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ORIGINAL PAPER

Physico-chemical Properties and Sensorial Appreciation of a New Fermented Probiotic Beverage Enriched with Pea and Rice Proteins

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Abstract

Objectives The purpose of this study was to evaluate the physico-chemical stability, the sensorial properties, and the microbial quality of a fermented beverage enriched with pea and rice proteins (PRF) during storage at 4 °C. To investigate the effect of the protein enrichment and fermentation, the PRF beverage quality was compared with non-fermented and non-enriched beverages.

Methods The beverage was supplemented with a 50/50 mixture of pea and rice protein concentrate to 13% concentration. Following inoculation with 10^8 CFU/mL of lactic acid bacteria, it was incubated at 37 °C for 14 h.

Results Results showed that the enrichment with protein induced an increase in pH, titratable acidity and viscosity of the PR products, while the fermentation led to a decrease of pH and viscosity. However, a significant increase of the viscosity of PRF from 39 to 57 cP was observed during the 143 days of storage ($P \leq 0.05$). The PRF beverage contained significantly more peptides < 200 Da than the non-fermented one (PRNF) and these small peptides were also released during the storage. Despite the physico-chemical modifications, the sensorial properties of the PRF product were appreciated over the storage, particularly for the texture. Furthermore, the beverage maintained a high concentration of viable probiotics during the entire storage with 8.4 log colony form unit (CFU)/mL after 143 days.

Conclusion Applying probiotics and the mixture of rice and pea proteins in the fermented beverage can enhance nutritional and nutraceutical value of the product.

Keywords Fermentation · Lactobacillus · Plant-based protein · Probiotic · Food analysis

Introduction

Functional foods like probiotic products and protein-enriched foods are increasingly popular among consumers of western countries. Probiotics are defined as "live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host". The prevention of diarrhea due to *Clostridioides difficile* [1], traveler's diarrhea [2] and antibiotic-associated diarrhea [3], the reduction of irritable bowel syndrome symptoms [4] and a general stimulation of the immune system [5] are among health benefits attributed to probiotic bacteria. Furthermore, products enriched with protein provide a high protein intake and more importantly they are usually low in fat and sugar. For these reasons, they are mainly consumed and appreciated by sportsmen, women, and individuals following a calorie-restrictive diet. Also, these products can be an interesting source of protein for elderly individuals by increasing their

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protein intake resulting in the reduced risk of frailty and the increased body mass and strength. Generally, probiotic and protein-enriched foods are found as dairy beverages such as drinkable yoghurt or milk, and whey is mainly used to enrich products with protein. But because of veganism, lactose intolerance or environmental risks, a growing number of consumers are looking for substitutes, like plant-based proteins. Pea and rice proteins are good alternatives to whey since they do not show any allerginicity [6] and they have a low environmental impact. Moreover, pea and rice proteins are comparable to whey concerning their beneficial effect on muscle strength and thickness [7, 8]. However, these proteins lack some essential amino acids (EAA). A combination of cereals and legumes, respectively deficient in lysine and methionine can however, establish equilibrium in the EAAs to achieve a complete intake of EAA. Nevertheless, a complete amino acid composition is not meant as good protein absorption or digestibility and vice versa. However, rice and pea proteins are known to have a high digestibility ranging from 92 to 99% for pea protein concentrate and 88% for rice protein concentrate [9]. Furthermore, bacteria of the genus *Lactobacillus* spp. are able to hydrolyze proteins and generate peptides and amino acids that are absorbed more efficiently compared to a native protein. Thus, the hydrolysis of proteins through fermentation can improve their digestibility and their absorption [10]. Also, storage time during fermentation process should have tangible impacts on the physico-chemical and the sensorial properties of fermented product.

The objective of this study was to develop a functional probiotic beverage enriched with pea and rice protein and to evaluate the effect of fermentation process and subsequent storage on physico-chemical properties, the microbial quality and the sensorial characteristics.

Materials and Methods

Materials and Beverage Preparation

A commercial probiotic beverage (Bio-K + ™ Blueberry), Bio-K + ™ ferment (composed of *Lactobacillus (L.) acidophilus* CL1285, *Lacticaseibacillus (L.) casei* LBC80R and *Lacticaseibacillus (L.) rhamnosus* CLR2), organic pea protein concentrate containing 80% protein (w/w) (Fying, Riverside, CA, USA) and organic brown rice protein containing 80% protein (w/w) (Fying) were kindly supplied by Bio-K Plus International Inc. (Laval, Québec, Canada).

