Microbiological and chemical characterization of fermented quinoa beverages obtained with kefir microorganisms

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Summary
Quinoa is an Andean pseudocereal considered a natural food resource of high nutritional value whose importance is recognized in food security for present and future generations. In this study we combined the nutritional power of quinoa with the potential beneficial effects of kefir. Microbiological and physico-chemical properties of fermented quinoa beverages with kefir grains, acid mother cultures thereof, or lactobacilli isolated from kefir, with reported potential probiotic effects, were evaluated. Fermented quinoa beverages obtained with *Lactiplantibacillus plantarum* CIDCA 83114 or acid mother cultures were comparable, although the *Lb. plantarum* strain demonstrated better growth at fermentation (to 8.63 ± 0.07 log CFU·ml^{-1} against 6.13 ± 0.07 log CFU·ml^{-1}, respectively). Furthermore, the quinoa suspensions fermented with *Lb. plantarum* CIDCA 83114 showed more significant improvements on total phenolics, antioxidant activity, proteolysis and amylolytic activity than those fermented with acid mother cultures. In contrast, *Lactobacillus kefiri* CIDCA 8348 and kefir grains CIDCA AGK1 were not able to ferment the substrate. Kefir grains lost biomass integrity during repeated inoculations into quinoa suspensions and the viability of the lactic acid bacteria decreased. The present study revealed suitable characteristics of *Lb. plantarum* CIDCA 83114 as a starter to obtain nutritious and potentially health-promoting fermented quinoa beverages.

Keywords
quinoa; kefir; *Lactiplantibacillus plantarum*; fermentation; plant-based beverages

In recent years, there has been a growing global trend towards consumption of nutritious foods with proteins from plant sources, known as "plant-based foods". An opportunity for sustainable ethnic crops with good yield properties and resiliency to extreme weather conditions could encourage a great market for pseudocereals-based food products in the coming years. The paradigm of the food innovations for special consumers, including vegans, lactose intolerants, celiac and flexitarians, as well as general consumers evidences a constant requirement of safe and functional foods. These include probiotics with a simultaneous interest in non-dairy foods that nourish and provide health benefits. Non-alcoholic cereal and pseudocereal fermentations added with lactic acid bacteria (LAB) are technologies usually used to ensure the food safety, prolong shelf life and to provide interesting profiles of bioactive metabolites [1, 2]. For a long time, the use of ferments in cereal-based products was limited to the tradition of Latin American, Asian and African cultures. Nevertheless, in recent years, the growth of commercial plant-based products extends the in-

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terest to study the benefits of fermenting non-traditional grains.

Quinoa (*Chenopodium quinoa* Willd) is an Andean pseudocereal considered to have an excellent nutritional value, mainly due to the content of vegetable proteins with high biological value (13–16 %) and fibre. Quinoa is the only plant-based food that contains important amounts of all essential amino acids, mostly lysine, methionine and threonine [3]. Moreover, quinoa seeds provide a health-promoting profile of unsaturated fatty acids, vitamins, minerals and bioactive compounds [4]. Although the Food and Agriculture Organization promotes the consumption of quinoa due to its contribution to the global food security, its potential in value-added products has not yet been fully developed. Quinoa beverages are a new commercial segment of plant-based foods and the addition of potentially probiotic lactobacilli to formulate fermented yogurt-like products has begun to be studied. A report based on the formulation of fermented quinoa beverages with *Lacticaseibacillus casei* revealed the presence of bioactive peptides with the ability to inhibit angiotensin-converting enzyme, which means a possible effect against hypertension [5]. Other study revealed that the fermentation of quinoa suspensions potentiated the hypoglycemic effect in Wistar rats, suggesting possible benefits in the prevention of type II diabetes [6].

Kefir is one of the most interesting natural fermented products. Kefir grains consist of symbiotic LAB and yeasts contained in a polysaccharide and protein matrix. It is noteworthy that the consumption of fermented milk with kefir confers health benefits, referred to the modulation of gut microbiota [7] and antioxidant properties [8], among others effects. Potential probiotics characteristics were previously described with both promising *Lactiplantibacillus plantarum* CIDCA 83114 and *Lactobacillus kefiri* CIDCA 8348 isolated from kefir grains CIDCA AGK1, including antimicrobial properties [9] and stimulation of the immune response [10]. While the fermentative properties of *Lb. plantarum* and *Lb. kefiri* isolated from kefir grains had been mainly characterized in cows' milk [11], some evidence in vegetable matrices was also reported. Recently, functional characteristics of kefir-fermented emulsions based on lupin [12] and soya [13] demonstrated the interesting versatility of kefir grains and their microorganisms in vegetable matrices.

Thus, combining quinoa and kefir in fermented food formulations could provide great nutritional and functional benefits. The present study analysed the performance of kefir grains, acid mother cultures thereof and two selected lactobacilli isolated from kefir to ferment quinoa suspensions. Microbiological and chemical properties useful to design nutritious and health-promoting fermented beverages were evaluated.

MATERIALS AND METHODS

Raw materials

An autochthonous variety of white quinoa seeds, known as Morrillos, cultivated in San Juan, Argentina, was utilized. Grains were stored in the dark at room temperature (20 °C) for a maximum of 1 week.

