LWT - Food Science and Technology 180 (2023) 114713

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Development and characterization of fermented soy beverages containing encapsulated or non-encapsulated vaginal probiotics

Margherita D'Alessandro [a,1], Davide Gottardi [a,d,*,1], Carola Parolin [b], Virginia Teresa Glicerina [c], Beatrice Vitali [b], Rosalba Lanciotti [a,d], Francesca Patrignani [a,d,**]

[a] Department of Agricultural and Food Sciences, University of Bologna, Piazza Gabriele Goidanich 60, 47521, Cesena, FC, Italy
[b] Department of Pharmacy and Biotechnology, University of Bologna, via San Donato 19/2, 40127, Bologna, BO, Italy
[c] Department of Agriculture, Forest and Food Sciences, University of Turin, 10095, Grugliasco, TO, Italy
[d] Interdepartmental Center for Industrial Agrifood Research, University of Bologna, via Quinto Bucci 336, 47521, Cesena, FC, Italy

ARTICLE INFO

Keywords:
Fermentation
Probiotics
Encapsulation
Sensory evaluation
Soy beverage

ABSTRACT

Human microbial niches such as the healthy vagina, are recently emerging as "unconventional" sources of candidate probiotics capable of preventing from different vaginal diseases. These microorganisms could be provided as oral preparations since they can reach the vaginal niche passing through the gastrointestinal tract. However, their use in food would be challenging. The aim of this work was to develop and characterize fermented soy beverages with encapsulated and non-encapsulated vaginal lactobacilli, namely *Lactobacillus crispatus* BC4 and *Lactobacillus gasseri* BC9, as future dietary strategies for vaginal dysbiosis. The viability of vaginal strains remained stable at 7 log CFU/mL of product during the entire 28 days of storage, despite the use of encapsulated or non-encapsulated bacteria. Samples containing encapsulated bacteria, especially E-BC4+BC9, showed higher Water Holding Capacity (62.29%), lactic acid content (1.43%), and a remarkable antagonistic activity against enteropathogens. Moreover, encapsulation protected the strains from simulated GIT conditions (>1 Log) but reduced the acceptability of the final products. Overall, strain BC4 and BC9, alone or in mix, demonstrated to be promising co-starter cultures providing a characteristic flavor (pleasant smell and taste) and aroma (lower hexanal, benzaldehyde and higher diacetyl, and 2,3-pentanedione, compared to control) to the fermented soy beverages.

1. Introduction

Research related to the microbiome has broadened the public perception of microorganisms as agents that can cause diseases but that, at the same time, have a beneficial role in human health. The resulting awareness and acceptance of probiotics in consumers will expand the probiotic market of 8% between 2023 and 2032 (Cunningham et al., 2021; Global Market Insights, 2022). However, while there is a common opinion on the beneficial effect of probiotics, there is still a common misunderstanding on their definition. In the scientific field, as recently established by the International Scientific Association for Probiotics and Prebiotics, safety and evidence of a health benefit are required as preliminary criteria for the selection of probiotics, also considering target sites, target populations, and way of administration (Hill et al., 2014). Besides gut and fermented foods, human microbial niches are recently emerging as "unconventional" sources of probiotic candidates. For instance, several studies conducted on vaginal microbiota of healthy women have reported *Lactobacillus* as the main genus present in this niche which can be used as an excellent source of beneficial microbes (Anahtar et al., 2018; Di Cerbo et al., 2016). Among the isolated lactobacilli, the most dominant species are *Lactobacillus crispatus*, *L. iners*, *L. jensenii* and *L. gasseri*. The beneficial effect of *Lactobacillus* spp. in the vaginal ecosystem is based on a mutualistic relationship between the vaginal microbiota and the human host. More specifically, these species

* Corresponding author. Department of Agricultural and Food Sciences, University of Bologna, Piazza Gabriele Goidanich 60, 47521, Cesena, FC, Italy.
** Corresponding author. Interdepartmental Center for Industrial Agri-food Research, University of Bologna, via Quinto Bucci 336, 47521, Cesena, FC, Italy.
E-mail addresses: margheri.dalessandr3@unibo.it (M. D'Alessandro), davide.gottardi2@unibo.it (D. Gottardi), carola.parolin@unibo.it (C. Parolin), virginiateresa.glicerina@unito.it (V.T. Glicerina), b.vitali@unibo.it (B. Vitali), rosalba.lanciotti@unibo.it (R. Lanciotti), francesca.patrignani@unibo.it (F. Patrignani).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.lwt.2023.114713
Received 5 February 2023; Received in revised form 14 March 2023; Accepted 27 March 2023
Available online 28 March 2023

can produce several antimicrobial compounds (e.g. hydrogen peroxide, lactic acid) and they can compete against pathogens for adhesion sites in the vaginal epithelium (Ahire et al., 2021), protecting the host from different diseases, including recurrent urinary infections, bacterial vaginosis, and vaginal candidiasis. In this sense, vaginal *L. crispatus* and *L. gasseri* strains, isolated by Parolin et al. (2015), exhibited activity towards several genital pathogens, including *Candida* (Calonghi et al., 2017; Parolin et al., 2015, 2021), *Chlamydia trachomatis* (Nardini et al., 2016; Parolin et al., 2018) *Neisseria gonorrhoeae* (Foschi et al., 2017) Group-B *Streptococcus* (Marziali et al., 2019) and HIV1 (Ñahui Palomino et al., 2019). Their metabolic aptitude (D'Alessandro et al., 2021) (a) as well as their safety and technological properties as functional adjunct cultures in the food sector were also tested (Siroli et al., 2017). The digestive fate of one of the most promising vaginal strains, namely *L. crispatus* BC4, carried in a soft cheese was also evaluated by using the SHIME® system (Patrignani et al., 2020). The strain was able to survive the stomach and small intestine simulated conditions, arriving in the gut with a minor reduction of living cells. This aspect is important since oral administered vaginal strains, once they have colonized the gut, can translocate from the large intestine to the vagina because of the spatial proximity of the two organs. However, although they could be provided as oral preparations, it would be very challenging to use them in foods as a dietary strategy to prevent eventually woman dysbiosis. In fact, from one hand they should be kept alive and functional during the entire shelf-life of the food. On the other hand, the food should be consumed constantly in proper amount so that the probiotics can reach and colonize the human gut. The development of suitable technologies for the maintenance of an adequate number of viable probiotic cells (>7 log colony-forming units [CFU]/g of product) is for sure a key step. On this regard, microencapsulation is one of the most widely used techniques that offers protection for heat-sensitive nutrients and probiotic microorganisms. In order to maximize the vaginal strains viability, assuming their potential use as functional adjunct cultures in the food sector, the use of spray-drying for microencapsulation was also evaluated (D'Alessandro et al., 2021) (b).

Traditionally probiotics have been consumed by humans through fermented dairy foods, mostly of bovine origin. However, due to the emergences of lactose intolerance, cow's milk protein allergy, issue with cholesterol-rich diet, veganism and negative environmental impacts of dairy productions, there is a growing demand for non-dairy-alternatives. Nowadays, the viability during storage of probiotics in plant-based beverages, such as the soy based one, has been reported as highly satisfactory (Rasika et al., 2021). Therefore, these milk substitutes may represent promising carriers for probiotics. Several studies have also pointed out that fermentation of soy beverages by lactic acid bacteria can: overcome the problem of beany flavor, increase the acceptability of these type of products by reducing their oligosaccharides (Otieno & Shah, 2007); improve the bioavailability of isoflavones, favor the digestion of proteins and intestinal health, enhance the antioxidant activity of the beverage, provide more soluble calcium, and supports immune system (Wang et al., 2003); increase the amount of vitamins, such as riboflavin (Bhushan et al., 2020).

The aim of this study was to develop functional fermented soy beverages using commercial starter cultures together with two encapsulated and non-encapsulated vaginal probiotics (*L. crispatus* BC4 and *L. gasseri* BC9, Department of Pharmacy and Biotechnology, University of Bologna, Italy). The two strains were applied individually or in combination to assess their potential synergistic effect on the final food product. The fermented soy beverages were then stored for 28 days at 4 °C (Cui et al., 2021), and analyzed for microbial cell loads and pH over time. Moreover, their antagonistic activity against intestinal pathogens and the survival of the vaginal strains through simulated gastro-intestinal juices were evaluated. Eventually, sensory characteristics (panel), aromatic compounds (GC-MS-SPME), and rheological measurements were determined on the final products at the end of storage.

2. Materials and methods

2.1. Bacterial strains

The functional strains used in this experimental work were *L. crispatus* BC4 and *L. gasseri* BC9, belonging to the collection of FABIT (Department of Pharmacy and Biotechnology, University of Bologna, Italy), isolated according to Parolin et al. (2015). *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* were provided by Sacco srl (Italy). Fresh cultures of each strain were obtained from frozen stocks by two consecutive refreshments in MRS broth (Oxoid, Basingstoke, United Kingdom) using a 1% (v/v) inoculum and incubated at 37 °C in anaerobic conditions overnight.

2.2. Production of microcapsules

L. crispatus BC4 and *L. gasseri* BC9 were individually cultivated overnight at 37 °C in 1 L of MRS broth supplemented with 0.05% L-cysteine and incubated in anaerobic jar with AnaeroGen sachet (Oxoid). This allowed to obtain a final concentration of at least 10^9 CFU/mL for each strain. The strain cell loads were determined after serial dilutions in 0.9% NaCl isotonic solution by plating on MRS agar with 0.05% L-cysteine and incubating at 37 °C for 48 h under anaerobiosis. One liter of each strain culture was centrifuged at 14514×*g* for 15 min at 4 °C (Avanti J-26 XP with Ja A-10 rotor, Beckman Coulter). After removing the supernatant, the microbial pellet was washed with 1 L of 0.9% NaCl isotonic solution and then resuspended in 500 mL of a commercial soy beverage having the following composition: 9.04% total solids, 9.8 °Brix, pH 6.64, 1.8% fats, 2.8% carbohydrates, 3% proteins, and 0.4% fibers. Spray-drying was conducted using a mini-spray-dryer (B191, Buchi - Labortechnik AG, Switzerland), which was a laboratory-scale spray-dryer equipped with a single fluid nozzle. Regarding the process conditions, inlet and outlet air temperatures were 110 and 70 °C, respectively. The pump rate was maintained between 19% and 36% aspiration, while the feed flow rate was 10 mL/min. For each culture, 100 mL of suspension was spray-dried to produce an average of 5.2 g of powder/100 mL of suspension. Spray-dried powder samples were collected from the cyclone, mixed gently, and vacuum-packed in nylon/polyethene, 102 μm high-barrier plastic bags (Tecnovac, San Paolo D'Argon, Bergamo, Italy) using an S100-Tecnovac device.

2.3. Preparation of fermented soy beverages

The production of fermented soy beverages was carried out in lab conditions. Commercial UHT soy beverage was split in 100 mL containers and each one was inoculated with starter cultures (*L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*) at level of 6 log CFU/mL while the functional strains, encapsulated or not, were inoculated at level of at least 7 log CFU/mL. The inoculated soy beverages were incubated at 42 °C until reaching pH 4.5. At the end of fermentation, the samples were stored at 4 °C up to 28 days. The analyses were performed after 1 (the day after production), 7, 14 and 28 days of refrigerated storage. Seven types of experimental fermented soy beverages were produced and defined as follows:

Control: fermented soy beverage containing starter cultures only;

BC4: fermented soy beverage containing starter cultures and *L. crispatus* BC4;

BC9: fermented soy beverage containing starter cultures and *L. gasseri* BC9;

BC4+BC9: fermented soy beverage containing starter cultures and the mix of *L. crispatus* BC4+*L. gasseri* BC9;

E-BC4: fermented soy beverage containing starter cultures and encapsulated *L. crispatus* BC4;

E-BC9: fermented soy beverage containing starter cultures and encapsulated *L. gasseri* BC9;

E-BC4+BC9: fermented soy beverage containing starter cultures and

the encapsulated mix of *L. crispatus* BC4+*L. gasseri* BC9.

