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# Functional goat milk cheese with feruloyl esterase activity

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ABSTRACT

The aim of this paper was to evaluate the effect of the intake of goat milk cheese manufactured with *Lactobacillus fermentum* CRL1446 on intestinal feruloyl esterase (FE) activity and oxidative status in Swiss albino mice. This strain was used as single-strain culture (CRL cheese) and in combination with starter culture (Mix cheese). In both cheeses, *L. fermentum* reached 8–9 log cfu/g and FE activity increased during ripening. Highest activity level was observed in Mix cheese. *In vivo* studies showed that total intestinal FE activity in mice fed with CRL and Mix cheeses increased 1.5 and 2-fold compared to non-treated mice, respectively. Administration of Mix cheese produced a 2-fold increase in FE activity in small and large intestine mucosa. Mice receiving this cheese also showed an approx. 2-fold decrease in plasmatic thiobarbituric acid-reactive substances (TBARS) levels and an approx. 3-fold increase in glutathione reductase (GR) activity.

Goat milk cheese elaborated with FE-producing strain *L. fermentum* CRL1446 could represent a novel functional food with FE activity, responsible for increasing intestinal FE activity and consequently the bioavailability of antioxidant ferulic acid in the gut, thus enhancing the oxidative status and providing protection against oxidative stress-related disorders.



## 1. Introduction

Goat milk and its products such as yoghurt and cheese are highly appreciated for their high nutritional value (Haenlein, 2004). Goat milk has been described as having a higher digestibility and lower allergenic properties than cow milk. In addition, goat milk has been attributed with certain therapeutic values in human nutrition (Alferez et al., 2001; Barrionuevo, Alferez, Lopez-Aliaga, Sanz-Sampelayo, & Campos, 2002; Díaz-Castro et al., 2012). The possibility to improve the nutritional benefits of these dairy products by enriching them with probiotic strains is of great interest for human health.

Functional dairy foods are currently restricted to beverages such as fermented milk and yoghurt, which have limited shelf life, in contrast to cheeses that have a higher temporary storage capacity (Stanton et al., 1998). Cheese is the most suitable dairy product for carrying probiotic strains, due to its higher pH value, high content of fat, and solid matrix, that protect

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*Abbreviations*: CBD, conventional balanced diet; CE, cinnamoyl esterase; CERELA, Centro de Referencia para Lactobacilos; CFU, colony-forming units; CRL, Culture Collection of Centro de Referencia para Lactobacilos; EtF, ethyl ferulate; FA, ferulic acid; FE, feruloyl esterase; GR, glutathione reductase; HA, hydroxycinnamic acid; LAB, lactic acid bacteria; LIC, large intestine content; LIM, large intestine mucosa; MRS, Man–Rogosa–Sharpe medium; MtF, methyl ferulate; NSLAB, non-starter lactic acid bacteria; NTG, non-treated group; PBS, phosphate buffer saline; RCA, Reinforced Clostridial Agar; SIC, small intestine content; SIM, small intestine mucosa; TBARS, thiobarbituric acid-reactive substances.

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bacteria more efficiently than fermented milk during intestinal transit (da Cruz, Buriti, de Souza, Fonseca, & Saad, 2009; Ranadheera, Baines, & Adams, 2010; Vinderola, Mocchiutti, & Reinheimer, 2002). There are numerous studies on probiotic cheeses, such as Cheddar (Phillips, Kailasapathy, & Tran, 2006), Gouda (Gomes, Malcata, Klaver, & Grande, 1995), Cottage (Araújo, de Carvalho, Leandro, Furtado, & de Moraes, 2010; Blanchette, Roy, Belanger, & Gauthier, 1996), Turkish Beyaz cheese (Başyiğit, Kuleaşan, Eralp, & Karahan, 2009), Argentinean cheese (Bergamini, Hynes, Quiberoni, Suárez, & Zalazar, 2005) and "Pikantne" goat cheeses (Songisepp et al., 2004).

Most probiotic cheeses carry bifidobacteria and non-starter lactic acid bacteria (NSLAB) such as *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* (Ong, Henriksson, & Shah, 2006) as probiotic strains. Up to date, there are few studies on probiotic cheese manufactured with *Lactobacillus fermentum* (Songisepp et al., 2004). Recently, Settanni and Moschetti (2010) discussed the health benefits attributable to NSLAB species involved in cheese production. Cheeses carrying NSLAB producers of bioactive substances such as peptides with angiotensin-converting-enzyme inhibitor activity (Pripp, Sorensen, Stepanek, & Sorhaug, 2006), conjugated linoleic acid with antioxidant properties (Van Nieuwenhove, Gauffin Cano, Pérez-Chaia, & González, 2011), and NSLAB with antigenotoxic properties (Corsetti et al., 2008) have been reported in the literature. In this paper we proposed that goat milk cheese could also be used to carry bacteria with enzymatic activities that play an important role in human health, such as feruloyl esterases.

Feruloyl esterases (FE) are carboxyl ester hydrolases that degrade esters of ferulic acid (FA). They belong to the group of enzymes known as cinnamoyl esterases (CE), that hydrolyze hydroxycinnamate esters, which are commonly found in cereals, fruits and vegetables, releasing hydroxycinnamic acids (HA) such as ferulic, sinapic, caffeic and *p*-coumaric acids (Fazary & Ju, 2007). These phenolic compounds are part of human and animal diets and may contribute to the beneficial effects derived from the consumption of cereal bran, due to their chemoprotective and antioxidant properties (Fazary & Ju, 2007; Vitaglione, Napolitano, & Fogliano, 2008). Ferulic acid (FA) induces intrinsic antioxidant mechanisms such as superoxide dismutase, catalase and glutathione reductase (GR) activities. Regular ingestion of this acid may provide substantial protection against oxidative stress-related ailments like cancer, diabetes, cardiovascular and neurodegenerative diseases, and in ageing (Srinivasan, Sudheer, & Menon, 2007).