Starter cultures

Four starters were examined to ferment quinoa suspensions: milk kefir grains CIDCA AGK1, *Lactiplantibacillus plantarum* CIDCA 83114, *Lactobacillus kefiri* CIDCA 8348, both isolated from kefir grains AGK1, and mother cultures obtained from quinoa suspensions acidified with kefir grains. *Lb. plantarum* and *Lb. kefiri* were grown in De Man, Rogosa, and Sharpe (MRS) broth (Difco, Sparks, Maryland, USA) at 37 °C for 24 h and 48 h, respectively. Bacterial sediment was washed with 1 $g \cdot l^{-1}$ tryptone water (Biokar, Beauvais, France), then collected by centrifugation at 4 185 ×*g* for 10 min and suspended in the diluent for the sample preparation.

Sample preparation

Raw quinoa grains were washed with distilled water for 10 times in order to remove saponins. Concentration of 75 $g \cdot l^{-1}$ of quinoa suspension was selected in order to obtain drinkable texture comparable to parameters of solids content previously assayed by us at the development of a commercial quinoa beverage (BIBA Quinoa, Conicet-Babasal, Buenos Aires, Argentina). The unsaponified quinoa grains were mixed with distilled water using a blender. Then, mixtures were homogenized at 170 Hz for 15 min with a rotor-stator disperser Polytron PT-3100 (Kinematica, Malters, Switzerland). To provide fermentable sugars from the starch hydrolysis, 0.3 $g \cdot l^{-1}$ of amylolytic enzymes added to quinoa suspensions were tested. Aliquots of α-amylase, EC 3.2.1.1 (BAN 800, Novozymes, Bagsvaerd, Denmark) plus glucan 1,4-α-glucosidase, EC 3.2.1.3 (AMG-BG, Novozymes) or maltogenic α-amylase, EC 3.2.1.1 (Novamyl L, Novozymes) were added before the inoculation with the respective fermentation starter. Homogenized mixtures were heated in a water bath for 30 min at 60–65 °C for the pur-

pose of enzyme activation. After that, the enzymes were inactivated by boiling of samples at 100 °C for 1 min. This was analogous to high-temperature-short-time processes to obtain drinkable textures. Then the samples were cooled down to room temperature (20 °C). Finally, quinoa suspensions were transferred to sterile bottles and inoculated with individual fermentation starters. Kefir grains were washed with distilled water, dried with tissue paper and weighed on a precision balance BPS51 (Boeco, Hamburg, Germany). To prepare the acid mother cultures, kefir grains were cultured in 100 $g \cdot l^{-1}$ of quinoa suspensions and incubated at 37 °C until pH < 4. Then, the acidified samples were filtered with a strainer and kefir grains were separated. Kefir grains or acid mother cultures were inoculated at 100 $g \cdot l^{-1}$ and 200 $ml \cdot l^{-1}$, respectively, to the quinoa suspensions. Likewise, inocula with 7.0 log $CFU \cdot ml^{-1}$ of *Lb. plantarum* CIDCA 83114 or *Lb. kefiri* CIDCA 8348 were added in quinoa suspensions. All samples were incubated at 37 °C up to endpoint of pH < 4.

Kefir grains biomass

To analyse the effect of the quinoa matrix on the kefir grains biomass, 100 $g \cdot l^{-1}$ of kefir grains were weighed (wet weight) and cultured daily at 37 °C in 75 $g \cdot l^{-1}$ of quinoa suspensions until final pH < 4 for fourteen days.

Microbial counts

Aliquots of samples were collected and 10-fold serial dilutions in 1 $g \cdot l^{-1}$ tryptone water (Biokar) were prepared. Viable cells were determined by the plate-count method. Total viable LAB were estimated on MRS agar medium (Difco), while yeast counts were determined on yeast-glucose-chloramphenicol (YGC) agar medium (Biokar). MRS and YGC plates were incubated at 37 °C and 30 °C, respectively, under aerobic conditions for 48 h. Cell counts were expressed as logarithm of colony forming units per millilitre.

Determination of pH

The pH values were measured at 25 °C using a digital pH-meter Meterlab PHM220 (Radiometer Analytical, Villeurbanne, France) with glass electrode Phoenix 577-3521 (Phoenix, Houston, Texas, USA). The values of pH during the acidification until final pH < 4 were registered.

Antibiotic sensitivity

Susceptibility to antibiotics of the isolated LAB strains utilized to ferment quinoa suspensions was determined in order to analyse any potential acquired resistance. An in vitro antimicrobial sensitivity test for Gram-positive bacteria (Brizuela-Lab, Córdoba, Argentina) consisting in agar disk-diffusion method was used [14]. Mueller-Hinton agar plates (Difco) were spot-inoculated with the strains and then, the antibiotic disks were put down on the agar surface. Plates were incubated at 37 °C for 18 h, the diameters of the inhibition zones were measured and the strains were interpreted as sensitive or resistant to the antibiotic with reference to the cut-off points of the test. The antibiotics tested were vancomycin (30 $\mu g \cdot ml^{-1}$), ciprofloxacin (5 $\mu g \cdot ml^{-1}$), amoxicillin–clavulanic acid (30 $\mu g \cdot ml^{-1}$), penicillin (10 $U \cdot ml^{-1}$), oxacillin (1 $\mu g \cdot ml^{-1}$), erythromycin (15 $\mu g \cdot ml^{-1}$), clindamycin (2 $\mu g \cdot ml^{-1}$), rifampicin (5 $\mu g \cdot ml^{-1}$), gentamicin (10 $\mu g \cdot ml^{-1}$) and trimethoprim-sulfamethoxazole (1.2/23.76 $\mu g \cdot ml^{-1}$).

