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Synbiotic yoghurt with *Lactiplantibacillus plantarum* and plant powder substrates

*Kristina Veselá, Milada Plocková, Eva Musilová, Blanka Vrchotová, Šárka Horáčková**

Department of Dairy, Fat and Cosmetics, University of Chemistry and Technology, Prague, Czech Republic

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

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Abstract: Enrichment of yoghurt with probiotic bacterial strains and prebiotic plant substrates has gained increasing interest among both consumers and food manufacturers. In this study, limited growth and fermentation activity of the commercial probiotic strain *Lactiplantibacillus plantarum* 299v were observed when cultured in ultra-high temperature (UHT) milk. Electron microscopy revealed the strain's ability to adhere to the surface of prebiotically active plant particles, specifically quinoa, lucuma, and baobab powders. Supplementation of these plant substrates at a concentration of 1% (w/v) slightly enhanced the growth of *L. plantarum* 299v in UHT milk and improved its viability over a 28-day storage period at 5 ± 1 °C. Co-cultivation of *L. plantarum* 299v with the yoghurt starter culture YC-381 (in a 1 : 0.5 ratio), along with the addition of 1% (w/v) of lucuma, quinoa, or baobab powders to UHT milk, was successfully achieved. These substrates positively influenced the stability of *L. plantarum* 299v during the 28-day storage and in the case of quinoa of *Lactobacillus delbrueckii* subsp. *bulgaricus* during 21-day storage at 5 ± 1 °C. Moreover, the addition of 1% (w/v) plant powders slightly stimulated the production of lactic and acetic acids in yoghurt containing *L. plantarum* 299v.

Keywords: baobab; quinoa; lucuma; prebiotic; probiotic; stability; viability

Lactiplantibacillus plantarum probiotic strains are currently of significant interest to both the scientific community and consumers due to their beneficial effects on human health and diverse functional properties (Echegaray et al. 2023). *L. plantarum* is a facultatively heterofermentative lactic acid bacterium that forms non-motile rods occurring singly, in pairs, or in short chains. Its genomic DNA has a G+C content ranging from 44% to 46%. This species efficiently ferments glucose, raffinose, xylose, cellobiose, mannitol, sucrose, and sorbitol. It is classified as a mesophilic organism, capable of growth at 15 °C, but not at 45 °C (Hammes and Hertel 2009). *L. plantarum* is a highly adaptable species found in a wide range of environments, including fermented plant materials, meat, fish or dairy products (Seddik et al. 2017), and also the human gastrointestinal tract (Zheng et al. 2020). It holds 'Qualified Presumption of Safety' status from the European Food Safety Authority and 'Generally Recognized as Safe' status from the United States Food and Drug Administration (Echegaray et al. 2023).

This species is well characterised in terms of its occurrence, applications in the food industry, as well as its production of functional metabolites and probiotic properties (Behera et al. 2018; Nath et al. 2020; Hang et al. 2022; Liu et al. 2022). The most well-known probiotic strain, *L. plantarum* 299v, has been extensively studied over the past three decades for its health-promoting effects and application in both dairy and non-dairy fermented products (Arvidsson Nordström et al. 2021). This strain was originally iso-

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lated from the intestinal mucosa of a healthy adult and was successfully re-isolated after consumption (Molin 2001).

The use of dairy products as a matrix for *L. plantarum* is limited due to its relatively poor ability to metabolise lactose and its weak proteolytic activity, which is essential for releasing free amino acids and peptides from milk proteins to support bacterial growth (Hang et al. 2020). As a result, co-cultivation with other lactic acid bacteria (LAB) that thrive in milk is often necessary. For instance, the probiotic strain *L. plantarum* NUC08 has been successfully used in synergy with traditional yoghurt starter cultures (Cai et al. 2023).

Previous studies have explored various additives aimed at enhancing *L. plantarum* viability in milk, with varying degrees of success. Examples include the addition of pineapple powder to influence fermentation dynamics and improve rheological and textural properties (Sah et al. 2016), the use of sea buckthorn as a prebiotic substrate (Gunenc et al. 2016), or the application of fresh and freeze-dried apples, raisins, and wheat grains to extend bacterial viability during yoghurt storage (Bosnea et al. 2017). Other studies have investigated pineapple and mung bean juice (Zhang et al. 2024), as well as a variety of plant-based meals such as walnut, sweet corn, peanut, soybean meal, malt extract, and oat extract (Hang et al. 2020).

