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Effect of fermentation in nutritional, textural and sensorial parameters of vegan-spread products using a probiotic folate-producing *Lactobacillus sakei* strain

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ABSTRACT

Folates are essential vitamins for human life and must be taken as part of the diet. It is known that some lactic bacteria are capable to produce folates during fermentation, which represents an interesting strategy as alternative to fortification with folic acid. The aim of this work was to study the effect of fermentation with a probiotic folate-producing *Lactobacillus sakei* strain in vegan spread products. Two products were manufactured with Andean potato and amaranth -with and without chia- and emulsified with other ingredients. Nutritional, textural, structural and sensorial analysis were performed. Products were also studied during 28 days of refrigerated storage. After 24 h fermentation bacterial growth achieved 11.0 log CFU/g and folate concentration reached 1900 ng/g, both values were kept during conservation period. No changes in firmness, viscosity, syneresis or particle size were observed after fermentation and storage, all parameters associated with products stability. Sensory analyses showed that the product without chia, fermented during 6 h, reached a good acceptability score, especially among vegetarian population. These results demonstrated the feasibility of bioenrich novel spread vegan products throughout lactic fermentation with no changes in technological parameters.

1. Introduction

Folate is a term referring to a family of compounds which belong to the B vitamin group and are essential elements for life. In human pathology, folate deficiency is associated with megaloblastic anemia, cardiac diseases and neural tube malformations in newborns, among others (Iyer & Tomar, 2009). This vitamin is necessary for basic cellular functions related with amino acid metabolism, nucleotide biosynthesis, and methylation cycle. Unlike plants and some microorganisms, humans are not able to synthesize folate *de novo*, and must be acquired entirely from dietary sources (Savoy & LeBlanc, 2018).

Folate deficiency is frequent worldwide, both in developing and developed countries (Youngblood et al., 2013). Folic acid, a synthetic form of folate, is used as oral supplement and is also incorporated in cereals for food fortification in over 57 countries. Nevertheless, high intake of this compound has been associated with some adverse secondary effects, such as masking symptoms of vitamin B12 deficiency (Liew, 2016). A new interesting strategy as alternative to fortification with folic acid is the use of folate-producing microorganisms to bioenrich fermented food (Savoy & LeBlanc, 2018).

In particular, it was demonstrated that certain lactic acid bacteria (LAB) are able to produce folate. However, this ability is a strain-dependent trait and varies considerably among strains. In addition, probiotic bacteria are good candidates, since they are able to survive through the gastrointestinal tract and adhere to the human intestinal cells, which may increase folate production *in situ* (Pompei et al., 2007). Thus, selection of folate-producing strains and optimization of the fermentation conditions are essential prerequisites to perform a proper biotechnological process to develop novel food products. Furthermore, studying the survival of probiotic bacteria in foods is important since the physicochemical and organoleptic properties of the product must remain stable during shelf-life as well as the probiotic microorganisms

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must stay viable (Soares et al., 2019).

Some vegetable substrates have been assessed to develop folate bio-enriched products, like oat, soy and wheat, among others (Saubade, Humblot, Hemery, & Guyot, 2018). In this way, Andean potato and amaranth are good starch sources, and the latter is also important due to its protein content (around 15%) with a good essential amino acid pattern, high lysine content and a biological value comparable to that of cheese (Bressani, de Martell, & de Godinez, 1993). In addition, chia seeds have an enormous potential of application in food since its mucilage can substitute emulsifiers and stabilizers and also provides omega 3, nevertheless some sensorial challenges are associated with its incorporation (Fernandes & Mellado, 2018).

A vegan-spread product was manufactured with the mentioned substrates and additives such as oil, using high speed shear to form an emulsion, which was fermented with a folate-producing *Lactobacillus sakei* strain. In general, fermentation can improve nutritional, functional and textural quality of foods. Nevertheless, some metabolites produced might be responsible of flavor and texture and fermentation parameters should be assessed to define optimal conditions.

Thus, the aim of this work was to study the effect of fermentation with a folate-producing *L. sakei* CRL2210 in two vegetable vegan spread products (with and without chia) in order to determine folate production and fermentation parameters. The effect of adding chia, in order to improve textural and nutritional values of these fermented foods, was also evaluated.

2. Materials y methods

2.1. Bacterial strain and manufacture of fermented products

A probiotic folate-producing *Lactobacillus sakei* CRL2210 was previously isolated from Tocosh (a traditional Andean fermented food) and selected based on its kinetics of growth, high folate production capacity and tolerance to gastrointestinal simulated conditions (Mosso, Jimenez, Vignolo, LeBlanc, & Samman, 2018). It belonged to the Culture Collection of CERELA-CONICET.

The strain was subcultured twice in MRS broth (Britania, Argentina) at 37 °C until the late exponential phase of growth was reached (ca. 10 h), washed twice in 50 mmol/L phosphate buffer, pH 7.0, re-suspended in saline solution and used to inoculate.

Andean potato (*Solanum tuberosum* ssp *andigena* - collareja variety) and amaranth grains (*Amarantus caudathus*) were provided by INTA-IPAF Posta de Hornillos (Jujuy, Argentina). Chia grains (*Salvia hispanica L.*), sunflower oil, NaCl and cheese flavor were purchased from a local market. Two vegan spread products (PA and PAC) were manufactured according to the protocol described in Fig. 1.

Samples were taken at 0, 6 and 24 h of fermentation and 28 d of cold storage.

Preliminarily, the amount of oil, salt and flavor employed for the products were screened based on sensory analysis. Final compositions of each product are listed in Table 1.

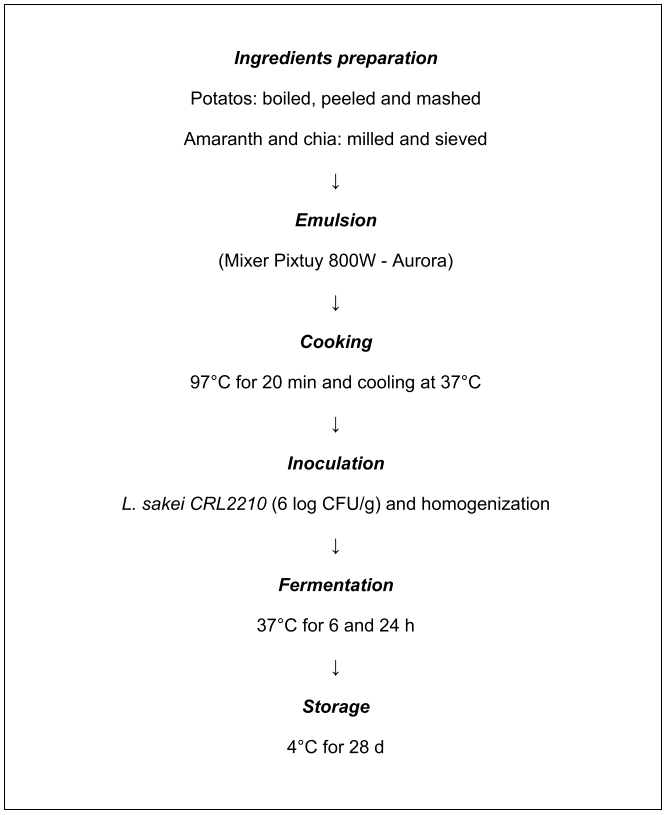

Fig. 1. Protocol for the manufacture of products.