Andreasen, Kroon, Williamson, and García-Conesa (2001) reported the release of sinapic and *p*-coumaric acids from rye and wheat brans by human colonic esterase(s). These authors demonstrated that intestinal CE activity may have an epithelial and a microbial origin.

CE activity is commonly found in different bacterial genera present in the human gut (Couteau, McCartney, Gibson, Williamson, & Faulds, 2001; Lai, Lorca, & González, 2009). CE activity has been demonstrated in the large intestine microbiota of rats and humans (Buchanan, Wallace, & Fry, 1996; Kroon, Faulds, Ryden, Robertson, & Williamson, 1997), and levels and specificity of these enzymes are critical factors influencing the bioavailability of HA (Buchanan et al., 1996).

Abeijón Mukdsi, Gauffin Cano, González, and Medina (2012) evaluated the effect of oral administration of *L. fermentum* CRL1446, strain with FE activity, in Swiss albino mice fed a conventional balanced diet containing hydroxycinnamate esters. These authors observed increases in total intestinal FE activity, decreases in plasmatic TBARS levels and increases in GR activity, compared to non-treated mice. Therefore, *L. fermentum* CRL1446 would enhance the bioavailability of FA, thus improving oxidative status of mice (Abeijón Mukdsi et al., 2012). Beneficial effects of lactic acid bacteria (LAB) with FE activity have also been reported by Bhathena et al. (2009). These authors found that the administration of encapsulated cells of *L. fermentum* ATCC11976 with FE activity to diet-induced hypercholesterolemic hamsters produced significant reductions in serum total cholesterol, LDL cholesterol and triglyceride levels.

At present, there is little information about functional foods carrying LAB with CE or FE activity. Moreover, the effect of the consumption of these products on intestinal esterase activity has never been studied. Guglielmetti et al. (2008) developed a fermented food with high total antioxidant and potential probiotic properties, manufactured with *Lactobacillus helveticus* MIMLh5 with strong CE activity, but these authors did not perform *in vivo* studies.

The aim of this paper was to evaluate the effect of the intake of goat cheeses manufactured with *L. fermentum* CRL1446 on intestinal FE activity and oxidative status in Swiss albino mice.

## 2. Materials and methods

### 2.1. Bacterial strain and growth conditions

*L. fermentum* CRL1446, strain isolated from Argentinean goat cheese (Oliszewski, Medina, González, & Pérez Chaia, 2007), was obtained from the Culture Collection of the Centro de Referencia para Lactobacilos (CERELA, Tucumán, Argentina). This strain presented *in vitro* FE activity and resistance to simulated gastrointestinal tract conditions (Abeijón Mukdsi, 2009). *L. fermentum* CRL1446 was maintained in Man–Rogosa–Sharpe broth (MRS; Britania, Buenos Aires, Argentina) containing 20% (v/v) glycerol at −70 °C, and propagated three times in MRS broth before each experimental use.

### 2.2. Goat milk cheese manufacture

Cheeses were manufactured according to the protocol of Abeijón Mukdsi et al. (2009). The process was carried out aseptically in a laminar air-flow unit (Cleanroom Products, Inc., New York, NY, USA). Sterile flasks were filled with 400 ml of raw goat milk, and it was then pasteurized in thermostatic bath at 65 °C for 30 min. After cooling to 38 °C, each batch was inoculated with 1% (v/v) (~$10^8$ cfu/ml) culture of the corresponding strains in sterilized reconstituted (10%, w/v) skim milk. Four cheeses were manufactured:

(i) Starter cheese, elaborated with a starter culture constituted by *Lactobacillus delbrueckii* subsp. *bulgaricus* CRL1447 and *Streptococcus thermophilus* CRL739 (1%, v/v)

(ii) CRL cheese, elaborated with *L. fermentum* CRL1446 (1%, v/v)
(iii) Mix cheese, elaborated with starter culture and *L. fermentum* CRL1446 (1%, v/v, each)
(iv) Curd, elaborated without culture addition and pH-adjusted to 5.2 with lactic acid.

Inoculated milk was then incubated at 37 °C until the pH dropped to 6.4. After 30 min, sterile calcium chloride (0.1 g/l of milk) and microfiltered (0.22 μm, white GSWP, 25 mm, Millipore Corp., Bedford, MA, USA) rennet solution from *Kluyveromyces lactis* (MAXIREN 150. 9000 mg/l, Gist-brocades, Delft, The Netherlands) were added. Coagulation time was controlled by rocking and turning the bottles gently to test casein adhesion to their sides. Clotting took place in 25 min, after which the curd was cut in nut size cubes with a sterile knife and each flask was upturned 10 times. The mass was cooked for 5 min at 45 °C, and it was placed in sterile flasks using sterile tablespoons.

The curd was first centrifuged at 1700*g* (Damon/IEC Division IEC B-20A, International Equipment Company, Needham Heights, MA, USA) for 10 min, and then at 5000*g* for 20 min. Whey was discarded in both cases. The curd was laid in 100 ml sterile glass flasks and allowed to rest until reaching pH 5.2 at 25 °C. Sterile brine was added to each flask (330 g/l NaCl, pH-adjusted to 5.2 with lactic acid) and discarded after 5 min. Cheeses were ripened inside the flasks for 60 days (12 °C and 80% relative humidity). Four cheeses of approx. 100 g were manufactured from the same batch of milk. Cheese manufacture was performed in triplicate.

Three samples (a cheese from each batch) were taken at 1, 30 and 60 days of ripening for FE activity determination and microbiological analyses.

