Journal of Dairy Research

cambridge.org/dar

Research Article

Cite this article: Alves CSJ, Floriano SAM, Voltarelli VP, de Rensis CMVB, Pimentel TC, Costa GN and Vianna PCB (2020). Effect of carbonation and probiotic addition on the physicochemical, microbiological and sensory characteristics of whey dairy beverage. *Journal of Dairy Research* **87**, 255–258. https://doi.org/10.1017/S0022029920000291

Received: 6 September 2019
Revised: 9 March 2020
Accepted: 9 March 2020
First published online: 13 May 2020

Keywords:
Carbonated beverages; dairy drink; *L. casei*; probiotics

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Effect of carbonation and probiotic addition on the physicochemical, microbiological and sensory characteristics of whey dairy beverage

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Abstract

This research communication addresses the impact of the addition of *Lactobacillus casei* and/or carbonation (CO_2) on the chemical composition, physicochemical characteristics, probiotic survival, and sensory acceptance of passion-fruit flavored whey dairy beverages (70% milk/30% whey) during storage (30 d/4°C). The addition of *Lactobacillus casei* and/or carbonation did not impact on the chemical composition, pH values, and acceptance (flavor and overall impression) of the products, but increased the acidity, and decreased the aroma acceptance. The carbonation process did not affect the probiotic survival but decreased the acidity of the products during storage. It can be concluded that it is possible to develop a probiotic passion-fruit flavored carbonated whey dairy beverage with suitable chemical composition, acidity, sensory acceptance (>6 in 9-point hedonic scale) and probiotic viability (>7 log cfu/ml) that could be refrigerated stored for 30 d. This is the first report considering a probiotic non-fermented carbonated whey dairy beverage.

Whey is a by-product of the dairy industry that is well known for its technological and nutritional properties (Ozorio *et al*., 2019). A total of 1.6 billion liters of whey are generated each year during the manufacture of cheeses and fermented dairy products with high protein content, such as Greek-style yogurt (Schäfer *et al*., 2019). Part of the produced whey is still used for low-value applications, such as pig feeding, land application or anaerobic digestion (Xu *et al*., 2019). Production of whey-based beverages appears to be the most economical and simplest solution for whey utilization in human nutrition (Barukčić *et al*., 2019).

Whey dairy beverages (WDB) are fermented or non-fermented products in which whey is used as an ingredient (Janiaski *et al*., 2016). This type of product has a promising food market, mainly because of the sustainability appeal and the putative health benefits associated with the bioactive compounds present in the whey (Coutinho *et al*., 2019). Furthermore, the demand for beverages containing functional components, such as probiotics, has increased and driven the market (Silva e Alves *et al*., 2018). Probiotics are living microorganisms that confer benefits to the health of the host when administered in adequate amounts, in general at least 10^6–10^7 cfu/ml in the product (Hill *et al*., 2014).

The development of carbonated beverages is increasing, mainly because of the thirst quenching and refreshing properties of the products, and due to the fact that carbonation is considered an inexpensive and safe process (Silva e Alves *et al*., 2018). However, the carbonation process can result in probiotic death (Ansari *et al*., 2013). Previous studies are mainly related to the processing of probiotic carbonated fermented whey dairy beverages (Shah and Prajapati, 2014; Sağlam *et al*., 2019) because the fermentation step helps in increasing the probiotic viability. As far as the authors know, there are no reports on probiotic carbonated non-fermented whey dairy beverages based on a mixture of whey and milk. Therefore, this study aimed to evaluate the impact of the addition of probiotic culture *Lactobacillus casei* and/or carbonation on the chemical composition, physicochemical characteristics, probiotic survival and sensory acceptance of passion-fruit flavored whey dairy beverages during refrigerated storage (30 d/4°C).

Table 1. Chemical composition of whey dairy beverages ($n = 9$)

	CONTROL	PRO	CARB	PRO-CARB
Total solids (g/100 ml)	14.2 ± 1.3^{a}	15.1 ± 0.2^{a}	13.3 ± 2.4^{a}	13.3 ± 2.6^{a}
Ash (g/100 ml)	0.8 ± 0.1^{a}	0.7 ± 0.1^{a}	0.7 ± 0.1^{a}	0.7 ± 0.1^{a}
Protein (g/100 ml)	2.4 ± 0.8^{a}	3.2 ± 1.4^{a}	3.1 ± 1.3^{a}	2.9 ± 1.6^{a}
Fat (g/100 ml)	2.0 ± 0.1^{a}	1.9 ± 0.1^{a}	2.0 ± 0.1^{a}	2.0 ± 0.1^{a}
Carbohydrates (g/100 ml)	9.0 ± 0.9^{a}	9.3 ± 0.8^{a}	7.4 ± 0.8^{a}	7.7 ± 0.7^{a}

Means ± standard deviations in the same row with the same letters indicate no differences among formulations of whey dairy beverages ($P > 0.05$). (CONTROL: CO_2-free and without *L. casei*; PRO: CO_2-free and with *L. casei*; CARB: with CO_2 and without *L. casei*; PRO-CARB: with both CO_2 and *L. casei*).