All the ingredients in powder form Bio-K + ™ Blueberry, plus a 50/50 blend of pea and brown rice protein concentrates, were weighed and hydrated with filtered water. The PR-based beverages contained pea and rice proteins for a total protein content of 13% (w/w) whereas the CF and CNF beverages contained only rice proteins for a total protein content of 3% (w/w). The beverages were then pasteurized at 90 ± 2 °C for 60 s, packaged in 98 g bottles, sealed, and then cooled to 37 °C. To produce the fermented beverages, a part of the beverages was inoculated with Bio-K + ™ ferment at 10^8 colony form unit (CFU)/mL, incubated at 37 ± 1 °C for 14 ± 2 h, and cooled to 4 °C.

Product Characterization

In order to analyze the effect of the fermentation and subsequent storage on all characteristics to be evaluated, fermented beverages enriched with pea and rice protein (PRF) were compared with non-fermented beverages enriched with pea and rice protein (PRNF). Furthermore, fermented and non-fermented commercial Bio-K + ™ Blueberry (CF and CNF respectively) were also evaluated as non-enriched control beverages.

The pH of samples was measured with a pH meter (Fisher Scientific, MA, USA). The titratable acidity (TA), expressed as g lactic acid/100 g, was determined by the titration method with 0.1 M NaOH solution. The viscosity was evaluated with a Brookfield DV-II viscometer (Brookfield Engineering, MA, USA). Due to the great difference of viscosity between beverages, measures of viscosity were made at 4 °C at 100 rpm with spindle 00 for non-enriched drinks (CF and CNF) and at 20 rpm with spindle 02 for protein-enriched drinks (PRF and PRNF). Color parameters were determined with a Color reader CR 10 (Konica Minolta Sensing, Inc, Mahwah, NJ, USA) and expressed as L^* (lightness), C^* (chroma), h^* (hue angle) values. By employing the equations of $\tan^{-1}(b^*/a^*)$ and $(a^{*2}+b^{*2})^{1/2}$, the color difference (ΔE) of the products from the beginning to the end of the storage was calculated as follows:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where a^* value is the green–red axis, b^* value is the blue-yellow axis and the L^* value is the lightness from white to black. All parameters were determined every 21 days during 143-day storage period at 4 °C based on the storage duration of the commercialized control beverage.

Determination of Peptides Molecular Weight Distribution by Size Exclusion-High Performance Liquid Chromatography (SEC-HPLC)

Molecular weight distribution of soluble fractions of fermented and non-fermented beverages were analyzed by SEC-HPLC. A Biosep-SEC 2000 column (300 × 7.8 mm, 5 mm particle size, pore size 145 Å) from Phenomenex (Torrance, CA, USA) connected to an Agilent 1260 HPLC Infinity system (Agilent Technologies, Saint-Laurent,

QC, Canada) was used. Samples were first centrifuged at 10,000 × *g* for 20 min at room temperature. Then, 10 μL of the supernatant filtered a (0.2 μm) was injected on the column, and eluded using 100 mM sodium phosphate buffer solution (pH 6.8) at a flow rate of 1 mL/min for 20 min. Detection was then performed using a diode array detector at 280 nm. Bovine thyroglobulin (670 kDa), IgA (300 kDa), IgG (150 kDa), ovalbumin (44 kDa), myoglobin (17 kDa) and uridine (244.2 Da) were used as standards. The total surface area of chromatograms of peptide profiles was integrated and separated into three ranges of molecular weight (> 2700 Da, high molecular weight; 200–2700 Da, medium molecular weight; < 200 Da, low molecular weight), expressed as a percentage of the total area. Analysis of peptide profiles were done on fermented and non-fermented control and PR-based beverages every 21 days during storage at 4 °C for 143 days.

Microbial Analysis

Total viable count of *Lactobacillus* spp. was determined by spread plate method on MRS agar. The plates were incubated at 37 °C for 48 h. Analyzes were done during the storage at 4 °C every 21 days for 143 days.

Sensory Analysis

The fermented control and PR-based beverages were tested at days 0, 42 and 84 of storage.