### 2.3. Determination of feruloyl esterase activity in cheese

Cheese samples (10 g) were dispersed in 90 ml of PBS pH 7.0 and homogenized for 2 min in a Stomacher (Laboratory Blender 400, Seward Medical, London, UK). Two hundred microlitres of homogenate were incubated in the presence of 1 mM methyl ferulate (MtF) as substrate at 37 °C for 18 h. Reactions were stopped by the addition of glacial acetic acid. Controls containing the reaction mixture plus glacial acetic acid were also incubated to test for the presence of background peaks. Samples and controls were centrifuged (13,000*g*, 10 min, 4 °C) and filtered (0.22 μm) prior to HPLC analysis of released FA. Results were expressed as units (U) of FE activity per gram of cheese. One unit was defined as the amount of enzyme releasing 1 mmol of FA per hour.

### 2.4. Determination of hydroxycinnamic acids by HPLC

Separations were performed on a Knauer system (Berlin, Germany) equipped with an UV detector, using a reverse-phase C-18 column (ReproSil-Pur ODS, 3.5 μm, 250 × 4.6 mm, Dr. Maisch GmbH, Ammerbuch, Germany). Twenty microlitres of sample were injected and eluted isocratically with a mixture of water/acetonitrile/acetic acid (69:30:1, v/v/v) at a flow rate of 1 ml/min. Compounds were monitored by absorbance at 320 nm. Released HA were quantified from the regression curve ($R^2 > 98\%$) of the corresponding standard (Apin Chemicals, Abingdon, OX, UK), using external standard calibration.

### 2.5. Cheese chemical composition

Total solids, fat and total nitrogen contents were analyzed according to the FIL-IDF standards 4A (1982); 5B (1986) and 20B (1993), respectively. Cheese pH was determined with pH-meter Metrohm 962 (Metrohm AG, Herisau, Switzerland).

### 2.6. Cheese microbiota counts

Cheese samples (10 g) were homogenized in 90 ml of peptone water (0.1%, w/v), and serial dilutions were plated in agarized media. MRS medium was used for starter culture counts and MRS-EtF [MRS without glucose, supplemented with 1 g/l ethyl ferulate (Sigma, St. Louis, MO, USA)] was used for *L. fermentum* CRL1446 counts, whose colonies were characteristically surrounded by a clear zone due to the hydrolysis of EtF. Plates were incubated for 48 h at 42 °C for starter culture, and at 37 °C for *L. fermentum*.

### 2.7. Animals and diet

Six-week-old male Swiss albino mice ($n = 60$) were obtained from the closed random-bred colony maintained at CERELA. They were housed in individual cages and acclimated to 22 ± 2 °C with a 12 h light/dark cycle. All mice received daily conventional balanced diet (CBD) and drinking water *ad libitum*. Composition of CBD was: 60.8% carbohydrates, 25.5% proteins, 3.8% fats, 3.4% raw fibre (from maize), 6.5% total minerals (Asociación de Cooperativas Argentinas, Buenos Aires, Argentina). This diet supplied ~0.60 mg of hydroxycinnamates per day per mouse.

All animal protocols were approved by the Animal Protection Committee of CERELA, and complied with current Argentinean laws.

### 2.8. Feeding procedures

Mice from each treated group were daily fed a mixture of CBD and 60-day-ripened cheese, adjusting the dose of *L. fermentum* CRL1446 to $10^7$ cfu/g of cheese. This food was prepared estimating that each mouse consumes approx. 5 g of food per day.

All cheeses had similar chemical composition (12–13% total nitrogen, 47–50% fat and 42–44% total solids).

The experimental protocol comprised four treated groups ($n = 12$ each) and a non-treated group ($n = 12$):

(i) Curd group, receiving CBD and curd
(ii) Starter group, receiving CBD and Starter cheese
(iii) CRL group, receiving CBD and CRL cheese
(iv) Mix group, receiving CBD and Mix cheese
(v) Non-treated group (NTG), receiving CBD

Animals were daily fed during 7 days and had access to water *ad libitum*. They were fasted for 16 h before sacrifice, and sacrificed by cervical dislocation at day 7. Twelve animals from each five groups were sacrificed to evaluate intestinal FE

activity ($n = 6$), microbiota ($n = 6$), TBARS levels ($n = 12$) and GR activity in plasma ($n = 12$). The Curd group was incorporated to evaluate whether the curd itself may have an effect on the evaluated parameters.

### 2.9. Preparation of intestinal extracts

Small and large intestines were aseptically removed and different intestinal sections (SIM, small intestine mucosa; LIM, large intestine mucosa; SIC, small intestine content; LIC, large intestine content) were obtained according to Abeijón Mukdsi et al. (2012). Samples were kept on ice prior to FE activity determination.

### 2.10. Determination of intestinal feruloyl esterase activity

Intestinal FE activity was determined in intestinal mucosa and content by incubation in PBS pH 7.0 containing 1 mM methyl ferulate as substrate at 37 °C for 18 h. Reactions were stopped by the addition of glacial acetic acid. Controls containing the reaction mixture plus glacial acetic acid were also incubated to test for the presence of background peaks. Samples and controls were centrifuged (13,000*g*, 10 min, 4 °C) and filtered (0.22 μm) prior to HPLC analysis of released FA. Results were expressed as units (U) of FE activity per gram of intestinal extract.

### 2.11. Intestinal microbiota counts

Large intestines were aseptically removed, weighted and homogenized in 5 ml of peptone water (0.1%, w/v). Serial dilutions of the homogenized samples were plated in agarized media (Britania, Buenos Aires, Argentina): Reinforced Clostridial (RCA) for total anaerobic bacteria; RCA containing 0.2% (w/v) LiCl, 4 mg/l colistin, 1% (w/v) aniline blue and adjusted to pH 5.0 with acetic acid after sterilization (RCA pH 5) for bifidobacteria; MRS for total lactobacilli; Brucella containing 20 ml/l blood and 7.5 μg/ml vancomycin for *Bacteroides*, and Mac Conkey for *Enterobacteriaceae*. Plates were anaerobically incubated at 37 °C for 48–96 h. At the end of the incubation period, colonies were counted and results were expressed as logarithm$_{10}$ of colony-forming units (cfu) per gram of intestine.