Table 1
Composition of vegan spread products with (PAC) and without (PA) chia.

Ingredients (g/100 g)	PA (% w.b.)	PAC (% w.b.)
Collareja Andean potato *(boiled, peeled and mashed)*	33.3	31.6
Amaranth flour	5.5	5.2
Sunflower oil	5.0	3.6
NaCl	1.0	1.0
Cheese flavor	1.5	1.5
Tap water	53.5	53.6
Chia flour	0.0	3.3
Inoculum	0.2	0.2

2.2. Determination of pH and bacterial growth

To determine pH and bacterial growth 10 g of each product were diluted in 90 mL of distilled water. The values of pH were measured with a digital pH meter (DALVO, MHS). Viable cell counts (log CFU/mL) of these solutions were assessed by serial dilutions and the standard plate method with MRS agar after 48 h of incubation at 37 °C.

2.3. Total folate content and vitamers distribution

2.3.1. Total folates by microbiological assay

The samples preparation procedure included heat extraction (97 °C–5 min) to precipitate proteins and release folates from binding proteins, centrifugation (10,000*g*–5 min) and enzymatic treatment with α-amylase, protease and polyglutamates (deconjugation of folate) by rat serum. Total folate concentrations were determined by a microbiological assay based on the growth of the indicator strain *L. rhamnosus* NCIMB 10463 (Laiño, LeBlanc, & Savoy, 2012). Briefly, samples and different concentrations of HPLC-grade folic acid (Fluka BioChemica, Sigma-Aldrich, Switzerland) were placed with the indicator strain and incubated for 48 h at 37 °C in 96-well microtiter plates containing folate-free culture media. The growth of the indicator was measured at an optical density of 580 nm with a microplate spectrophotometer (EPOCH, BioTech). Folate concentrations of the samples were calculated from the calibration curve obtained with folic acid.

2.3.2. Folate vitamers distribution by UPLC

Samples were suspended into 10 mL of bicarbonate buffer −0.5 mmol/L-with 0.5% of dieritrotietol (DTT) and 1% of ascorbic acid, at pH 7.2. Samples were then boiled for 10 min and centrifuged in an Amicon filter (5 kDa), at 13,000*g*, during 50 min at 4 °C. To determine the vitamers distribution, three standards were used: 5-methyltetrahydrofolate (5-MTHF), 10-formyltetrahydrofolate (10-CHOTHF) and 5-formyltetrahydrofolate (5-CHOTHF) (Schircks Laboratories, Jona, Switzerland). To perform this experiment an Ultra Performance Liquid

Chromatography - tandemmass spectrometer (UPLC -MS/MS, Thermo Fisher, Waltham, USA) was used. The chromatographic separation was achieved using an HSS T3 1.8 μm 2.1 × 150 mm column (Waters Co.) at 45 °C, 0.35 mL/min flow rate of 0.1% formic acid solution in water (A) and 0.1% formic acid solution in acetonitrile (B) in gradient elution. The proportion of B was increased to 25% in 4 min, and finally increased to 70% and held for 1 min. Subsequently, the mobile phase was adjusted to its initial composition (0.5% B) and held for 2 min.

2.4. Fatty acids analysis

The Bligh and Dyer method was used for the lipid extraction (Bligh & Dyer, 1959). A 40 g sample was mixed with 120 mL methanol/chloroform (2:1; v/v), shaken (120 r/min) for 30 min and then the solvent was removed. Following that, the residue was washed five times with methanol/chloroform and dried under a N_2 stream. The composition of the fatty acid methyl esters was determined by capillary gas chromatography (CGC 6890 System Plus, Agilent Technologies, Canada) with a flame ionization detector (FID). The analytical conditions were: injector temperature 220 °C, detector temperature 220 °C; oven temperature 60 °C (1 min) programmed to increase to 210 °C at a rate of 6 °C/min and maintained at this temperature for a further 20 min; carrier gas: N_2-UAP; and make-up gas: N_2-UAP. The individual fatty acid methyl esters were identified using the Lipid Standard Sigma 189-1 (Sigma Chemical, UK).

2.5. Texture, viscosity, syneresis and color analysis

For textural and viscosity analyses, products were put in rigid plastic containers (45 mm internal diameter) before fermentation, so the process took place in this final recipient. Textural analysis was conducted in an INSTRON texturometer (Universal Testing Machine 3342, USA). Two tests were performed to assess consistency and firmness (Colombo, León, & Ribotta, 2011). For back extrusion test, measurements were carried out in these containers, in which a 35 mm diameter compression disk with an extension bar, moved 35 mm at a speed of 1 mm/s. After the test was completed, maximum positive area (consistency) of extrusion was obtained from recorded graphs. Gel firmness was determined through a single compression test using a cylinder (25 mm diameter; 10 mm distance; 10 mm/s speed). Firmness was considered as the maximum penetration force. All textural analyses were performed in triplicate at 25 °C. Viscosity was determined in triplicate in a rotational viscometer (Fungilab Expert Series) with concentric cylinders and samples placed in the stationary cup. Measurements were performed at 25 °C with spindle velocity of 0.5 rpm. Statistical analysis was performed comparing between PA and PAC series and intra-series for each fermentation and conservation time.

To determine syneresis at 0, 6 and 24 h fermentation and 28 d of cold storage, samples were centrifuged at 1500*g* for 10 min at 20 °C, weighed and subsequently the percentage of water expelled was quantified.

Color was measured by a colorimeter (Hunterlab, Miniscan). Illuminant D65 and 10° observer angle were used. The results reported are averages of measurements of three portions, using CIE L*, a*, b* coordinates. And also, the total color difference (ΔE) and browning index (BI) were calculated to describe the color change between fermented and not-fermented samples, using the following equations (Maskan, 2001).

$$\Delta E = [(L_0 - L_n)^2 + (a_0 - a_n)^2 + (b_0 - b_n)^2]^{1/2}$$

$$BI = \frac{100\ (X - 0.31)}{0.17}$$

$$X = \frac{(a + 1.75L)}{(5.645L + a - 3.012b)}$$

2.6. Particle size distribution and microstructure

The particle size distribution was determined using laser diffraction (Horiba Partica LA-950) with a refractive index of 1.54. A small volume of sample was added to the flow system and pumped through the optical chamber for measurements (Timgren, Rayner, Dejmek, & Marku, 2013).

