and beverage products and has extensively boosted their market requirement (Belwal et al., 2020). Also, they have many health benefits such as prophylactic to cardiovascular disease, anti-inflammatory, anti-carcinogenic, anti-obesity, and anti-diabetic (Yousuf et al., 2016).

Pullulan is a polysaccharide produced by *Aureobasidium pullulans* (Haghighatpanah et al., 2020); it is used as a coating material in food and pharmaceutical because of its water-soluble, tasteless, odorless, colorless, and heat-stable properties. Also, whey protein is widely used as a polymer for microencapsulation of bioactive compounds using different techniques (de Araújo Etchepare et al., 2020). Pullulan and whey protein mixtures have been studied to form edible films and coatings to reduce moisture loss and extend the shelf life in food applications (Gounga et al., 2008).

Previous studies did the best to develop probiotic microencapsulation in different functional food. While, anthocyanin-colored microencapsulated probiotic bacteria within a single biocomposite matrix in fruit juices have not been studied yet. Most of the published researches focused on used capsule material, i.e., alginate (Rodrigues et al., 2012; Shinde et al., 2014), alginate-chitosan (De Prisco et al., 2017; Malmo et al., 2013), whey protein isolate-gum arabic (Bosnea et al., 2014), pectin-starch (Dafe et al., 2017), inulin (dos Santos et al., 2019), soy protein (González-Ferrero et al., 2020), and starch (Murúa-Pagola et al., 2021). However, few studies utilized pullulan in microencapsulation (Ma et al., 2021; Sun et al., 2021). There are no literatures related to the impact of juice additives and processing conditions on the variability of probiotic microcapsules. Also, the effect of probiotic microcapsules on the sensory acceptability of food products remains a challenge. Therefore, our research proposes to develop a protection system and attractive tools for simultaneously delivering these two bioactive compounds to the gastrointestinal tract. These results add evidence that microencapsulation of anthocyanins and probiotic bacteria may be considered as a suitable approach to develop functional compounds and value-added foods, with preventive attributes against diseases.

Therefore, the aim of this study was to (I) produce a functional strawberry (*F. × ananassa*, cultivar Camarosa) nectar fortified with microencapsulated *L. rhamnosus* GG; (II) investigate the stability of probiotic microcapsules under gastrointestinal, thermal processing, and juice

additives; and (III) evaluate the influence of *L. rhamnosus* GG microcapsules on quality parameters and sensory properties (difference-preference test) of strawberry nectar during storage.

Materials and Methods

Chemicals and Additives

Bile salt, sodium alginate, tri-sodium citrate, phosphate buffer, Tween 80, HCl, NaOH, KCl, CaCl₂, anthocyanin, whey protein, porcine pepsin, and pancreatin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Citric acid, sodium benzoate, potassium sorbate, and CMC (El-Naser Co., Cairo, Egypt), carmoisine, and strawberry flavor (Kamena Co., Cairo, Egypt), sugar, and cocoa butter were purchased from a local market in Cairo, Egypt. Pullulan was obtained from the Hayashibara Company (Okayama, Japan).

Culture Activation

The *Lactobacillus rhamnosus* GG (American Type Culture Collection [ATCC] 53,103; Manassas, VA, USA) culture was maintained on the MRS agar (MRS-A; Biolife, Italy). The bacteria were activated twice in MRS broth (MRS-B; Biolife, Italy) and incubated at 37 °C/48 h. The cells were harvested using centrifugation (Thermo Fisher Scientific Megafuge 8R, UK) at 3500 × g under-cooling for 10 min. The pellets were washed twice with sterile peptone water (0.1%; BPW; Biolife, Italy), then re-suspended in 5 mL sterile BPW to the final population ~ 7.5 log₁₀ CFU mL⁻¹.

Microencapsulation of Probiotic Bacteria

The microencapsulation of probiotic bacteria was prepared according to the method described by Mokhtari et al. (2017) with some modifications. Briefly, different solutions of sodium alginate (2%; w/v), whey protein (2 or 3%; w/v), and pullulan (2%; w/v) were dissolved in distilled water, then autoclaved (121 °C for 15 min); after cooling, the anthocyanin (0.1%; w/v) was added. The 5 mL of *L. rhamnosus* GG pellets (~ 7.5 log₁₀ CFU mL⁻¹) and cocoa butter-Tween 80 (1%; w/v) (core material) were suspended in 100 mL of sterile-colored alginate (wall material). The probiotic suspension was dispensed gently using a 5-mL syringe (Nipro, Japan) into 500 mL calcium chloride (0.155 M) with stirring at 300 rpm and held for 30 min for gelation. Afterward, the beads were harvested using filter paper (Whatman No. 4), then washed with sterilized distilled water, and kept subsequently in a sterile peptone solution (0.1%) at 4 °C until use (Morsy & Elsabagh, 2021).

Preparation of Strawberry Nectar

Strawberry (*Fragaria × ananassa*, cultivar Camarosa) concentrate (65°Brix) was purchased from Döhler Co. (Giza, Egypt), then diluted for the preparation of strawberry nectar, according to the General Standards for Fruit Juices and Nectars (Codex, 2005). The nectar recipe was fruit juice (40%; v/v), citric acid (0.3%; w/v), preservatives (0.1%; w/v), carmoisine (0.01%; w/v), flavor (0.05%; v/v), CMC (0.1%; w/v), and sugar (TSS 7.5 °Brix). The nectar was placed in a glass bottle of 200 mL (Middle East Co. of glass, Cairo, Egypt). The nectar was divided depending on microcapsule supplementation into six groups as follows; first (FC; free cells [non-encapsulated]), second (F1; alginate 2% + anthocyanin 0.1% + *L. rhamnosus* GG), third (F2; alginate 2% + anthocyanin 0.1% + cocoa butter 1% + *L. rhamnosus* GG), fourth (F3; alginate 2% + anthocyanin 0.1% + whey protein 3% + cocoa butter 1% + *L. rhamnosus* GG), fifth (F4; alginate 2% + anthocyanin 0.1% + whey protein 2% + pullulan 2% + cocoa butter 1% + *L. rhamnosus* GG), and last one without cells (C; control). All treatments were kept at 4 ± 1 °C and 25 ± 1 °C for 4 weeks. The nectar samples were checked at intervals 0, 1, 2, 3, and 4 weeks.