Sensorial analysis was performed with 10 selected judges using a 9-point hedonic scale (1: extremely unpleasant and 9: extremely good) [11]. The parameters of color/appearance, odor, texture, flavor and overall appreciation were assessed. Selection of members of the sensory panel was based on their capacity to discriminate samples with good reproducibility, the repeatability of the results.

Statistical Analysis

The experiments were done in triplicate and for each replicate, three samples were analyzed. The data were reported as mean ± standard deviation. Data were subjected to one-way analysis of variance (ANOVA) by SPSS 22.0 software (IBM, NY, USA). Differences among mean values were examined by the Duncan's multiple comparison test at a $P \leq 0.05$.

Results and Discussion

Physico-chemical Properties of Fermented Milks during Storage

The results of pH are presented in Fig. 1S (supplementary material). T0 in the Fig. 1S is the starting point of storage for the beverage which has gone through fermentation process and it is going to be stored for 143 days. Results showed that pH of 6.2 was maintained throughout the storage period for the non-fermented beverages (CNF and PRNF) (data not shown). The pH of PRF beverage was higher than that of CF beverage for the entire storage period. The results corresponding to the pH of PRF and CF based beverages showed a decrease from 4.9 to 3.8 and from 4.2 to 3.4, respectively, during the entire storage period. The rate of pH decrease was higher for the PRF beverage than for the CF beverage with slope values of -0.024 and -0.010, respectively. After 42 days of storage, a stabilisation at pH 4.0 was observed for the PRF beverage. A stabilisation of pH was also observed for the CF beverage, after 84 days of storage, with a pH of 3.4.

The titratable acidity (TA) results are shown in Fig. 1S. The results showed that the TA of PRNF was three times higher than the TA of CNF with values of 0.3 and 0.1 g lactic acid/100 g, respectively (data not shown) and both were stable during the entire storage. Also, the TA of PRF increased from 0.73 to 1.70 g lactic acid/100 g from day 0 to day 63, then, kept going up with a gentle slope to 1.90 g lactic acid/100 g at the day 143. While the CF followed a gradual increasing trend during the whole storage period, from 0.37 to 1.23 g lactic acid/100 g. Similarly, the slope of TA corresponding to the PRF beverage was higher than that of the CF beverage with values of 0.015 and 0.006, respectively. The pH and the TA are both involved in the measurement of the acidity of the product but they give different information. The pH is related to the dissociation capacity of acids present in food and the TA value corresponds to the total quantity of acids. The higher pH of the PRF beverage compared to the CF beverage could be related to the buffering effect of the proteins whilst its higher TA corresponds to a higher lactic acid concentration. Pereira de Antoni (2015) [12] observed that the enrichment of a probiotic drink with whey protein increased the TA from 3.0 to 7.7% lactic acid while the pH remained stable [12]. The increased TA as a consequence of protein addition can be explained by the improved growth of bacteria contained in the fermented beverage.

The addition of proteins increased also significantly the viscosity from 3.8 cP for CF to 39.1 cP for PRF ($P \leq 0.05$) (Table 1) which can be attributed to the increased total solid content of the beverages upon protein addition [13].