Table 2. Physicochemical and microbiological characteristics of the whey dairy beverages during refrigerated storage ($n = 9$)

	Storage time (days)	CONTROL	PRO	CARB	PRO-CARB
pH	1	5.7 ± 0.2^{aA}	5.5 ± 0.1^{aA}	5.4 ± 0.1^{aA}	5.4 ± 0.1^{aA}
	7	6.0 ± 0.3^{aA}	5.9 ± 0.3^{aA}	5.9 ± 0.4^{aA}	5.8 ± 0.3^{aA}
	15	5.5 ± 0.1^{aA}	5.4 ± 0.1^{aA}	5.5 ± 0.1^{aA}	5.3 ± 0.3^{aA}
	30	5.6 ± 0.4^{aA}	5.5 ± 0.9^{aA}	5.4 ± 0.9^{aA}	5.3 ± 0.9^{aA}
Acidity (% lactic acid)	1	0.40 ± 0.02^{cA}	0.47 ± 0.06^{bA}	0.54 ± 0.03^{aA}	0.55 ± 0.03^{aA}
	7	0.40 ± 0.08^{cA}	0.42 ± 0.02^{bcA}	0.48 ± 0.03^{bB}	0.57 ± 0.03^{aA}
	15	0.41 ± 0.02^{cA}	0.46 ± 0.05^{bcA}	0.48 ± 0.03^{abB}	0.52 ± 0.03^{aAB}
	30	0.46 ± 0.09^{aA}	0.48 ± 0.06^{aA}	0.48 ± 0.03^{aB}	0.49 ± 0.03^{aB}
L. casei (log cfu/ml)	1	–	7.2 ± 0.6^{aB}	–	7.7 ± 0.4^{aB}
	7	–	7.5 ± 0.7^{aB}	–	7.7 ± 0.3^{aB}
	15	–	7.7 ± 0.5^{aB}	–	7.6 ± 0.5^{aB}
	30	–	8.0 ± 0.8^{aA}	–	8.2 ± 0.8^{aA}

Means ± standard deviations in the same row with the same small letters indicate no differences among formulations of whey dairy beverages ($P > 0.05$). Means ± standard deviations in the same row with the same capital letters indicates no differences during refrigerated storage ($P > 0.05$). (CONTROL: CO_2-free and without *L. casei*; PRO: CO_2-free and with *L. casei*; CARB: with CO_2 and without *L. casei*; PRO-CARB: with both CO_2 and *L. casei*).

Materials and methods

Whole UHT milk (3% fat, Parmalat, São Paulo, SP) and whey powder (reconstituted to 14% total solids, Confepar, Londrina, PR) were mixed in the ratio of 70 : 30, heated at 90°C/5 min in a water bath and cooled to 10°C. Then, 5% sugar (União, Rio de Janeiro, RJ) and 5% passion fruit pulp (Redondo, Cambé, PR) were added. The passion fruit pulp was previously heated at 90°C/1 min and cooled to room temperature (25°C). The WDB was cooled to 4°C and divided into four portions, corresponding to the following treatments: CO_2-free and without *L. casei* (CONTROL); CO_2-free with *L. casei* (PRO); CO_2 addition and without *L. casei* (CARB); and CO_2 and *L. casei* addition (PRO-CARB).

For the carbonated beverages, CO_2 (food grade) was bubbled directly into the samples (4°C) with the aid of a sanitized hose. The CO_2 concentration added was the same for all formulations and controlled by the decrease in 0.3 units in the pH value, defined in a preliminary test based on the sensory aspect. The lyophilized probiotic culture *Lactobacillus casei* (Chr Hansen Valinhos, Brasil) was activated at 37°C for 3 h in sterilized skim milk and added to the products at a concentration of 2 ml/100 ml (7.8 log cfu/ml), after the carbonation step. Then, the beverages were packaged in sterilized polyethylene terephthalate (PET) bottles (240 ml) and stored at 4°C.

The pH, titratable acidity, total solids, protein, ash, fat, and carbohydrates were determined according to AOAC (2005). *L. casei* was determined in Man, Rogosa and Sharpe (MRS) Agar (Becton, Dickinson, and Company Sparks, USA) incubated at 37°C for 72 h under anaerobic condition (Shah and Prajapati, 2014). The chemical composition was evaluated on the first day of storage, while the pH, titratable acidity and probiotic survival were performed on the 1, 7, 15 and 30 d of storage, which is the general shelf life of whey dairy beverages.