Some plant substrates have also demonstrated protective effects on probiotics during storage or upon exposure to harsh gastrointestinal conditions (Echegaray et al. 2023). This protection is often attributed to the immobilisation of bacterial cells on the substrate surface (Terpou et al. 2017). A recent study by Paredes-Toledo (2021) reported improved viability and gastrointestinal resistance of *L. plantarum* 299v when immobilised on edible seeds such as almonds, pumpkin seeds, and roasted chickpeas.

The combination of fermented dairy products with probiotics and plant-based substrates rich in fibre, vitamins, essential amino acids, and fatty acids is increasingly popular among health-conscious consumers. This trend is driven by the growing belief that food can play a preventative role in maintaining health and preventing disease (Hang et al. 2020).

The aim of this study was to enhance the growth of *L. plantarum* 299v using selected non-traditional plant substrates with presumed prebiotic effects, and to propose a symbiotic yoghurt formulation incorporating this well-known probiotic strain alongside beneficial plant ingredients.

MATERIAL AND METHODS

Microorganisms. *L. plantarum* 299v – commercial probiotic strain (Probi AB, Lund, Sweden) was routinely cultivated in MRS broth (pH 5.6, Merck, Darmstadt, Germany), using 2% v/v inoculum, for 18 h at 37 °C in 5% v/v CO_2 atmosphere. It was also inoculated into milk from MRS broth (2% v/v) and cultured aerobically at 37 °C for 18 h. Yoghurt culture YC-381 – commercial culture (Ch. Hansen, Horsholm, Denmark) was cultivated at UHT skim milk, aerobically at 30 °C for 16 h.

Plant substrates. Lucuma bio powder 100% from *Pouteria lucuma* (Biovita bvba–Purasana, Gullegem, Belgium).

Quinoa flour from *Chenopodium quinoa* (Adveni Medical, Mělčany, Czech Republic).

Baobab bio powder 100% from *Adsonia digitatis* (Iswari Superfoods Ltd., Setúbal, Portugal).

HPLC analysis of organic acids. Determination of lactic and acetic acids was performed using Polymer IEX H (205 mm × 8 mm) column with pre-column (50 mm × 8 mm, Watrex, Prague, Czech Rep.) connected to DAD detector (210 nm, Agilent Technologies, Waldbronn, Germany). Nine mmol·L^{-1} H_2SO_4 was used as mobile phase, flow 0.6 mL·min^{-1}. The column temperature was 60 °C.

Adhesion of *L. plantarum* 229v cells to plant substrates. To evaluate the adhesion of cells to plant substrates, scanning electron microscopy (VEGA3 LMU, TESCAN, Brno, Czech Rep.) equipped with an EDS analyser (INCA 350, Oxford Instruments, Abingdon, UK) was used. The strain was cultivated in MRS broth with 1 % (w/v) plant substrate at 37 °C for 18 h at 5 % (v/v) CO_2 atmosphere. After incubation, cells were centrifuged (8 784 × g, 5 min, 4 °C), applied onto glass slides, and fixed with 3% (v/v) glutaraldehyde at 4 °C for 2 h. Prior to microscopy, the samples were coated with a 3 µm gold layer.

Model fermented milk and yoghurt preparation. Model fermented milk products were prepared using UHT milk supplemented with 1% (w/w) plant substrates. After heat treatment at 90 °C for 10 minutes, the milk was inoculated with either 1% (v/v) *L. plantarum* 299v alone, or with a mixture of 1% (v/v) *L. plantarum* 299v and 0.5% (v/v) yoghurt culture YC-381. Fermentation was carried out at 30 °C for 16 h, followed by storage at 5 ± 1 °C for up to 28 days.

Microbial counts. Microbial counts of *L. plantarum*, *Streptococcus thermophilus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus*, as well as pH values, were assessed immediately after fermentation and

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at the end of the storage period. The value of pH was measured using a pH meter Jenway 3020 (Jenway Ltd., UK) provided with a combined electrode. The fermentation process was performed in duplicate with two parallel replicates (n = 4). Microbial enumeration was carried out using standard plate count methods based on decimal dilutions. For the cultivation of *L. plantarum* 299v alone in UHT milk, MRS agar (Merck, Germany) was used. Plates were incubated at 37 °C for 48 h in a 5% (v/v) CO_2 atmosphere. For the co-cultivation of *L. plantarum* 299v with the yoghurt culture, microbial differentiation and enumeration were performed according to the method described by Veselá et al. (2019). This approach allows the selective and accurate quantification of *L. plantarum*, *S. thermophilus*, and *L. delbrueckii* subsp. *bulgaricus* in mixed cultures.