The ultrastructure was evaluated by scanning electron microscopy (SEM). Samples were prepared by freezing aliquots to −80 °C and freeze drying. Dry samples were cutted and mounted on SEM stubs with conductive carbon cement (Leit-C, Neubauer Chemikalien, Germany), and sputter coated with a 5 nm layer of gold/palladium (80:20) (Polaron E5150 Sputter Coating Unit, Quorum Technologies, UK). Samples were then examined with a Scanning Electron Microscope and images were analyzed using the open source software ImageJ (v1.51s, National Institutes of Health, USA).

2.7. Sensory analysis

Sensory analysis was carried out with the aim of determine optimal fermentation time according to the perception of 78 consumers (between 18 and 65 years of age, equal gender distribution). After 6 and 24 h fermentation, PA and PAC samples were refrigerated for 24 h, randomly coded and served (20 g) at 20 °C, according to Pramudya and Seo (2018). Sensory acceptance tests were performed using a structured 9-point hedonic scale (1 = dislike a lot; 9 = like a lot) for overall acceptability (Matias, Bedani, Castro, & Saad, 2014). For CATA (Check-All-That-Apply) test, sensory attributes considered belonged to odor, flavor, texture and appearance categories.

2.8. Statistic analysis

All experiments were conducted in triplicate. Results were expressed as mean ± SD (standard deviation). Statistical analyses were performed comparing intra- and inter- PA and PAC series, with the software Statistica 7.0 (StatSoft, Inc., USA) using ANOVA followed by a Tukey's test, and differences were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Cell growth, acidification and total folate production

Products with and without chia (PAC and PA respectively) were fermented with *L. sakei* CRL2210 and microbial counts were assessed. During fermentation, bacterial population increased from initial value of ca. 6.0 to achieve 8.0 log CFU/g in 6 h and above 11.0 log CFU/g after 24 h in both cases, as shown in Fig. 2. After 28 days of storage at 4 °C, the cell number decreased 0.8 log CFU/g in PAC and 1.8 log CFU/g in PA. The pH drop was concomitant with bacterial growth and indicated an active metabolism, even during refrigerated conservation. Folate production in PA sample was 890 ng/g at 6 h, reached 1890 ng/g in 24 h and dropped ($P < 0.05$) to 1600 ng/g after 28 d. Regarding the addition of chia, it caused *L. sakei* CRL2210 to produce higher folate concentration compared with PA: 1800 ng/g in the first 6 h of fermentation, reached 2098 ng/g after 24 h and remained constant ($P > 0.05$) during the conservation period. Albuquerque, Bedani, LeBlanc, and Saad (2017) obtained similar folate production values with *S. thermophilus* ST-M6 in 24 h-fermented soymilk + FOS (1325 ng/mL) and with *S. thermophilus* TH-4 (1250 ng/mL) in 24 h-fermented soymilk + FOS + passion fruit. Whereas, in the same work, the four studied *Lactobacillus* strains (*L. acidophilus* LA-5, *L. rhamnosus* LGG, *L. fermentum* PCC and *L. reuteri* RC-14) were not able to produce large amounts of folate in 24 h fermentation of different substrates. Indeed, some of them consumed matrix folates. Folate concentrations in PA and PAC after 6 h fermentation overcome values found by Bassett and

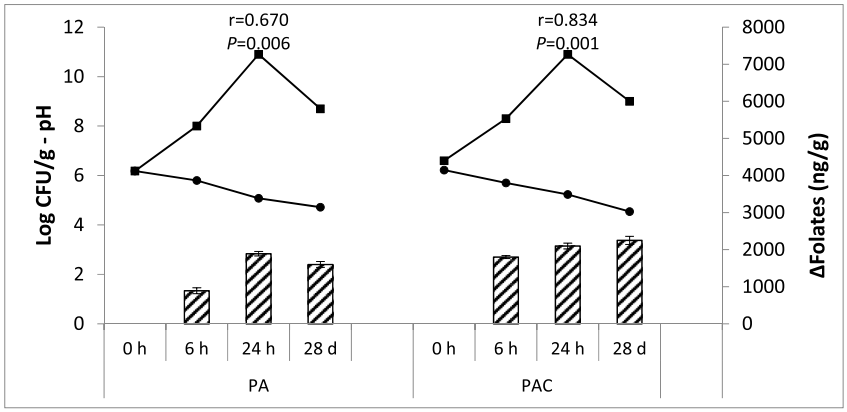

Fig. 2. Cell growth, acidification and total folate production of *L. sakei* CRL2210 in PA and PAC formulations ●pH ■ log CFU/g ▨ folates (ng/g).

Sammán (2010) in broccoli (1110 ng/g), spinach (1450 ng/g) and lentils (970 ng/g), which are considered high folate foods.

As shown in Fig. 2, initial folate content of the formulations did not downregulate folate synthesis as was also observed by Albuquerque, Bedani, Vieira, LeBlanc, and Saad (2016) in different fermented substrates, including amaranth and soybean okara combined with passion fruit, orange, acerola and mango by-products. In the same way, Kariluoto et al. (2014) observed that glucose addition did not affect folate production in oat flour fermented with *S. thermophilus* ABM5097. In the present work, folate synthesis was not straightforwardly associated with final cell concentrations, which is in accordance with other authors (Kariluoto et al., 2014; Laiño et al., 2012). Although in both PA and PAC series strain was able to grow and produce folates, the highest initial folate production and the more stable production rate during 28 d was PAC product. This result may be related with the presence of some nutrients and/or bioactive compounds in chia (for example, dietary fiber) which could stimulate folate production by *L. sakei* CRL2210. A 50 g serving of 6 h-fermented PAC formulation contains 90 μg of folate, which could represent a 45% of the RDA for an adult (400 μg/d).

3.2. Folate vitamers and fatty acids distribution

Microbiological assay is the standard method to quantify total folate content, nevertheless vitamers differentiation should be determined by UPLC–MS/MS. Also, the short run time makes it advantageous when analyzing very labile compounds, such as folates. Several authors have used this technique to identify vitamers in fortified wheat, rice, corn starch and tapioca breads (Chandra-Hioe, Bucknall, & Arcot, 2011; Kariluoto et al., 2010). Vitamer distribution in PA and PAC (Fig. 3) reflected those produced by *L. sakei* CRL2210 since the largest part of folate derived from microbial synthesis rather than the matrix itself. Indeed, the same patterns remained even if the distributions were calculated on a net basis. Differences were found between formulations, however, the dominant vitamers were the same in each PA and PAC fermentation series. The main vitamer in both formulations was 5-methyltetrahydrofolate, which is required for several methylation reactions including the methionine biosynthesis (Kariluoto et al., 2010). This is the most common natural form and the principal form of folate that occurs in the blood and does not accumulate in plasma, such as folic acid for example. In PA sample, *L. sakei* CRL2210 produced 5-methyltetrahydrofolate in the first 6 h and vitamer profile was kept during the rest of fermentation and storage process. The amount of 10-formyltetrahydrofolate increased with fermentation in PAC series, this vitamer is necessary for purine biosynthesis. The role of 5-formyltetrahydrofolate is not completely clear. This very stable form of vitamer could act as a storage molecule for one-carbon compounds and its metabolism could indirectly regulate several biosynthetic pathways because it inhibits folate-dependent enzymes. Although it does not act directly as a donor of carbon groups, it can be converted into 5,10-methenyltetrahydrofolate, which in turn acts as a coenzyme in the synthesis of thymidylate (Kariluoto et al., 2010).