2.4. Starter cultures and probiotic strain viability in fermented soy beverages

The viability of starters cultures and vaginal strains was assessed after 1, 14 and 28 days of storage. Five grams of fermented soy beverage were placed into 45 mL of PBS and homogenized in a stomacher (Labblender 80, Pbi International, Milan, Italy) for 3 min. Decimal dilutions of the homogenates were made in 0.9% NaCl and 0.1 mL of appropriate dilutions was spread onto the surface of different agar media. *S. thermophilus* was counted on M17 agar (Oxoid, Basingstoke, Hampshire, UK) while *L. delbrueckii* subsp. *bulgaricus* was counted anaerobically on MRS agar (Oxoid) acidified with glacial acetic acid (Merck, Darmstadt, Germany) at pH 5.4. Both M17 and MRS plates were incubated at 45 °C to preclude the growth of the two vaginal strains. On the other hand, *L. crispatus* BC4 and *L. gasseri* BC9 were anaerobically counted onto MRS supplemented with 0.2% (w/v) Lithium chloride, 0.3% (w/v) Sodium propionate and 0.05% L-cysteine or MRS with 0.05% L-cysteine, respectively, incubated at 30 °C to preclude the growth of the *L. delbrueckii* subsp. *bulgaricus*. The be sure that the proper bacteria were counted, the morphology of each strain was checked.

2.5. Physico-chemical analysis

Physico-chemical analyses (soluble solids (°Brix), water holding capacity (%), lactic acid (%) and pH) were measured in the fermented soy beverages after 1 day of storage, according to Torrico et al. (2019). Total soluble solids were measured using a digital refractometer for °Brix determination (DBR 95 digital refractometer, XS Instruments, Carpi, Italy). The refractometer was calibrated with distilled water. The mean values of six replicates and the standard deviations were calculated. The water holding capacity (WHC %) of the samples was determined using a refrigerated centrifuge (Avanti J-26 XP with Ja A-10 rotor, Beckman Coulter). Fermented soy beverages (5 g) were centrifuged at 4500×*g* for 15 min at 4 °C. After centrifugation, the clear supernatant formed was collected and weighed. Triplicates were measured for each sample and the mean was calculated. The extent of whey separation of the fermented samples was calculated from the weight of the supernatant and the fermented soy beverage using the following equation: WHC (%) = (1- (weight of supernatant (g) × weight of fermented soy beverage (g)) × 100. Titratable acidity (lactic acid %) was measured according to the methods of Shori et al. (2013). Approximately 10 g of the fermented soy beverage were diluted with the same amount of distilled water and titrated with 0.1 M NaOH using a 0.5% phenolphthalein indicator until the end point of a faint pink colour was reached. The titratable acidity was expressed as % lactic acid based on the weight of the sample using the following formula: lactic acid (%) = (V × 0.009)/(W × 100), where V is the volume of 0.1 M NaOH (mL) and W is the weight of fermented product (g). Titratable acidity was determined from the average of three replicates for each sample. The decrease in pH during fermentation and its evolution during 28 days of storage at 4 °C was monitored using the Basic 20 pH meter (Crison Instruments, Modena, Italy).

2.6. Survival to simulated gastric and intestinal juices of the vaginal strains present in the fermented soy beverage

To evaluate the resistance of the vaginal strains, encapsulated or not, to simulated gastric and intestinal juices when carried in the fermented soy beverages, the method described by D'Alessandro et al. (2021) (b) was performed with some modifications after 14 days of storage at refrigerated conditions. Briefly, the first sample was mixed with the same volume of a "saliva–gastric" solution. The saliva–gastric solution contained $CaCl_2$ (0.22 g/L), NaCl (16.2 g/L), KCl (2.2 g/L), $NaHCO_3$ (1.2 g/L), and 0.3% (w/v) porcine pepsin (Sigma-Aldrich, Milan, Italy). The sample was quickly brought to pH 3 with HCl 1 M and then incubated for 90 min at 37 °C in a water bath (WB-MF, Falc Instruments, Treviglio, Italy). After this, 1 mL of sample was collected to assess microbial cell viability (acid step). Instead, 2 mL of the sample were centrifuged (13800×*g*, 4 min and 4 °C) and the resulting pellet was washed with 2 mL of NaCl 0.9% isotonic solution (13800×*g*, 4 min and 4 °C) before resuspending it in 2 mL of simulated intestinal juice (PBS solution with 0.3% bile and 0.1% pancreatin 100 units/mg porcine pancreatin from porcine pancreas, Sigma-Aldrich, Milan, Italy). The incubation time in the thermostatic bath was 90 min at 37 °C. Then, 100 μL was taken from the sample in order to assess the resulting cell viability by microbiological sampling.

2.7. Antagonistic activity against intestinal pathogens

All the investigated fermented soy beverages were tested for their antagonistic activity towards the intestinal pathogens enterotoxigenic *Escherichia coli* H10407, *Salmonella choleraesuis* serovar *typhimurium*, *Yersinia enterocolitica* (Department of Pharmacy and Biotechnology, University of Bologna, Italy, Giordani et al., 2018) by overlay assay, as described in (D'Alessandro et al. (2022) with minor modifications. Pathogenic strains were routinely grown in BHI broth, at 37 °C with gentle agitation, pathogens were subcultures twice before being used in the experiments. Five μl of each fermented beverage were spotted over the surface of MRS plates (containing 0.05% L-cysteine and 1.2% agar) and incubated in anaerobic conditions at 37 °C for 24 h. Then 100 μl (corresponding to approximately 7–8 log CFU) of overnight subcultures of the pathogenic strains were inoculated into 10 mL of BHI 0.7% agar and poured over the spots. The plates were further incubated at 37 °C for additional 24 h, then checked to evaluate the presence of a growth inhibition zone. The inhibition halos were measured from the outer perimeter of the spots in four directions and the average was considered. The antagonistic activity was expressed in relation to the observed zone of inhibition: +, inhibition <10 mm; ++, inhibition between 10 and 13 mm; + + +, inhibition between 13 and 16 mm; + + ++, inhibition >16 mm.

2.8. Sensory evaluation

The sensory evaluations of all the fermented soy beverages were carried out by ten untrained panelists, aged 20–60 years, according to the Likert scale (from 1: strongly agree to 5: strongly disagree) considering color, smell, taste, texture and body, overall acceptability, general appearance, at the beginning (1 day) and at the end of designed shelf-life (28 days).

2.9. Aroma profiles

Volatile compounds of fermented soy beverages were monitored after 24 h from coagulation and after 28 days of refrigerated storage using gas chromatography-mass spectrometry (GC–MS) coupled with solid phase micro extraction (SPME) using the method described by Patrignani et al., (2017a).

2.10. Rheological measurements

Rheological measurements on fermented samples were carried out at 4 °C using a controlled stress–strain rheometer mod. MCR 300 (Physica/Anton Paar, Ostfildern, Germany) equipped with a bob and cup geometry (CC27) and a Peltier system. The rheological behavior was analyzed in steady state conditions. After a pre-shearing of 500 s at 0.1 s^{-1}, viscosity was measured increasing shear rate from 0.1 to 100 s^{-1} within 500 s, taking 50 measurements points. The maximum shear was chosen in order to avoid considerable loss of viscosity that can be observed when ìhigh shear values are used (Lucey, 2004). To better highlight differences between samples, viscosity curves were fitted according with the Casson model (Ramaswamy & Basak, 1991; Lee & Lucey, 2006;

Glicerina et al., 2015) (1)

$$\text{Casson model}: \sigma^{0.5} = \sigma_0^{0.5}\ \eta_{PL}\gamma^{0.5} \quad (1)$$

where: σ_0 is the yield stress and η_{PL} is the so-called "plastic viscosity".

2.11. Statistical analysis

The microbiological and physico-chemical data are the mean of 3 repetitions. The obtained data were analyzed by Statistica software (version 8.0; StatSoft, Tulsa, OK, USA) adopting the analysis of variance (ANOVA) and Tukey's test for data comparisons.

3. Results and discussion

3.1. Viability of the bacteria in fermented soy beverages during the refrigerated storage

Concerning starters viability, the microbial cell loads of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* measured in fermented soy beverages during refrigerated storage are reported in Fig. 1.

The data clearly showed that *L. delbrueckii* subsp. *bulgaricus* decreased significantly after 14 days of storage and particularly when non-encapsulated *L. gasseri* BC9 was added alone or mixed with *L. crispatus* BC4. This behavior was confirmed at the end of the storage period (28 days). According to Patrignani et al. (2017), decrease of *L. delbrueckii* subsp. *bulgaricus* could be due to the interaction with the vaginal strains. On the contrary, with respect to the control, *S. thermophilus* viability was not affected by the presence of the probiotic strains, either encapsulated or not, during the entire shelf-life of the product. Actually, an increase in *S. thermophilus* was observed, especially in presence of *L. gasseri* BC9, and it was confirmed in the samples collected during the shelf-life. A similar behavior was reported by other studies. In fact, according to Minervini et al. (2012), the addition of probiotic lactobacilli increased the survival of S. thermophilus in a Fior di Latte cheese. Also Patrignani et al. (2017) reported a higher concentration of *S. thermophilus* in fermented milks supplemented with encapsulated probiotic lactobacilli, even after 56 days of storage at 4 °C. In a more recent study, Cui et al. (2021) showed a significantly higher amount of *S. thermophilus* in soy beverage fermented by starter cultures and probiotics compared to the same product not supplemented with adjunct cultures. Several aspects should be considered when discussing the survival of probiotic strains in foods. The most important one is for sure the probiotic survival during food production and storage process. In fact, many factors can impact the viability of these bacteria in food products, such as intrinsic parameters (like pH, titratable acidity, oxygen, water activity, presence of salt, sugar, hydrogen peroxide, bacteriocins, artificial flavoring and coloring agents etc.), processing parameters (e.g. incubation temperature, heat treatment, cooling and storage conditions of the product, packaging materials, scale of production), and microbiological parameters (strain of probiotics employed, rate and proportion of inoculation) (Palanivelu et al., 2022; Putta et al., 2018). Therefore, the number of viable cells of *L. crispatus* BC4 and *L. gasseri* BC9 per gram of fermented soy beverage were determined in all the products during the 28 days of storage at 4 °C (Table 1).