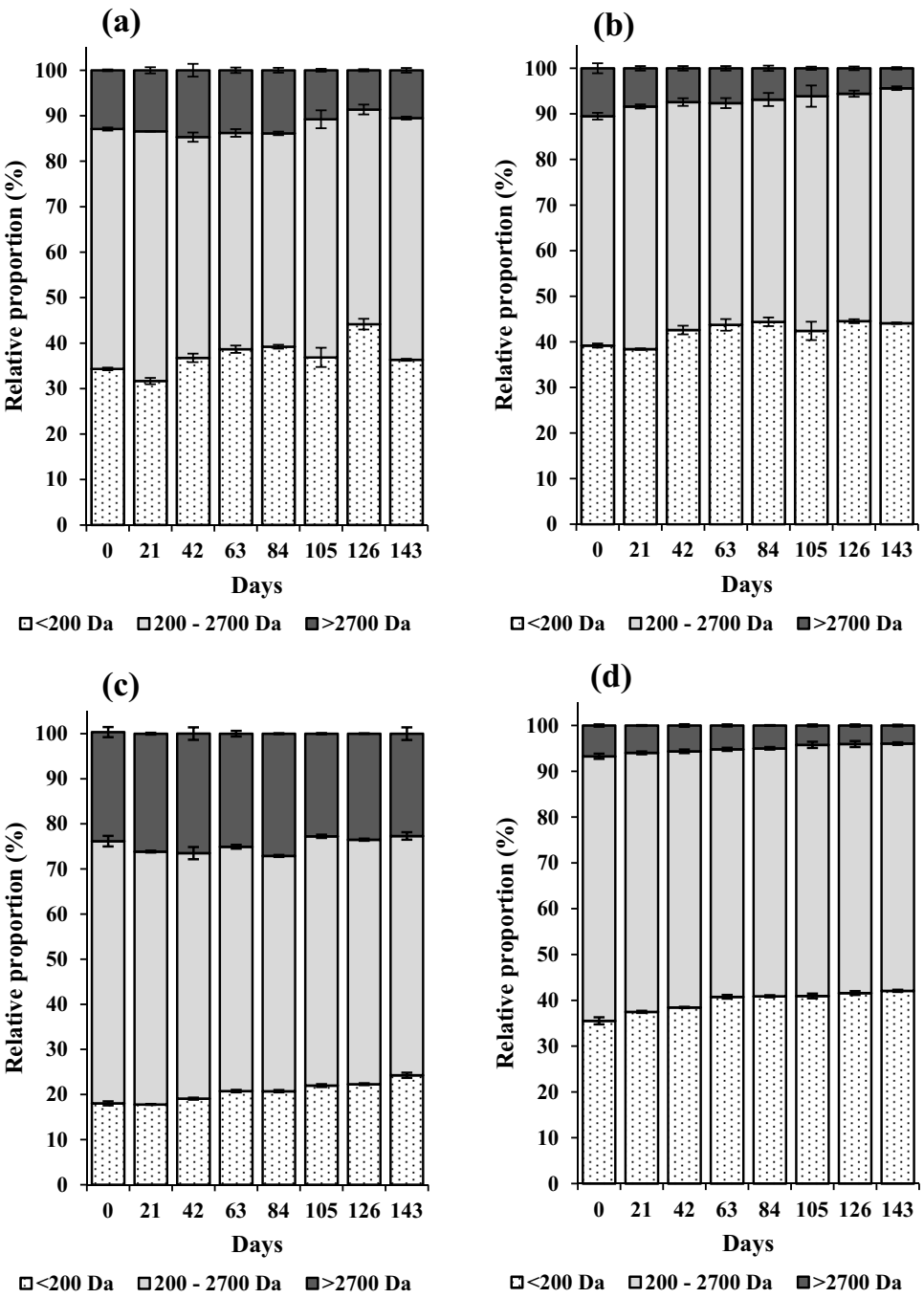

Fig. 1 Relative proportion (%) of molecular weight distribution (⁘ < 200 Da; □ 200–2700 Da; ■ > 2700 Da) of (a) non-fermented control (CNF), (b) fermented control (CF), (c) non-fermented pea-rice (PRNF) and (d) fermented pea-rice (PRF) beverages during storage at 4 °C for 143 days. Values are means of three replicates experiments. Error bars indicates standard error of three replicates measurements

The fermentation process had a decreasing effect on the PR-based beverage viscosity so that the viscosity of PRNF beverage at day 0 was 83.7 cP, compared to 39.1 cP for PRF beverage. The effect of the fermentation on the decrease of the viscosity can be due to the hydrolysis of protein by bacterial enzymes reducing the molecular size of proteins and peptides, thereby leading to more soluble peptides. Hayta, Alpaslan & Köse [14] observed also an increase of the protein solubility of fermented boza (a cereal-based beverage) during fermentation and a lower viscosity of fermented boza compared to the same non-fermented boza. Manus et al [15] also observed a similar effect of protein hydrolysis due to lactic acid fermentation and the release of small peptides (< 200 Da) for beverages enriched with pea and rice proteins compared to non-enriched beverages [15]. It is clear from Table 1, that the storage time had a significant increment on the viscosity of both control and PR-based beverages but this increase was more notable in PR-based beverages. The increase of viscosity observed during storage of PR-based beverages was 77 and 46% for PRNF and PRF beverages,