### 2.12. Preparation of plasma

Blood was collected by cardiac puncture and transferred into tubes containing anticoagulant EDTA (Wiener Lab, Rosario, Argentina). Plasma was obtained by centrifugation (5000*g*, 5 min), and used for the TBARS assay and determination of GR activity.

### 2.13. Thiobarbituric acid-reactive substances assay

Lipid peroxidation was estimated spectrophotometrically by measuring thiobarbituric acid-reactive substances (TBARS) concentrations according to Okawa, Ohsihi, and Yagi (1979). Results were expressed as nmol of TBARS per mg of protein.

### 2.14. Determination of glutathione reductase activity

Glutathione reductase (GR) activity was determined according to Esterbauer and Grill (1978), by following the rate of NADPH oxidation at 340 nm. Results were expressed as units (U) of GR activity per mg of protein. One unit was defined as the amount of enzyme producing 1 nmol of oxidized NADP per minute.

### 2.15. Protein determination

Protein concentrations were determined according to the method of Bradford (1976), using a commercial kit (Bio-Rad, Hercules, CA, USA) and bovine serum albumin (Sigma, St. Louis, MO, USA) as standard.

### 2.16. Statistical analysis

Results are means of three independent experiments ± standard deviation (SD). After the analysis of variance (ANOVA), Tukey's test was used to identify statistically significant differences ($p < 0.05$). These analyses were performed with the software package Minitab14 (Minitab Inc., State College, PA, USA).

## 3. Results

### 3.1. Feruloyl esterase activity, microbiota counts and end pH of goat cheeses

FE activity was determined in cheeses manufactured with starter culture (Starter cheese), *L. fermentum* CRL1446 (CRL cheese), starter culture plus *L. fermentum* CRL1446 (Mix cheese) and cheese elaborated without addition of cultures (curd) at day 1, 30 and 60 of ripening (Table 1). FE activity was observed in CRL and Mix cheeses since the beginning of ripening, and increased until day 60. FE activity in Mix cheeses was approx. 2-fold higher than in CRL cheeses at both day 30 and 60 of ripening. No FE activity was detected in curd and Starter cheese until day 60.

In Starter cheese, microbiota counts decreased 2.5 log units throughout ripening (Table 1). In curd, microbiota reached approx. 2 log cfu/g at the end of ripening. Microbiota of CRL and Mix cheeses increased approx. 1 log unit during the 60 days of ripening, when bacterial growth was evaluated in MRS-EtF medium. In Mix cheese, *L. fermentum* CRL1446 reached higher counts (9.38 ± 0.09 log cfu/g) than in CRL cheese (8.73 ± 0.07 log cfu/g). At day 60, the behaviour of starter culture microbiota was similar in Mix and Starter cheeses (data not shown). In all cheeses, pH values decreased approx. 0.10 units after 60 days of ripening.

### 3.2. Effect of goat cheese administration on intestinal FE activity

FE activity in each intestinal fraction is shown in Table 2. In NTG, intestinal FE activity was mainly localized in mucosa, being 1.5-fold higher in SIM than LIM. Regarding intestinal contents, FE activity was 2-fold higher in LIC than SIC.

**Table 1 – Feruloyl esterase activity, microbiota and end pH in cheese.**

| Ripening time (days) | Starter cheese | | | CRL cheese | | | Mix cheese | | | Curd | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | FE activity[A] | Log cfu/g[B] | pH | FE activity | Log cfu/g[C] | pH | FE activity | Log cfu/g[C] | pH | FE activity[A] | Log cfu/g[B] | pH |
| 1 | n.d. | $7.39 \pm 0.40^{a}$ | $4.95 \pm 0.08^{a}$ | $0.05 \pm 0.01^{a}$ | $8.05 \pm 0.22^{a}$ | $5.70 \pm 0.06^{a}$ | $0.06 \pm 0.01^{a}$ | $8.83 \pm 0.16^{a}$ | $4.81 \pm 0.08^{a}$ | n.d. | $2.40 \pm 0.13^{a}$ | $5.25 \pm 0.08^{a}$ |
| 30 | n.d. | $5.92 \pm 0.15^{b}$ | $4.80 \pm 0.07^{a}$ | $0.09 \pm 0.01^{b}$ | $8.30 \pm 0.05^{a}$ | $5.64 \pm 0.05^{a}$ | $0.23 \pm 0.02^{b}$ | $9.01 \pm 0.12^{a}$ | $4.88 \pm 0.09^{a}$ | n.d. | $2.32 \pm 0.15^{a}$ | $5.08 \pm 0.07^{a}$ |
| 60 | n.d. | $4.80 \pm 0.32^{c}$ | $4.84 \pm 0.06^{b}$ | $0.15 \pm 0.01^{c}$ | $8.73 \pm 0.07^{b}$ | $5.59 \pm 0.05^{b}$ | $0.34 \pm 0.01^{c}$ | $9.38 \pm 0.09^{b}$ | $4.72 \pm 0.07^{b}$ | n.d. | $1.80 \pm 0.12^{b}$ | $5.10 \pm 0.07^{b}$ |

Data are mean ± SD ($n = 3$). Means in the same column without a common superscript letter (a–c) differ significantly ($p < 0.05$). n.d., not detected.

[A] Results are expressed as units (U) of feruloyl esterase (FE) activity per gram of cheese. U = mmol ferulic acid released per hour.

[B] cfu/g of starter culture detected in MRS agar medium.

[C] cfu/g of *L. fermentum* CRL1446 detected in MRS-EtF agar medium.