The viability of vaginal strains remained at around 7 log CFU/mL of product from the beginning of the refrigerated storage till the end, despite the use of encapsulated or non-encapsulated bacteria with no significant differences, also considering the type of vaginal strain (*L. crispatus* BC4, *L. gasseri* BC9). This is in line with the requirements for probiotic functional foods concerning the minimum recommended level of probiotics (~ 6–7 log CFU/mL or g of product) (Kumari et al., 2022; Roobab et al., 2020). Some authors reported that lactic acid bacteria (LAB), also probiotics, can reach up to 8–9 log CFU/g (Li et al., 2012;

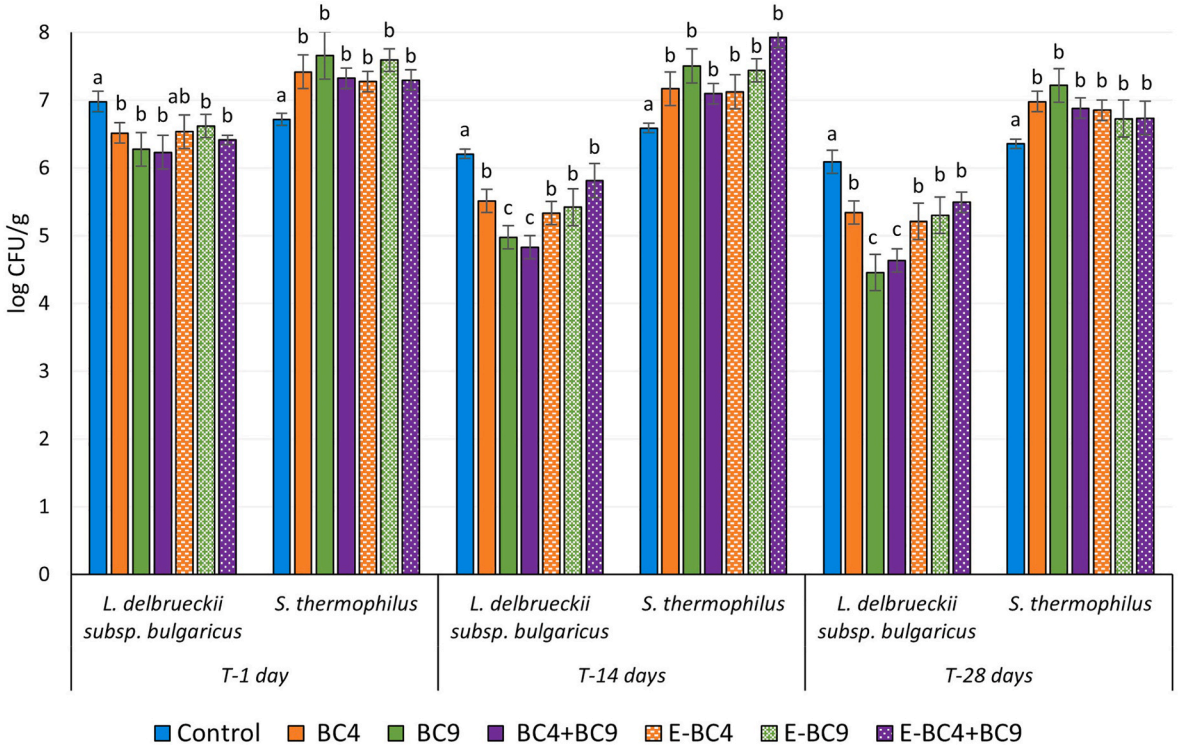

Fig. 1. Cell load viability (log CFU/g) of starter cultures (*L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*) in fermented soy beverages considering the presence of probiotic strains, the use of microencapsulation and refrigerated shelf-life. Control: fermented soy beverage; BC4: fermented soy beverage with *L. crispatus* BC4; BC9: fermented soy beverage with *L. gasseri* BC9; BC4+BC9: fermented soy beverage with the mix of *L. crispatus* BC4+*L. gasseri* BC9; E-BC4: fermented soy beverage with encapsulated *L. crispatus* BC4; E-BC9: fermented soy beverage with encapsulated *L. gasseri* BC9; E-BC4+BC9: fermented soy beverage with the encapsulated mix of *L. crispatus* BC4+*L. gasseri* BC9. Within the same group, data with the same superscript letter must be considered not statistically different ($p > 0.05$).

Table 1
Cell load viability (log CFU/g) of vaginal strains (*Lactobacillus crispatus* BC4 and *Lactobacillus gasseri* BC9) in fermented soy beverages considering the presence of probiotic strains, the use of microencapsulation and refrigerated shelf-life (observed after 1, 14 and 28 days of storage). BC4 and E-BC4: respectively fermented soy beverage with *L. crispatus* BC4 unencapsulated and encapsulated; BC9 and E-BC9: respectively fermented soy beverage with *L. gasseri* BC9 unencapsulated and encapsulated; BC4+BC9 and E-BC4+BC9: respectively fermented soy beverage with the mix of *L. crispatus* BC4+*L. gasseri* BC9 unencapsulated and encapsulated. Within the same column data with the same superscript letter must be considered not statistically different ($p > 0.05$).

Target bacterium	*Fermented soy beverage*	Cell load viability (log CFU/g)		
		1day	14day	28day
L. crispatus BC4	BC4	7.17 ^{a} ± 0.3	7.25 ^{a} ± 0.4	6.81 ^{a} ± 0.4
	E-BC4	7.49 ^{a} ± 0.2	7.38 ^{a} ± 0.3	6.99 ^{a} ± 0.3
	BC4 + BC9	7.08 ^{a} ± 0.4	6.97 ^{a} ± 0.3	6.72 ^{a} ± 0.4
	E-BC4 + BC9	7.24 ^{a} ± 0.3	7.31 ^{a} ± 0.3	6.71 ^{a} ± 0.3
L. gasseri BC9	BC9	6.95 ^{a} ± 0.5	6.88 ^{a} ± 0.5	6.78 ^{a} ± 0.3
	E-BC9	7.12 ^{a} ± 0.4	6.95 ^{a} ± 0.4	6.84 ^{a} ± 0.3
	BC4 + BC9	7.23 ^{a} ± 0.3	7.16 ^{a} ± 0.2	7.14 ^{a} ± 0.2
	E-BC4 + BC9	7.39 ^{a} ± 0.3	7.07 ^{a} ± 0.3	6.95 ^{a} ± 0.3

İçier et al., 2015) of viable cells in soy beverage without the need of additional carbohydrates. According to Kumari et al. (2022), this beverage is commonly used as a cheap and appropriate medium for the growth of several LAB, also intended as a suitable delivery carrier for various probiotic strains (Kumari et al., 2021).

3.2. Physico-chemical properties observed in fermented soy beverages

Selected physico-chemical properties, such as soluble solids (°Brix), water holding capacity (%), lactic acid (%) and pH, of the fermented soy beverages after 1 day of refrigerated storage are shown in Table 2. The soluble solids values of the control (3.03°Bx) and the fermented products with non-encapsulated vaginal strains did not differ significantly ($p \geq 0.05$). The remaining samples (E-BC4, E-BC9, E-BC4+9) not only differed from the previous ones, but also from each other, showing marginal differences. The highest value was determined in the sample containing E-BC4+BC9 (6.53°Bx). Water-holding capacity (WHC), which is thought to be an important attribute to measure the ability of food structure to bind water in its protein matrix (Torrico et al., 2019; Zayas, 1997), was also analyzed. In general, WHC values of the beverages fermented with non-encapsulated vaginal strains were not significantly different and were ranging from 37 to 39%. Interestingly, the samples containing the capsules had higher WHC values (50–62%), suggesting that the addition of the capsules may have increased the water retention capacity of the sample, an important aspect, especially given the type of food we are looking at. Lactic acid concentration increased significantly ($p < 0.05$) after one day of storage, especially in samples containing encapsulated bacteria, such as E-BC4+9 in which lactic acid reached 1.43%. This had an impact also on the pH value of the products. Over time, the pH value of control sample decreased slowly during the first 3 weeks, while it reduced of about 0.6–0.7 units during the fourth week. Excluding day 1, the pH values of the control over 28 days at 4 °C were always significantly different from the other samples (Fig. 2). In fact, already after the first week of storage, pH drops were observed for the remaining samples, more moderate for E-BC9, E-BC4, BC9, BC4 and more noticeable for BC4+BC9 and E-BC4+BC9. In general, the pH of fermented soy beverages produced with non-encapsulated probiotic cultures decreased within the first 21 days of storage, while they remained quite stable up to 28 days with pH values that never went below pH 4.39. On the contrary, samples containing encapsulated probiotics showed a more constant pH reduction during the entire 28 days of storage, reaching values below 4.35 at the end of incubation. The stronger acidification of the samples containing encapsulated bacteria can depend on the capsule that protect better the cells from inhibiting factors (e.g. low pH). Indeed, a higher efficient fermentation was also reported by Simó et al. (2017) for encapsulated bacteria in red wine compared to the use of non-encapsulated ones. The decrease in pH, resulting from chemical interactions that occur in fermented soy beverage in presence of vaginal strains can have an impact on the sensory panel and the aroma profiles of the final products.

3.3. Survival to simulated gastric and intestinal juices of the vaginal strains present in the fermented soy beverages

To achieve the highest beneficial effects, probiotics must survive during food processing, storage, and consumption, as well throughout the GIT, retaining their bioactivity at their target site. Also, for the present study this aspect is fundamental. In fact, even if the final target is the vaginal environment, these orally administered vaginal strains need first to survive trough the passage in the gastrointestinal tract. Then, from there they can transfer to the vagina due to the spatial proximity of the two organs and they can colonize the gynecological niche thanks to their physiological trophism for the vaginal mucosa. According to several studies (Basu et al., 2018; Putta et al., 2018; Roobab et al., 2020; Shori, 2016) an important role in the stability of probiotics and resistance to GIT conditions could be provided by the food matrix. However, which role the matrix plays is still not clear. In addition, microencapsulation could improve the survival rate of probiotics, using several encapsulation methods, such as extrusion, emulsion, and freeze or spray-drying (Rashidinejad et al., 2022). In this framework, the performances of both the fermented soy beverage and the microencapsulation in maintaining strain viability were tested after 14 days of refrigerated storage and subsequent passage through simulated gastric

Table 2
Physico-chemical analyses (°Brix, water holding capacity (%), lactic acid (%) and pH) performed on all the investigated fermented soy beverages, after 1 day of refrigerated storage. Control: fermented soy beverage; BC4: fermented soy beverage with *L. crispatus* BC4; BC9: fermented soy beverage with *L. gasseri* BC9; BC4+BC9: fermented soy beverage with the mix of *L. crispatus* BC4+*L. gasseri* BC9; E-BC4: fermented soy beverage with encapsulated *L. crispatus* BC4; E-BC9: fermented soy beverage with encapsulated *L. gasseri* BC9; E-BC4+BC9: fermented soy beverage with the encapsulated mix of *L. crispatus* BC4+*L. gasseri* BC9. Within the same observed parameter (°Brix, water holding capacity (%), lactic acid (%) and pH) data with the same superscript letter must be considered not statistically different ($p > 0.05$).

Sample	Soluble solids (°Brix)	WHC (%)	Lactic acid (%)	pH
Control	3.03±0.06^{a}	39.17±1.18^{a}	0.58±0.01^{a}	4.55±0.01^{a}
BC4	3.30±0.1^{a}	37.21±0.47^{a}	0.65±0.02^{a}	4.52±0.02^{b}
BC9	3.00±0.1^{a}	38.04±2.11^{a}	0.50±0.03^{a}	4.56±0.01^{a}
BC4+9	3.23±0.25^{a}	39.07±2.81^{a}	0.59±0.01^{a}	4.51±0.02^{b}
E-BC4	4.13±0.21^{b}	50.50±2.12^{b}	0.83±0.03^{b}	4.55±0.01^{a}
E-BC9	5.23±0.12^{c}	52.33±2.11^{b}	0.86±0.02^{b}	4.56±0.01^{a}
E-BC4+9	6.53±0.06^{d}	62.29±2.11^{c}	1.43±0.06^{c}	4.49±0.02^{c}