The sensory evaluation was performed by 51 consumers. The evaluation was conducted in individual booths under white light and the samples were served at 4°C in 50 ml plastic cups coded with random three-digit numbers, presented in a monadic form. The beverages were evaluated for overall acceptance, flavor, and aroma, using a 9-point hedonic scale ranging from '1 – disliked very much' to '9 – liked very much'.

All physicochemical and microbiological analyses were performed in triplicate and the experiment was repeated three times. The experimental design was a split-plot, in which the main factor was the formulation and the second factor was the

https://doi.org/10.1017/S0022029920000291 Published online by Cambridge University Press

Table 3. Sensory acceptance of the whey dairy beverages ($n = 51$)

Attributes	Acceptance scores			
	CONTROL	PRO	CARB	PRO-CARB
Aroma	7.8 ± 1.1^a	7.3 ± 1.3^b	7.2 ± 1.4^b	7.1 ± 1.4^c
Flavor	7.2 ± 1.4^a	7.0 ± 1.6^a	6.9 ± 1.7^a	7.0 ± 1.6^a
Overall impression	7.1 ± 1.6^a	6.9 ± 1.6^a	6.9 ± 1.6^a	7.0 ± 1.5^a

Means ± standard deviations in the same row with the same letters indicate no differences among formulations of whey dairy beverages ($P > 0.05$). (CONTROL: CO_2-free and without *L. casei*; PRO: CO_2-free and with *L. casei*; CARB: with CO_2 and without *L. casei*; PRO-CARB: with both CO_2 and *L. casei*).

storage time. The results were submitted to Analysis of Variance (ANOVA) and Tukey test ($P = 0.05$) using the Statistica 8.0 software (Stat Soft, Inc).

Results and discussion

The chemical composition of the products (g/100 mL) was in the following range: 13.3–14.2 for total solids, 0.7–0.8 for ash, 2.4–3.2 for protein, 1.9–2.0 for fat and 7.4–9.3 for carbohydrates (Table 1). The addition of *L. casei* and/or carbonation did not impact the chemical composition (total solids, protein, ash, fat and carbohydrate contents, $P > 0.05$). Our data are similar to those reported in a previous study for whey dairy beverages (Janiaski *et al.*, 2016). Therefore, the addition of *L. casei* and/or carbonation had no negative influence on the nutritional value of the products.

Table 2 presents the pH and acidity of the products during refrigerated storage. The whey dairy beverages presented pH values of 5.3–6.0 and acidity of 0.40–0.57% lactic acid, corroborating a previous study (Janiaski *et al.*, 2016). The addition of *L. casei* and/or carbonation did not impact on the pH values of the whey dairy beverages ($P > 0.05$) but increased the acidity ($P \leq 0.05$). The maintenance of the pH values is related to the buffering capacity of the whey proteins (McCarthy *et al.*, 2017). The initially increased acidity of the probiotic whey beverage can be related to the addition and/or adaptation of the probiotic culture to the medium, with the production of organic acids, mainly lactic acid (Sperry *et al.*, 2018). The CO_2 addition increases the acidity due to the presence of carbonic acid in the medium (Ansari *et al.*, 2013). The higher acidity of the probiotic and carbonated products can increase the shelf life of the products but can have an impact on the probiotic survival or consumer acceptance of the products (Sperry *et al.*, 2018). During storage, the control and probiotic formulations behaved similarly, with the maintenance of the pH and acidity values ($P > 0.05$). However, the carbonated products had a decrease in the acidity values during storage ($P < 0.05$). Probably, the carbonic acid could have been hydrolyzed during the storage time, reducing the acidity of the products. Furthermore, there may have been CO_2 losses during the storage period, although the products did remain carbonated until the end of the refrigerated storage (30 d).

The carbonation did not impact on the probiotic survival in the whey dairy beverages ($P > 0.05$, Table 2). Both evaluated products presented an increase in the probiotic viability at the end of the storage period ($P \leq 0.05$), which is related to a possible proteolytic activity of the probiotic cultures upon the whey proteins and release of important compounds for the probiotic survival (Sperry *et al.*, 2018). *L. casei* remained viable and stable in both products during the 30 d of refrigerated storage ($P > 0.05$), presenting satisfactory populations (7 log cfu/ml). Therefore, the total number of probiotic microorganisms in the whey dairy beverages exceeded 10^6 cfu/mL throughout the storage time, which is the minimum probiotic population required in food at the time of consumption (Hill *et al.*, 2014; Silva e Alves *et al.*, 2018). The results suggest that the carbonated whey dairy beverage is a good matrix for the addition of *L. casei* as a probiotic culture.