RESULTS AND DISCUSSION

Initially, the growth and organic acid production of *L. plantarum* 299v were evaluated in UHT milk (initial pH 6.4). The results are presented in Figure 1 and Table 1. The strain exhibited only moderate growth in milk, with an increase of less than 1 log CFU·mL^{-1} observed after 16 h of incubation at 37 °C. Lactic acid production reached 2.5 g·L^{-1}, while acetic acid levels remained negligible.

The limited growth of *L. plantarum* strains in milk has been reported previously. For instance, Veselá et al. (2021) assessed the milk fermentation performance of 11 *L. plantarum* strains of various origins and found that, after 24 h of aerobic cultivation at 37 °C, the cell counts increased by approximately 1 log CFU·mL^{-1}. Lactic acid production ranged from 1.6 g·L^{-1} to 4.8 g·L^{-1}, while acetic acid remained at negligible levels.

This low growth performance in milk has been attributed to the high nutritional demands of *L. plantarum*, particularly its requirement for free amino acids, which are not sufficiently released by its limited proteolytic system. However, the species does possess a robust set of intracellular peptidases, which supports its use as an adjunct culture for accelerating cheese ripening (Duan et al. 2019).

More recently, a combined supplementation strategy, for example involving mung bean and pineapple has shown promising results in promoting the growth of the probiotic strain *L. plantarum* STIII in milk (Zhang et al. 2024). It is widely accepted that plant-based substrates can enhance the survival of probiotic bacteria during product manufacturing and storage. This protective effect is primarily attributed to the immobilisation of bacterial cells on the plant matrix (Terpou et al. 2017). The subsequent phase of this study focused on evaluating the potential adhesion of *L. plantarum* 299v to plant-derived substrates. The plant substrates lucuma, baobab, and quinoa powders were selected from an initial screening of twelve plant-derived materials based on several criteria: the ability of *L. plantarum* 299v to adhere to their surfaces, their capacity to stimulate the strain's growth and fermentation activity in milk, and their cholesterol-binding potential in the medium (data not shown, Veselá 2021).

Electron microscopy was used to assess the adhesion of bacterial cells to particles of lucuma, baobab, or quinoa powders. In addition, the growth of *L. plantarum* 299v in UHT milk supplemented with 1% (w/v) of each plant

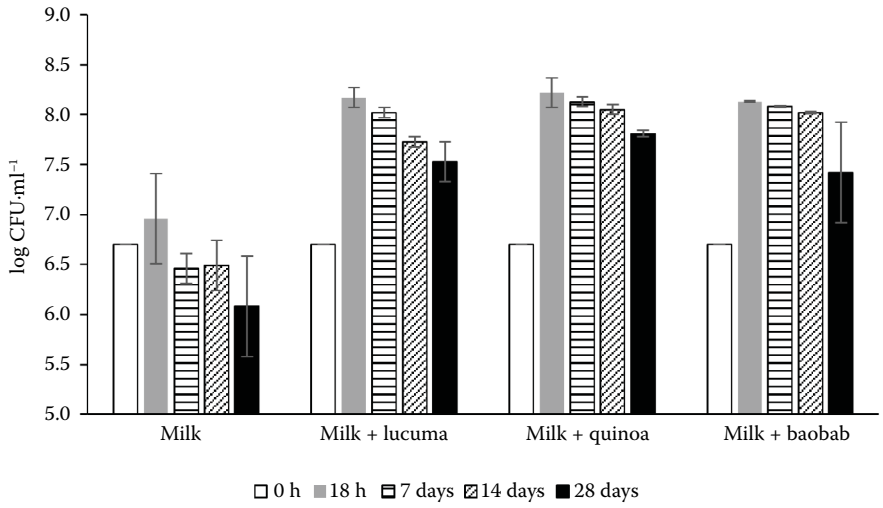

Figure 1. Cell count of *L. plantarum* 299v after cultivation in ultra high temperature (UHT) milk and in UHT milk with 1% (w/w) addition of lucuma, quinoa and baobab powder at 37 °C, 18 h and during storage at 5 ± 1 °C