Morphology, Bead Size, and Color

The morphology of the microencapsulated beads (*L. rhamnosus* GG) was evaluated using scanning electron microscopy (SEM; JSM-6510-LA Ja, Japan). The distribution of microbead size in dispersion medium was measured by a Malvern Mastersizer 3000 (Malvern Instruments, Germany) (de Araújo Etchepare et al., 2020). The color intensity L^* , a^* , and b^* of microencapsulated beads was measured by a Minolta spectrophotometer (model: CM-508d Minolta Corp., Ramsey, NJ, USA). The instrument values are L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness). The chroma (C^*) were calculated by Eq. (1).

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

Encapsulation Efficiency

The EE (%) was determined using the method described by Afzaal et al. (2019) with slight modifications. The microencapsulated beads were centrifuged at 3000 × g (Thermo Fisher Scientific Megafuge 8R, UK) to remove the thin film of peptone solution. A 10 g of microencapsulated beads was dissolved in 100 mL phosphate buffer

(pH 7.2; 37 °C; 60 min), and thereafter, viable bacterial cells were diluted and counted on MRS agar (expressed as $[\log_{10} \text{CFU g}^{-1}]$). The EE (%) was calculated according to Eq. (2).

$$EE(\%) = 100 \frac{M}{M0} \quad (2)$$

where M is the number of viable cells ($\log_{10} \text{CFU g}^{-1}$) released from the microcapsules and $M0$ is the number of free viable cells ($\log_{10} \text{CFU g}^{-1}$) before the encapsulation process.

Stability of Microencapsulated *L. rhamnosus* GG

Heat Treatments and Storage Conditions

The stability of *L. rhamnosus* GG encapsulated beads against different heat treatments was assessed as described by (Zhang et al., 2015) with slight modifications. A 10 g of microparticles and free cells was transferred to 90 mL of peptone water (0.1%; w/v) in falcon tubes. These contents were exposed to various temperatures in a water bath (Thermo Scientific, UK), i.e., 25 ± 1 °C for 60 min and 72 ± 1 °C for 15 min as well as refrigeration temperature 4 ± 1 °C for 60 min. All aliquots were collected, and probiotic viable cells were counted on MRS agar (as in the “Enumeration of Entrapped *L. rhamnosus* GG Cells” section).

Juice Additives

The viability of the microencapsulated *L. rhamnosus* GG in different juice additives was evaluated. Ten grams of microparticles and free cells was transferred to 90 mL of sugar solution (7.5°Brix), citric acid 0.3%, sodium benzoate 0.1%, potassium sorbate 0.1%, carmoisine 0.01%, strawberry flavor 0.05%, CMC 0.1%, and mix of additives. The probiotic viable cells were counted on MRS agar.

Simulated Gastrointestinal Conditions

The viability of *L. rhamnosus* GG under simulated gastric juice (SGJ) was determined according to Krasaekoopt et al. (2008). A 10 g of freshly prepared beads and free cells was placed in 90 mL of sterile SGJ (0.08 M HCl containing 0.2% NaCl, pH 1.5), then incubated at 37 °C for 0, 30, 120, and 240 min with agitation at 50 rpm using a shaker incubator (New Brunswick™ Excella® E25/E25R, USA). The beads were collected by filtration (Whatman No. 4), and free cells were collected by centrifugation under-cooling (Thermo Fisher Scientific Megafuge 8R, UK). Both beads and free cells were placed individually in 90 mL of sterile simulated intestinal juice (SIJ; 0.05 M KH_2PO_4 , 0.6% bile salt, pH 7.4),

then incubated at 37 °C for 150 min. The viable bacterial cells were enumerated in MRS medium.

Enumeration of Entrapped *L. rhamnosus* GG Cells

Ten grams of *L. rhamnosus* GG beads was placed into 90 mL of 0.1 M sterile phosphate buffer (~pH 7) and stomached for 10–15 min (Chaikham, 2015). Certainly, tenfold serial dilutions were done with sterile 0.1% peptone solution. *L. rhamnosus* was enumerated on MRS agar (Biolife, Milan, Italy) at 37 °C for 48–72 h under anaerobic conditions; also free cells enumerated similarly. The populations converted to $\log_{10}$ and expressed as $\log_{10} \text{CFU g}^{-1}$ or mL^{-1} .

pH and Anthocyanin in Strawberry Nectar

The pH value of strawberry (*F. × ananassa*, cultivar Camarosa) nectar was carried out using a digital pH meter (Consort, model P107, Belgium). Anthocyanin content was determined using the spectrophotometer method (CE 599 Universal, USA) at 535 nm (AOAC, 2016).

Sensory Evaluation and Questionnaire

The sensory evaluation was done using a difference-preference test as exploratory and preliminary responses. Ten-member trained panelists aged (20–45 years) from the Department of Food Technology, Benha University, asked to evaluate the strawberry nectar containing free cells and microencapsulated probiotic during the refrigerated storage. The nectar samples were homogenous, served in 50 mL covered plastic cups coded with 3-digit random numbers. The panelists were judged to evaluate the nectar samples using a 7-point hedonic scale for color, odor, taste, texture, and overall acceptability with scores being as follows: 7 = like extremely, 6 = like moderately, 5 = like slightly, 4 = neither like nor a dislike, 3 = dislike slightly, 2 = dislike moderately, and 1 = dislike extremely (Krasaekoopt & Kitsawad, 2010). While, the questionnaire was done according to Van der Merwe et al. (2014) with minor modification. The questionnaire performed by juice companies in Egypt using 11 team or committee (every committee included 15 member; total 165 member); these committees from trained panelists at different departments in the company such as production staff, quality staff, and R&D staff. The panelists asked through a questionnaire to evaluate the strawberry nectar containing microencapsulated probiotic using a 3-point scale (3 = agree, 2 = undecided, and 1 = disagree) and expressed as a percentage.

Statistical Analysis

The obtained data were analyzed using SPSS software (Version 18, Chicago, IL, USA) and presented as mean $\pm$ SD. One-way analysis of variance (ANOVA) and Tukey's tests at $P < 0.05$ of means were carried out. All experiments in the study were performed in triplicate, while the sensory evaluation (difference-preference test) was run with 10 panelists and questionnaire 11-juice companies. Two factors (free cells and microencapsulated) of probiotics were applied. For microcapsules, evaluation and analysis (morphological, bead size, encapsulation efficiency, and stability) were done. Strawberry nectar analysis (pH, anthocyanin, sensory properties, and questionnaire) and factorial design ANOVA with two factors, six treatments (control, FC, F1, F2, F3, F4) and storage time with five points at 0, 1, 2, 3, and 4 weeks, were applied for each parameter (Steel et al., 1980).