Table 1 Effect of the storage at 4 °C on the viscosity (cP) of CNF, CF, PRNF and PRF beverages

	Viscosity (cP)			
Storage days	CNF	CF	PRNF	PRF
0	4.0 ± 0.2^{aA}	3.8 ± 0.1^{aA}	83.7 ± 1.6^{aB}	39.1 ± 2.2^{aA}
21	4.1 ± 0.2^{aA}	4.2 ± 0.2^{bA}	96.7 ± 1.7^{bB}	37.4 ± 2.2^{aA}
42	4.6 ± 0.1^{bA}	4.5 ± 0.1^{cdA}	145.3 ± 2.3^{cB}	47.3 ± 0.8^{bA}
63	4.8 ± 0.4^{bB}	4.4 ± 0.1^{bcA}	148.7 ± 1.5^{cB}	53.4 ± 1.8^{cA}
84	5.8 ± 0.2^{dB}	5.2 ± 0.3^{eA}	156.2 ± 5.1^{cdB}	47.4 ± 1.4^{bA}
105	5.3 ± 0.3^{cB}	4.4 ± 0.1^{bcA}	164.8 ± 12.6^{dB}	58.7 ± 2.2^{deA}
126	5.1 ± 0.1^{cB}	4.5 ± 0.3^{cdA}	157.5 ± 11.8^{cdB}	61.4 ± 3.4^{eA}
143	4.7 ± 0.1^{bA}	4.8 ± 0.2^{dA}	148.5 ± 2.4^{cB}	57.0 ± 2.1^{cdA}

Data expressed as mean ± standard deviation ($n = 3$). CNF: non-fermented control beverage. CF: fermented control beverage. PRNF: non-fermented pea-rice beverage. PRF: fermented pea-rice beverage. Mean values with different lowercase letters within the same column are significantly different ($P \leq 0.05$). Mean values with different uppercase letters within the same row for the same base product are significantly different ($P \leq 0.05$)

respectively. While, this increase was estimated to be 17 and 26% for CNF and CF beverages, respectively. The increase of viscosity during the storage can be explained by molecular interactions like polysaccharide-polysaccharide interactions or protein-polysaccharide interactions present in PR proteins [16, 17]. Another explanation can be expressed for the increased viscosity during storage is that pea proteins are able to form fine-stranded networks at pHs away from isoelectric point. Figure 1S showed a pH loss to below isoelectric point of pea protein (5). On contrary to coarse networks formed at isoelectric point of proteins, more water is held inside the gel network formed at farther pHs and for this reason the viscosity has increased during the storage in the beverages supplemented by the proteins [18]. Bernat, Cháfer, Chiralt & González-Martínez [19] observed a significant increase ($P \leq 0.05$) in the apparent viscosity of a fermented hazelnut-based milk after 28 days of storage from 0.69 Pa.s at day 0 to 1.8 Pa.s.

The impact of the protein enrichment and the fermentation process on the beverage color is presented in Table 1S (Supplementary Material). The color of the beverage is characterized by three parameters. The L^* value represents lightness (100) and blackness (0); C^* value corresponds to the chroma (+: high color intensity; −: low color intensity) and h^* value corresponds to hue angle (0°: red, 90°: yellow, 180°: green, 270°: blue). The results showed that the decrease of the L^* value after fermentation (day 0) in the samples containing protein is followed by a gradual increase during the time of preservation. The enrichment with protein is accompanied by the presence of the particles benefiting from light absorbing chromophores leading to the decreased values of the L^* coordinates. L^* coordinate is related to the ability of sample to reflect and diffuse light; hence the decreased reflected light is accompanied by L^* loss. The increase in lightness after the fermentation process can be attributed to protein denaturation caused by the acidity increase during fermentation [20] resulting in aggregate formation and light reflection and consequently the increment of the L*values. In this study, the L^* value increased from 38.0 to 43.0 in CNF and CF and from 34.0 to 39.7 in PRNF and PRF, respectively. The L^* value during storage time showed that the clarity all beverages was mostly stable during the whole storage. For example, in PRF, the L^* value increased slightly from 39.7 at day 0 to 41.2 at day 143 ($PP > 0.05$).