No statistically significant differences were observed in total intestinal FE activities between NTG and the groups fed with curd and Starter cheese. Mice fed with CRL and Mix cheeses presented a total intestinal FE activity 1.5 and 2-fold higher than NTG, respectively. In SIM, FE activity was approx. 2-fold higher in CRL and Mix groups than in NTG, whereas no statistically different activities were observed between Curd, Starter and non-treated groups. In LIM, FE activity was 1.8 and 2.3-fold higher in Curd and Mix groups than in NTG, respectively. No statistically different activities in LIM were observed in Starter and CRL groups. FE activity in SIC in all treated groups was slightly lower than in NTG. No statistical differences in FE activity in LIC were observed between treated and non-treated groups.

### 3.3. *Effect of goat cheese administration on intestinal microbiota*

To determine whether administration of goat cheese with FE activity quantitatively altered intestinal microbiota, log cfu/g of main intestinal bacterial genera was evaluated in large intestine homogenates at day 7 of feeding. Counts of main colonic bacterial genera in treated and non-treated mice are shown in Table 3. In Curd group, statistically significant increase (approx. 1 log cfu/g) in anaerobic bacteria population was detected. No significant changes in intestinal bacterial population were observed in Starter, CRL and Mix groups at the end of treatment, compared to NTG.

### 3.4. *Effect of goat cheese administration on lipid peroxidation*

To assess whether administration of goat cheeses with FE activity could affect the oxidative status of mice, plasmatic TBARS concentration, a marker of lipid peroxidation, was determined in treated and non-treated mice (Fig. 1). TBARS concentrations were significantly lower in mice from CRL and Mix group (1.3-fold and 1.5-fold, respectively), compared to the NTG. However, no significant statistical differences in TBARS levels were observed between Curd, Starter and non-treated groups.

### 3.5. *Effect of goat cheese administration on plasmatic glutathione reductase activity*

GR activity determined in plasma of mice fed with goat cheeses and non-treated mice is shown in Fig. 2. Mice fed with CRL and Mix cheeses showed a statistically significant increase in GR activity (1.4 and 2.7-fold, respectively), compared to NTG. No significant statistical differences in GR activity levels were observed between Curd, Starter and non-treated groups.

## 4. Discussion

Fermented dairy foods constitute 90% of the products in the probiotic market (Meyer, 2007) and a growing sector in functional food market (Ozer & Kirmaci, 2010). They have revealed good abilities to protect bacteria against digestive stresses (Ranadheera et al., 2010). Numerous studies have demonstrated that the

**Table 2 – Intestinal feruloyl esterase activity in mice fed with goat milk cheeses.**

| Source of enzyme | FE activity[A] | | | | |
|---|---|---|---|---|---|
| | NTG | Treated groups | | | |
| | | Curd | Starter | CRL | Mix |
| SIM | 5.91 ± 1.11[a] | 5.62 ± 1.04[a] | 6.47 ± 0.89[a] | 10.42 ± 1.48[b] | 11.44 ± 1.33[b] |
| LIM | 3.91 ± 0.51[a] | 7.21 ± 0.88[b] | 5.31 ± 0.93[a] | 4.89 ± 0.63[a] | 9.17 ± 1.00[c] |
| SIC | 0.46 ± 0.09[a] | 0.33 ± 0.05[b] | 0.37 ± 0.09[b] | 0.33 ± 0.04[b] | 0.33 ± 0.03[b] |
| LIC | 0.78 ± 0.12[a] | 0.59 ± 0.10[a] | 0.58 ± 0.07[a] | 0.71 ± 0.10[a] | 0.84 ± 0.13[a] |
| Total intestinal FE activity[B] | 11.06 ± 1.61[a] | 13.74 ± 1.50[a] | 12.72 ± 1.16[a] | 16.35 ± 1.70[b] | 21.78 ± 1.69[c] |

Data are presented as mean ± SD ($n$ = 6/group for each treatment). SIM, small intestine mucosa; LIM, large intestine mucosa; SIC, small intestine content; LIC, large intestine content. Means in the same row without a common superscript letter (a–c) differ significantly ($p < 0.05$). NTG, non-treated group.

[A] Results are expressed as units (U) of feruloyl esterase activity (FE) per gram of intestinal mucosa or content. U = mmol ferulic acid released per hour.

[B] Total intestinal FE activity = sum of FE activity of all intestinal fractions.

**Table 3 – Cell counts of intestinal bacterial microbiota in mice fed with goat milk cheeses and non-treated mice.**

| Treated groups | Microorganisms[A] | | | | |
|---|---|---|---|---|---|
| | Lactobacilli | Bifidobacteria | Bacteroides | Anaerobic bacteria | Enterobacteria |
| Curd | 10.67 ± 0.95[a] | 10.03 ± 0.78[a] | 11.24 ± 0.64[a] | 11.44 ± 0.20[b] | 7.02 ± 0.39[a] |
| Starter | 9.76 ± 0.72[a] | 9.02 ± 0.56[a] | 10.88 ± 0.24[a] | 10.67 ± 0.13[a] | 6.74 ± 0.23[a] |
| CRL | 9.58 ± 0.67[a] | 9.68 ± 0.40[a] | 10.48 ± 0.78[a] | 10.50 ± 0.62[a] | 5.83 ± 1.10[a] |
| Mix | 9.52 ± 1.04[a] | 9.23 ± 0.78[a] | 9.79 ± 0.90[a] | 9.83 ± 0.60[a] | 5.50 ± 0.90[a] |
| NTG | 10.68 ± 0.56[a] | 9.85 ± 0.99[a] | 10.58 ± 0.45[a] | 10.62 ± 0.26[a] | 6.25 ± 0.90[a] |

Data are mean ± SD ($n$ = 6/group at each treatment). Means in the same column without a common superscript letter (a and b) differ significantly from NTG ($p < 0.05$). NTG, non-treated group.

[A] Results are expressed as $\log_{10}$ cfu/g of large intestine.