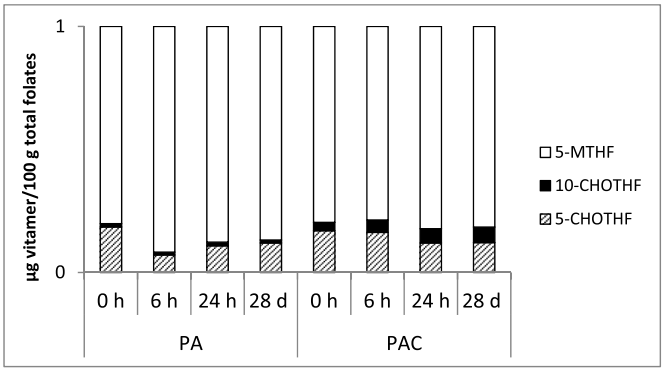

Fig. 3. Vitamer distribution in fermented PA and PAC products.

It is known that microbial metabolism during fermentation can generate interconversions of fatty acids, caused by isomerase enzymes and hydrolases that in some cases such as those described by Lee, Hwang, Kim, Chung, and Kim (2019) hydrolyze polyunsaturated fatty acids into others of lower molecular mass, which could be related with the formation of characteristic aroma compounds and lactones in some fermented products. Profiles of the fatty acids in PA and PAC formulations revealed the presence of saturated fatty acids, (e.g., myristic, pentadecanoic, palmitic, stearic, margaric, arachidic, behenic and lignoceric acids) and unsaturated fatty acids (e.g., palmitoleic, oleic, cis-vaccenic linoleic and linolenic acids, among others) (Table 2). Results indicated that the main fatty acid in both was linoleic acid (18:2 9c12c), principally from sunflower oil, which remained constant after fermentation and storage periods. In addition, in PAC samples, the second major lipid was alpha linolenic, which was also maintained during fermentation and storage. This fatty acid is characteristic of chia and associated with health beneficial properties, therefore its conservation during fermentation is an important aspect to be considered. In both matrices the consumption of long chain fatty acids (22 and 24 carbon atoms) and the increase in palmitic acid (16:0) were recorded. In PA there was also an increase in the concentration of oleic acid (18:1 9c) and in PAC of linoleic acid (18:2 9c12c). Similar results were found by Jia, Chen, and Ding (2016) in fermented goat milk with a *L. rhamnosus* GG strain, where long-chain fatty acids were hydrolyzed into short-chain and medium-chain by a lipoprotein lipase, which improved absorptivity and health function of goat milk.

Table 2
Fatty acids profile in PA and PAC products.

Fatty acid		mg/100 mg methyl ester							
		PA				PAC			
		0 h	6 h	24 h	28 d	0 h	6 h	24 h	28 d
14:00	miristic	0.04	0.06	0.05	0.05	0.04	0.07	0.05	0.05
15:00	pentadecanoico	0.01	0.02	0.01	0.00	0.01	0.02	0.01	0.01
16:00	palmitic	5.75	6.73	6.80	7.03	5.53	6.90	7.20	7.82
16:1 9t	trans-9-hexadecenoic	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.00
16:1 9c	palmitoleic	0.07	0.08	0.07	0.07	0.06	0.07	0.06	0.06
17:00	margaric	0.05	0.05	0.02	0.00	0.04	0.03	0.20	0.00
17:1 10c	cis-10-heptadecenoic	0.03	0.03	0.02	0.00	0.02	0.03	0.20	0.00
18:00	stearic	3.81	3.73	3.60	3.85	3.56	3.02	3.00	2.90
18:1 9c	oleic	37.01	37.36	38.90	39.43	30.18	28.79	29.20	29.29
18:1 11c	cis-vaccenic	0.40	0.55	0.50	0.56	0.58	0.07	0.00	0.00
18:2 9c12c	linoleic	49.02	48.83	49.00	49.47	42.60	43.62	44.72	44.89
18:3 9c12c15c	alpha linolenic	0.46	0.44	0.40	0.45	15.22	15.35	15.00	15.80
20:00	araquidonic	0.42	0.38	0.30	0.18	0.41	0.38	0.20	0.12
20:1 11c	gadoleic	0.19	0.18	0.19	0.19	0.18	0.18	0.18	0.19
22:00	behenic	1.02	0.81	0.60	0.33	0.70	0.62	0.45	0.28
24:00	lignoceric	0.37	0.13	0.08	0.00	0.20	0.17	0.00	0.00
24:1 5c	cis-5-tetracosenoic	1.34	1.02	0.80	0.00	0.68	0.43	0.20	0.00

3.3. Effect of fermentation in texture, syneresis, structure and color

3.3.1. Textural analysis

The texture of samples was evaluated by two tests: penetration and back extrusion. Firmness is an indicator of resistance to penetration by a probe and is greater as the force required for penetration increases. Texture parameters of PA and PAC fermented and stored series are presented in Table 3. Firmness values were similar ($P > 0.05$) in PA and PAC series, indicating stability during the entire fermentation and conservation period in both cases. Previously it was demonstrated that *L. sakei* CRL2210 lacks of amylolytic enzymes which could be related with stability through fermentation due to the prevention of amylose cleavage and consequent retrogradation.