Results and Discussion

Morphology, Size, and Color of Microparticles

The microcapsule morphology for different formulations (F1–F4), i.e., alginate, whey protein, and pullulan, showed spherical, uniform, smooth, and free from cracks under scanning electron microscopy. Results revealed that formula F4, quite spherical shape, red color, and homogenous probiotic cells, spread throughout the microcapsules compared to other formulations. Therefore, sodium alginate (2%; w/v) in combination with hydrocolloids, i.e., whey protein and/or pullulan, had formed a good matrix for protective layers of microencapsulated probiotic. To verify the alginate presence (wall material) and microorganisms (core material) in the entire interior of the microbeads, characterizing it as a matrix, verifying that the core material is not only sited in the center but also inside the beads (de Araújo Etchepare et al., 2020). The hydrocolloids and alginate multilayer did not change the appearance of the analyzed microcapsules (Fig. 1). From scanning electron microscopy (SEM) images, it was noticed that treatments F1 and F2 have very few pores with potential penetration on the surface of the beads (Fig. 1). While other formulations (F3 and F4) revealed that beads free from pores may be due to hydrocolloid compounds and were integrated into the polymer network, and beads entrapped the cells. Similar findings were reported by Nami et al. (2020) who found that microencapsulated *Lactococcus lactis* ABRIINW-N19 with alginate-Persian gum (ALG-PG) exhibited spherical shape beads. The spherically shaped microcapsules have advantages such as ease of production, consumption, application, and packaging. Other studies confirmed that spherical beads with multilayer were observed in alginate-psyllium (Zeashan et al., 2020)

Fig. 1 Photo of colored microcapsules of *L. rhamnosus* GG (a) and scanning electron microscopy (SEM) images of encapsulated *L. rhamnosus* GG at different formulations (F1–F4). F1: alginate 2%+anthocyanin 0.1%+*L. rhamnosus* GG), F2: alginate 2%+anthocyanin 0.1%+cocoa butter 1%+*L. rhamnosus* GG), F3: alginate 2%+anthocyanin 0.1%+whey protein 3%+cocoa butter 1%+*L. rhamnosus* GG), F4: alginate 2%+anthocyanin 0.1%+whey protein 2%+pullulan 2%+cocoa butter 1%+*L. rhamnosus* GG)

and alginate-chitosan (Praepanitchai et al., 2019; Xu et al., 2016).

As shown in Table 1, the mean size of *L. rhamnosus* GG microcapsules was ranged between 406 and 504 μm . The maximum bead size was in F4, while the minimum was recorded in F1. A significant difference in particle size ($P < 0.05$) was observed between all formulations. However, alginate multilayer has the potential to keep residence at the application site as a juice supplement delivery system. The variation in bead size could be related to (i) hydration capacity of the polysaccharides (pullulan) through hydrogen bonds (Boudou et al., 2010), (ii) combination between whey protein and alginate that forms viscose solution effect of the size (Zeashan et al., 2020), (iii) technological process such as varying agitation rate and water/oil ratio, and (iv) structural configuration of the microcapsule layers. Similar findings have been reported by Praepanitchai et al. (2019) who found that encapsulation material and multilayer type affect the bead size.

The color parameters, i.e., L^* , a^* , and c^* , of the multilayer microparticles are shown in Table 1. All the microparticles showed a red color (as color reference of strawberry nectar 40% fruit juice), indicating a good homogeneity. No significant difference ($P > 0.05$) in color was recorded

Table 1 Bead size, color, and encapsulation efficiency of the *L. rhamnosus* GG microparticles with different multilayer (mean $\pm$ SD)

Formula	Bead size (μm)	Tristimulus color values			Encapsulation efficiency (EE; %)		
		L^*	a^*	C^*	Numbers before encapsulation ($\log_{10}$ CFU g^{-1})	Numbers after encapsulation ($\log_{10}$ CFU g^{-1})	EE%
F1	406.00 $\pm$ 19.31 d	30.94 $\pm$ 0.71 a	39.27 $\pm$ 0.29 a	49.99 $\pm$ 0.77 a	7.83 $\pm$ 0.15 a	7.05 $\pm$ 0.17 d	89.95 $\pm$ 0.91 d
F2	425.33 $\pm$ 13.87 c	31.11 $\pm$ 0.28 a	39.92 $\pm$ 0.35 a	50.61 $\pm$ 0.45 a	7.83 $\pm$ 0.15 a	7.20 $\pm$ 0.12 c	91.87 $\pm$ 0.23 c
F3	454.67 $\pm$ 13.32 b	31.50 $\pm$ 1.08 a	40.03 $\pm$ 0.62 a	50.94 $\pm$ 1.24 a	7.83 $\pm$ 0.15 a	7.41 $\pm$ 0.14 b	94.66 $\pm$ 0.69 b
F4	504.00 $\pm$ 22.34 a	31.67 $\pm$ 0.73 a	40.09 $\pm$ 0.45 a	51.09 $\pm$ 0.86 a	7.83 $\pm$ 0.15 a	7.72 $\pm$ 0.11 a	98.59 $\pm$ 0.41 a

F1: alginate 2% + anthocyanin 0.1% + *L. rhamnosus* GG, F2: alginate 2% + anthocyanin 0.1% + cocoa butter 1% + *L. rhamnosus* GG, F3: alginate 2% + anthocyanin 0.1% + whey protein 3% + cocoa butter 1% + *L. rhamnosus* GG, F4: alginate 2% + anthocyanin 0.1% + whey protein 2% + pullulan 2% + cocoa butter 1% + *L. rhamnosus* GG. $n=3$. Same small letter indicates that there is no significant difference between any two means “in the same column” ($P > 0.05$)

L^* lightness, a^* redness/greenness, C^* chroma, EE encapsulation efficiency

between all formulations. The color of microbeads was very parallel to strawberry nectar. This red color of microparticles was due to anthocyanin pigment and matched with the structure of strawberry nectar.

Encapsulation Efficiency

Table 1 shows that the encapsulation efficiency of different formulations of the alginate microcapsule was 89.95%, 91.87, 94.66, and 98.59% for F1, F2, F3, and F4, respectively. According to the outcomes, there were critical contrasts for encapsulation efficiency in all these four formulations. High EE ($> 98\%$) in F4 indicated successful multilayer entrapping of viable cells in prepared beads. Consequently, efficient probiotic viable cells ($7.72 \log_{10}$ CFU g^{-1}) could be released at the site of impact. As indicated in the structure of microbeads, the core material and multilayer wall were hydrophobic/hydrophilic (O/W), respectively; thus, the phospholipid-based multilayer acted as a reservoir for viable cells. These results were in agreement with those reported by de Araújo Etchepare et al. (2020) who found that better microcapsules contain alginate and whey protein because of their functional properties and direct impact on the encapsulation efficiency. Another study by Yasmin et al. (2019) explained that the microparticle containing WPC and pectin as a wall material demonstrated high EE ($> 85\%$) and have a protective impact on bacterial cells.