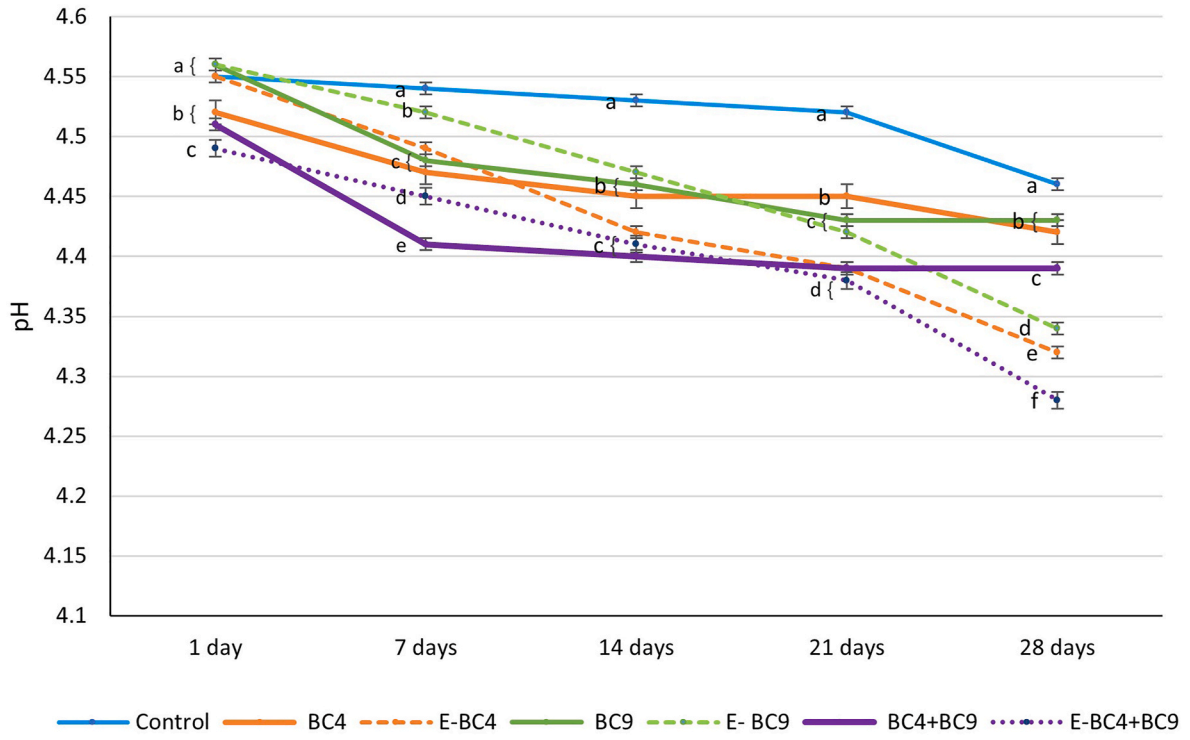

Fig. 2. pH values recorded during the refrigerated shelf-life (1,7, 14 and 28 days) of all the investigated fermented soy beverages. Control: fermented soy beverage; BC4: fermented soy beverage with *L. crispatus* BC4; BC9: fermented soy beverage with *L. gasseri* BC9; BC4+BC9: fermented soy beverage with the mix of *L. crispatus* BC4+*L. gasseri* BC9; E-BC4: fermented soy beverage with encapsulated *L. crispatus* BC4; E-BC9: fermented soy beverage with encapsulated *L. gasseri* BC9; E-BC4+BC9: fermented soy beverage with the encapsulated mix of *L. crispatus* BC4+*L. gasseri* BC9. Within the same timepoint, samples with the same superscript letter must be considered not statistically different ($p > 0.05$).

and intestinal phase (Fig. 3).

The presence of the microcapsule protected the vaginal strains from the different simulated digestive steps. In fact, at the end of the intestinal incubation bacteria were at least 6 log CFU/ml, compared to the cell load of 5 log CFU/mL for the strain non-encapsulated. On this regard the fermented beverages containing encapsulated probiotics met the requirements for probiotic functional foods (Kumari et al., 2022). In fact, spray-drying provided a greater protection from the natural physiological barriers present in the GIT compared to the use of the food matrix alone. The good performances of this kind of microencapsulation for these specific vaginal strains were recently observed by D'Alessandro et al. (2021) (b). More specifically, the authors studied the production of

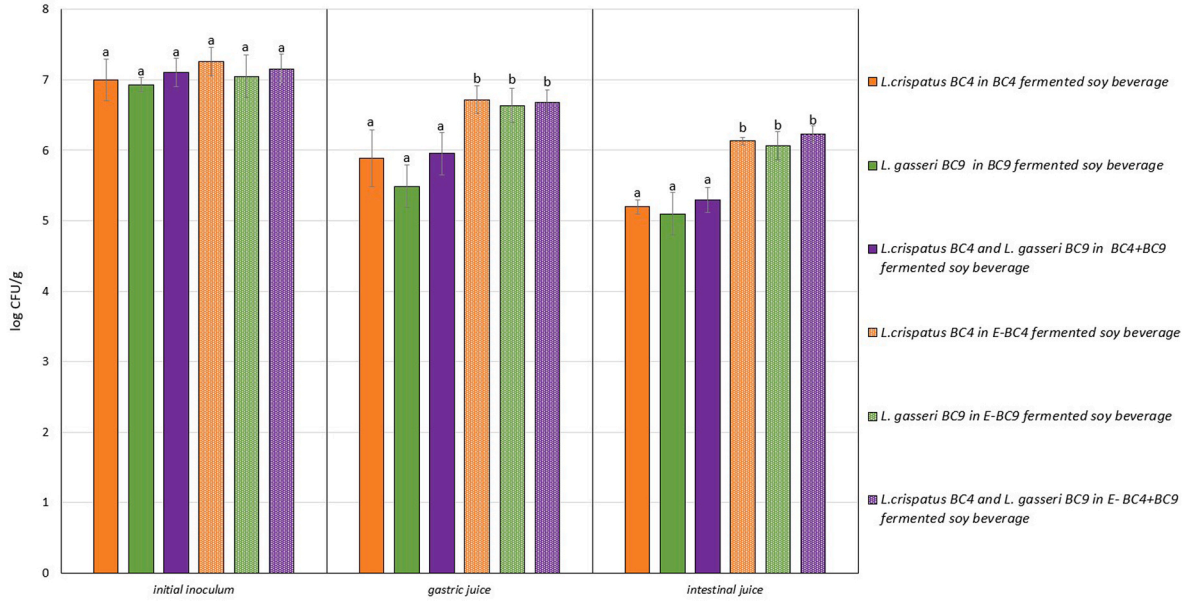

Fig. 3. Cell load viability (log CFU/g) of vaginal strains (*Lactobacillus crispatus* BC4 and *Lactobacillus gasseri* BC9) in fermented soy beverages when subjected to a gastro-intestinal juices simulation, considering the use of microencapsulation, and refrigerated shelf-life (T14). Within the sampling point (initial inoculum, gastric juice or intestinal juice), data with the same superscript letter must be considered not statistically different ($p > 0.05$).

soy beverage microcapsules of *L. crispatus* BC4 and *L. gasseri* BC9, which resulted with suitable morphology, good technological features in terms of water activity and moisture content. Moreover, the encapsulated bacteria demonstrated a better viability over time (1 year of storage) and upon simulated GIT conditions. As already mentioned, fermented soy beverages produced with encapsulated vaginal strains were able to withstand the simulated conditions of the GIT, meeting the minimum requirements for probiotic foods (~ 6–7 log CFU/mL or g of product), even though the initial inoculation of vaginal strains into the food product was lower than in D'Alessandro et al. (2021) (b). It is also important to mention that the viability was assessed through plate counting. Sublethal injuries may turn cells into a viable but non-culturable state (Vinderola et al., 2019). Therefore, the encapsulation may have protected the cells also from this event and at the same time a higher number of metabolic active cells may be present in the final product.

3.4. Antagonistic activity of fermented soy beverages against intestinal pathogens

Fermented soy beverages were tested for their antagonistic activity towards the intestinal pathogens enterotoxigenic *E. coli* H10407, *S. choleraesuis* and *Y. enterocolitica* by overlay assay (Table 3). All samples reduced enteropathogens growth, especially *E. coli* H10407 and *S. choleraesuis,* as indicated by the formation of inhibition halos. Those containing starter cultures only (control) displayed a moderate antagonistic activity towards the three enteropathogens, and such activity was kept stable over 21 days of refrigerated storage. For fermented products containing non-encapsulated *L. crispatus* BC4, *L. gasseri* BC9 or their mixture, the activity against *E. coli* H10407 and *Y. enterocolitica* was maintained and showed a slight increase after 14 and 21 days of storage. The addition of non-encapsulated vaginal lactobacilli into soy beverage enhanced the antagonistic activity against *S. choleraesuis*, especially for samples at 14 days of storage. Interestingly, samples added with encapsulated vaginal lactobacilli displayed the best profile of pathogen growth inhibition, especially after 14 and 21 days of refrigeration. The overall better performances of fermented soy beverages containing vaginal probiotics may depend on the metabolites that bacteria can produce and accumulate in the product, such as lactic acid, hydrogen peroxide or even bacteriocin. In addition, previous literature data had clearly indicated that the vaginal lactobacilli considered in this study, especially *L. crispatus* strains, were characterized by production of organic acids as demonstrated by the pH values of their cell free supernatants, and the volatile molecule profiles when inoculated in milks, registering the highest abundances of acetic acid and short chain fatty acids (Siroli et al., 2017). Also in this study, the observed reduction in pH of the samples containing vaginal probiotics with respect to the control suggest a higher production of acids. However, the lower pH measured cannot explain alone the superior properties of the samples containing encapsulated bacteria. According to Siroli et al., 2017, these bacteria should not produce bacteriocins. Therefore, the superior activity observed in the fermented products containing encapsulated bacteria could derive from the protection exerted by the capsule on the bacterial cells. In fact, encapsulation can enhance, or allow to maintain, better fermentation performances (production of more metabolites) compared to non-encapsulated cells which, on the contrary, are more sensitive to environmental stressors (Simó et al., 2017).

3.5. Sensory evaluation

The results of the sensory evaluation of all the investigated samples carried out at the beginning (1 day) and at the end of the designed shelf-life (28 days) are shown in Figs. 4 and 5, respectively.

In the first test performed after 1 day of storage, fermented soy beverages containing encapsulated probiotics showed significant lower scores in terms of texture and body. At the end of storage (28 days) the same samples received lower marks for more descriptors (such as taste, texture and body, overall acceptability, and general appearance), especially for those made with the encapsulated mixed probiotics. On the contrary, better evaluations were provided for samples containing non-encapsulated bacteria after 28 days of storage. Notably, although in terms of overall acceptability and texture and body, the preferred sample was the control, the differences with the remaining samples containing the non-encapsulated vaginal strains were limited. Furthermore, if in terms of general appearance, no differences were highlighted between the control and the samples mentioned above (BC4, BC9 and BC4 + BC9) in terms of taste, the preferred sample was BC9 while in terms of smell it was BC4. Even in terms of colour, no differences emerged between control, BC4 and BC9, while the sample containing the mix of the two non-encapsulated vaginal strains differed more, scoring a lower value than the samples mentioned above. More in general, fermentation of soy beverage or soy-based foods could significantly impact the sensory features of the final products in a positive way. In fact, even though soy beverage is a great nutrient supplement, its acceptance by consumers could be limited due to disagreeable beany flavour, the presence of antinutritional factor, that compromises mineral natural availability, and indigestible oligosaccharides, such as raffinose and stachyose, which cause flatulence (Lin et al., 2023; Theodoropoulos et al., 2018). To address these limitations, fermentation by LAB, especially when probiotics, has been proposed as a useful tool for improving nutritional bioavailability, supplying health benefits and enhancing physicochemical and sensory properties. In fact, soy natural flavor is generally described as beany and astringent and it is not well accepted by consumers. Fermentation provides a characteristic aroma and the use of probiotics to ferment soy beverage has been reported to significantly increase the final odor and flavor of the product (Ara et al., 2002). This can be related to the production of organic acids and flavoring agents, as well as through the metabolism of n-hexanal which is generally

Table 3
Evaluation of the antagonistic activity of fermented soy beverages against enterotoxigenic *E. coli* H10407, *S. choleraesuis*, and *Y. enterocolitica* over refrigerated storage. T1, day1; T7, day 7; T14, day 14; T21, day21. +, inhibition halo <10 mm; ++, inhibition between 10 and 13 mm; + + +, inhibition between 13 and 16 mm; + + ++, inhibition >16 mm. The diameter of inhibition, for each strain, was the average of three replicates. Control: fermented soy beverage; BC4: fermented soy beverage with *L. crispatus* BC4; BC9: fermented soy beverage with *L. gasseri* BC9; BC4+BC9: fermented soy beverage with the mix of *L. crispatus* BC4+*L. gasseri* BC9; E-BC4: fermented soy beverage with encapsulated *L. crispatus* BC4; E-BC9: fermented soy beverage with encapsulated *L. gasseri* BC9; E-BC4+BC9: fermented soy beverage with the encapsulated mix of *L. crispatus* BC4+*L. gasseri* BC9.