Total phenolic compounds and antioxidant activity

Phenolic compounds were determined by Folin-Ciocalteu method with some modifications [15]. Phenolic compounds of fermented quinoa samples were extracted by centrifugation with a methanol solution. Aliquots of supernatants recovered from samples were added to 1 : 10 solution of Folin-Ciocalteu phenolic reagent (Merck, Darmstadt, Germany). Mixtures were allowed to stand at room temperature for 8 min. Then, mixtures were mixed with 75 $g \cdot l^{-1}$ sodium carbonate solution and were stored in the dark for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer Metrolab 1700 (Wiener Lab, Buenos Aires, Argentina). A standard curve was prepared with gallic acid and the results were expressed as milligrams of gallic acid equivalents (GAE) per litre in fermented samples.

The antioxidant activity was evaluated using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$) [16]. $ABTS^{\bullet+}$ working solution was prepared by mixing equal proportions of 0.007 $mol \cdot l^{-1}$ ABTS stock solution with 0.0025 $mol \cdot l^{-1}$ of potassium persulfate and incubating them in the dark at room temperature (20 °C) for 16 h. The ABTS solution was diluted with methanol adjusting to an absorbance of 0.70 ± 0.02 at 734 nm. Methanolic extracts of fermented quinoa samples were obtained by centrifugation. Aliquots of supernatants were mixed with radical ABTS working solution and the decrease in absorbance was measured by spectrophotometry at 734 nm, after incubation at room temperature (20 °C) in the dark for 8 min. Ascorbic acid was used as an antioxidant standard and antioxidant activity was expressed as milligrams of vitamin C equivalent per litre in fermented samples.

Proteolytic and amylolytic activity

Degree of protein hydrolysis (*DH*), defined as the proportion of cleaved peptide bonds, was determined by the *o*-phthaldialdehyde test (OPA) [17]. The OPA solution was prepared by combining 25 ml of sodium tetraborate buffer (0.1 mol·l^{-1}, pH 9.3, Anedra, Buenos Aires, Argentina), 2.5 ml of sodium dodecyl sulphate (200 g·kg^{-1}, Bio-Rad, Hercules, California, USA), 40 mg·ml^{-1} of OPA reagent (Sigma Aldrich, St. Louis, Missouri, USA) dissolved in methanol (Anedra), and 100 μl of β-mercaptoethanol (Bio-Rad) and diluted in 50 ml of distilled water. Aliquots of fermented quinoa samples were mixed with 1.5 ml of OPA solution and absorbance was measured by spectrophotometry at 340 nm, after incubation at room temperature (20 °C) for 2 min. Serine S4500 (Sigma Aldrich) was used for the standard curve. *DH* was determined using Eq. 1 and expressed in percent.

$$DH = \frac{h}{h_{tot}} \times 100 \quad (1)$$

where, h_{tot} is the total number of peptide bonds per protein equivalent, for gluten, the h_{tot} value was 8.3 g·kg^{-1} equivalents per protein [18] and h is the number of hydrolysed bonds, which was determined using Eq. 2.

$$h = \frac{S - \beta}{\alpha} \quad (2)$$

where, S is serine-NH_2, α (1.0) and β (0.40) are the constants for cereals and pseudocereals. The value of S, expressed in grams per kilogram serine equivalents per protein, was determined using Eq. 3.

$$S = \frac{(A_S - A_B)}{(A_{St} - A_B)} \times C \times \frac{V \times 1000}{X \times P} \quad (3)$$

where A_S is absorbance of sample, A_B is absorbance of blank, A_{St} is absorbance of standard, C is the concentration of serine equivalents standard (expressed as milligrams per litre), V is final made-up volume of samples (in litres), X is the weight of quinoa sample (expressed in grams) and P is the protein content of quinoa (expressed in grams per kilogram).

Starch degradation in fermented quinoa samples was examined by spot inoculation on modified MRS agar containing 10 g·kg^{-1} of soluble starch [19]. Spotted plates were allowed to incubate at 37 °C for 48 h. All the analyses were carried out in triplicate. After incubation, the plates were covered with iodine solution (40 g·l^{-1}) and the appearance of a clear zone around the colonies indicated positive enzymatic reaction.

Chemical composition

Proteins, lipids, fatty acid profile and dietary fibre were determined according to the AOAC methods [20]. Moisture was estimated based on loss by drying at 105 °C until constant weight in a heating oven. Proteins concentration was determined through nitrogen content estimation by Kjeldahl method (AOAC 2001.11) [20]. A factor of 6.25 was used to convert nitrogen to proteins concentration, which was expressed as grams per litre of total proteins in fermented samples. Petroleum ether was used as a solvent for the extraction of lipids and fat concentration was quantified using Soxhlet method (AOAC 2003.05) [20]. Fatty acid profile was analysed from preparation of fatty acid methyl esters (FAME) by capillary gas-liquid chromatography (according to AOAC 996.06) [20] using a Dani Master GC (Dani Instruments, Cologno Monzese, Italy) equipped with a flame-ionization detector and a HP-88 capillary column (length 100 m, inner diameter 0.25 mm, film thickness 0.2 μm; Agilent Technologies, Santa Clara, California, USA). Identification of FAME was performed with the standard mixture Supelco 37 component FAME Mix (Supelco, Bellefonte, Pennsylvania, USA). Total carbohydrates (*TC*) were calculated by difference according to Eq. 4.

$$TC = 100 - (P + L + A + DF + M) \quad (4)$$

where, P are proteins, L are lipids, A is ash, DF is dietary fibre and M is moisture.