Assessment of the Survival *L. rhamnosus* GG Microencapsulated During Heat Treatments and Storage Conditions

Probiotic bacteria must survive in different processing temperatures to be beneficial and remain viable in food products. The survival of *L. rhamnosus* free cells and encapsulated was evaluated under different heat treatments at 72, 25, and

4 °C (Table 2). A significant difference ($P < 0.05$) in the stability of microcapsule formulations at different temperatures was found. Generally, a slight decrease in the number of bacterial cells at refrigeration and ambient temperature was observed. However, the free cells (FCs) were more sensitive to thermal shock than microencapsulated cells at 72 °C. The FC was unviable when exposed to pasteurization temperature (72 °C, 15 min). While, bead encapsulated cells were 5.88, 6.18, 6.88, and $7.31 \log_{10}$ CFU g^{-1} , in F1, F2, F3, and F4, respectively. The formulations F3 and F4 showed significantly higher resistance at 72 °C compared to the others, with a reduction of 0.71 and $0.28 \log_{10}$ CFU g^{-1} , respectively. This may be because the encapsulated probiotic cells using alginate, whey protein, and/or pullulan can protect the cells from thermal pasteurization. These results were promising and in agreement with those reported by Rather et al. (2017) who found that microencapsulated probiotics by double alginate microencapsulation have higher stability to heat treatment (75 °C for 10 min). The obtained results are also in line with the experiments carried out by Zeashan et al. (2020). Thus, the survival of the probiotic microcapsules upon exposure to a thermal process depends on many factors, i.e., encapsulation technique, the architecture of microcapsules, and suitable polymers (Vemmer & Patel, 2013).

Viability of Encapsulated *L. rhamnosus* GG Exposed to Juice Additives

Many additives are placed in the nectar during the processing to enhance its quality and shelf life. The impact of juice additives, i.e., sugar, citric acid, sodium benzoate, potassium sorbate, carmoisine, flavor, and CMC, on the survival of free and encapsulated *L. rhamnosus* GG was evaluated (Table 2). Results demonstrated a significant difference ($P < 0.05$) in the stability of probiotic microcapsule formulations in juice

Table 2 Stability and viability of free and encapsulated *L. rhamnosus* GG on thermal processing and juice additives (mean $\pm$ SD)

Factors	Items	Function	<i>L. rhamnosus</i> GG cells					
			Initial	FC	F1	F2	F3	F4
Thermal conditions	4 °C	Refrigeration	7.67 $\pm$ 0.16 aA	5.38 $\pm$ 0.17 eA	6.11 $\pm$ 0.16 dA	6.51 $\pm$ 0.09 cA	7.26 $\pm$ 0.12 bA	7.46 $\pm$ 0.22 aA
	25 °C	Ambient condition	7.72 $\pm$ 0.18 aA	5.21 $\pm$ 0.15 eA	5.99 $\pm$ 0.15 dB	6.41 $\pm$ 0.07 cA	7.12 $\pm$ 0.10 bB	7.40 $\pm$ 0.17 aA
	72 °C	Pasteurization	7.59 $\pm$ 0.15 aA	0.0 fB	5.88 $\pm$ 0.14 eB	6.18 $\pm$ 0.14 dB	6.88 $\pm$ 0.17 cC	7.31 $\pm$ 0.10 bB
Juice additives	Sugar solution 7.5°Bx	Sweeteners	7.63 $\pm$ 0.09 aA	4.53 $\pm$ 0.23 dB	5.81 $\pm$ 0.14 cB	6.29 $\pm$ 0.15 bC	7.37 $\pm$ 0.11 aA	7.54 $\pm$ 0.05 aA
	Citric acid 0.3%	Acidify	7.73 $\pm$ 0.15 aA	4.17 $\pm$ 0.09 dC	6.36 $\pm$ 0.16 cA	6.57 $\pm$ 0.09 cB	7.28 $\pm$ 0.06 bA	7.56 $\pm$ 0.08 aA
	Sodium benzoate 0.1%	Antimicrobial	7.65 $\pm$ 0.15 aA	0 $\pm$ 0.11 dE	5.77 $\pm$ 0.19 cB	6.27 $\pm$ 0.09 bC	6.40 $\pm$ 0.17 bB	7.43 $\pm$ 0.12 aA
	Potassium sorbate 0.1%	Antimicrobial	7.57 $\pm$ 0.08 aA	2.64 $\pm$ 0.20 eD	5.80 $\pm$ 0.19 dB	6.37 $\pm$ 0.08 cB	6.96 $\pm$ 0.25 bB	7.46 $\pm$ 0.16 aA
	Carmoisine 0.01%	Color	7.71 $\pm$ 0.11 aA	6.61 $\pm$ 0.17 eA	6.47 $\pm$ 0.24 dA	7.03 $\pm$ 0.36 cA	7.45 $\pm$ 0.17 bA	7.61 $\pm$ 0.15 aA
	Strawberry flavor 0.05%	Flavor	7.64 $\pm$ 0.09 aA	6.50 $\pm$ 0.11 dA	6.66 $\pm$ 0.15 dA	7.10 $\pm$ 0.22 cA	7.43 $\pm$ 0.15 bA	7.59 $\pm$ 0.13 aA
	CMC 0.1%	Thickener	7.55 $\pm$ 0.10 aA	6.72 $\pm$ 0.15 cA	6.88 $\pm$ 0.11 cA	7.22 $\pm$ 0.17 bA	7.40 $\pm$ 0.13 aA	7.48 $\pm$ 0.07 aA
	Additives mixture	Quality	7.77 $\pm$ 0.13 aA	0 $\pm$ 0.10 eE	5.68 $\pm$ 0.31 dA	6.21 $\pm$ 0.12 cC	6.52 $\pm$ 0.10 bB	7.61 $\pm$ 0.16 aA

FC free cell (non-encapsulated), F1: alginate 2% + anthocyanin 0.1% + *L. rhamnosus* GG, F2: alginate 2% + anthocyanin 0.1% + cocoa butter 1% + *L. rhamnosus* GG, F3: alginate 2% + anthocyanin 0.1% + whey protein 3% + cocoa butter 1% + *L. rhamnosus* GG, F4: alginate 2% + anthocyanin 0.1% + whey protein 2% + pullulan 2% + cocoa butter 1% + *L. rhamnosus* GG. Same small letter indicates that there is no significant difference between any two means “in the same row” ($P > 0.05$). Same capital letter indicates that there is no significant difference between any two means “in the same column” ($P > 0.05$). $n = 3$

additives. Generally, the FC of probiotics was decreased after exposure to most juice additives, while being absent after exposure to sodium benzoate (0.1%). On the other hand, the microencapsulated cells in beads were more resistant to all juice additives. The formulations F3 and F4 were higher stability against juice additives; this may be related to the materials and/or multilayer of differing chemical nature which

provide better tolerance to probiotics microcapsules, mainly with sodium benzoate and potassium sorbate. Therefore, the whey protein and polymer multilayer inserted into the probiotic cell can maintain the bacterial structure better than the free cells or even with a polymeric coating, thus improving the stability against additives (de Araújo Etchepare et al., 2020).