The fermentation increased the chroma values meaning that the intensity of the color of fermented products was higher than the non-fermented. The chroma values increased by 3 to 4 units for fermented beverages, whether enriched with protein or not. It should be noted that the storage time did not affect the chroma values of all samples. Likewise, the fermentation did not affect the hue parameters but they were affected by the protein enrichment. The hue values were significantly higher ($P \leq 0.05$) in control beverages (80–81) as compared to the PR-based beverages (72–73) as the color of control beverages was closer to yellow while hue values of PR beverages were closer to orange. The dark yellow color of the rice protein can be effective in the reducing hue values [21]. The hue values were also stable in all samples during storage except the PRF sample. The release of some pigments naturally associated with plant proteins following conformational alterations of proteins during storage owing to pH loss can be the possible reason of the increased values of hue coordinate during storage. When the pigments are released, their spectral properties will change in comparison with the associated ones. The color difference (ΔE) among samples after 143 days of storage was lower than 3 for all beverages suggesting color differences were not easily detectable by the human eye. The highest values of ΔE were observed for PRNF and PRF beverages (2.97 and 2.18, respectively) [22, 23]. This indicates the preservative action of lactic acid fermentation on the color parameters [24].

Peptides Molecular Weight Distribution in Fermented Beverages

In order to characterize the peptides produced under the effect of the fermentation and storage time, the peptide profiles of the beverages were analyzed every 21 days by SEC-HPLC (Fig. 1). Results showed that at day 0, fermented products (CF and PRF) contained significantly more ($P \leq 0.05$) low molecular weight (LMW) peptides (< 200 Da) than non-fermented products (CNF and PRNF) with an augmentation of 14% for control products and an augmentation of 97% for PR products as compared to their respective non-fermented products. Furthermore, the fermented products possessed a significant lower quantity ($P \leq 0.05$) of high molecular weight (HMW) peptides (> 2700 Da) showing a decrease of 18% in control product (CF) and a decrease of more than 72% in PR-based product (PRF) as compared to their respective non-fermented products. These results suggest that fermentation had a greater impact on the molecular weight of peptides of PR products as compared to the control products as an increased release of LMW peptides corresponding mainly to di-peptides. Similarly, the degradation of HMW peptides into smaller ones was observed for the beverage enriched with cricket proteins under fermentation process [25].

The effect of the storage on the peptides molecular weight showed that the proportion of low molecular weight peptides of PRF beverage increased by 20% over the course of the storage period as compared to an increase by 12% for the CF beverage. The level of medium molecular weight peptides was relatively stable during the storage for all beverages with small variations (< 10%). The quantity of high molecular weight peptides decreased during the storage for all beverages, but the decrease was more pronounced for the fermented beverages, CF and PRF, with a respective reduction of 58 and 41%. The release of low molecular weight peptides during fermentation and subsequent storage could be due to the proteolytic activity of the specific combination of probiotics *L. acidophilus* CL1285, *L. casei* LBC80R and *L. rhamnosus* CLR2 of the Bio-K + ferment during the process and over the storage time implying that proteolytic activity of lactic acid bacteria (LAB) can be maintained during the storage [26]. The ability of lactic acid bacteria in degrading protein into peptides and amino acids during fermentation process is due to their disability to synthesize all of amino acids needed for their growth. Since all generated peptides are not consumed by the bacteria, the rest can promote various physiological functions in consumers. Small peptides can be easily absorbed in the gastrointestinal tract and cardiovascular circulation system and finally exhibit physiological-regulating properties [15]. It is worth mentioning that bacteria belonging to the genus *Lactobacillus* spp. are able to hydrolyze proteins and generate peptides that are absorbed more efficiently compared to a native protein. Thus, the hydrolysis of proteins through fermentation can improves their digestibility and their absorption [10]. Additionally, the bioactivity of the peptides resulted from rice and pea proteins through lactic acid fermentation have already been proved [27, 28] due to their strong biological effects such as antiobesity, antioxidant and antihypertensive. However, further research is needed to determine the potentially antioxidant activity of peptides released by the action of Bio-K + ferment.