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Table 1. Organic acid production ($g \cdot l^{-1}$) and pH after cultivation of *L. plantarum* 299v in UHT milk and in UHT milk with 1% (w/w) addition of lucuma, quinoa and baobab powders at 37 °C, 18 h and during storage at 5 ± 1 °C

		Fermentation		Storage		
		0 h	18 h	7 days	14 days	28 days
Milk	pH	6.5 ± 0.0	5.5 ± 0.1	5.5 ± 0.0	5.5 ± 0.0	5.5 ± 0.0
	lactic acid	nd	2.5 ± 0.0	2.5 ± 0.1	2.5 ± 0.0	2.5 ± 0.1
	acetic acid	nd	nd	nd	nd	nd
Milk + lucuma	pH	6.4 ± 0.1	5.1 ± 0.1	5.2 ± 0.0	4.9 ± 0.0	4.9 ± 0.1
	lactic acid	nd	3.5 ± 0.4	3.3 ± 0.1	3.3 ± 0.4	4.7 ± 0.3
	acetic acid	nd	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.9 ± 0.1
Milk + quinoa	pH	6.4 ± 0.0	5.2 ± 0.0	4.9 ± 0.1	4.8 ± 0.1	4.6 ± 0.1
	lactic acid	nd	3.8 ± 0.2	4.5 ± 0.4	4.6 ± 0.2	5.2 ± 0.1
	acetic acid	nd	0.7 ± 0.1	1.8 ± 0.1	1.5 ± 0.1	1.7 ± 0.1
Milk + baobab	pH	6.4 ± 0.1	5.0 ± 0.0	5.1 ± 0.1	5.2 ± 0.1	4.7 ± 0.0
	lactic acid	nd	3.5 ± 0.2	3.0 ± 0.4	3.0 ± 0.2	4.5 ± 0.1
	acetic acid	nd	1.6 ± 0.1	2.2 ± 0.2	1.8 ± 0.4	2.1 ± 0.1

nd – not detected

substrate, along with the stability of viable cells during 28 days of storage at 5 ± 1 °C, was investigated. Electron microscopy results (Figure 2) revealed that *L. plantarum* 299v exhibited notable adhesion to the surfaces of lucuma and baobab powder particles, which were significantly larger than those of quinoa powder. This suggests that particle size and surface structure may play important roles in facilitating microbial attachment.

The effect of plant substrates addition into milk on the growth and storage stability of *L. plantarum* 299v in UHT milk is presented also in Figure 1 and Table 1. As was concluded above, bacterial growth in non-supplemented UHT milk was minimal. However, the addition of 1% (w/v) plant substrates significantly enhanced cell proliferation, resulting in an approximate 1.5 log $CFU \cdot mL^{-1}$ increase after 16 h of fermentation at 37 °C. Among the substrates tested, lucuma and quinoa powders were particularly effective in supporting post-fermentation cell viability, as there was no significant decline in viable counts during refrigerated storage at 5 ± 1 °C for 28 days. These findings indicate that certain plant-based additives, in this case especially lucuma and quinoa powders, can serve dual roles as prebiotic substrates and protective matrices. They enhance not only the initial growth but also the long-term survival and metabolic stability of probiotic *L. plantarum* 299v in dairy systems.

As illustrated in Table 1, the fermentation activity in milk supplemented with plant substrates, particu-