Table 3 Survival of free and encapsulated *L. rhamnosus* GG under simulated gastrointestinal conditions (SGC), in 0, 30, 120, and 240 min (mean $\pm$ SD)

Time (min.)	<i>L. rhamnosus</i> GG cells				
	FC	F1	F2	F3	F4
0	7.64 $\pm$ 0.17 aA	7.57 $\pm$ 0.25 aA	7.61 $\pm$ 0.13 aA	7.88 $\pm$ 0.09 aA	7.74 $\pm$ 0.17 aA
30	4.80 $\pm$ 0.18 cB	6.34 $\pm$ 0.16 bB	6.67 $\pm$ 0.13 bB	7.21 $\pm$ 0.19 aA	7.54 $\pm$ 0.27 aA
120	3.52 $\pm$ 0.24 eC	5.54 $\pm$ 0.15 dC	6.34 $\pm$ 0.26 cB	6.87 $\pm$ 0.12 bB	7.26 $\pm$ 0.13 aB
240	1.89 $\pm$ 0.15 eD	3.96 $\pm$ 0.21 dD	5.11 $\pm$ 0.26 cC	6.79 $\pm$ 0.10 bB	7.11 $\pm$ 0.26 aB
Reduction rate (%)	75.26	47.69	32.85	13.83	8.14

FC free cell (non-encapsulated), F1: alginate 2% + anthocyanin 0.1% + *L. rhamnosus* GG, F2: alginate 2% + anthocyanin 0.1% + cocoa butter 1% + *L. rhamnosus* GG, F3: alginate 2% + anthocyanin 0.1% + whey protein 3% + cocoa butter 1% + *L. rhamnosus* GG, F4: alginate 2% + anthocyanin 0.1% + whey protein 2% + pullulan 2% + cocoa butter 1% + *L. rhamnosus* GG. Same small letter indicates that there is no significant difference between any two means “in the same row” ($P > 0.05$). Same capital letter indicates that there is no significant difference between any two means “in the same column” ($P > 0.05$). $n = 3$

Stability and Viability of Free and Encapsulated *L. rhamnosus* GG Exposed to Simulated Gastrointestinal Conditions

In order to provide health benefits and functional properties, probiotics must be able to be viable under gastrointestinal conditions (between 10^6 and 10^8 CFU g^{-1} of intestinal content) (Mays & Nair, 2018). As shown in Table 3, the count of free culture and probiotic microparticles at the beginning of the experiment ranged from 7.57 to 7.88 log CFU g^{-1} . However, the free cell count indicated a higher reduction of (5.75 log CFU g^{-1} ; 75.26%) after exposure to simulated gastrointestinal, whereas viable count of *L. rhamnosus* encapsulated in formulations, F1, F2, F3, and F4 showed a decrease of (3.61 log CFU g^{-1} ; 47.69%), (2.5 log CFU g^{-1} ; 32.85%), (1.09 log CFU g^{-1} ; 13.83%), and (0.63 log CFU g^{-1} ; 8.14%), respectively. These results revealed that multilayer encapsulation has a positive protection and shielding impact toward probiotics in simulated gastrointestinal. The formulations F2, F3, and F4 showed higher survive ability than F1 after exposure to simulated gastrointestinal conditions ($P < 0.05$), but the best outcome was noticed in F4. Similar findings and loss of free cells in simulated gastrointestinal conditions were in agreement with investigations by Gandomi et al. (2016) and Nami et al. (2020). The use of proteins and polysaccharides in the microencapsulation process provided substantially better protection. Previous studies by Su et al. (2018) and de Araújo Etchepare et al. (2020) have reported that the use of polymer as an encapsulating wall material provided better protection to probiotic cells as compared to free cultures when exposed to gastrointestinal conditions. Whey protein has been exhibited to be a proper and efficient wall material because of its buffering ability and tolerance of low pH. Also, the pullulan has high protection ability in microencapsulated cells that may be due to higher density and strong structure (Ma et al., 2021).

Viability of Free and Encapsulated *L. rhamnosus* GG in Strawberry Nectar After Storage

Table 4 shows the viable count of free cells and microbeads *L. rhamnosus* GG in strawberry (*F. × ananassa*, cultivar Camarosa) nectar after 4 weeks of storage. The viable cell counts of *L. rhamnosus* GG decreased ($P < 0.05$) after 4 weeks of storage at both 4 and 25 °C by 57.9% and 78.63%, respectively. The extreme decrease of free *L. rhamnosus* GG cell number in apple juice ~87.5% after 4 weeks of storage was also confirmed by Ding and Shah (2008). Results demonstrated that the free *L. rhamnosus* GG reduced 100% ($P < 0.05$) under the simulated gastrointestinal at both 4 and 25 °C for 4 weeks of storage. These results indicated that the *L. rhamnosus* GG was sensitive to the conditions in strawberry

nectar and during the gastrointestinal transit. These findings were in agreement with those reported by Lai et al. (2020) where free *L. rhamnosus* GG cells in hawthorn berry tea reduced by 100% after exposure to gastrointestinal for 120 min. Another report by Chávarri et al. (2012) found 100% reduction of free *Lactobacillus gasseri* and *Bifidobacterium bifidum* cells after exposing to gastrointestinal.

The viability of microencapsulated *L. rhamnosus* GG formulations was quite stable in strawberry nectar and even after exposure to simulate gastrointestinal. Formulations F1, F2, F3, and F4 showed a viability decrease of 53.07, 51.04, 19.97, and 8.48%, respectively, at 25 °C and 41.31, 36.1, 18.05, and 6.81%, respectively, at 4 °C.