In PAC samples, addition of 3.3% chia has no influence in firmness compared with PA ($P > 0.05$). Chia mucilage, composed mainly by dietary fibers, combined with water forms a gel with emulsifying properties. Fernandes and Mellado (2018) studied the addition of different amounts of chia mucilage in mayonnaise and found that firmness was kept with 15 and 25% of mucilage. Notwithstanding this, firmness values decreased about 20% when its content was 35 and 45%. Along with the penetration test, back extrusion is also a common technique used for measuring texture parameters in spreadable foods. Consistency values are reported in Table 3. No significant differences ($P > 0.05$) were found between PA and PAC in each series, and between formulations. Regarding viscosity, the were no significant differences ($P > 0.05$) between fermentation times in each sample set, which means that the process had no effect in this parameter, even after 28 d of cold storage with an active bacterial metabolism. On the other hand, viscosity significantly ($P < 0.05$) differentiated between formulations: PAC samples showed higher values, which could be attributed to the presence of chia mucilage. Timilsena, Adhikari, Kasapis, and Adhikari (2016) confirmed that emulsion viscosity increased when the concentration of chia mucilage was higher, which led to a reduced rate of droplet coalescence that prevented flocculation. Emulsion stability is an important technological parameter since it is associated with the prevention of coalescence of the oil droplets, flocculation and cream formation. Many factors, such as pH, added amount of salts and spices and oil concentration, among others, may contribute to emulsion stability. In this way, gums and starches are typical thickering agents added to enhance stability in spread foods (Mun, Kim, Kang, & Park, 2009).

Table 3
Texture, viscosity and syneresis of PA and PAC products.

	Firmness (N)	Consistency (N.s)	Viscosity (mPa)	Syneresis
PA 0	1.1 ± 0.3 [a]	2.6 ± 0.5 [a]	15543.7 ± 626.8 [a]	0
PA 6	1.2 ± 0.2 [a]	2.5 ± 0.3 [a]	16073.0 ± 714.2 [a]	0
PA 24	1.1 ± 0.1 [a]	2.3 ± 0.4 [a]	15232.7 ± 1160.7 [a]	0
PA 28	1.3 ± 0.4 [a]	2.7 ± 0.5 [a]	17511.2 ± 694.3 [a]	0
PAC 0	1.3 ± 0.1 [a]	2.9 ± 0.1 [a]	19844.4 ± 680.3 [b]	0
PAC 6	1.5 ± 0.2 [a]	2.9 ± 0.5 [a]	20213.3 ± 1739.9 [b]	0
PAC 24	1.4 ± 0.2 [a]	3.1 ± 0.3 [a]	18438.5 ± 896.8 [b]	0
PAC 28	1.4 ± 0.4 [a]	2.8 ± 0.4 [a]	19190.6 ± 747.3 [b]	0

Average of three values with standard deviation, same letter in the column indicates that there was no significant difference between the means by Tukey's test ($P < 0.05$).

Another important technological parameter is syneresis. Water release by contraction of a gel network containing starch is called syneresis and occurs due to the reorganization of the molecules or retrogradation. In general, this is considered a quality defect in food (Masotti, Cattaneo, Stuknyte & de Noni, 2018). Syneresis degree of samples was studied by centrifugation, and no water release was recorded in any case (Table 3), even after the 28 d period of refrigerated conservation. This may be due the high starch content and its low rate of retrogradation, which was concomitant with firmness, consistence and viscosity values that remained constant in both PA and PAC products throughout the study period.

3.3.2. Particle size distribution

Particle size distribution was measured by light scattering and results are shown in Fig. 4. This assay performed in complex material systems -like PA and PAC- allows to know if changes in particle size occurred due mainly to fermentation or other phenomena such as oil drops coalescence. In PA formulation two peaks were recorded and in samples with chia a third peak appeared. Nevertheless, in both cases, the first peak between 2 and 12 μm could represent the starch granules and the amaranth endosperm fraction that was solubilized when heated. Similar sizes were reported by Leal-Castañeda et al. (2018) for amaranth starch granules. The second peak corresponded to the endosperm particles that remain linked to starch and emulsified particles, formed by small drops of oil and starch. Formulations contained 5% of oil and the manufacturing method included high shear force, which led to small oil drops formation. To prevent coalescence and maintain the stability of emulsions, amphiphilic molecules are usually added to lower the interfacial tension, including starch and hydrocolloids (Dickinson, 2010). Particularly, oil drops stabilized by dispersed

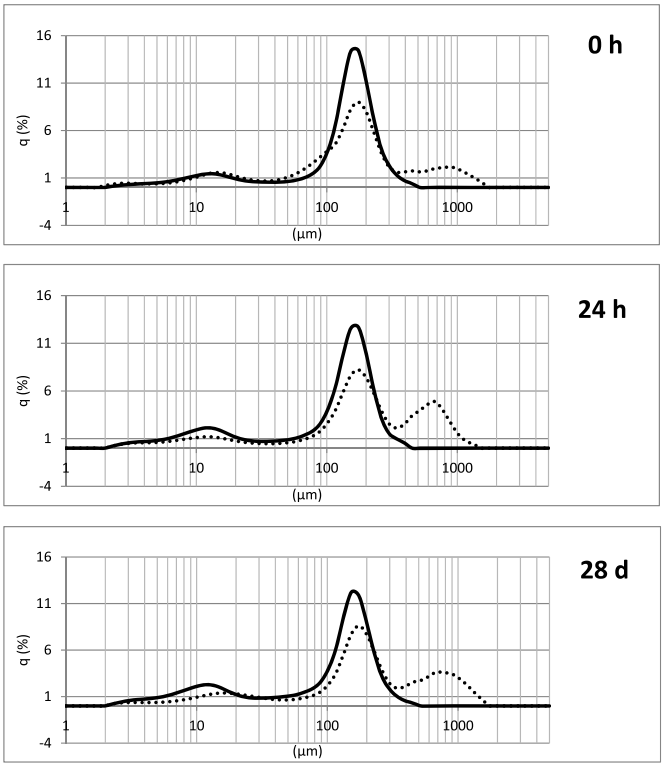

Fig. 4. Particle size distribution. PAC (•••) PA (−).

particles are known as Pickering emulsions. Some authors, such as Rayner, Timgren & Dejmek (2012) suggested that amaranth as well as quinoa contain solid particles that stabilize emulsions when placed on the interface, especially when the oil droplets are small, preventing coalescence and directly improving stability. In this way, PA and PAC emulsions could be stabilized by amaranth particles located at the interface which after cooking were gelatinized, and leaded to further increase the stability of the systems. This is in accordance with other parameters recorded in Table 3, such as viscosity, texture and syneresis, and could explain the stability of the formulations over time. The third peak in the PAC series corresponded to chia flour, of heterogeneous granulometry, which explained the slight variations in sizes. Notwithstanding this, this peak remained constant through fermentation and storage time.

3.3.3. Microstructure

Microscopy photographs obtained by SEM are shown in Fig. 5. Samples exhibited a compact structure with a spot of big open spaces. Differences are evident between the microstructures of the PA and PAC series: in samples with chia the presence of filaments was notorious (Arrows in Fig. 5). These formed cavities, probably composed of fibers and chia mucilage, which resulted in the sample lacking a homogeneous and smooth appearance. PA formulation was characterized by more homogeneous and softer surfaces than PAC, with some open spaces unevenly distributed on the surface. Regarding the effect of *L. sakei* CRL2210 in both sample sets, as fermentation and storage times elapsed, the structures become more irregular and porous. Espírito-Santo et al. (2014) carried out lactic fermentations of yogurts supplemented with passion fruit and the results of the samples taken at different fermentation times also indicated that the matrices become more heterogeneous as time passed by, and even towards the end of the process the cavities were larger.