Table 3 presents the sensory scores of whey dairy beverages. All formulations presented scores higher than 6 in a 9-point hedonic scale, indicating that the consumers liked at least slightly the products. The addition of *L. casei* and/or carbonation did not impact on the acceptance of the whey dairy beverages in flavor or overall impression ($P > 0.05$) but decreased the aroma acceptance ($P \leq 0.05$). The results indicate that the higher acidity of the probiotic and carbonated products (Table 2) did not impact on the consumer acceptance in the flavor. The presence of the probiotic culture and/or of CO_2 may have altered the volatile profile of the products, resulting in the appearance of compounds with negative influence on the aroma, and/or disappearance of compounds that contribute positively to it. Future studies should evaluate the volatile compounds of the products to confirm the results of the present study.

In conclusion, this is the first report of a carbonated non-fermented whey dairy beverage and the results demonstrate that it is possible to develop a probiotic passion-fruit flavored carbonated whey dairy beverage with suitable chemical composition, sensory acceptance (>6 in 9-point hedonic scale) and probiotic viability (>7 log cfu/ml) that could be refrigerated stored for 30 d.

Acknowledgments. This work was supported by Fundação Nacional de Desenvolvimento de Ensino Superior Particular/FUNADESP.

References

Ansari T, Hesari J, Ahmadi A, Giasifar S and Aiaseh A (2013) Effects of CO_2 addition to raw milk on microbial, physiochemical and sensory properties of probiotic set yoghurt. *Journal of Agricultural Science and Technology* **15**, 253–263.

AOAC (2005) *Official Methods of Analyses of the Association Analytical Chemists, 18th*. Gaithersburg, MD: Association of Official Analytical Chemistry.

Barukčić I, Jakopović KL and Božanić R (2019) Valorisation of whey and buttermilk for production of functional beverages–an overview of current possibilities. *Food Technology and Biotechnology* **57**, 4.

Coutinho NM, Silveira MR, Fernandes LM, Moraes J, Pimentel TC, Freitas MQ, Silva MC, Raices RSL, Ranadheera CS, Borges FO, Neto RPC, Tavares MIB, Fernandes FAN, Fonteles TV, Nazzaro F, Rodrigues S and Cruz AG (2019) Processing chocolate milk drink by low-pressure cold plasma technology. *Food Chemistry* **278**, 276–283.

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC and Sanders ME (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**, 506–514.

Janiaski DR, Pimentel TC, Cruz AG and Prudencio SH (2016) Strawberry-flavored yogurts and whey beverages: what is the sensory profile of the ideal product?. *Journal of Dairy Science* **99**, 5273–5283.

McCarthy CM, Wilkinson MG and Guinee TP (2017) Effect of coagulant type and level on the properties of half-salt, half-fat Cheddar cheese made with or without adjunct starter: improving texture and functionality. *International Dairy Journal* **75**, 30–40.

Ozorio L, Silva LP, Prates MV, Bloch Jr C, Takeiti CY, Gomes DM, da Silva-Santos JE, Deliza R, Brígida AIS, Furtado A, Mellinger-Silva C and Cabral LMC (2019) Whey hydrolysate-based ingredient with dual functionality: from production to consumer's evaluation. *Food Research International* **122**, 123–128.

Sağlam H, Sarioglu T, Karahan AG and Oner Z (2019) An investigation for the development of whey-based probiotic beverages. *Romanian Biotechnological Letters* **24**, 1097–1106.

Schäfer J, Sebald K, Dunkel A, Hofmann T, Rosenthal I, Schuster R, Atamer Z and Hinrichs J (2019) A feasibility study on the pilot scale manufacture of fresh cheese from skim milk retentates without acid whey production: effect of calcium content on bitterness and texture. *International Dairy Journal* **93**, 72–80.

Shah N and Prajapati JB (2014) Effect of carbon dioxide on sensory attributes, physico-chemical parameters and viability of probiotic *L. helveticus* MTCC 5463 in fermented milk. *Journal of Food Science and Technology* **51**, 3886–3893.

Silva e Alves A, Spadoti L, Zacarchenco P and Trento F (2018) Probiotic functional carbonated whey beverages: development and quality evaluation. *Beverages* **4**, 49.

Sperry MF, Silva HL, Balthazar CF, Esmerino EA, Verruck S, Prudencio ES, Neto RPC, Tavares MIB, Peixoto JC, Nazzaro F, Rocha RS, Moraes J, Gomes ASG, Raices RSL, Silva MC, Granato D, Pimentel TC, Freitas MQ and Cruz AG (2018) Probiotic Minas Frescal cheese added with *L. casei* 01: physicochemical and bioactivity characterization and effects on hematological/biochemical parameters of hypertensive overweighted women–A randomized double-blind pilot trial. *Journal of Functional Foods* **45**, 435–443.

Xu J, Hao J, Guzman JJ, Spirito CM, Harroff LA and Angenent LT (2019) Temperature-phased conversion of acid whey waste into medium-chain carboxylic acids *Via* Lactic acid: no external e-donor. *Joule* **3**, 885–888.