Among the formulations, the F4 microbeads stored at 4 °C had the lowest reduction of 13.62% when compared to other formulations up to 4 weeks of storage. Moreover, encapsulated *L. rhamnosus* GG with alginate–whey protein–pullulan formulations (F3 and F4) provided better protection of entrapped *L. rhamnosus* GG cells, with 6.52 log CFU mL^{-1} and 6.72 log CFU mL^{-1} maintained count for 25 and 4 °C, respectively, at the end of 4-week storage. This indicated that the incorporation of pullulan into the encapsulating material exhibited a synergistic effect that protects the cells against the low pH of strawberry nectar. A previous study by Gandomi et al. (2016) reported that entrapped *L. rhamnosus* GG cells in apple juice exhibited higher viable counts after 3 months of storage at 4 °C than 25 °C.

Physical Properties of Strawberry Nectar Fortified with Probiotic Microcapsules

pH Value

In Fig. 1, the initial pH value of the strawberry (*F. × ananassa*, cultivar Camarosa) nectar was 3.57; then, the pH decreased significantly ($P < 0.05$) in most treatments that contained probiotic cells during storage. It was observed that the pH of strawberry nectar containing free cells decreased to 1.99 and 1.57 after 4 weeks of storage at 4 and 25 °C, respectively, while the pH of strawberry nectar containing formulations (F1 and F2) significantly ($P < 0.05$) showed a decline (2.47 and 2.68) at storage time. While, the pH in control, F3, and F4 remained relatively constant during the storage at 4 °C, since the final pH recorded was 3.33, 3.2, and 3.3, respectively. A free probiotic cell used carbohydrates and released small amounts of lactic acid, thus lowering the pH of the product during storage. Furthermore, some free cells did not survive at later stages of storage, although the lost probiotic cells could release enzymes for hydrolyzing sugars in the fruit nectar, thus lowering the pH. These results demonstrated that microencapsulated probiotic bacteria create

Table 4 Viability of free and encapsulated *L. rhamnosus* GG in strawberry nectar in simulated gastrointestinal conditions (SGC) during storage at 4 and 25 °C for 4 weeks (mean $\pm$ SD)

Storage time (week)	Temperature (°C)	Formula	Viable cell count (log ₁₀ CFU mL ⁻¹)			
			Time zero	After SGC 30 min	After SGC 120 min	After SGC 240 min
0	4	FC	7.72 $\pm$ 0.14 aA	5.64 $\pm$ 0.21 bC	4.32 $\pm$ 0.1 cD	2.99 $\pm$ 0.20 dE
		F1	7.65 $\pm$ 0.15 aA	6.34 $\pm$ 0.11 bB	5.62 $\pm$ 0.18 cC	4.83 $\pm$ 0.11 dD
		F2	7.70 $\pm$ 0.09 aA	6.86 $\pm$ 0.06 bB	6.66 $\pm$ 0.11 bB	5.78 $\pm$ 0.12 cC
		F3	7.81 $\pm$ 0.12 aA	7.46 $\pm$ 0.16 aA	7.02 $\pm$ 0.11 bA	6.96 $\pm$ 0.12 cB
		F4	7.78 $\pm$ 0.19 aA	7.65 $\pm$ 0.13 aA	7.68 $\pm$ 0.19 aA	7.56 $\pm$ 0.14 aA
	25	FC	7.72 $\pm$ 0.14 aA	5.37 $\pm$ 0.19 bC	3.86 $\pm$ 0.14 cE	2.26 $\pm$ 0.25 dE
		F1	7.65 $\pm$ 0.15 aA	6.14 $\pm$ 0.12 bB	4.94 $\pm$ 0.09 cD	4.37 $\pm$ 0.28 dD
		F2	7.70 $\pm$ 0.09 aA	6.49 $\pm$ 0.15 bB	6.23 $\pm$ 0.14 bB	5.35 $\pm$ 0.22 cC
		F3	7.81 $\pm$ 0.12 aA	7.39 $\pm$ 0.17 aA	6.74 $\pm$ 0.18 bB	6.77 $\pm$ 0.23 bB
		F4	7.78 $\pm$ 0.19 aA	7.44 $\pm$ 0.10 aA	7.47 $\pm$ 0.16 aA	7.33 $\pm$ 0.14 aA
1	4	FC	5.50 $\pm$ 0.21 aC	4.24 $\pm$ 0.17 bC	3.17 $\pm$ 0.13 cD	2.41 $\pm$ 0.21 dD
		F1	6.53 $\pm$ 0.13 aB	5.66 $\pm$ 0.24 bB	4.27 $\pm$ 0.12 cC	3.35 $\pm$ 0.18 dD
		F2	6.86 $\pm$ 0.06 aB	6.65 $\pm$ 0.14 aA	5.71 $\pm$ 0.15 bB	4.94 $\pm$ 0.10 cC
		F3	7.41 $\pm$ 0.17 aA	7.16 $\pm$ 0.13 aA	6.74 $\pm$ 0.15 bA	6.52 $\pm$ 0.09 bB
		F4	7.61 $\pm$ 0.10 aA	7.4 $\pm$ 0.27 aA	7.33 $\pm$ 0.13 aA	7.19 $\pm$ 0.16 aA
	25	FC	4.52 $\pm$ 0.12 aD	3.13 $\pm$ 0.08 bD	2.48 $\pm$ 0.18 cE	1.68 $\pm$ 0.12 dE
		F1	5.47 $\pm$ 0.14 aC	4.63 $\pm$ 0.15 bC	3.48 $\pm$ 0.28 cD	2.68 $\pm$ 0.14 dD
		F2	5.99 $\pm$ 0.26 aC	5.72 $\pm$ 0.19 aB	4.78 $\pm$ 0.2 bC	4.46 $\pm$ 0.24 bC
		F3	7.17 $\pm$ 0.10 aA	6.62 $\pm$ 0.21 aA	6.48 $\pm$ 0.19 bA	6.33 $\pm$ 0.18 bB
		F4	7.45 $\pm$ 0.11 aA	7.26 $\pm$ 0.23 aA	7.2 $\pm$ 0.07 aA	7.08 $\pm$ 0.13 aA
2	4	FC	4.52 $\pm$ 0.12 aD	3.57 $\pm$ 0.15 bC	2.23 $\pm$ 0.15 cC	1.75 $\pm$ 0.17 dC
		F1	5.69 $\pm$ 0.18 aB	4.58 $\pm$ 0.14 bB	2.45 $\pm$ 0.21 cC	2.24 $\pm$ 0.25 cB
		F2	5.81 $\pm$ 0.12 aB	4.79 $\pm$ 0.12 bB	3.45 $\pm$ 0.16 cB	2.86 $\pm$ 0.31 dB
		F3	7.29 $\pm$ 0.15 aA	7.08 $\pm$ 0.09 aA	6.86 $\pm$ 0.17 aA	6.68 $\pm$ 0.12 aA
		F4	7.42 $\pm$ 0.07 aA	7.38 $\pm$ 0.14 aA	7.12 $\pm$ 0.09 aA	7.08 $\pm$ 0.11 aA
	25	FC	2.43 $\pm$ 0.21 aE	1.49 $\pm$ 0.13 bD	0.51 $\pm$ 0.11 cD	0 $\pm$ 0 dD
		F1	4.17 $\pm$ 0.06 aC	3.59 $\pm$ 0.16 bC	2.57 $\pm$ 0.15 cC	1.49 $\pm$ 0.16 dC
		F2	4.56 $\pm$ 0.29 aC	4.27 $\pm$ 0.22 aB	3.68 $\pm$ 0.09 bB	2.54 $\pm$ 0.19 cB
		F3	7.16 $\pm$ 0.08 aA	7.06 $\pm$ 0.22 aA	6.8 $\pm$ 0.1 aA	6.44 $\pm$ 0.10 aA
		F4	7.26 $\pm$ 0.09 aA	7.18 $\pm$ 0.08 aA	7.11 $\pm$ 0.1 aA	7.03 $\pm$ 0.16 aA
4	4	FC	3.25 $\pm$ 0.17 aD	2.58 $\pm$ 0.12 bD	1.42 $\pm$ 0.18 cC	0 $\pm$ 0 dE
		F1	4.49 $\pm$ 0.14 aC	3.53 $\pm$ 0.19 bC	2.26 $\pm$ 0.09 cB	1.12 $\pm$ 0.08 dC
		F2	4.92 $\pm$ 0.15 aC	3.82 $\pm$ 0.17 bC	2.67 $\pm$ 0.15 cB	1.53 $\pm$ 0.13 dC
		F3	6.40 $\pm$ 0.18 aB	6.23 $\pm$ 0.11 aB	6.13 $\pm$ 0.11 aA	6.11 $\pm$ 0.08 aA
		F4	7.25 $\pm$ 0.12 aA	7.12 $\pm$ 0.08 aA	6.84 $\pm$ 0.19 aA	6.72 $\pm$ 0.25 aA
	25	FC	1.65 $\pm$ 0.16 aE	0 $\pm$ 0 bE	0 $\pm$ 0 bD	0 $\pm$ 0 bE
		F1	3.59 $\pm$ 0.10 aD	2.28 $\pm$ 0.17 bD	1.31 $\pm$ 0.1 cC	0 $\pm$ 0 dE
		F2	3.77 $\pm$ 0.14 aD	3.27 $\pm$ 0.16 aC	2.25 $\pm$ 0.15 bB	0.58 $\pm$ 0.11 cD
		F3	6.25 $\pm$ 0.13 aB	6.07 $\pm$ 0.05 aB	6.02 $\pm$ 0.03 aA	5.71 $\pm$ 0.08 bB
		F4	7.12 $\pm$ 0.07 aA	7.03 $\pm$ 0.09 aA	6.77 $\pm$ 0.12 aA	6.52 $\pm$ 0.25 aA