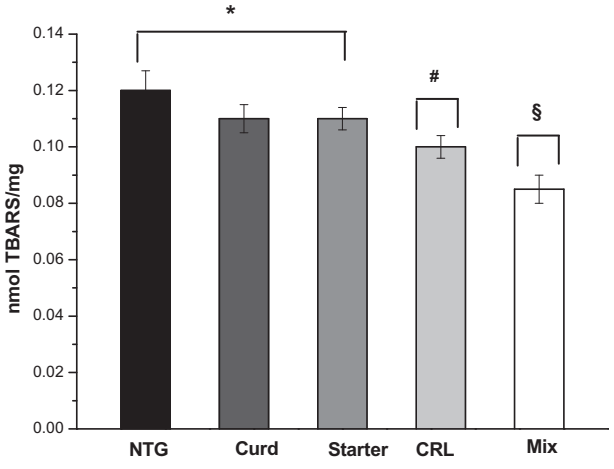


**Fig. 1 – Thiobarbituric acid-reactive substances (TBARS) levels in plasma of mice fed with goat milk cheeses with feruloyl esterase activity. NTG, non-treated group; Curd, mice fed with curd for 7 days; Starter, mice fed with Starter cheese for 7 days; CRL, mice fed with CRL cheese for 7 days; Mix, mice fed with Mix cheese for 7 days. Data are presented as mean ± SD ($n$ = 12/group). Means for each value without any common symbols (*, #, §) differ significantly ($p < 0.05$).**

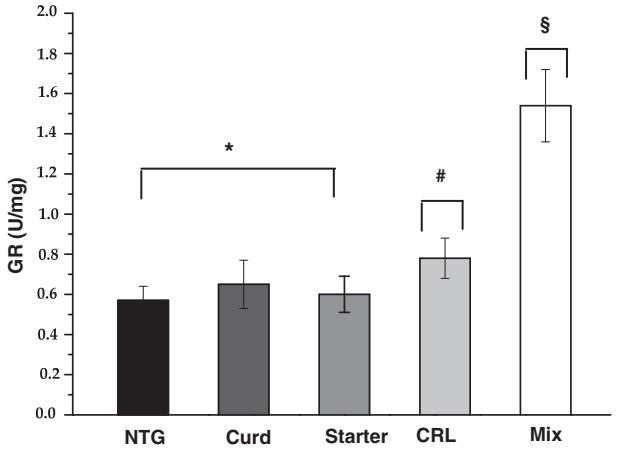


**Fig. 2 – Glutathione reductase (GR) activity in plasma of mice fed with goat milk cheeses with feruloyl esterase activity. NTG, non-treated group; Curd, mice fed with curd for 7 days; Starter, mice fed with Starter cheese for 7 days; CRL, mice fed with CRL cheese for 7 days; Mix, mice fed with Mix cheese for 7 days. Data are presented as mean ± SD ($n$ = 12/group). Means for each value without any common symbols (*, #, §) differ significantly ($p < 0.05$).**

addition of probiotic bacteria such as bifidobacteria and lactobacilli in functional foods has beneficial effects for human health; therefore, these foods have become the primary choice for consumers (Ranadheera et al., 2010; Saxelin, Tynkkynen, Mattila-Sandholm, & de Vos, 2005).

Goat cheese is highly nutritious in terms of its high protein content in relation to calorie and fat contents, and might be further improved by the addition of probiotic cultures (Gomes & Malcata, 1998). Songisepp et al. (2004) reported a goat cheese "Pikantne" with antimicrobial and antioxidant properties manufactured with *L. fermentum* ME-3. Kullisaar et al. (2003) demonstrated that the consumption of fermented milk containing this *L. fermentum* strain exhibited antioxidant and antiatherogenic effects in healthy volunteers.

In this paper we studied for the first time the effect of the intake of goat milk cheese with FE activity on the intestinal esterase activity and oxidative status in mice. Goat cheeses were manufactured with *L. fermentum* CRL1446, strain that presented strong FE activity, and was able to tolerate the gastrointestinal tract conditions (acid pH and presence of bile salts) (Abeijón Mukdsi, 2009). This strain also had carboxyl esterase activity involved in the production of flavour compounds *in vitro* and in goat cheeses (Abeijón Mukdsi, 2009; Abeijón Mukdsi, Medina, Álvarez, & González, 2009; Oliszewski et al., 2007).

*L. fermentum* CRL1446 was used as single-strain culture (CRL cheese) or as adjunct culture (Mix cheese). In both cheeses, FE activity increased during ripening, reaching the highest level in Mix cheese at the end of ripening. These results suggested that in the presence of starter culture, FE activity of CRL1446 strain would be stimulated. Starter culture strains did not present FE activity (data not shown), which was in agreement with the absence of FE activity in Starter cheese. In addition, *L. fermentum* CRL1446 cell counts were similar (~9 log cfu/g) in CRL and Mix cheeses, whereas the starter counts decreased ~2.5 log cfu/g at day 60 of ripening. These results would indicate that the starter culture could lyse and then promote the growth of *L. fermentum* CRL1446, or stimulate its FE activity in Mix cheese.

Minimal concentration of probiotic that should be present at the moment of intake to assure a favourable impact on consumer's health is about $10^7$ cfu/g or ml of food (De Vuyst, 2000). In our experimental protocol, CBD was mixed with 60-day-ripened cheese to obtain this concentration of *L. fermentum* CRL1446 in daily portion.

In general, FE enzymes of LAB present optimal activity at pH values of 6–8 and temperatures of 20–37 °C (Lai et al., 2009; Wang, Geng, Egashira, & Sanada, 2004). Results obtained in this study showed that at pH values of ~5 (cheese pH at the end of ripening) and 12 °C (ripening temperature), FE of *L. fermentum* CRL1446 remained active for 60 days. Therefore, goat cheese elaborated with *L. fermentum* CRL1446 and ripened for 60 days, presented suitable number of viable cells and FE activity to positively impact on the host.