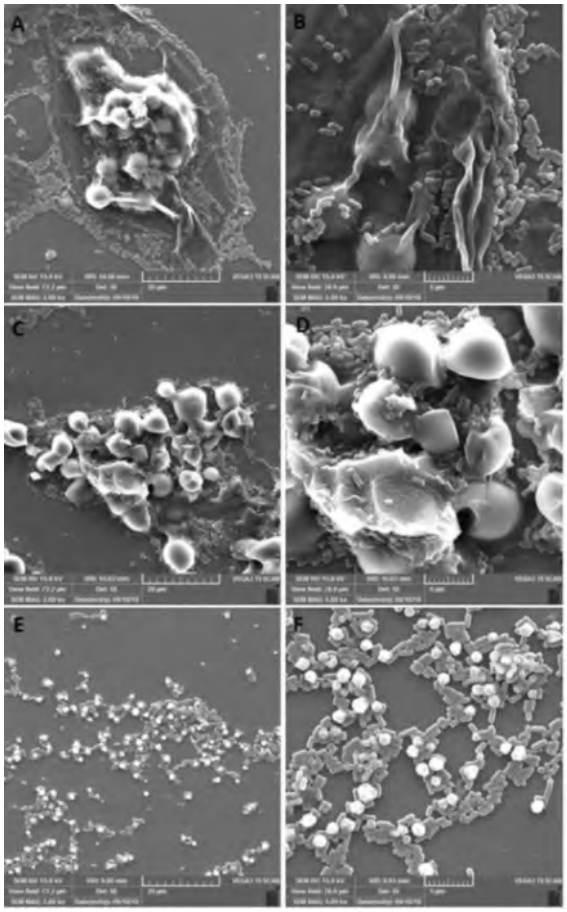

Figure 2. Microscopic imaging of *L. plantarum* 299v adhesion on plant powder substrates: A, B – lucuma powder; C, D – baobab powder; E, F – quinoa powder

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larly quinoa, was stimulated. The results demonstrate that, following fermentation, the pH values of milk samples supplemented with plant substrates were 0.3–0.5 units lower than those of the control (fermented milk by *L. plantarum* 299v without plant additions). All samples enriched with plant substrates exhibited enhanced lactic acid production. Notably, the sample containing baobab powder showed increased acetic acid production compared to the other substrates. Acetic acid was not detected during fermentation in milk without the addition of plant substrates. All samples containing plant powders exhibited greater post-acidification during storage compared to the control.

The compositional richness of these three substrates suggests they may serve not only as prebiotic carriers for probiotic bacteria but also as functional food ingredients capable of enhancing the nutritional and sensory profiles of fermented dairy products. Their roles in promoting cell adhesion, improving fermentation dynamics, and contributing to product stability highlight their potential for use in the development of novel synbiotic formulations (Kwon et al. 2019; Foltz et al. 2021; Glez et al. 2025).

To develop a functional yoghurt incorporating above mentioned prebiotic plant substrates and combination of *L. plantarum* 299v with the yoghurt culture YC-381 (in a 1 : 0.5 ratio) was tested.

The effect of adding 1% (w/w) plant substrate to UHT milk was evaluated based on LAB growth during 16 h of fermentation and on the viability of *L. plantarum* 299v, *S. thermophilus*, and *L. delbrueckii* subsp. *bulgaricus* during 28 days of storage at 5 ± 1 °C (Figure 3). The addition of plant substrates had no significant impact on yoghurt culture growth during fermentation; however, it clearly contributed to the stability of *L. plantarum* 299v and *L. delbrueckii* subsp. *bulgaricus* during cold storage for 28 resp. 14 or 21 days. Even after 28 days of storage, *L. plantarum* 299v counts remained 1 to 1.5 log CFU·mL^{-1} higher in the samples with added plant powders compared to the control yoghurt. The plant substrates had no adverse effect on the number of *L. delbrueckii* subsp. *bulgaricus*, although its counts decreased by approximately two log units in all samples during storage. The growth and stability of *S. thermophilus* were not affected. In this case, the symbiotic effect between the yoghurt culture and *L. plantarum* 299v, as previously reported in the study by Cai et al. (2023), could not be confirmed. However, the possibility of combining *L. plantarum* with yoghurt culture has been verified by other authors (Dan et al. 2019).