Sample	*E. coli* H10407				*S. choleraesuis*				*Y. enterocolitica*			
	T1	T7	T14	T21	T1	T7	T14	T21	T1	T7	T14	T21
Control	++	++	++	++	++	++	++	++	+	+	++	++
BC4	++	++	+++	++	+++	+++	++++	+++	+	+	+++	++
BC9	++	++	+++	+++	+++	+++	+++	++	+	+	++	++
BC4+9	++	++	++	+++	+++	++	++++	++	+	++	++	++
E-BC4	++	++	++++	+++	+++	+++	++++	++	+	+	++	++
E-BC9	+	++	++++	++	++	++	++++	++++	+	+	++	++
E-BC4+9	+	++	+++	++++	++	+++	++++	++++	++	++	+++	+++

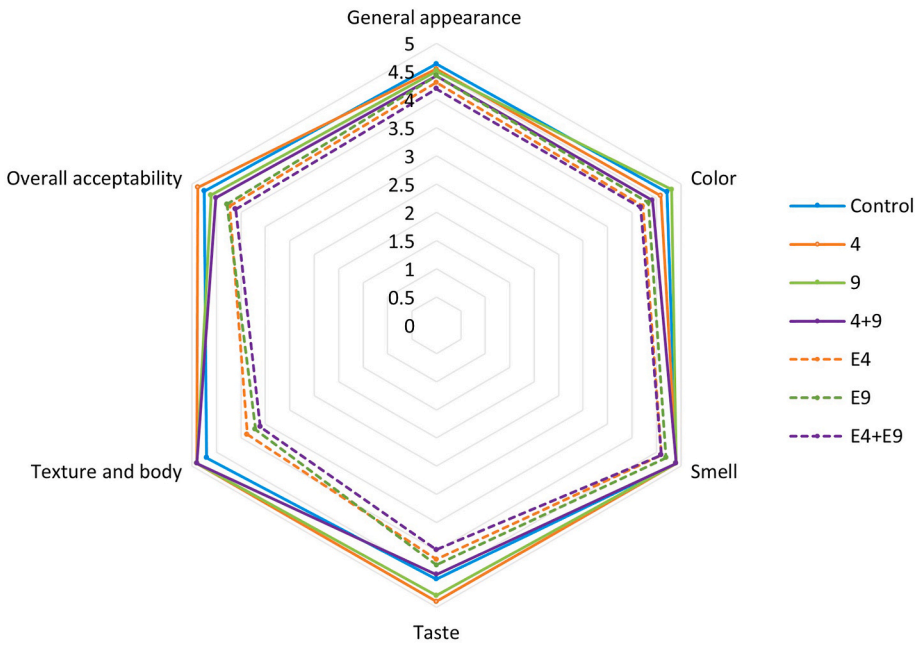

Fig. 4. Sensory analysis of all the investigated formulated soy beverages performed at 1 day of refrigerated storage considering six descriptors such as color, smell, taste, texture and body, overall acceptability, and general appearance. Control: fermented soy beverage; 4: fermented soy beverage with *L. crispatus* BC4; 9: fermented soy beverage with *L. gasseri* BC9; 4 + 9: fermented soy beverage with the mix of *L. crispatus* BC4+*L. gasseri* BC9; E-4: fermented soy beverage with encapsulated *L. crispatus* BC4; E-9: fermented soy beverage with encapsulated *L. gasseri* BC9; E-4+9: fermented soy beverage with the encapsulated mix of *L. crispatus* BC4+*L. gasseri* BC9. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

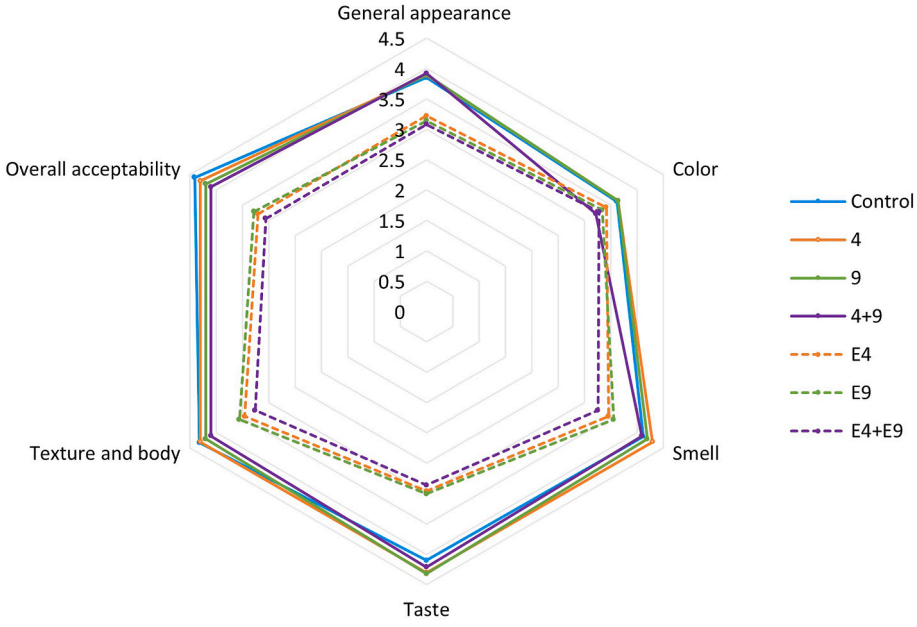

Fig. 5. Sensory analysis of all the investigated formulated soy beverages performed after 28 days of refrigerated storage considering six descriptors such as color, smell, taste, texture and body, overall acceptability, and general appearance. Control: fermented soy beverage; 4: fermented soy beverage with *L. crispatus* BC4; 9: fermented soy beverage with *L. gasseri* BC9; 4 + 9: fermented soy beverage with the mix of *L. crispatus* BC4+*L. gasseri* BC9; E-4: fermented soy beverage with encapsulated *L. crispatus* BC4; E-9: fermented soy beverage with encapsulated *L. gasseri* BC9; E-4+9: fermented soy beverage with the encapsulated mix of *L. crispatus* BC4+*L. gasseri* BC9. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

associated with the bean flavor in soy beverages.

3.6. Volatile compounds

The sensory property of soy beveragesis a crucial aspect, affecting the consumers' choices. Identifying volatile compounds can help to understand their flavor characteristics and the impact of fermentation on the final products (de Ovalle et al., 2018; Madjirebaye et al., 2022). Volatile compounds of fermented soy beverages measured and identified after 1 and 28 days of refrigerated storage are presented in Tables 4 and 5, respectively. Even if each fermented product was characterized by a specific aroma profile, molecules belonging to alcohols, aldehydes, ketones, and organic acids were detected in all the samples. Based on our results (Table 4) there were apparent changes in flavor substances after fermentation. In fact, hexanal, the main volatile compound in soy beverage (1.468 ppm) responsible for the characteristic beany flavor was remarkably reduced not only by the starters (0.109 ppm), but also by the vaginal strains. In fact, the lowest hexanal concentration (0.067 ppm) was reached with BC4 (Table 4). After fermentation, a similar trend of reduction was observed in the above mentioned sample (BC4) for other aldehydic compounds, such as benzaldehyde (from 0.865 to 0.458 ppm) and nonal (from 0.885 to 0.048 ppm), frequently associated with the beany unwanted flavor, also known as "grassy flavor" and "oxidized oil" flavor. In addition, soy beverages fermented by starters or mixed cultures with vaginal strains contained desirable aroma compounds, including diacetyl (between 1.048 and 2.911 ppm), 2,3-pentanedione (between 0.148 and 0.356 ppm), acetoin (between 0.202 and 0.573 ppm) and 2-heptanone (between 0.041 and 0.127 ppm), as recently reported by other studies on these food products. These compounds have suitable buttery, creamy or fruity aromas and are mainly

Table 4
Volatile compounds (reported as ppm) in soy beverage and in all the investigated fermented products, after 1 day of refrigerated storage detected by GC–MS coupled with a solid phase micro extraction (SPME). Control: fermented soy beverage; BC4: fermented soy beverage with *L. crispatus* BC4; BC9: fermented soy beverage with *L. gasseri* BC9; BC4+BC9: fermented soy beverage with the mix of *L. crispatus* BC4+*L. gasseri* BC9; E-BC4: fermented soy beverage with encapsulated *L. crispatus* BC4; E-BC9: fermented soy beverage with encapsulated *L. gasseri* BC9; E-BC4+BC9: fermented soy beverage with the encapsulated mix of *L. crispatus* BC4+*L. gasseri* BC9.

Molecules expressed in ppm	Soy beverage	Control T1 day	BC4 T1 day	E-BC4 T1 day	BC9 T1 day	E-BC9 T1 day	BC4+BC9 T1 day	E-BC4+BC9 T1 day
Diacetyl	n.d.	1.547	1.792	1.048	2.911	1.144	1.514	0.627
Isobutyl ketone	0.248	0.217	0.088	0.175	0.137	0.140	0.152	0.149
2,3-Pentanedione	n.d.	0.168	0.249	0.200	0.356	0.273	0.232	0.148
Acetoin	n.d.	0.253	0.202	0.279	0.342	0.573	0.275	0.227
2-Hexanone	0.390	0.144	0.071	0.102	0.102	0.100	0.136	0.321
4-Heptanone	n.d.	0.189	0.063	0.192	0.107	0.332	0.260	0.157
2-Heptanone	n.d.	0.056	0.041	0.082	0.069	0.127	0.077	0.068
2-Nonanone	n.d.	0.029	0.014	0.027	0.023	0.061	0.021	0.038
Ethanol	1.134	1.190	0.998	1.035	1.313	1.554	1.023	1.047
1-Pentanol	n.d	0.302	0.327	0.365	0.365	0.566	0.295	0.352
1-Hexanol	0.537	1.094	1.262	2.263	1.508	4.786	1.371	2.872
2-Hexanol	0.089	0.063	0.041	0.074	0.063	0.060	0.074	0.112
Ciclohexanol	0.866	0.900	0.899	0.682	0.892	1.191	0.905	1.034
1-Octanol	0.118	0.089	0.149	0.134	0.126	0.239	0.128	0.301
Hexanal	1.468	0.109	0.067	0.024	0.113	0.053	0.100	0.206
Nonanal	0.885	0.119	0.048	0.092	0.081	0.138	0.115	0.151
Acetic acid	0.317	0.723	0.409	1.019	1.243	1.404	2.035	2.354
Hexanoic acid	n.d.	0.039	0.031	0.041	0.046	0.086	0.070	0.035
Benzaldehyde	0.865	0.624	0.458	1.449	0.700	0.975	0.812	1.211
Benzene	0.476	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Xylene	0.070	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-Pentylfuran	0.223	0.021	0.009	0.008	0.008	0.011	0.008	0.010

Note: "n.d" means no determined.

Table 5
Volatile compounds (reported as ppm) in all the investigated fermented soy beverages, after 28 days of refrigerated storage, detected by GC–MS coupled with a solid phase micro extraction (SPME). Control: fermented soy beverage; BC4: fermented soy beverage with *L. crispatus* BC4; BC9: fermented soy beverage with *L. gasseri* BC9; BC4+BC9: fermented soy beverage with the mix of *L. crispatus* BC4+*L. gasseri* BC9; E-BC4: fermented soy beverage with encapsulated *L. crispatus* BC4; E-BC9: fermented soy beverage with encapsulated *L. gasseri* BC9; E-BC4+BC9: fermented soy beverage with the encapsulated mix of *L. crispatus* BC4+*L. gasseri* BC9.