3.3.4. Instrumental color

Color plays an important role in both the quality and consumer's preference in foods, especially those containing chia flour because its oxidation leads to undesirable colors. Table 4 shows the results obtained for the instrumental color evaluation parameters, total color difference (ΔE) and browning index (BI). For both, PA and PAC series, ΔE increased during fermentation and conservation periods, suggesting that there is a relationship between these changes and the presence of the *L. sakei* CRL2210. In PA color change was due to the increment ($P < 0.05$) of luminosity (L*) after 6 h fermentation. Also an increase in a* and b* values were observed, indicating a tendency towards red and yellow colors, respectively. Samples with chia showed lower ($P < 0.05$) luminosity values than PA samples. During PAC fermentation, there was a progressive darkening (increased BI) ($P < 0.05$) which continued after 28 days. Component a* was maintained, while b* increased significantly ($P < 0.05$) with fermentation and then during refrigerated storage time, which is consistent with the browning index, and could be related with chia oxidation.

3.4. Sensorial analysis

To determine optimal fermentation time (6 or 24 h), a sensorial analysis was performed. Also the impact of chia addition in sensorial perception was assessed.

Results from a 78-consumers study are summarized in Fig. 6. As shown, 53.59% liked the PA6 product (38.2% liked + 15.4% liked a lot). In addition, this is the only sample that reached an acceptance score of 6 (6.3 for PA6; 5.2 for PA24; 4.5 for PAC6 and 4.2 for PAC24).

The study also included CATA questions, since the choice of the attributes that describe each of the samples can explain the relationship with some instrumentally determined parameters. To obtain CATA attributes, previously, a descriptive flash profile study was performed with 10 semi-trained sensorial judges to characterize samples and determine descriptors for appearance, texture and flavor.

After CATA study with 78 consumers, a correspondence factor analysis was carried out, and results showed that PA6 was described as follows: 51.3% of consumers agreed that the product had “adequate salt level”, 25.6% “adequate acidity level”; 28.2% “fermented flavor”; 44.9% “good taste”; 47.4% “mild taste”; 29.9% “rancid flavor”; 47.4% “luminous”; 58.9% “good appearance”; 61.5% “homogeneous appearance”; 76.9% “good spreadability”; and 58.9% “homogeneous texture”. The samples containing chia (PAC6 and PAC24) were perceived with some negative attributes in CATA analysis, especially related to appearance and taste, which may explain their lower acceptability (e.g. for PAC24: 49.2% marked “dark” and 49.7% “bad aspect”). This is evidence of the importance of appearance in spreadable products, since the products without chia were better accepted than those containing this flour for all the other attributes evaluated. Sensory analysis lead to define that the optimal fermentation time was 6 h, after that metabolites produced during fermentation generated unpleasant flavors. On the other hand, from the sociodemographic data collected, 23% of the consumers who participated in the study was vegetarian or vegan (since a part of the study was performed in a vegetarian food store). Within this group, acceptability of PA6 product was 12.7% higher than the score reached by the general media, getting a value of 7.1.

4. Conclusions

The two formulations containing Andean potato and amaranth, with and without chia, resulted adequate substrates for fermentation with the folate-producing *L. sakei* CRL2210 strain. After 6 h of fermentation folate production reached 1800 ng/g and 890 ng/g in products with and without chia, respectively. Studies were carried out to assess the effect of fermentation in nutritional, textural, structural and sensorial features. There were no major changes in fatty acids profile in both products due to fermentation. Specially, in the product containing chia, the content of linolenic acid was maintained after fermentation and 28 days of cold storage. No differences in firmness, consistency and viscosity were registered in products during fermentation and storage.

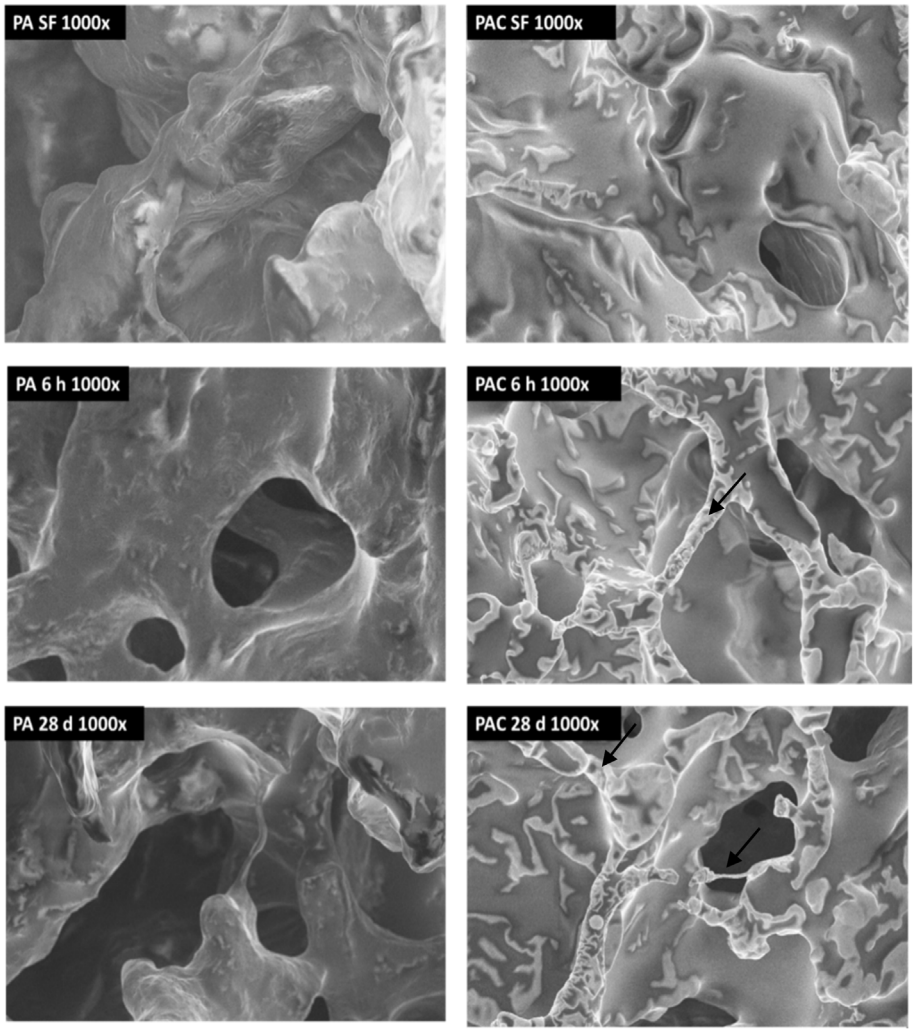

Fig. 5. Effects of chia incorporation and fermentation process in scanning electron micrographs (magnification 1000x).