Dietary fibre was determined by enzymatic-gravimetric method (AOAC 985.29) [20] using a total dietary fibre assay kit (Megazyme, Wicklow, Ireland). Ash concentration was determined by incinerating aliquots of samples at 525 °C for 5 h using a muffle furnace. The concentration of reducing sugars was determined using 3,5-dinitrosalicylic acid reagent (DNS). A reagent solution containing 10 g·l^{-1} DNS (Sigma Aldrich), 16 g·l^{-1} sodium hydroxide (Anedra), 2 g·l^{-1} phenol (Biopack, Buenos Aires, Argentina), 5 g·l^{-1} sodium bisulphite (Cicarelli, Santa Fe, Argentina) and 400 g·l^{-1} sodium potassium tartrate (Merck) in distilled water was used. Fermented quinoa samples were centrifuged at 4 185 ×g for 15 min. Aliquots of supernatant were mixed vigorously with DNS solution (at 1 : 1) and heated in a shaking bath at 100 °C for 5 min. Then, the mixture was cooled down and 10 ml of distilled water was added. Absorbance was measured by spectrophotometry at 540 nm and D-glucose (Merck) was used as a standard for the construction of a calibration curve.

Minerals concentration was determined by inductively coupled plasma atomic emission spec-

troscopy (ICP-OES) using an ICAP 7400 Duo Analyser (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Analysis was performed in axial mode. Fermented samples were added with 5 ml of 650 $g \cdot l^{-1}$ HNO_3 (Biopack, Buenos Aires, Argentina) and placed in polytetrafluoroethylene (PTFE) vessels (Parr Instruments, Moline, Illinois, USA) for acid digestion. The PTFE vessels were heated in a conventional microwave oven. Digested samples obtained were diluted with deionized water (conductivity ≤ 5 $\mu S \cdot m^{-1}$). The TraCERT 92091 multielement standard (Sigma Aldrich) was used to construct the calibration curve. The wavelengths were 589 nm for sodium, 766 nm for potassium, 279 nm for magnesium, 259 nm for iron, 206 nm for zinc, 324 nm for copper, 257 nm for manganese, 177 nm for phosphorus, and 184 nm for calcium. Results were expressed as milligrams per litre of fermented samples. The energy density was calculated from proteins, carbohydrates and fat converted by the coefficients provided for each nutrient. Energy coefficients of 16 747.2 J for protein and carbohydrates, and 37 681.2 J for fat were used.

Gliadins concentration in quinoa grains and fermented samples were determined by R5 enzyme immunoassay test kit (Ridascreen ELISA, R-Biopharm, Darmstadt, Germany) with absorbance read at 450 nm using a microplate spectrophotometer (Rida Absorbance 96, R-Biopharm). Results were expressed as milligrams per litre of fermented samples.

Total saponins

Total saponins concentration was determined by spectrophotometric quantification based on vanillin-sulfuric acid method. Quinoa seeds were mixed with methanol (100 $g \cdot l^{-1}$, Anedra). Mixtures were centrifuged at 4 185 $\times g$ for 15 min to separate the supernatant. A 0.25 ml aliquot of the methanolic extract was mixed with 0.25 ml of 80 $mg \cdot l^{-1}$ vanillin solution (Sigma Aldrich) plus 2.5 ml of 720 $ml \cdot l^{-1}$ of sulfuric acid (Cicarelli). Samples were incubated in a water bath at 60 °C for 10 min. The absorbance measurement was performed at 544 nm and oleanolic acid (Sigma Aldrich) was used as a standard for construction of the calibration curve. Results obtained were expressed as milligrams per litre of oleanolic acid equivalents (OAE) in fermented quinoa samples.

Statistical analysis

Results were presented as mean and standard deviation. Analysis of variance (ANOVA) and post-hoc Tukey's test were carried out in order to evaluate differences between the means in every group of samples and variables. Pearson's correlation analyses between the total phenolic compounds and the antioxidant activity were carried out. All the data were analysed with 95% confidence interval, by SPSS statistical package version 27.0 (IBM, Armonk, New York, USA). All experiments were performed at least in duplicate.

RESULTS AND DISCUSSION

pH and acidification kinetics

Acidification kinetics of quinoa suspensions inoculated with *Lb. plantarum* CIDCA 83114, *Lb. kefiri* CIDCA 8348, kefir grains and acid mother cultures are shown in Fig. 1. An endpoint of pH < 4 at samples was considered. The addition of kefir grains caused a rapid drop in pH, reaching a mean value of 3.52 ± 0.06 after 1 h of incubation. Quinoa suspensions inoculated with *Lb. plantarum* CIDCA 83114 exhibited a mean value of pH equal to 3.69 ± 0.03 after 4 h of incubation, while *Lb. kefiri* CIDCA 8348 caused no acidification under the same conditions. When acid mother cultures were used, the pH value decreased gradually below 4.00 after 20 h of incubation. In comparison with acid mother cultures, the acidification rate of quinoa suspensions fermented with *Lb. plantarum* CIDCA 83114 would mean an advantage in terms of the contribution to safety with the substrate used and to the optimal time parameters related to the production of yogurt-like products in food industry. The degree of fermentation by *Lb. plantarum* CIDCA 83114 was consistent with the short times of acidification obtained in a study of Ludena Urquizo et al. [21] with a multi-strain starter proposed for the development of fermented quinoa beverages.