Molecules expressed in ppm	Control T28days	BC4 T28 days	E-BC4 T28 days	BC9 T28 days	E-BC9 T28 days	BC4+BC9 T28 days	E-BC4+BC9 T28 days
Diacetyl	0.335	0.382	0.226	0.391	0.055	0.342	0.032
Isobutyl ketone	0.029	0.031	0.042	0.028	0.054	0.039	0.051
2,3-Pentanedione	0.114	0.066	0.084	0.118	0.028	0.068	0.026
Acetoin	0.231	0.534	0.253	0.267	0.118	0.243	0.281
2-Hexanone	0.054	0.026	0.049	0.023	0.071	0.029	0.027
4-Heptanone	0.139	0.287	0.415	0.123	1.072	0.141	0.035
2-Heptanone	0.058	0.025	0.079	0.025	0.056	0.022	0.043
2-Nonanone	0.046	0.020	0.055	0.028	0.069	0.082	0.028
Ethanol	0.156	0.252	0.177	0.180	0.487	0.222	0.166
1-Pentanol	0.418	0.418	0.473	0.370	0.176	0.062	0.151
1-Hexanol	0.359	0.291	0.436	0.376	0.525	0.485	0.975
2-Hexanol	0.045	0.024	0.086	0.026	0.023	0.030	0.048
Ciclohexanol	0.034	0.144	0.023	0.017	0.026	0.486	0.024
1-Octanol	0.053	0.136	0.058	0.063	0.112	0.965	0.094
Hexanal	0.066	0.007	0.045	0.034	0.020	0.049	0.032
Nonanal	0.049	0.035	0.068	0.032	0.066	0.050	0.061
Acetic acid	1.752	1.688	3.494	1.271	4.286	1.436	5.711
Hexanoic acid	0.024	0.046	0.054	0.084	0.044	0.030	0.046
Benzaldehyde	3.295	2.159	2.142	0.632	1.502	0.952	2.394
2-Pentylfuran	0.021	0.010	0.047	0.010	0.042	0.042	0.033

derived from the biotransformation of linoleic acid (Poliseli-Scopel et al., 2013). According to Yuan and Chang (2007) furans are associated with an unpleasant flavor and indicate color changes in soy beverages. Fermentation probably affects their contents, which is why furan compounds have drastically decreased after fermentation (from 0.223 up to 0.008 ppm) (Table 4). Our results are in line with what observed by Madjirebaye et al. (2022) and Yi et al. (2021) who found a decrease in furans (2-Pentylfuran) upon LAB fermentation. Besides this, acetic acid contents detected increased significantly, especially with the progress of the shelf-life (Table 5) for the samples containing encapsulated vaginal strains.

To better understand the effects of the variables adopted (presence of probiotic, encapsulation), on the aroma profiles of the fermented products, a principal component analysis (PCA) was performed with the volatilome data. The projection of the samples and the related molecules, analyzed after 1 and 28 days of storage (Figs. 6 and 7), were able to explain respectively 72.39% and 58.41% of the total variance among the samples. As reported above, the main volatile compounds in soy beverage were hexanal, nonal and 2-pentylfuran while molecules such as diacetyl or 2,3-pentanedione, recognized as desirable aroma compounds in fermented milk-type products, were characterizing control and products with non-encapsulated strains (Fig. 6). Encapsulation influenced the distribution of the samples in the space more than the type of vaginal strain both at the beginning and at the end of the shelf-life (Figs. 6 and 7).

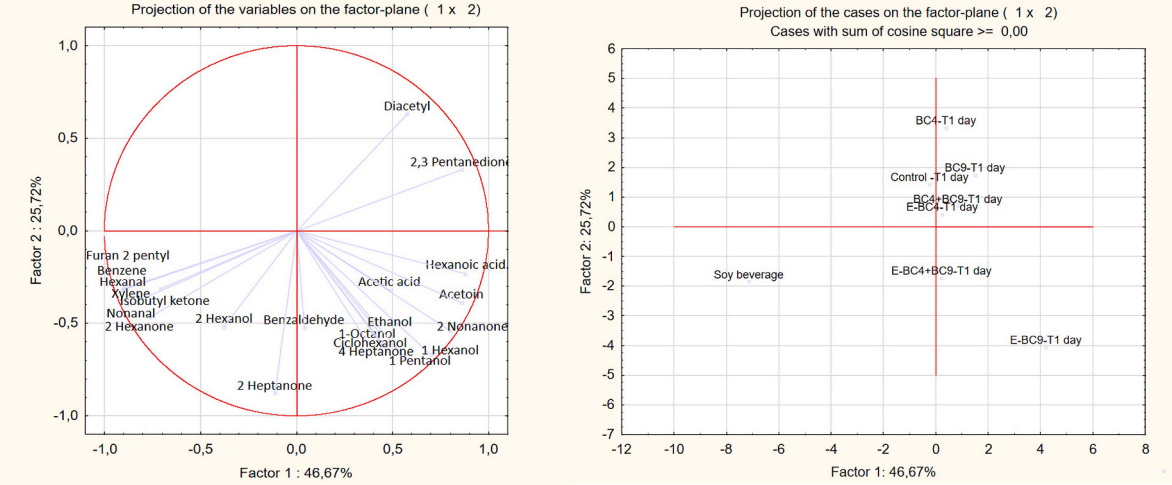

Fig. 6. Plot of cases (a) and variables (b) obtained by PCA elaboration of the total volatile molecules characterizing the soy beverage (unfermented) and the fermented products after 1 day of refrigerated storage. Control: fermented soy beverage; BC4: fermented soy beverage with *L. crispatus* BC4; BC9: fermented soy beverage with *L. gasseri* BC9; BC4+BC9: fermented soy beverage with the mix of *L. crispatus* BC4+ *L. gasseri* BC9; E-BC4: fermented soy beverage with encapsulated *L. crispatus* BC4; E-BC9: fermented soy beverage with encapsulated *L. gasseri* BC9; E-BC4+BC9: fermented soy beverage with the encapsulated mix of *L. crispatus* BC4+ *L. gasseri* BC9.

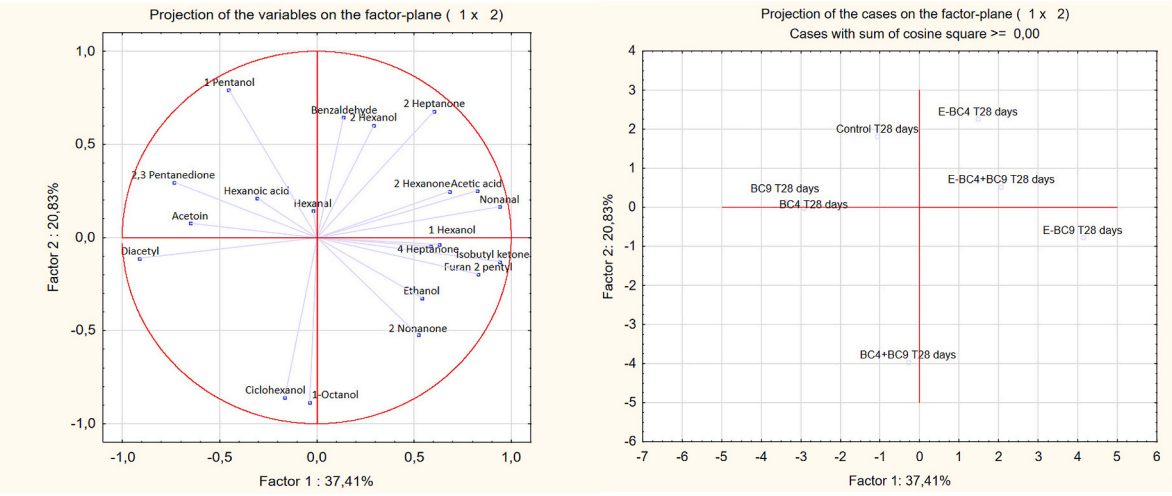

Fig. 7. Plot of cases (a) and variables (b) obtained by PCA elaboration of the total volatile molecules characterizing the fermented soy beverages after 28 days of refrigerated storage. Control: fermented soy beverage; BC4: fermented soy beverage with *L. crispatus* BC4; BC9: fermented soy beverage with *L. gasseri* BC9; BC4+BC9: fermented soy beverage with the mix of *L. crispatus* BC4+*L. gasseri* BC9; E-BC4: fermented soy beverage with encapsulated *L. crispatus* BC4; E-BC9: fermented soy beverage with encapsulated *L. gasseri* BC9; E-BC4+BC9: fermented soy beverage with the encapsulated mix of *L. crispatus* BC4+*L. gasseri* BC9.

3.7. Rheological properties of fermented soy beverages

Flow properties could be useful to better understand the gel structure of fermented soy beverage or yogurt (Kumari et al., 2022). In Fig. 8 the viscosity curves of different samples at different time of storage are shown. To reduce the number of variables, it was decided to analyze only the control sample and the fermented soy beverage made with the mix of vaginal probiotics (BC4+BC9 and E-BC4+BC9).

All samples exhibit a typical non-Newtonian behavior (*shear thinning*), characterized by a decrease of viscosity with the shear rate increase in agreement with the studies of Lee & Lucey (2006); Li et al. (2014) and Zhu et al. (2020). This behavior can be explained by the structural breakdown of the molecules due to the hydrodynamic forces generated and to the increased alignment of the constituent molecules (Glicerina et al., 2013). Moreover, to better explain results obtained from the curves, the yield stress, classically defined as the minimum shear stress that must be applied to the material to initiate flow (Sun & Gunasekaran, 2009), and Casson plastic viscosity, expressed as the force required to maintain a constant flow in the product, are showed in Figs. 9 and 10, respectively. All samples were well fitted by the Casson model, providing high correlation coefficients (R^2) that ranged from 0.85 to 0.99.

As shown in Figs. 9 and 10, both rheological parameters seem to show a similar trend when comparing the various samples and considering the shelf-life progress. The sample containing the encapsulated vaginal strains showed the highest values in terms of yield stress and viscosity after 1 day of storage (T1) which was followed by a decrease with the progress of the shelf-life. At the final time (T28), no significant differences were observed among the samples considered for both the parameters. More in general, as reported by Zhu et al. (2020) fermentation has a significant effect on the rheological properties of soy beverage, converting it from a Newtonian fluid into a non-Newtonian fluid. In the same study, significantly higher viscosity values were reported for soy beverage fermented by riboflavin producing lactobacilli

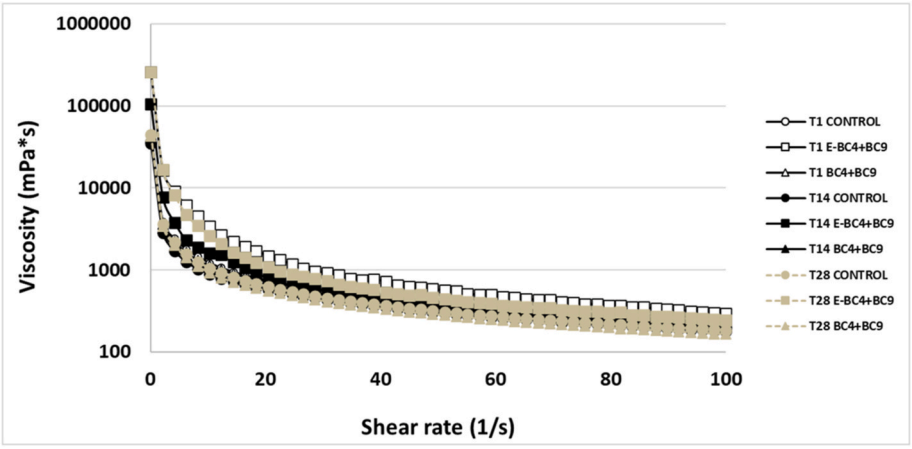

Fig. 8. Viscosity curves of control, E-BC4+BC9, BC4+BC9 samples during the refrigerated storage (T1 means 1 day of storage at 4 °C, T14 means 14 days of storage at 4 °C, T28 means 28 days of storage).