Table 4
Instrumental color determination.

		SF	6 h	24 h	28 d
ΔE	PA	–	54.9	177.5	279.4
	PAC	–	0.7	48.2	151.0
L*	PA	76.5 ± 2.3 ^{aA}	80.4 ± 1.6 ^{bA}	81.8 ± 3.6 ^{bA}	83.2 ± 2.4 ^{cA}
	PAC	58.7 ± 4.2 ^{aB}	59.0 ± 2.6 ^{aB}	61.8 ± 4.8 ^{aB}	62.6 ± 5.6 ^{aB}
a*	PA	−0.1 ± 0.0 ^{aA}	0.4 ± 0.0 ^{bA}	0.5 ± 0.0 ^{bA}	0.6 ± 0.0 ^{bA}
	PAC	2.4 ± 0.1 ^{aB}	2.6 ± 0.1 ^{aB}	3.1 ± 0.1 ^{aB}	2.9 ± 0.0 ^{aB}
b*	PA	15.3 ± 1.8 ^{aA}	17.8 ± 2.7 ^{bA}	17.2 ± 1.2 ^{bA}	18.2 ± 2.9 ^{bA}
	PAC	11.3 ± 0.9 ^{aB}	12.4 ± 2.1 ^{bB}	12.7 ± 2.3 ^{bB}	14.2 ± 1.6 ^{cB}
BI	PA	21.7 ± 1.6 ^{aA}	24.8 ± 0.9 ^{bA}	23.5 ± 1.4 ^{bA}	24.6 ± 1.8 ^{bA}
	PAC	24.0 ± 0.8 ^{aB}	26.4 ± 1.7 ^{bA}	26.2 ± 2.1 ^{bA}	28.6 ± 1.3 ^{cB}

a–c: different letters in the same row denote differences ($P < 0.05$) in the same formulation (PA or PAC) during fermentation/storage.
A–C: different letters in the same column denote differences ($P < 0.05$) between PA and PAC for each parameter at different fermentation/storage time.

Neither syneresis nor changes in particle size distribution were detected. These parameters indicated stability in structures, particularly amaranth particles could be exerting an emulsion stabilizing role. The results of the sensory analysis indicated that the product without chia and fermented for 6 h had the highest acceptability score, highlighting appearance and texture attributes. Even though chia incorporation improved alpha linoleic acid content and was associated with an increase in folate production, the prototype product was not accepted by the consumers due to its dark color and general bad aspect which are consistent with the use of chia flour. Further modifications of the formulation will be required to mask these undesirable traits before commercialization, but the fermentation was clearly beneficial in improving the nutritional, textural and structural aspects of this novel food.

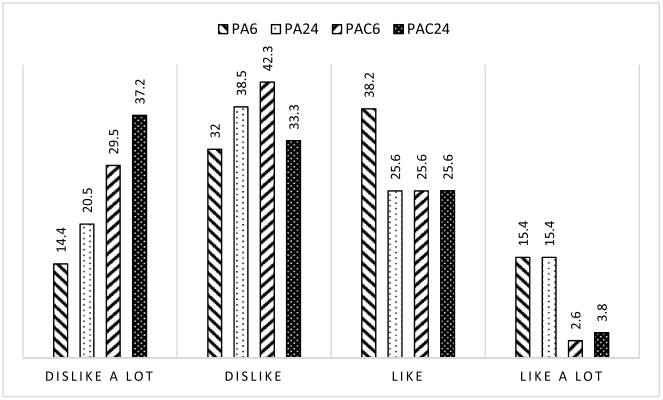

Fig. 6. Consumers aceptability of fermented PA and PAC products.

The application of bio-fortification of vegan products using folate-producing probiotic LAB is an interesting alternative to the use of synthetic folic acid in foods and provides a strategy for the development of functional foods with increased nutritional value.

CRediT authorship contribution statement

Ana Laura Mosso: Methodology, Conceptualization, Formal analysis, Writing - original draft. **Jean Guy LeBlanc:** Methodology, Conceptualization, Resources, Writing - original draft. **Carla Motta:** Validation, Methodology. **Isabel Castanheira:** Methodology, Validation, Resources. **Pablo Ribotta:** Methodology, Validation, Resources. **Norma Sammán:** Conceptualization, Investigation, Resources, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

None.

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References

Albuquerque, M. A. C., Bedani, R., LeBlanc, J. G., & Saad, S. M. I. (2017). Passion fruit by-product and fructooligosaccharides stimulate the growth and folate production by starter and probiotic cultures in fermented soymilk. *International Journal of Food Microbiology, 261*, 35–41. https://doi.org/10.1016/j.ijfoodmicro.2017.09.001.

Albuquerque, M. A. C., Bedani, R., Vieira, A. D. S., LeBlanc, J. G., & Saad, S. M. I. (2016). Supplementation with fruit and okara soybean by-products and amaranth flour increases the folate production by starter and probiotic cultures. *International Journal of Food Microbiology, 236*, 26–32. https://doi.org/10.1016/j.ijfoodmicro.2016.07.008.

Bassett, M. N., & Sammán, N. C. (2010). Folate content and retention in selected raw and processed foods. *Archivos Latinoamericanos de Nutricion, 60*(3), 298–305.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*, 911–917.

Bressani, R., de Martell, E., & de Godinez, C. (1993). Protein quality evaluation of amaranth in adult humans. *Plant Foods for Human Nutrition, 43*, 123–143. https://doi.org/10.1007/BF01087917.

Chandra-Hioe, M., Bucknall, M., & Arcot, J. (2011). Folate analysis in foods by UPLC-MS/MS: Development and validation of a novel, high throughput quantitative assay; folate levels determined in Australian fortified breads. *Analytical and Bioanalytical Chemistry, 401*, 1035–1042. https://doi.org/10.1007/s00216-011-5156-3.

Colombo, A., León, A. E., & Ribotta, P. D. (2011). Rheological and calorimetric properties of corn-, wheat-, and cassava- starches and soybean protein concentrate composites. *Starch, 63*, 83–95. https://doi.org/10.1002/star.201000095.

Dickinson, E. (2010). Food emulsions and foams: Stabilization by particles. *Current Opinion in Colloid & Interface Science, 15*, 40–49. https://doi.org/10.1016/j.cocis.2009.11.001.

Espírito-Santo, A., Mouquet-Riviera, C., Humblot, C., Cazevieille, C., Icard-Verniere, C., Soccol, C., et al. (2014). Influence of cofermentation by amylolytic *Lactobacillus* strains and probiotic bacteria on the fermentation process, viscosity and microstructure of gruels made of rice, soy milk and passion fruit fiber. *Food Research International, 57*, 104–113. https://doi.org/10.1016/j.foodres.2014.01.028.