*In vivo* studies described in this paper, about the effect of the administration of goat cheese with FE activity, showed that total intestinal FE activity in mice from CRL and Mix groups increased 1.5 and 2-fold, respectively, compared to NTG. However, in Curd and Starter groups, no significant differences were observed. These results were consistent with the high FE activity found in CRL and Mix cheeses.

In a previous study we observed a 2-fold increase in total intestinal FE activity in mice receiving *L. fermentum* CRL1446, at dose $10^7$ cfu/ml in drinking water for 7 days, compared to NTG (Abeijón Mukdsi et al., 2012). This increase was similar to that we observed when administering cheese as a vehicle for the probiotic strain, as reported in this paper.

In order to determine whether the administration of cheeses with FE activity could affect epithelial or microbial FE activity in the gut, this activity was assessed in content and mucosa of small and large intestines. Abeijón Mukdsi et al. (2012) reported that in mice administered with *L. fermentum* CRL1446 in drinking water, higher levels of FE activity were detected in LIC and SIM, compared to NTG. However, the administration of this bacterium, carried in Mix cheese, only produced activity increase in mucosa (SIM and LIM). This fact may indicate that *L. fermentum* CRL1446 could stimulate the epithelial FE activity. Slight increase in FE activity observed in LIM in the Curd group was not in accordance with the absence of activity in the curd. This result could be explained by the increase (~1 log cfu/g) in total anaerobe population observed in Curd group. This microbiota, located primarily in the colon, may stimulate FE activity of mucosal cells. However, the activity increase in LIM in Mix group was significantly higher than in Curd group. There were no significant quantitative changes in intestinal microbiota in Starter, CRL and Mix groups.

Lipid peroxidation is one of the harmful consequences of the formation of reactive-oxygen species, and is widely used as a biomarker of oxidative status. In our study, the level of TBARS, a typical byproduct of lipid peroxidation, served as an index of lipid peroxidation in plasma. Our results showed that plasmatic TBARS concentrations decreased in CRL and Mix groups (1.3 and 1.5-fold, respectively), compared to NTG. Moreover, GR activity increased in CRL and Mix groups (1.4 and 2.7-fold, respectively). These results indicated that feeding mice with cheeses with high FE activity improved the oxidative status of animals, and were in agreement with the higher levels of total intestinal FE activity observed in both treated groups. An increased intestinal FE activity would enhance the hydrolysis of dietary hydroxycinnamates, releasing FA, which then could be absorbed and exert its *in vivo* antioxidant effect.

In a previous study we observed that the administration of *L. fermentum* CRL1446 in drinking water produced an approx. 30–40% decrease in the basal levels of plasmatic TBARS, and an approx. 2-fold increase in GR activity from day 5 of feeding at dose $10^7$ cells per day per mouse (Abeijón Mukdsi et al., 2012). Kullisaar et al. (2003) evaluated in 21 healthy subjects the effect of consumption of goat milk fermented with *L. fermentum* ME-3 on markers of oxidative stress and atherosclerosis determined in blood and urine. That milk consumption increased LDL oxidation resistance and decreased lipoperoxide levels and glutathione redox ratio (oxidized glutathione/reduced glutathione). GR activity stimulation is of great relevance since GR is involved in the regeneration of reduced glutathione (GSH), which is essential for the endogenous antioxidant defence. GR can maintain GSH concentrations in the cell, which is involved in glutathione

peroxidase-catalyzed elimination of $H_2O_2$ and lipoperoxides (Halliwell & Gutteridge, 1999).

## 5. Conclusions

The results obtained in this study showed that goat milk cheeses elaborated with *L. fermentum* CRL1446, as single-strain (CRL cheese) or adjunct culture (Mix cheese), presented FE activity until the end of ripening (60 days). Supplementing the conventional balanced diet with these cheeses for 7 days, increased total intestinal FE activity and improved oxidative status, mainly in mice fed with Mix cheese. Therefore, goat cheese elaborated with *L. fermentum* CRL1446 as adjunct culture could represent a novel dairy functional food with FE activity, responsible for increasing the bioavailability of antioxidant ferulic acid in the gut, and thus enhancing the consumer's oxidative status. This functional cheese would represent an innovative product providing protection against oxidative stress-related disorders.

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## REFERENCES

Abeijón Mukdsi, M. C. (2009). Esterases of lactic acid bacteria in fermented foods. Ph.D. Thesis, Universidad Nacional de Tucumán, Tucumán, Argentina. pp. 1–206.

Abeijón Mukdsi, M. C., Gauffin Cano, M. P., González, S. N., & Medina, R. B. (2012). Administration of *Lactobacillus fermentum* CRL1446 increases intestinal feruloyl esterase activity in mice. *Letters in Applied Microbiology,* 54, 18–25.

Abeijón Mukdsi, M. C., Medina, R. B., Álvarez, M. F., & González, S. N. (2009). Ester synthesis by lactic acid bacteria isolated from goat's and ewe's milk and cheeses. *Food Chemistry,* 117, 241–247.

Abeijón Mukdsi, M. C., Medina, R. B., Katz, M. B., Pivotto, R., Gatti, P., & González, S. N. (2009). Contribution of lactic acid bacteria esterases to the release of fatty acids in miniature ewe's milk cheese models. *Journal of Agricultural and Food Chemistry,* 57, 1036–1044.

Alferez, M. J., Barrionuevo, M., Lopez-Aliaga, I., Sanz-Sampelayo, M. R., Lisbona, F., Robles, J. C., & Campos, M. S. (2001). Digestive utilization of goat and cow milk fat in malabsorption syndrome. *Journal of Dairy Research,* 68, 451–461.