FC free cell (non-encapsulated), F1: alginate 2% + anthocyanin 0.1% + *L. rhamnosus* GG, F2: alginate 2% + anthocyanin 0.1% + cocoa butter 1% + *L. rhamnosus* GG, F3: alginate 2% + anthocyanin 0.1% + whey protein 3% + cocoa butter 1% + *L. rhamnosus* GG, F4: alginate 2% + anthocyanin 0.1% + whey protein 2% + pullulan 2% + cocoa butter 1% + *L. rhamnosus* GG. Same small letter indicates that there is no significant difference between any two means “in the same row” ($P > 0.05$). Same capital letter indicates that there is no significant difference between any two means “in the same column” ($P > 0.05$). $n = 3$

Fig. 2 Changes in pH value of strawberry nectar supplemented with free and encapsulated *L. rhamnosus* GG during storage at (a) 4 °C and (b) 25 °C. Error bars indicate standard deviation (n=3). Control: without probiotic cells, FC: free cells (non-encapsulated), F1: alginate 2% + anthocyanin 0.1% + *L. rhamnosus* GG), F2: alginate 2% + anthocyanin 0.1% + cocoa butter 1% + *L. rhamnosus* GG), F3: alginate 2% + anthocyanin 0.1% + whey protein 3% + cocoa butter 1% + *L. rhamnosus* GG), F4: alginate 2% + anthocyanin 0.1% + whey protein 2% + pul-lulan 2% + cocoa butter 1% + *L. rhamnosus* GG)

a stable acidic environment in strawberry nectar (Fig. 2a). Similar findings were reported by Kailasapathy (2006) in yogurts. The pH changes in control and different formulations of strawberry nectar stored at 25 °C were a similar trend to those at 4 °C (Fig. 2b). While the nectar samples stored at 25 °C have more pH reduction than those stored at 4 °C (Gandomi et al., 2016).

Anthocyanin Content

As seen in Fig. 3, the total anthocyanin in strawberry (*F. × ananassa*, cultivar Camarosa) nectar at time zero was 75.55 mg 100 g⁻¹, while in treatments (F1–F4) ranged from 77.32 to 77.91 mg 100 g⁻¹. Generally, a significant reduction

($P < 0.05$) was observed in the anthocyanin content in strawberry nectar after 4 weeks of storage. Total anthocyanin in strawberry nectar-containing free cells decreased to 61.77 and 53.86 mg 100 g⁻¹ after 4 weeks of storage at 4 and 25 °C, respectively. While the anthocyanin of strawberry nectar containing formulations (F1, F2, and F3) significantly ($P < 0.05$) showed a reduction of 6.75, 4.89, and 4.52 mg 100 g⁻¹, respectively, F4 has a slight decline of 2.23 mg 100 g⁻¹ at storage time (Fig. 3a). Anthocyanin change would indicate that free probiotic cells used the sugars and produced lactic acid in the strawberry nectar if compared to cells that were trapped inside microcapsules. The total anthocyanin changes in the control sample and different formulations of strawberry nectar stored at 25 °C were in a similar trend to