Fernandes, S., & Mellado, M. (2018). Development of mayonnaise with substitution of oil or egg yolk by the addition of chia (*Salvia Hispanica* L.) mucilage. *Journal of Food Science, 83*(1), 74–83. https://doi.org/10.1111/1750-3841.13984.

Iyer, R., & Tomar, S. K. (2009). Folate: A functional food constituent. *Journal of Food Science, 74*(9), 114–122. https://doi.org/10.1111/j.1750-3841.2009.01359.

Jia, R., Chen, A., & Ding, Y. (2016). Effects of fermentation with *Lactobacillus rhamnosus* GG on product quality and fatty acids of goat milk yogurt. *Journal of Dairy Science, 99*, 221–227. https://doi.org/10.3168/jds.2015-10114.

Kariluoto, S., Edelmann, M., Herranen, M., Lampi, A. M., Shmelev, A., Salovaara, H., et al. (2010). Production of folate by bacteria isolated from oat bran. *International Journal of Food Microbiology, 143*, 41–47. https://doi.org/10.1016/j.ijfoodmicro.2010.07.026.

Kariluoto, S., Edelmann, M., Nyström, L., Sontag-strohm, T., Salovaara, H., Kivelä, R., et al. (2014). *In situ* enrichment of folate by microorganisms in beta-glucan rich oat and barley matrices. *International Journal of Food Microbiology, 176*, 38–48. https://doi.org/10.1016/j.ijfoodmicro.2014.01.018.

Laiño, J. E., LeBlanc, J. G., & Savoy, G. (2012). Production of natural folates by lactic acid bacteria starter cultures isolated from artisanal Argentinean yogurts. *Canadian Journal of Microbiology, 58*, 581–588. https://doi.org/10.1139/w2012-026.

Leal-Castañeda, E. J., García-Tejeda, Y., Hernández-Sánchez, H., Alamilla-Beltrán, L., Téllez-Medina, D. I., Calderón-Domínguez, G., et al. (2018). Pickering emulsions stabilized with native and lauroylated amaranth starch. *Food Hydrocolloide, 80*, 177–185. https://doi.org/10.1016/j.foodhyd.2018.01.043.

Lee, S., Hwang, Y., Kim, M., Chung, M., & Kim, Y. (2019). Comparison of volatile and nonvolatile compounds in rice fermented by different lactic acid bacteria. *Molecules, 24*(6), 1183. https://doi.org/10.3390/molecules24061183.

Liew, S. C. (2016). Folic acid and diseases - supplement it or not? *Revista de la Asociación Médica Brasilera, 62*, 90–100.

Maskan, M. (2001). Kinetics of colour change of kiwifruits during hot air and microwave drying. *Journal of Food Engineering, 48*, 169–175. https://doi.org/10.1016/S0260-8774(00)00154-0.

Masotti, F., Cattaneo, S., Stuknytė, M., & De Noni, I. (2018). Status and developments in analogue cheese formulations and functionalities. *Trends in Food Science & Technology, 74*, 158–169. https://doi.org/10.1016/j.tifs.2018.02.016.

Matias, N. S., Bedani, R., Castro, I. A., & Saad, S. M. I. (2014). A probiotic soy-based innovative product as an alternative to petit-suisse cheese. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 59*, 411–417. https://doi.org/10.1016/j.lwt.2014.05.054.

Mosso, A. L., Jimenez, M. E., Vignolo, G., LeBlanc, J. G., & Samman, N. C. (2018). Increasing the folate content of tuber based foods using potentially probiotic lactic acid bacteria. *Food Research International, 109*, 168–174. https://doi.org/10.1016/j.foodres.2018.03.073.

Mun, S., Kim, Y., Kang, C., & Park, K. (2009). Development of reduced-fat mayonnaise using 4αGTase-modified rice starch and xanthan gum. *International Journal of Biological Macromolecules, 44*(5), 400–407. https://doi.org/10.1016/j.ijbiomac.2009.02.008.

Pompei, A., Cordisco, L., Amaretti, A., Zanoni, R., Matteuzzi, A., & Rossi, A. (2007). Folate production by bifidobacteria as a potential probiotic property. *Applied and Environmental Microbiology, 73*, 179–185. https://doi.org/10.1128/AEM.01763-06.

Pramudya, R., & Seo, H. (2018). Using check-all-that-apply (CATA) method for determining product temperature-dependent sensory-attribute variations: A case study of cooked rice. *Food Research International, 105*, 724–732. https://doi.org/10.1016/j.foodres.2017.11.075.

Rayner, M., Sj, M., Timgren, A., & Dejmek, P. (2012). Quinoa starch granules as stabilizing particles for production of Pickering emulsions. *Faraday Discussions, 158*, 139–155. https://doi.org/10.1039/c2fd20038d.

Saubade, Humblot, Hemery, & Guyot (2018). Influence of fermentation and other processing steps on the folate content of a traditional African cereal-based fermented food. *International Journal of Food Microbiology, 266*, 79–86. https://doi.org/10.1016/j.ijfoodmicro.2017.11.015.

Savoy, G., & LeBlanc, J. G. (2018). Folate production by lactic acid bacteria. *Canadian Journal of Microbiology, 58*(5), 581–588. https://doi.org/10.1016/B978-0-12-813008-7.00002-3 Polyphenols: Prevention and Treatment of Human Disease.

Soares, M. B., Martinez, R. C. R., Pereira, E. P. R., Balthazar, C. F., Cruz, A. G., Ranadheera, C. S., et al. (2019). The resistance of *Bacillus, Bifidobacterium*, and *Lactobacillus* strains with claimed probiotic properties in different food matrices exposed to simulated gastrointestinal tract conditions. *Food Research International, 125*, 108542. https://doi.org/10.1016/j.foodres.2019.108542.

Timgren, A., Rayner, M., Dejmek, P., & Marku, D. (2013). Emulsion stabilizing capacity of intact starch granules modified by heat treatment or octenyl succinic anhydride. *Food Sciences and Nutrition, 1*(2), 157–171. https://doi.org/10.1002/fsn3.17.

Timilsena, Y., Adhikari, R., Kasapis, S., & Adhikari, B. (2016). Molecular and functional characteristics of purified gum from Australian chia seeds. *Carbohydrate Polymers, 136*, 128–136. https://doi.org/10.1016/j.carbpol.2015.09.035.

Youngblood, M. E., Williamson, R., Bell, K. N., Johnson, Q., Kancherla, V., & Oakley, G. P. (2013). Update on global prevention of folic acid-preventable spina bifida and anencephaly. *Birth Defects Research Part A. Clinical and Molecular Teratology, 97*(10), 658–663. https://doi.org/10.1002/bdra.23166.