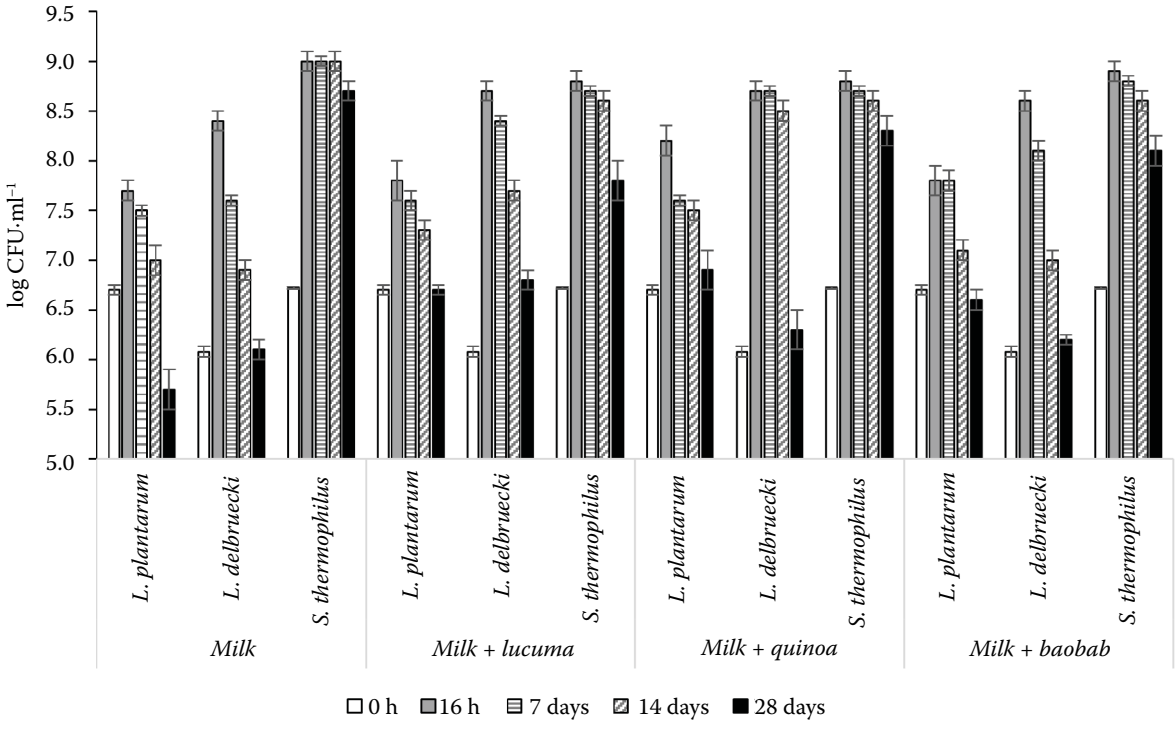

Figure 3. Cell count of *L. plantarum* 299v and yoghurt culture after co-cultivation in UHT milk and in UHT milk with 1% (w/w) addition of lucuma, quinoa or baobab powders at 30 °C, 16 h and during storage at 5 ± 1 °C

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Table 2. Organic acid production ($g \cdot l^{-1}$) and pH after *L. plantarum* 299v and yoghurt culture co-cultivation in UHT milk and in UHT milk with 1% (w/w) addition of lucuma, quinoa or baobab powders at 30 °C, 16 h and during storage at 5 ± 1 °C

		Fermentation		Storage		
		0 h	16 h	7 days	14 days	28 days
Milk	pH	6.5 ± 0.0	4.3 ± 0.0	4.4 ± 0.0	4.2 ± 0.0	4.2 ± 0.0
	lactic acid	nd	9.3 ± 0.2	9.0 ± 0.2	9.4 ± 0.1	9.3 ± 0.4
	acetic acid	nd	0.9 ± 0.0	0.9 ± 0.1	0.9 ± 0.0	0.9 ± 0.0
Milk + lucuma	pH	6.4 ± 0.1	4.2 ± 0.0	4.3 ± 0.0	4.2 ± 0.0	4.1 ± 0.0
	lactic acid	nd	11.3 ± 0.2	10.9 ± 0.1	10.9 ± 0.0	11.3 ± 0.2
	acetic acid	nd	1.0 ± 0.1	0.9 ± 0.0	0.9 ± 0.1	0.9 ± 0.0
Milk + quinoa	pH	6.4 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.1	4.1 ± 0.1
	lactic acid	nd	11.8 ± 0.1	11.7 ± 0.1	11.5 ± 0.2	11.4 ± 0.1
	acetic acid	nd	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.0	0.9 ± 0.0
Milk + baobab	pH	6.4 ± 0.1	4.2 ± 0.1	4.2 ± 0.0	4.2 ± 0.0	4.1 ± 0.0
	lactic acid	nd	10.8 ± 0.2	10.3 ± 0.3	10.3 ± 0.1	10.8 ± 0.2
	acetic acid	nd	1.1 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1

Although the active acidity (Table 2) of all samples was similar, slightly higher lactic acid production was observed in the samples supplemented with plant powders. In this case, no post-acidification changes occurred during storage.

CONCLUSION

Novel synbiotic fermented milk products incorporating non-traditional probiotics and plant-based prebiotics represent an emerging trend, offering health-conscious consumers a valuable alternative. Based on the present findings, the combination of *L. plantarum* 299v and yoghurt culture YC-381 in UHT milk supplemented with 1% (w/w) lucuma, quinoa, or baobab powders shows promise for the development of synbiotic products with improved functional and stability characteristics.

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