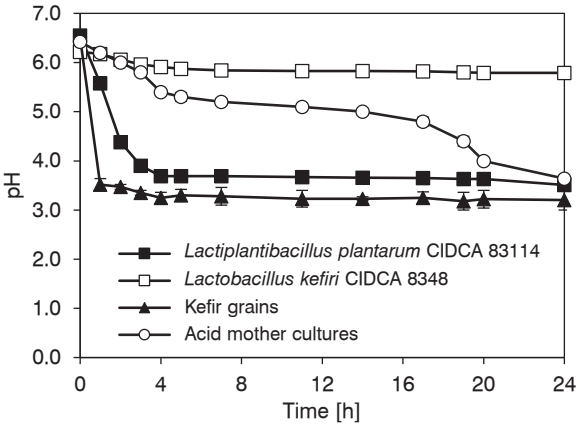

Fig. 1. Acidification kinetics of quinoa suspensions inoculated with starter cultures.

Tab. 1. Concentration of viable microorganisms in quinoa suspensions inoculated with starter cultures with or without addition of amylolytic enzymes.

Starter	Type	Initial suspension		Final suspension					
				Without added enzymes		With α-amylase and glucan 1,4-α-glucosidase		With maltogenic α-amylase	
		N [log CFU·ml^{-1}]	pH	*N* [log CFU·ml^{-1}]	pH	*N* [log CFU·ml^{-1}]	pH	*N* [log CFU·ml^{-1}]	pH
Lactiplantibacillus plantarum CIDCA 83114	LAB	7.22 ± 0.06 ^{a}	6.55 ± 0.08	8.63 ± 0.07 ^{b}	3.69 ± 0.03	8.87 ± 0.12 ^{b}	3.77 ± 0.03	8.84 ± 0.08 ^{b}	3.84 ± 0.02
Lactobacillus kefiri CIDCA 8348	LAB	7.19 ± 0.04 ^{a}	6.30 ± 0.01	7.24 ± 0.05 ^{a}	5.89 ± 0.08	7.36 ± 0.13 ^{a}	5.96 ± 0.15	7.13 ± 0.14 ^{a}	6.10 ± 0.03
Acid mother cultures	LAB	5.28 ± 0.12 ^{c}	6.22 ± 0.10	6.13 ± 0.07 ^{d}	3.64 ± 0.03	6.36 ± 0.09 ^{d}	3.70 ± 0.07	6.52 ± 0.18 ^{d}	3.59 ± 0.07
	Yeasts	6.42 ± 0.07 ^{d}		6.53 ± 0.10 ^{d}		6.65 ± 0.07 ^{d}		6.40 ± 0.15 ^{d}	
Kefir grains	LAB	7.20 ± 0.13 ^{a}	6.49 ± 0.01	5.81 ± 0.20 ^{c}	3.52 ± 0.06	5.52 ± 0.10 ^{c}	3.88 ± 0.10	5.86 ± 0.05 ^{c}	3.63 ± 0.04
	Yeasts	6.44 ± 0.10 ^{d}		6.40 ± 0.04 ^{d}		7.25 ± 0.06 ^{a}		7.16 ± 0.04 ^{a}	

Data are presented as mean ± standard deviation. Different superscript letters in the same row and column indicate significant differences between the mean values ($p < 0.05$).
N – microbial viable counts, LAB – lactic acid bacteria.

Microbial growth

Tab. 1 summarizes the total viable LAB and yeasts counts in the quinoa suspensions at initial and final pH revealing the growth of the tested starter cultures during fermentation. Counts of *Lb. plantarum* CIDCA 83114 increased by 1.4 log unit ($p < 0.05$), from 7.22 ± 0.06 log CFU·ml^{-1} to 8.63 ± 0.07 log CFU·ml^{-1}. LAB from acid mother cultures, where kefir grains were previously separated, showed an increment ($p < 0.05$) of 0.85 log unit, although the final counts were relatively low (6.13 ± 0.07 CFU·ml^{-1}). Counts of *Lb. kefiri* CIDCA 8348 remained unchanged, which indicated inability of the strain to ferment quinoa. Although the kefir grains quickly acidified the quinoa suspensions, the sudden drop in pH meant ineffectiveness of fermentation. In effect, viability of LAB in kefir grains was affected during the acidification of quinoa evidencing a significant decrease ($p < 0.05$) of more than one log unit. Meanwhile, yeasts concentrations at using kefir grains or acid mother cultures remained without change during the quinoa acidification.

According to a previous report of Valero-Cases et al. [22], our outcomes support the idea that microbial viability and integrity of kefir grains are more difficult to preserve in non-dairy matrices that in typical dairy matrices. In this sense, low counts of LAB strains in kefir grains AGK1 fermenting a non-typical source (soymilk), in comparison with those usually identified in kefir from cows' milk [13], could explain the detected loss of LAB viability.

Only quinoa suspensions fermented with *Lb. plantarum* CIDCA 83114 demonstrated a growth performance to achieve viability levels ≥ 7 log CFU·ml^{-1}, that are recognized as a minimum standard for fermented milks [23]. According to previous reports [24], *Lb. plantarum* CIDCA 83114 demonstrated better abilities to ferment quinoa suspensions in contrast to cows' milk. Additionally, the results were in agreement with the selective effectiveness of strains of *Lb. plantarum* to ferment quinoa suspensions reported by Ayub et al. [25] and Lorusso et al. [26].