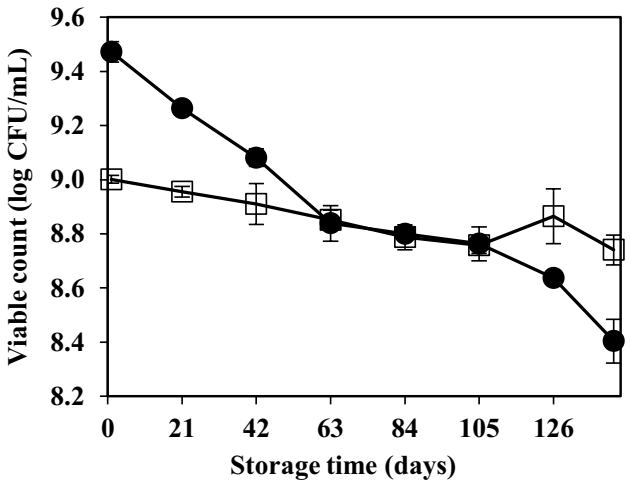

Fig. 2 Concentration of *Lactobacillus* spp. of fermented control (□ CF) and fermented pea-rice (● PRF) beverages during storage at 4 °C for 143 days. Values are means of three replicates experiments. Error bars indicates standard error of three replicates measurement

Microbiological Analysis

Since the viability of probiotics could be challenged during storage, it is important to follow this parameter to ensure an acceptable number of bacteria for the entire storage period. The concentration of *Lactobacillus* spp. of PRF and CF was followed during 143 days of storage and results are presented in Fig. 2. According to our results, despite both beverages were inoculated with the same concentration of bacteria, PRF exhibited higher concentration of *Lactobacillus* (9.45 log CFU/mL) bacteria at the end of fermentation (day 0) compared to the CF (9 log CFU/mL) at the same condition. The incorporation of proteins into the product apparently has provided peptide and amino acids for the bacterial growth and improved partly the viability of bacteria [29]. Greater values of TA in protein-enriched beverages are in good agreement with these results. The level of *Lactobacillus* spp. was almost stable during the 143 days of storage for the CF beverage. Indeed, there was a slight difference of 0.26 log CFU/mL between the beginning and the end of the storage, which is less than 1 log and is not considered as a

Table 2 Sensory analysis during 84 days of storage of CF and PRF beverages

	CF			PRF		
Parameters	0 d	42 d	84 d	0 d	42 d	84 d
Color/Appearance	6.4 ± 1.7^{aA}	6.9 ± 0.7^{aA}	6.4 ± 1.1^{aA}	7.4 ± 1.2^{abBC}	8.3 ± 0.9^{bC}	8.5 ± 0.5^{bB}
Odor	6.6 ± 0.7^{abA}	7.0 ± 0.7^{bA}	6.4 ± 1.7^{abA}	5.3 ± 1.4^{aA}	6.4 ± 1.7^{abAB}	6.6 ± 1.6^{abA}
Texture	6.3 ± 1.6^{aA}	7.2 ± 1.5^{aA}	6.6 ± 1.5^{aA}	8.0 ± 0.6^{aC}	7.6 ± 1.5^{aBC}	6.9 ± 1.7^{aA}
Flavor	7.6 ± 0.9^{cA}	7.4 ± 1.2^{cA}	7.6 ± 1.3^{cA}	5.8 ± 1.9^{abA}	5.2 ± 1.3^{aA}	6.7 ± 1.4^{bcA}
Global appreciation	7.4 ± 0.9^{bcA}	7.6 ± 0.7^{cA}	7.3 ± 1.3^{bcA}	6.2 ± 1.2^{abAB}	5.3 ± 1.3^{aA}	6.6 ± 1.5^{bcA}

Data expressed as mean ± standard deviation ($n = 3$). CNF: non-fermented control beverage. CF: fermented control beverage. PRNF: non-fermented pea-rice beverage. PRF: fermented pea-rice beverage. Scores vary between 1 (extremely disliked) and 9 (extremely liked). Scores are means ± SD. Mean values with different lowercase letters within the same raw are significantly different ($P \leq 0.05$). Mean values with uppercase lower letters within the same column are significant

microbiological significant difference. A reduction of 1 log was observed at the end of the storage period of the PRF beverage, from 9.47 to 8.40 log CFU/mL. Higher concentration of probiotics at the beginning of the storage in the PRF beverage gave rise to the production of higher concentration of lactic acid during storage leading to a drop in the concentration of *Lactobacillus* bacteria. The more pronounced pH loss in PRF compared to CF confirms the tangible loss of probiotics viability in PRF due to acid accumulation [30]. However, the level of microorganisms after 143 days is still considered sufficient in terms of nutraceutical properties [31].