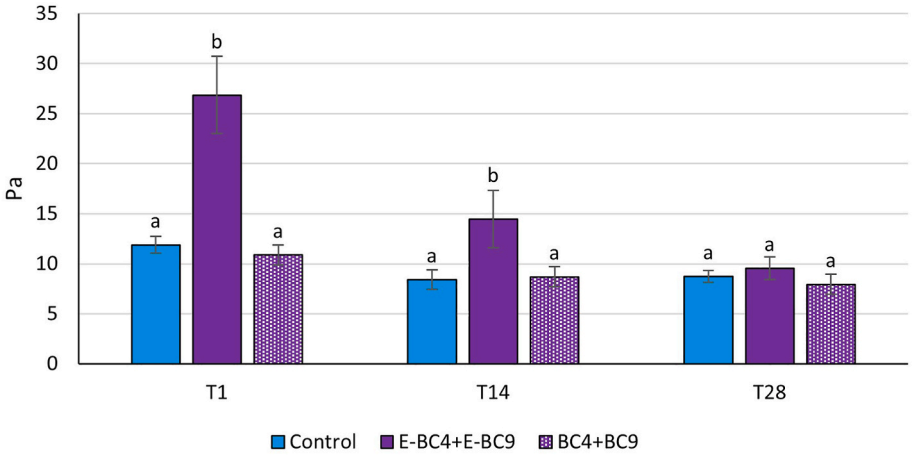

Fig. 9. Yield stress (Pa) values reported for control, E-BC4+BC9, BC4+BC9 during the refrigerated storage (T1 means 1 day of storage at 4 °C, T14 means 14 days of storage at 4 °C, T28 means 28 days of storage. Within the same timepoint, data with the same superscript letter must be considered not statistically different ($p > 0.05$).

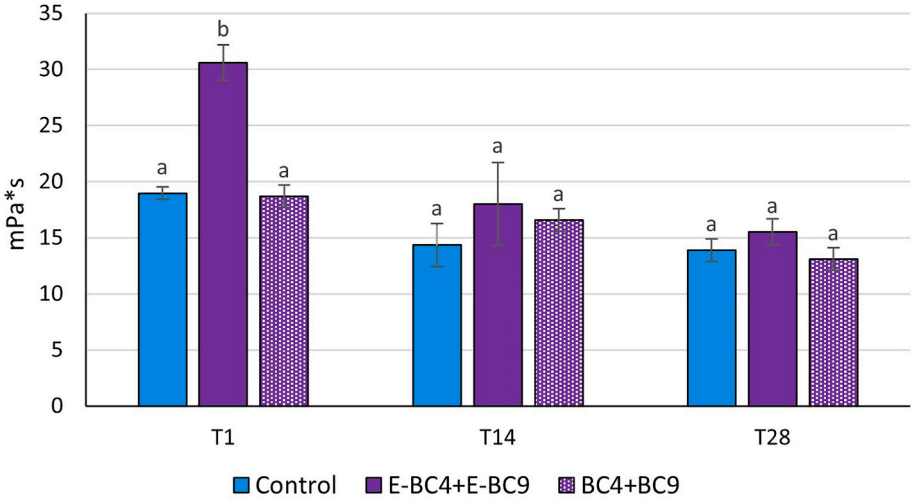

Fig. 10. Viscosity (mPa*s) values reported for control, E-BC4+BC9, BC4+BC9 during the refrigerated storage (T1 means 1 day of storage at 4 °C, T14 means 14 days of storage at 4 °C, T28 means 28 days of storage. Within the same timepoint, data with the same superscript letter must be considered not statistically different ($p > 0.05$).

compared to the non-fermented soy beverage. Moreover, the reduction observed in yield stress and plastic viscosity during storage can be related to a partial whey separation, however not perceived on the sensory test, or a decreased charge, which weakens colloidal stability caused by the post acidification phenomenon. According to Walstra & Wouters (2006) and Han et al. (2012), this involves also a strong acid taste, as highlighted indeed by volatile and sensory analysis. However, other studies reported a decrease in fermented soy beverage viscosity by increasing the shear rate, showing non-Newtonian fluid behavior (Li et al., 2014; Zhu et al., 2020). On the basis of these considerations, the resulting rheological properties of fermented soy beverages could be influenced by many factors such as composition of the beverage, strains of lactic acid bacteria and fermentation conditions.

4. Conclusions

The results obtained in this work showed that *L. crispatus* BC4 and *L. gasseri* BC9 were able to grow well in soy beverage, also increasing the viable cells of *S. thermophilus* during the refrigerated storage. Their viability remained stable at around 7 log CFU/mL of product during the entire 28 days of refrigerated storage, despite the use of encapsulated or non-encapsulated bacteria. However, the use of encapsulation had multiple effects. From one hand, encapsulated bacteria resulted more active as demonstrated by lactic acid production, pH reduction and their tolerance to this food matrix. Moreover, encapsulated bacteria survived better to *in vitro* GIT conditions fulfilling the desirable criteria for probiotic functional foods. On the contrary, their presence during storage reduced the sensorial acceptability of the products but provided the best inhibitory activity against intestinal pathogens. Non-encapsulated vaginal strains improved the release of aroma compounds (such as diacetyl or 2,3-pentanedione), typical of fermented dairy products, and reduced the undesired ones which provide beany flavour. In conclusion, *L. crispatus* BC4 and *L. gasseri* BC9 resulted promising strains to be used as co-starters to produce fermented soy beverage. The use of encapsulation seems to be a useful technology to improve the survival and activity of these strains. However, further studies are required to optimise the encapsulation process also considering the sensorial impact of the final product.

CRediT authorship contribution statement

Margherita D'Alessandro: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, All the authors read and approved the final version of the manuscript. **Davide Gottardi:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing, All the authors read and approved the final version of the manuscript. **Carola Parolin:** Investigation, Writing – original draft, Writing – review & editing, All the authors read and approved the final version of the manuscript. **Virginia Teresa Glicerina:** Investigation, Writing – original draft, Writing – review & editing, All the authors read and approved the final version of the manuscript. **Beatrice Vitali:** Writing – review & editing, Resources, All the authors read and approved the final version of the manuscript. **Rosalba Lanciotti:** Conceptualization, Resources, All the authors read and approved the final version of the manuscript. **Francesca Patrignani:** Conceptualization, Writing – review & editing, Resources, All the authors read and approved the final version of the manuscript.

Declaration of competing interest

All the authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All the data are presen tin the manuscript

References

Ahire, J. J., Sahoo, S., Kashikar, M. S., Heerekar, A., Lakshmi, S. G., & Madempudi, R. S. (2021). In vitro assessment of *Lactobacillus crispatus* UBLCp01, *Lactobacillus gasseri* UBLG36, and *Lactobacillus johnsonii* UBLJ01 as a potential vaginal probiotic candidate. *Probiotics and antimicrobial proteins*, 1–12. https://doi.org/10.1007/s12602-021-09838-9

Anahtar, M. N., Gootenberg, D. B., Mitchell, C. M., & Kwon, D. S. (2018). Cervicovaginal microbiota and reproductive health: The virtue of simplicity. *Cell Host and Microbe, 23*(2), 159–168. https://doi.org/10.1016/j.chom.2018.01.013

Ara, K., Yoshimatsu, T., Ojima, M., Kawai, S., & Okubo, K. (2002). Effect of new lactic acid fermentation on sensory taste of fermented soymilk. *Journal of the Japanese Society for Food Science and Technology ((Japan)*.

Basu, S., Banerjee, D., Chowdhury, R., & Bhattacharya, P. (2018). Controlled release of microencapsulated probiotics in food matrix. *Journal of Food Engineering, 238*, 61–69.

Bhushan, B., Kumkum, C. R., Kumari, M., Ahire, J. J., Dicks, L. M., & Mishra, V. (2020). Soymilk bio-enrichment by indigenously isolated riboflavin-producing strains of *Lactobacillus plantarum*. *Lebensmittel-Wissenschaft & Technologie, 119*, Article 108871. https://doi.org/10.1016/j.lwt.2019.108871

Calonghi, N., Parolin, C., Sartor, G., Verardi, L., Giordani, B., Frisco, G., Marangoni, A., & Vitali, B. (2017). Interaction of vaginal Lactobacillus strains with HeLa cells plasma membrane. *Beneficial Microbes*. https://doi.org/10.3920/BM2016.0212

Cui, L., Chang, S. K. C., & Nannapaneni, R. (2021). Comparative studies on the effect of probiotic additions on the physicochemical and microbiological properties of yoghurt made from soymilk and cow's milk during refrigeration storage (R2). *Food Control, 119*, Article 107474. https://doi.org/10.1016/j.foodcont.2020.107474. July 2020.

D'Alessandro, M., Pisanu, F., Baldo, D., Parolin, C., Filippini, G., Vitali, B., Lanciotti, R., & Patrignani, F. (2021). Unravelling the functional and technological potential of soy milk based microencapsulated Lactobacillus crispatus and Lactobacillus gasseri. *Journal of Functional Foods, 87*, Article 104745. https://doi.org/10.1016/j.jff.2021.104745 (b

Cunningham, M., Azcarate-Peril, M. A., Barnard, A., Benoit, V., Grimaldi, R., Guyonnet, D., & Gibson, G. R. (2021). Shaping the future of probiotics and prebiotics. *Trends in Microbiology, 29*(8), 667–685.

D'Alessandro, M., Parolin, C., Bukvicki, D., Siroli, L., Vitali, B., De Angelis, M., Lanciotti, R., & Patrignani, F. (2021). Probiotic and metabolic characterization of vaginal lactobacilli for a potential use in functional foods. *Microorganisms, 9*(4). https://doi.org/10.3390/microorganisms9040833 (a

D'Alessandro, M., Parolin, C., Patrignani, S., Sottile, G., Antonazzo, P., Vitali, B., Lanciotti, R., & Patrignani, F. (2022). Human breast milk: A source of potential probiotic candidates. *Microorganisms, 10*(7). https://doi.org/10.3390/microorganisms10071279

Di Cerbo, A., Palmieri, B., Aponte, M., Morales-Medina, J. C., & Iannitti, T. (2016). Mechanisms and therapeutic effectiveness of lactobacilli. *Journal of Clinical Pathology, 69*(3), 187–203. https://doi.org/10.1136/jclinpath-2015-202976

Foschi, C., Salvo, M., Cevenini, R., Parolin, C., Vitali, B., & Marangoni, A. (2017). Vaginal lactobacilli reduce Neisseria gonorrhoeae viability through multiple strategies: An in vitro study. *Frontiers in Cellular and Infection Microbiology*. https://doi.org/10.3389/fcimb.2017.00502

Giordani, B., Maria, L., Parolin, C., Foschi, C., Marangoni, A., Abruzzo, A., Dalena, F., Cerchiara, T., Bigucci, F., Luppi, B., & Vitali, B. (2018). Vaginal Bi fi dobacterium breve for preventing urogenital infections : Development of delayed release mucoadhesive oral tablets. *International Journal of Pharmaceutics, 550*(1–2), 455–462. https://doi.org/10.1016/j.ijpharm.2018.09.003

Glicerina, V., Balestra, F., Dalla Rosa, M., & Romani, S. (2013). Rheological, textural and calorimetric modifications of dark chocolate during process. *Journal of Food Engineering, 119*(1), 173–179.

Glicerina, V., Balestra, F., Dalla Rosa, M., & Romani, S. (2015). Effect of manufacturing process on the microstructural and rheological properties of milk chocolate. *Journal of Food Engineering, 145*, 45–50.

Global Market Insights. (2022). *Probiotics market size by ingredients (lactobacilli, bifidobacterium, Streptococcus, Bacillus), by end-use (human, animal), by application (food and beverages {dairy products, non-dairy products, cereals, baked food, fermented meat products, dry food}, dietary supplements {food, nutritional, specialty nutrients, infant formula}, animal feed), COVID-19 impact analysis, regional outlook, growth potential.* Competitive Market Share & Forecast.

Han, X., Zhang, L., Du, M., Yi, H., Li, J., & Zhang, L. (2012). Effects of copper on the post acidification of fermented milk by St. thermophilus. *Journal of Food Science, 77*, M25–M28.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology, 11*(8), 506–514. https://doi.org/10.1038/nrgastro.2014.66

İçier, F., Gündüz, G. T., Yılmaz, B., & Memeli, Z. (2015). Changes on some quality characteristics of fermented soy milk beverage with added apple juice. *LWT-Food Science & Technology, 63*(1), 57–64.