As shown in Tab. 2, kefir grains showed a progressive loss of biomass until 58 % of their initial weight after 14 days of successive inoculations in the quinoa suspensions. Consequently, the balance of microorganisms in the acidified quinoa suspensions was affected evidencing a constant decrease of LAB ($p < 0.05$), while progressive increase in yeasts was detected.

The activity of kefir microorganisms would depend on availability of sugar sources for fermentation. Therefore, addition of exogenous enzymes

Tab. 2. Evolution of biomass of kefir grains and viable microbial counts in quinoa suspensions after inoculation with them.

Day of fermentation	Kefir grains weight [g]	LAB [log CFU·ml^{-1}]	Yeasts [log CFU·ml^{-1}]	pH
0	2.56 ± 0.03 ^{a}	5.96 ± 0.10 ^{a}	6.37 ± 0.05 ^{a}	3.63 ± 0.07 ^{a}
1	2.39 ± 0.07 ^{a}	5.90 ± 0.05 ^{a}	6.49 ± 0.06 ^{a}	3.58 ± 0.05 ^{a}
3	2.26 ± 0.05 ^{a}	5.81 ± 0.07 ^{a}	6.50 ± 0.03 ^{a}	3.51 ± 0.02 ^{a}
6	2.01 ± 0.05 ^{a}	5.39 ± 0.11 ^{a}	6.52 ± 0.05 ^{a}	3.56 ± 0.07 ^{a}
9	1.58 ± 0.08 ^{b}	5.28 ± 0.02 ^{a}	6.63 ± 0.07 ^{a}	3.60 ± 0.04 ^{a}
14	1.07 ± 0.12 ^{b}	5.12 ± 0.06 ^{b}	6.70 ± 0.04 ^{a}	3.58 ± 0.06 ^{a}

Data are presented as mean ± standard deviation. Different superscript letters in the same column indicate significant differences between the mean values ($p < 0.05$).
LAB – lactic acid bacteria.

was studied in order to provide fermentable sugars from starch (Tab. 1). No increase in viable cell counts was detected with both enzymatic treatments of the quinoa suspensions inoculated with individual lactobacilli or with acid mother cultures. According to the results, the effect of hydrolysis due to enzymatic activity would not affect the fermentation and the degree of acidification. In contrast, the use of the enzymes in quinoa suspensions before the inoculation of kefir grains encouraged a significant growth of yeasts ($p < 0.05$), while their LAB populations showed a loss of viability comparable to that identified in the fermented samples without enzymes. The selective growth of yeasts when starch-converting enzymes are used could be interpreted as a competitive metabolism of sugars available among LAB and yeasts of the kefir grains, involving a microbial imbalance. Moreover, the proliferation of yeasts above LAB population would provide undesirable effects on organoleptic characteristics of the fermented products obtained with kefir grains due to the natural production of volatile compounds, namely, ethanol and carbon dioxide. It has been shown that endogenous enzymes produced by metabolic activity of the starter microorganisms could contribute as adjuvants for fermentation, as consumption of reducing sugars showed potential correlation with viability and growth of LAB in fermented quinoa suspensions [21]. Moreover, the addition of exogenous enzymes contributes technologically to hydrolysis of quinoa starch and improves rheological properties of the beverage. Nevertheless, the use of the enzymes in kefir production must be carefully evaluated due to the potential competing interference with the microorganisms' fermentative activity itself.

Resistance to antibiotics

The analysis of the diameters of inhibition zones demonstrated that *Lb. plantarum* CIDCA 83114 was susceptible to the full spectrum of antibiotics (Tab. 3). On the contrary, *Lb. kefiri* CIDCA 8348 exhibited resistance to vancomycin

Tab. 3. Susceptibility of the strains to antibiotics.

	Antibiotics concentration	*Lactiplantibacillus plantarum* CIDCA 83114	*Lactobacillus kefiri* CIDCA 8348
Vancomycin	30 μg·ml^{-1}	S	R
Ciprofloxacin	5 μg·ml^{-1}	S	R
Amoxicillin-clavulanic acid	30 μg·ml^{-1}	S	S
Penicillin	10 U·ml^{-1}	S	S
Oxacillin	1 μg·ml^{-1}	S	I
Erythromycin	15 μg·ml^{-1}	S	S
Clindamycin	2 μg·ml^{-1}	S	S
Rifampicin	5 μg·ml^{-1}	S	S
Gentamicin	10 μg·ml^{-1}	S	S
Trimethoprim-sulfamethoxazole	1.2/23.76 μg·ml^{-1}	S	S

R – resistant, S – sensitive, I – intermediate.

and ciprofloxacin, with an intermediate resistance pattern against oxacillin. The latter result may not be relevant because some LAB are intrinsically resistant to vancomycin, leading to exclusion of this antibiotic from testing for this purpose [27]. The susceptibility found for *Lb. plantarum* CIDCA 83114 as a potential probiotic starter culture to ferment quinoa foods was expected, as it is required from safety point of view. This regards healthy consumers and immunodeficient individuals, thus ruling out any risk of transmitting resistance in the gut and facilitating natural elimination.