Despite the viability of probiotics during storage, the majority of reports observed a decrease of 1 log or more during the storage of probiotic beverages. In addition, the storage period employed was often of 3 to 5 weeks [32–34], contrary to this study where the analyses were done during 143 days of storage. According to the Canadian Food Inspection Agency (CFIA), a portion of a food must contain at least 9 log CFU of CFIA-recognized probiotic bacteria to be considered as a probiotic food [31]. The results showed that the protein-enriched beverage PRF still contained at least 9 log CFU per serving defined by the CFIA. So that a 98-g bottle retained 10.4 log CFU per bottle after 143 days of storage.

Sensory Analysis

The results of the sensorial evaluation of the developed beverage are presented in Table 2. The comparison of PRF and CF showed that the global appreciation and the flavor were the most appreciated parameters in CF beverage and were negatively affected by the protein addition to the beverage ($P \leq 0.05$). The loss of acceptability of protein-enriched drinks can be originated from rice odor and bitter taste of some short chain peptides released during the fermentation of rice and pea proteins [11]. Furthermore, it could also be related to the pronounced vegetal taste and the beany flavor compounds of pea proteins. In this study, the earthy and powdery notes associated with peas lowered the flavor scores for the PRF beverage. The enhancing role of lactic acid fermentation in flavor was revealed during storage of PRF so that flavor values increased from 5.8 to 6.7 (meaning between "neither liked nor disliked" and "moderately liked") but it was still less than those of the samples free of protein (values over 7). It has been proved that lactic acid fermentation process reduces aldehyde and ketone content which are volatile compounds in pea and responsible for off-flavor [35].

Two parameters of odor and texture did not show significant difference in CF and PRF ($P > 0.05$). For the PRF beverage, the product was appreciated mainly for its color-appearance and its texture. Values from 7 to 8.5 were noticed, meaning from "moderately liked" to "extremely liked".

Regarding color, an insignificant increase was observed for protein-enriched drinks that this appreciation increased at the end of storage. The increase of alcohol and lactic acid after fermentation and subsequent storage can affect protein configuration and consequently the color parameters [35]. Moreover, these conformational alterations will result in pigment release and color changes of the final product.

Overall, the global appreciation scores adopted the same values as those of flavor. Indeed, the CF beverage was preferred, with values between 7.3 to 7.6, compared to PRF beverage, which had values between 5.3 and 6.6. It is worth mentioning that the CF beverage is the actual commercial probiotic product which contains an aroma specific to its formula. This could explain the highest appreciation of the CF beverage. Although further studies will be needed to develop a specific aroma or apply masking agents to improve the sensory quality of the PR-based beverage.

Conclusion

This research has focused on the development of a fermented beverage supplemented with plant proteins (pea and rice) and viable probiotic bacteria. The evaluation of physico-chemical properties showed that color was only property

that was not significantly affected by the fermentation and the length of storage period as the color difference among fermented and non-fermented samples was not detectable by the human eye. The pronounced viscosity increment resulted from protein addition diminished by half as a result of fermentation process. Nevertheless, getting far from isoelectric point of protein during storage increased viscosity due probably to gel networks formed at low pH with greater water absorption capability than those formed at near isoelectric point. Fermented beverages enriched by PR proteins were especially appreciated for its texture and its appearance in comparison with the control. However, some improvements should be done in order to improve the flavor appreciation of the product. The storage induced, however, some physicochemical variations but these variations did not affect the sensorial quality. The viability of high number of microorganisms over 10.4 log CFU per portion of 98 g in PRF beverages after 143 days of storage indicates achieving a probiotic drink despite having high titratable acidity. More importantly, the release of low molecular weight peptides (< 200 Da) during fermentation and subsequent storage improves health-promoting properties of the final product. Therefore, applying probiotics and the mixture of rice and pea proteins in the fermented beverage can enhance nutritional and nutraceutical of the product.

Authorship contribution statement

MJ, ABR, ZA: formal analysis, data elaboration and writing original draft. MM: experimental design planning, review and editing. SS: experimental design planning, review. ML: experimental design planning, review, validation and funding raising.

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Data Availability The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflicts of interest MM is paid employee of Bio-K Plus International Inc.

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