Kumari, M., Bhushan, B., Kokkiligadda, A., Kumar, V., Behare, P., & Tomar, S. K. (2021). Vitamin B12 biofortification of soymilk through optimized fermentation with extracellular B12 producing Lactobacillus isolates of human fecal origin. *Current Research in Food Science, 4*, 646–654.

Kumari, M., Kokkiligadda, A., Dasriya, V., & Naithani, H. (2022). Functional relevance and health benefits of soymilk fermented by lactic acid bacteria. *Journal of Applied Microbiology, 133*(1), 104–119. https://doi.org/10.1111/jam.15342

Lee, W. J., & Lucey, J. A. (2006). Impact of gelation conditions and structural breakdown on the physical and sensory properties of stirred yogurts. *Journal of Dairy Science, 89*(7), 2374–2385.

Li, C., Li, W., Chen, X., Feng, M., Rui, X., Jiang, M., & Dong, M. (2014). Microbiological, physicochemical and rheological properties of fermented soymilk produced with exopolysaccharide (EPS) producing lactic acid bacteria strains. *LWT - Food Science and Technology, 57*(2), 477–485. https://doi.org/10.1016/j.lwt.2014.02.025

Li, H., Yan, L., Wang, J., Zhang, Q., Zhou, Q., Sun, T., Chen, W., & Zhang, H. (2012). Fermentation characteristics of six probiotic strains in soymilk. *Annals of Microbiology, 62*(4), 1473–1483.

Lin, W., Kuang, H., Bai, M., Jiang, X., Zhou, P., Li, Y., & Guan, Y. (2023). Multiplex genome editing targeting soybean with ultra-low anti-nutritive oligosaccharides. *The Crop Journal*.

Lucey, J. A. (2004). Cultured dairy products: An overview of their gelation and texture properties. *International Journal of Dairy Technology, 57*, 77–84.

Madjirebaye, P., Peng, F., Huang, T., Liu, Z., Mueed, A., Pahane, M. M., Guan, Q., Xiao, M., Du, T., Wei, B., Xiong, S., Zhang, L., Xiong, T., & Peng, Z. (2022). Effects of fermentation conditions on bioactive substances in lactic acid bacteria-fermented soymilk and its storage stability assessment. *Food Bioscience, 50*, Article 102207. https://doi.org/10.1016/j.fbio.2022.102207

Marziali, G., Foschi, C., Parolin, C., Vitali, B., & Marangoni, A. (2019). In-vitro effect of vaginal lactobacilli against group B Streptococcus. *Microbial Pathogenesis*. https://doi.org/10.1016/j.micpath.2019.103692

Minervini, F., Siragusa, S., Faccia, M., Dal Bello, F., Gobbetti, M., & De Angelis, M. (2012). Manufacture of Fior di Latte cheese by incorporation of probiotic lactobacilli. *Journal of Dairy Science, 95*(2), 508–520. https://doi.org/10.3168/jds.2011-4150

Ñahui Palomino, R. A., Vanpouille, C., Laghi, L., Parolin, C., Melikov, K., Backlund, P., Vitali, B., & Margolis, L. (2019). Extracellular vesicles from symbiotic vaginal lactobacilli inhibit HIV-1 infection of human tissues. *Nature Communications*. https://doi.org/10.1038/s41467-019-13468-9

Nardini, P., Nâhui Palomino, R. A., Parolin, C., Laghi, L., Foschi, C., Cevenini, R., Vitali, B., & Marangoni, A. (2016). Lactobacillus crispatus inhibits the infectivity of Chlamydia trachomatis elementary bodies. *Scientific Reports*. https://doi.org/10.1038/srep29024. in vitro study.

Otieno, D. O., & Shah, N. P. (2007). A comparison of changes in the transformation of isoflavones in soymilk using varying concentrations of exogenous and probiotic-derived endogenous β-glucosidases. *Journal of Applied Microbiology, 103*(3), 601–612.

de Ovalle, S., Cavello, I., Brena, B. M., Cavalitto, S., & González-Pombo, P. (2018). Production and characterization of a β-glucosidase from Issatchenkia terricola and its use for hydrolysis of aromatic precursors in Cabernet Sauvignon wine. *Lebensmittel-Wissenschaft & Technologie, 87*, 515–522.

Palanivelu, J., Thanigaivel, S., Vickram, S., Dey, N., Mihaylova, D., & Desseva, I. (2022). Probiotics in functional foods: Survival assessment and approaches for improved viability. *Applied Sciences, 12*(1). https://doi.org/10.3390/app12010455

Parolin, C., Croatti, V., Laghi, L., Giordani, B., Tondi, M. R., De Gregorio, P. R., Foschi, C., & Vitali, B. (2021). Lactobacillus biofilms influence anti-Candida activity. *Frontiers in Microbiology, 12*(October), 1–11. https://doi.org/10.3389/fmicb.2021.750368

Parolin, C., Frisco, G., Foschi, C., Giordani, B., Salvo, M., Vitali, B., Marangoni, A., & Calonghi, N. (2018). Lactobacillus crispatus BC5 interferes with Chlamydia trachomatis infectivity through integrin modulation in cervical cells. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2018.02630

Parolin, C., Marangoni, A., Laghi, L., Foschi, C., Palomino, R. AÑ., Calonghi, N., Cevenini, R., & Vitali, B. (2015). Isolation of vaginal lactobacilli and characterization of anti-candida activity. *PLoS One*. https://doi.org/10.1371/journal.pone.0131220

Patrignani, F., Parolin, C., D'Alessandro, M., Siroli, L., Vitali, B., & Lanciotti, R. (2020). Evaluation of the fate of Lactobacillus crispatus BC4, carried in Squacquerone cheese, throughout the simulator of the human intestinal microbial ecosystem (SHIME). *Food Research International*. https://doi.org/10.1016/j.foodres.2020.109580

Patrignani, F., Siroli, L., Serrazanetti, D. I., Braschi, G., Betoret, E., Reinheimer, J. A., & Lanciotti, R. (2017a). Microencapsulation of functional strains by high pressure homogenization for a potential use in fermented milk. *Food Research International*. https://doi.org/10.1016/j.foodres.2017.04.020

Patrignani, F., Siroli, L., Serrazanetti, D. I., Braschi, G., Betoret, E., Reinheimer, J. A., & Lanciotti, R. (2017b). Microencapsulation of functional strains by high pressure homogenization for a potential use in fermented milk. *Food Research International, 97*(April), 250–257. https://doi.org/10.1016/j.foodres.2017.04.020

Poliseli-Scopel, F. H., Gallardo-Chacón, J.-J., Juan, B., Guamis, B., & Ferragut, V. (2013). Characterisation of volatile profile in soymilk treated by ultra high pressure homogenisation. *Food Chemistry, 141*(3), 2541–2548. https://doi.org/10.1016/j.foodchem.2013.05.067

Putta, S., Yarla, N. S., Lakkappa, D. B., Imandi, S. B., Malla, R. R., Chaitanya, A. K., Chari, B. P. V., Saka, S., Vechalapu, R. R., & Kamal, M. A. (2018). Probiotics: Supplements, food, pharmaceutical industry. In *Therapeutic, probiotic, and unconventional foods* (pp. 15–25). Elsevier.

Ramaswamy, H. S., & Basak, S. (1991). Rheology of stirred yogurts. *Journal of Texture Studies, 22*(2), 231–241.

Rashidinejad, A., Bahrami, A., Rehman, A., Rezaei, A., Babazadeh, A., Singh, H., & Jafari, S. M. (2022). Co-encapsulation of probiotics with prebiotics and their application in functional/synbiotic dairy products. *Critical Reviews in Food Science and Nutrition, 62*(9), 2470–2494.

Rasika, D., Vidanarachchi, J. K., Rocha, R. S., Balthazar, C. F., Cruz, A. G., Ana, A. S. S., & Ranadheera, C. S. (2021). *ScienceDirect Plant-based milk substitutes as emerging probiotic carriers* (pp. 8–20). https://doi.org/10.1016/j.cofs.2020.10.025

Roobab, U., Batool, Z., Manzoor, M. F., Shabbir, M. A., Khan, M. R., & Aadil, R. M. (2020). Sources, formulations, advanced delivery and health benefits of probiotics. *Current Opinion in Food Science, 32*, 17–28. https://doi.org/10.1016/j.cofs.2020.01.003

Shori, A. B. (2016). Influence of food matrix on the viability of probiotic bacteria: A review based on dairy and non-dairy beverages. *Food Bioscience, 13*, 1–8.

Shori, A. B., Baba, A. S., & Chuah, P. F. (2013). The effects of fish collagen on the proteolysis of milk proteins, ACE inhibitory activity and sensory evaluation of plain- and Allium sativum-yogurt. *Journal of the Taiwan Institute of Chemical Engineers, 44*(5), 701–706.

Simó, G., Vila-Crespo, J., Fernández-Fernández, E., Ruipérez, V., & Rodríguez-Nogales, J. M. (2017). Highly efficient malolactic fermentation of red wine using encapsulated bacteria in a robust biocomposite of silica-alginate. *Journal of Agricultural and Food Chemistry, 65*(25), 5188–5197.

Siroli, L., Patrignani, F., Serrazanetti, D. I., Parolin, C., Palomino, R. A. ñahu, Vitali, B., & Lanciotti, R. (2017). Determination of antibacterial and technological properties of vaginal lactobacilli for their potential application in dairy products. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2017.00166

Sun, A., & Gunasekaran, S. (2009). Yield stress in foods: measurements and applications. *International Journal of Food Properties, 12*(1), 70–101.

Theodoropoulos, V. C. T., Turatti, M. A., Greiner, R., Macedo, G. A., & Pallone, J. A. L. (2018). Effect of enzymatic treatment on phytate content and mineral bioacessability in soy drink. *Food Research International, 108*, 68–73.

Torrico, D. D., Tam, J., Fuentes, S., Gonzalez Viejo, C., & Dunshea, F. R. (2019). D-tagatose as a sucrose substitute and its effect on the physico-chemical properties and acceptability of strawberry-flavored yogurt. *Foods, 8*, 256. https://doi.org/10.3390/foods8070256

Vinderola, G., Reinheimer, J., & Salminen, S. (2019). The enumeration of probiotic issues: From unavailable standardised culture media to a recommended procedure? *International Dairy Journal, 96*, 58–65.

Walstra, P., & Wouters, J. T. M. (2006). *Dairy Science and Technology*. Boca Raton, FL: CRC Press.

Wang, Y.-C., Yu, R.-C., Yang, H.-Y., & Chou, C.-C. (2003). Sugar and acid contents in soymilk fermented with lactic acid bacteria alone or simultaneously with bifidobacteria. *Food Microbiology, 20*(3), 333–338.

Yi, C., Li, Y., Zhu, H., Liu, Y., & Quan, K. (2021). Effect of Lactobacillus plantarum fermentation on the volatile flavors of mung beans. *Lebensmittel-Wissenschaft & Technologie, 146*, Article 111434. https://doi.org/10.1016/j.lwt.2021.111434

Yuan, S. H., & Chang, S. K. C. (2007). Selected odor compounds in cooked soymilk as affected by soybean materials and direct steam injection. *Journal of Food Science, 72*(7), 5–10. https://doi.org/10.1111/j.1750-3841.2007.00461.x

Zayas, J. F. (1997). Water holding capacity of proteins. In *Functionality of proteins in food* (pp. 76–133). Springer.

Zhu, Y.-Y., Thakur, K., Feng, J.-Y., Cai, J.-S., Zhang, J.-G., Hu, F., & Wei, Z.-J. (2020). B-Vitamin enriched fermented soymilk: A novel strategy for soy-based functional foods development. *Trends in Food Science & Technology, 105*, 43–55. https://doi.org/10.1016/j.tifs.2020.08.019