Total phenolic compounds and antioxidant activity

Lb. plantarum CIDCA 83114 and acid mother cultures were selected to carry out all following studies. Tab. 4 shows data on changes of potentially bioactive compounds and on the hydrolytic activity in fermented quinoa samples with individual starter cultures. Both total phenolic compounds and antioxidant activity significantly increased ($p < 0.05$) due to fermentation of quinoa suspensions with *Lb. plantarum* CIDCA 83114, while no appreciable changes ($p > 0.05$) were observed with acid mother cultures. It has been described that the metabolism of phenolic compounds by LAB during fermentation has a physiological role that is necessary for their viability and growth. This also means production of biologically active metabolites that provide benefit to human health through antioxidant, antiproliferative and uninflammatory properties [28]. Therefore, viability and growth of *Lb. plantarum* CIDCA 83114 could be responsible for the improvement in total phenolic compounds and antioxidant activity observed in the fermented quinoa samples, as also previously observed in other studies by Ayyash et al. [29] and Lorusso et al. [26]. Additionally, the results showed a positive correlation ($r = 0.95$) between total phenolic compounds and antioxidant activity. According to Melini and Melini [30], bound phenolic compounds can be biologically converted from their linked or conjugated forms to their free ones due to the metabolic activity of fermenting microorganisms. In their free form, the released phenolic compounds could become available and have the potential for increasing the antioxidant activity.

Degree of protein hydrolysis and amylolytic activity

Proteolytic activity during fermentation of quinoa suspensions with *Lb. plantarum* CIDCA 83114 and acid mother cultures was determined. The results showed different degree of protein hydrolysis, 49.8 % and 19.1 %, respectively (Tab. 4). According to Dallas et al. [31], peptidome analyses revealed that lactobacilli of kefir contribute to the release of functional peptides by proteolytic activity during fermentation, except for kefir yeasts, which probably do not possess extracellular proteolytic activity.

Formation of clear halos greater than 1 mm around the colonies on MRS starch agar were taken as indicative of amylolytic activity by quinoa samples fermented with *Lb. plantarum* CIDCA 83114 (halos of 2–3 mm radius) and acid mother cultures from kefir (halos of 1–2 mm radius). It is known that fermentative LAB and yeasts of kefir require a source of sugars for fermentation. Reducing sugars analysed by the DNS method showed mean values of 2.6 ± 0.9 g·l^{-1} in unfermented quinoa suspensions. After fermentation with *Lb. plantarum* CIDCA 83114 or acid mother cultures, reducing sugars decreased strongly, by 82 % and 54 %, respectively (Tab. 5). These results may indicate that LAB utilized the starch-derived reducing sugars for their growth

Tab. 4. Total phenolic compounds, antioxidant, proteolytic and amylolytic activity of unfermented and fermented quinoa beverages.

	Unfermented quinoa beverages	Fermented quinoa beverages	
		Lactiplantibacillus plantarum CIDCA 83114	Acid mother cultures
Total phenolic compounds [mg·l^{-1}]	7 118.5 ± 1 525.4 ^{a}	23 131.1 ± 1 473.4 ^{b}	12 127.2 ± 2 124.8 ^{a}
Antioxidant activity [mg·l^{-1}]	86.8 ± 10.3 ^{a}	224.5 ± 33.4 ^{b}	101.2 ± 10.1 ^{a}
Degree of protein hydrolysis [%]	1.0 ± 2.4 ^{a}	49.8 ± 4.1 ^{b}	19.1 ± 1.6 ^{c}
Amylolytic activity	–	++	+

Data are presented as mean values ± standard deviation. Different superscript letters in the same column indicate significant differences between the mean values ($p < 0.05$).
Total phenolic compounds are expressed as concentration of gallic acid equivalents. Antioxidant activity is expressed as ascorbic acid equivalents. Amylolytic activity: (+) – indicates starch hydrolysis with halos of 1–2 mm radius, (++) – indicates starch hydrolysis with halos of 2–3 mm radius, (–) – indicates non starch hydrolysis.

during the fermentation of quinoa. Additionally, our results were consistent with the ability of the strain CIDCA 83114 to ferment starch-derived sugars, such as maltose and glucose [32]. According to FILANNINO et al. [28], the capability to ferment plant-based foods is interpreted as bacterial species-specific and strain-specific, depending on their intrinsic enzyme portfolio in synergy with plant enzyme activities, enabling LAB to execute the metabolic pathways successfully.

Chemical composition

As shown in Tab. 5, the concentration of nutrients was similar among the quinoa suspensions fermented by *Lb. plantarum* CIDCA 83114 or acid mother cultures, showing a profile of low energy density and relatively high proteins and dietary fibre concentrations. The proteins concentration of fermented quinoa samples (approximately 14 $g \cdot l^{-1}$) was comparable to amounts present in a nutritive unfermented quinoa commercial beverage previously designed by us (i.e., 16 $g \cdot l^{-1}$ proteins in BIBA quinoa beverage, CONICET-Babasal, Argentina). The concentration of proteins is an attribute carefully considered in plant-based formulations, due to those occasionally revealing poor nutritional balance and low concentration (below 5 $g \cdot l^{-1}$), with the exception of soya-based milk analogues [33]. In contrast with the reports of some authors [26, 34], there was no increase in proteins concentration due to the fermentation respect to the initial proportions in the quinoa suspensions. The dietary fibre concentration remained unchanged in both formulations. On the contrary, LI et al. [34] described a significant decrease in the fibre after fermentation of quinoa suspensions with a strain of Lacticaseibacillus casei. Moreover, the use of quinoa whole grains favoured the availability of dietary fibre, although the perception of astringency is usually a limitation regarding the sensory acceptance of beverages. Total fat and concentration of reducing sugars showed low values, which contributed to the indication that the product was beneficial to health. Seventeen fatty acids were identified in both fermented beverages by gas-chromatographic analysis. Unsaturated fatty acids profiles predominated, especially 18:2*n*6*c* linoleic acid (from 504.9 ± 0.9 $g \cdot kg^{-1}$ to 522.2 ± 0.8 $g \cdot kg^{-1}$ of total fats), 18:1*n*9*c* oleic acid (from 197.8 ± 0.9 $g \cdot kg^{-1}$ to 204.3 ± 0.6 $g \cdot kg^{-1}$ of total fats) and 18:3*n*3 α-linolenic acid (from 51.8 ± 0.7 $g \cdot kg^{-1}$ to 55.2 ± 0.8 $g \cdot kg^{-1}$ of total fats). Meanwhile, saturated 16:0 palmitic acid ranged from 90.9 ± 0.6 $g \cdot kg^{-1}$ to 101.8 ± 0.3 $g \cdot kg^{-1}$ of total fats. Copper and manganese were determined as the main contributors to minerals of the fermented quinoa beverages, covering 40 % and 21 %, respectively, of the recommended daily intake (*RDI*) in adults. Iron, zinc and magnesium detected in second place would cover 5 % to 7 % of *RDI* in adults, while the other dietary minerals were found at minor levels.