Fig. 3 Changes in total anthocyanin content in strawberry nectar supplemented with free and encapsulated *L. rhamnosus* GG during storage at (a) 4 °C and (b) 25 °C. Error bars indicate standard deviation ($n=3$). Control: without probiotic cells, FC: free cells (non-encapsulated), F1: alginate 2% + anthocyanin 0.1% + *L. rhamnosus* GG, F2: alginate 2% + anthocyanin 0.1% + cocoa butter 1% + *L. rhamnosus* GG, F3: alginate 2% + anthocyanin 0.1% + whey protein 3% + cocoa butter 1% + *L. rhamnosus* GG, F4: alginate 2% + anthocyanin 0.1% + whey protein 2% + pul-lulan 2% + cocoa butter 1% + *L. rhamnosus* GG)

those at 4 °C (Fig. 3b). While the nectar samples stored at 25 °C had more anthocyanin reduction than those stored at 4 °C. Similar findings were reported in the previous study by Colín-Cruz et al. (2019) in blackberry juice. One study has established that probiotic culture produces hydrogen peroxide (H_2O_2) at a safe level, but could accelerate the degradation of anthocyanin pigment and maybe controlled of the normal microflora (Felten et al., 1999).

Sensory Evaluation and Questionnaire of Strawberry Nectar Fortified with Probiotic Microcapsules

The sensory evaluation was based on a difference-preference test as exploratory and preliminary responses. The sensory

properties, i.e., color, odor, taste, texture, and overall acceptability of the strawberry (*F. × ananassa*, cultivar Camarosa) nectar containing free and encapsulated *L. rhamnosus* GG after 4 weeks of storage, were evaluated (Fig. 4). Results showed that probiotic microcapsules improved significantly all sensory characteristics ($P < 0.05$). The color scores decreased in FC and F1 samples with storage time; however, no significant difference ($P > 0.05$) was observed during the storage in formulations (F2–F4). It was noticed that the addition of red probiotic beads had a positive effect on the color of the strawberry nectar. This agrees with the previous report by Krasaekoopt and Kitsawad (2010) who found that the chitosan-alginate beads in fruit juices positively impacted its sensory parameters. Although whey protein-pullulan-alginate beads were added to strawberry nectar samples,

Fig. 4 Sensory evaluation parameters, i.e., (a) color, (b) odor, (c) taste, (d) texture, and (e) overall acceptability of strawberry nectar supplemented with free and encapsulated *L. rhamnosus* GG during 4-week storage at 4 °C (n = 10). (f) A questionnaire of 11-juice companies for fortified strawberry nectar with microencapsulated probiotic. Control: without probiotic cells, FC: free cells (non-encapsulated), F1: alginate 2% + anthocyanin 0.1% + *L. rhamnosus* GG, F2: alginate 2% + anthocyanin 0.1% + cocoa butter 1% + *L. rhamnosus* GG, F3: alginate 2% + anthocyanin 0.1% + whey protein 3% + cocoa butter 1% + *L. rhamnosus* GG, F4: alginate 2% + anthocyanin 0.1% + whey protein 2% + pul-lulan 2% + cocoa butter 1% + *L. rhamnosus* GG)

they remained invisible and made a better mouth feel for the panelists. A significant difference in odor and taste ($P < 0.05$) was observed between FC treatment and formulations (F1–F4) in nectar samples. The strawberry nectar with free probiotic cells was scored to have sour, undesirable, and but-tery flavor. It could be related to the fermentation metabolites

produced from the free cells (Pimentel et al., 2015), while the microcapsules limit the acidification (Sohail et al., 2012). No significant difference ($P > 0.05$) in texture was recorded between all formulations (F1–F4). However, the overall acceptability of nectar samples substantially decreasing in FC, F1, and F2, the sensory compound's intensity reaches

a plateau, indicated a possibility of rejection. While F3 and F4 were more accepted till the end of the storage period. These results were in agreement with Sohail et al. (2012) who reported that the addition of probiotic microcapsules (*L. acidophilus* NCFM and *L. rhamnosus* GG) in orange juice improved the sensory properties. Similar findings were reported by Krasaekoopt and Kitsawad (2010) in grape and orange juices, Gandomi et al. (2016) in apple juice. One study found that oligosaccharide extracts from *Eleutherine americana* have been proposed to keep the sensory scores of juices (Phoem et al., 2015). Another study by Fonseca et al. (2021) demonstrated that the probiotic *L. plantarum* CCMA 0743 strain confers unique sensory properties to passion fruit juice. According to the respondent of juice companies regarding strawberry nectar fortified with probiotic microcapsules (Fig. 4f), about 81.82% agreed, 9.09% undecided, and 9.09% disagreed. A significant difference in responses of juice companies ($P < 0.05$) was observed, but most juice companies agreed of fortified nectar because (i) encapsulated probiotic is a new approach in fruit nectar, (ii) enhances the functional properties of nectar, and (iii) develops novel products with acceptable characteristics. Based on sensory evaluation, questionnaire, and pH, the strawberry nectar fortified with probiotic microcapsules (F4) was more accepted and stable during the storage period, as well as recommended applying in food products.

Conclusions

In conclusion, the probiotic *L. rhamnosus* GG cells were successfully microencapsulated using four alginate-whey protein-pullulan blends in a proper size, color, and shape. Results demonstrated that microencapsulation of *L. rhamnosus* GG had a higher survival rate during thermal (4 °C and 72 °C), juice additives (chemical materials), and gastrointestinal (intestinal juice) treatments compared to the free bacterial cell. All formulations exhibited high encapsulation efficiency (> 89%), medium bead size (406–504 µm), and proper color (red color). F4 (alginate 2% + anthocyanin 0.1% + whey protein 2% + pullulan 2% + cocoa butter 1% + *L. rhamnosus* GG) showed the greatest viability ($7 \log \text{CFU mL}^{-1}$) in nectar during storage. A significant decrease ($P < 0.05$) in pH and anthocyanin values was observed in strawberry (*F. × ananassa*, cultivar Camarosa) nectar including free cells. The nectar samples fortified with probiotic microcapsules exhibited high sensory scores (difference-preference test as exploratory and preliminary responses) up to 4 weeks, while those included free probiotic cells were rejected (fermented flavor). The questionnaire response showed highly accepted in commercial companies (> 80% agreed). However, more

research is needed to evaluate the sensory parameters by consumer acceptance tests and questionnaires on a large scale, as well as a feasibility study of fortified nectar. In general, the results demonstrated that the strawberry nectar may be used as an appropriate vehicle for delivering encapsulated probiotic cells to humans.

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Data Availability The datasets supporting the conclusions of this article are included within the article and its additional files.

Declarations

Conflict of Interest The authors declare no competing interests.

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