Tab. 5. Energetic value and chemical composition of fermented quinoa beverages.

	Fermented quinoa beverages	
	Lactiplantibacillus plantarum CIDCA 83114	Acid mother cultures
Energy [$kJ \cdot l^{-1}$]	962.7 ± 4.6^{a}	910.0 ± 1.2^{a}
Moisture [$g \cdot l^{-1}$]	928.9 ± 0.2^{a}	931.2 ± 3.6^{a}
Ash [$g \cdot l^{-1}$]	1.0 ± 0.3^{a}	1.2 ± 0.7^{a}
Protein [$g \cdot l^{-1}$]	13.7 ± 0.5^{a}	14.9 ± 1.4^{a}
Fat [$g \cdot l^{-1}$]	1.9 ± 0.1^{a}	2.6 ± 0.4^{a}
Total carbohydrates [$g \cdot l^{-1}$]	39.7 ± 0.3^{a}	33.6 ± 0.5^{a}
Dietary fibre [$g \cdot l^{-1}$]	14.9 ± 0.4^{a}	17.0 ± 0.2^{a}
Reducing sugars [$g \cdot l^{-1}$]	0.5 ± 0.1^{a}	1.2 ± 2.8^{a}
Na [$mg \cdot l^{-1}$]	70.0 ± 8.3^{a}	119.8 ± 16.2^{a}
K [$mg \cdot l^{-1}$]	142.7 ± 14.4^{a}	206.1 ± 88.1^{a}
P [$mg \cdot l^{-1}$]	495.2 ± 58.6^{a}	597.7 ± 14.6^{a}
Mg [$mg \cdot l^{-1}$]	200.1 ± 24.1^{a}	217.2 ± 11.3^{a}
Mn [$mg \cdot l^{-1}$]	5.9 ± 0.1^{a}	3.9 ± 0.4^{a}
Ca [$mg \cdot l^{-1}$]	164.1 ± 13.3^{a}	204.3 ± 11.6^{a}
Fe [$mg \cdot l^{-1}$]	10.3 ± 1.3^{a}	10.5 ± 1.2^{a}
Zn [$mg \cdot l^{-1}$]	4.7 ± 0.5^{a}	4.8 ± 1.4^{a}
Cu [$mg \cdot l^{-1}$]	4.0 ± 1.9^{a}	3.3 ± 0.7^{a}
Gliadins [$mg \cdot l^{-1}$]	< 10	< 10
Saponins [$mg \cdot l^{-1}$]	23.8 ± 5.1^{a}	26.9 ± 2.1^{a}

Data are presented as mean ± standard deviation.

Safety analysis of the fermented quinoa beverages comprised quantification of saponins (Tab. 5). Quinoa grains selected for the study containing 1.6 $g \cdot kg^{-1}$ of total saponins were characterized as a semi-bitter genotype. Since the quinoa grains were previously cleaned and washed, less than 0.03 $g \cdot l^{-1}$ of residual saponins in the fermented quinoa beverages were found. Although there is no regulatory consensus, a maximum of 1.2 $g \cdot kg^{-1}$ of total saponins in scarified quinoa grains for human consumption is defined by the Codex Alimentarius [35]. Additionally, total gliadins were not detected by immunoassay in any of the fermented quinoa suspensions, suggesting that these gluten-free formulations were suitable for people with celiac disease.

CONCLUSIONS

Lb. plantarum CIDCA 83114 and acid mother cultures, both obtained from kefir grains CIDCA AGK1, were able to ferment quinoa suspensions. Nevertheless, *Lb. plantarum* CIDCA 83114 demonstrated good bacterial growth for preparation of a fermented quinoa beverage and greater production of phenolic compounds, antioxidants, proteolytic activity and amylolytic activity than the acid mother cultures. In contrast, *Lb. kefiri* CIDCA 8348 was not able to ferment the substrate and the kefir grains lost integrity and viability of their microorganisms. The results demonstrated suitable technological capabilities of the potentially probiotic strain *Lb. plantarum* CIDCA 83114 as a starter culture to obtain nutritious and potentially functional fermented quinoa beverages. Further studies in order to analyse organoleptic characteristics and shelf life at refrigeration are warranted. Results encourage pilot-scale industrial trials of packaged fermented quinoa beverages for future innovative developments.

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