Andreasen, M. F., Kroon, P. A., Williamson, G., & García-Conesa, M. T. (2001). Esterase activity able to hydrolyze dietary antioxidant hydroxycinnamates is distributed along the intestine of mammals. *Journal of Agricultural and Food Chemistry,* 49, 5679–5684.

Araújo, E. A., de Carvalho, A. F., Leandro, E. S., Furtado, M. M., & de Moraes, C. A. (2010). Development of a symbiotic cottage cheese added with *Lactobacillus delbrueckii* UFV H2b20 and inulin. *Journal of Functional Foods,* 2, 85–89.

Barrionuevo, M., Alferez, M. J. M., Lopez-Aliaga, I., Sanz-Sampelayo, M. R., & Campos, M. S. (2002). Beneficial effect of goat milk on nutritive utilization of iron and copper in malabsorption syndrome. *Journal of Dairy Science,* 85, 657–664.

Başyiğit, G., Kuleaşan, H., Eralp, I., & Karahan, A. G. (2009). Manufacture of Turkish Beyaz cheese added with probiotic strains. *LWT – Food Science and Technology,* 42, 1003–1008.

Bergamini, C. V., Hynes, E. R., Quiberoni, A., Suárez, V. B., & Zalazar, C. A. (2005). Probiotic bacteria as adjunct starters: Influence of the addition methodology on their survival in a semi-hard Argentinean cheese. *Food Research International,* 38, 597–604.

Bhathena, J., Martoni, C., Kulamarva, A., Urbanska, A. M., Malhotra, M., & Prakash, S. (2009). Orally delivered microencapsulated live probiotic formulation lowers serum lipids in hypercholesterolemic hamsters. *Journal of Medicinal Food,* 12, 310–319.

Blanchette, L., Roy, L., Belanger, G., & Gauthier, S. F. (1996). Production of Cottage cheese using dressing fermented by bifidobacteria. *Journal of Dairy Science,* 79, 8–15.

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry,* 72, 248–255.

Buchanan, C. J., Wallace, G., & Fry, S. C. (1996). *In vivo* release of $^{14}$C-labelled phenolic groups from intact dietary spinach cell walls during passage through the rat intestine. *Journal of the Science of Food and Agriculture,* 71, 459–469.

Corsetti, A., Caldini, G., Mastrangelo, M., Trotta, F., Valmorri, S., & Cenci, G. (2008). Raw milk traditional Italian ewe cheeses as a source of *Lactobacillus casei* strains with acid-bile resistance and antigenotoxic properties. *International Journal of Food Microbiology,* 125, 330–335.

Couteau, D., McCartney, A. L., Gibson, G. R., Williamson, G., & Faulds, C. B. (2001). Isolation and characterization of human colonic bacteria able to hydrolyse chlorogenic acid. *Journal of Applied Microbiology,* 90, 873–881.

da Cruz, A. G., Buriti, F. C. A., de Souza, C. H. B., Fonseca, J. A. F., & Saad, S. M. I. (2009). Probiotic cheese: Health benefits, technological and stability aspects. *Trends in Food Science & Technology,* 20, 344–354.

De Vuyst, L. (2000). Technology aspects related to the application of functional starter cultures. *Food Technology and Biotechnology,* 38, 105–112.

Díaz-Castro, J., Pérez-Sánchez, L. J., Ramírez López-Frías, M., López-Aliaga, I., Nestares, T., Alférez, M. J., Ojeda, M. L., & Campos, M. S. (2012). Influence of cow or goat milk consumption on antioxidant defence and lipid peroxidation during chronic iron repletion. *British Journal of Nutrition,* 108, 1–8.

Esterbauer, H., & Grill, D. (1978). Seasonal variation of glutathione and glutathione reductase in needles of *Picea abies*. *Plant Physiology,* 61, 119–121.

Fazary, A. E., & Ju, Y. H. (2007). Feruloyl esterases as biotechnological tools: Current and future perspectives. *Acta Biochimica et Biophysica Sinica,* 39, 811–828.

FIL-IDF (1982). *Standard 4A. Cheese and processed cheese. Determination of the total solids content*. Brussels, Belgium: International Dairy Federation.

FIL-IDF (1986). *Standard 5B. Cheese and processed cheese products. Determination of fat content*. Brussels, Belgium: International Dairy Federation.

FIL-IDF (1993). *Standard 20B. Milk. Determination of nitrogen content (Kjeldahl method)*. Brussels, Belgium: International Dairy Federation.

Gomes, A. M. P., & Malcata, F. X. (1998). Development of probiotic cheese manufactured from goat milk: Response surface

analysis via technological manipulation. *Journal of Dairy Science, 81*, 1492–1507.
Gomes, A. M. P., Malcata, F. X., Klaver, F. A. M., & Grande, H. J. (1995). Incorporation and survival of *Bifidobacterium* sp. strain Bo and *Lactobacillus acidophilus* strain Ki in a cheese product. *Netherlands Milk and Dairy Journal, 49*, 71–95.
Guglielmetti, S., De Noni, I., Caracciolo, F., Molinari, F., Parini, C., & Mora, D. (2008). Bacterial cinnamoyl esterase activity. Screening for the production of a novel functional food product. *Applied and Environmental Microbiology, 74*, 1284–1288.
Haenlein, G. F. W. (2004). Goat milk in human nutrition. *Small Ruminant Research, 51*, 155–163.
Halliwell, B., & Gutteridge, J. M. C. (1999). Oxidative stress and antioxidant protection: Some special cases. In B. Halliwell & J. M. C. Gutteridge (Eds.), *Free radical in biology and medicine* (pp. 617–783). New York: Oxford University Press.
Kroon, P. A., Faulds, C. B., Ryden, P., Robertson, J. A., & Williamson, G. (1997). Release of covalently bound ferulic acid from fiber in human colon. *Journal of Agricultural and Food Chemistry, 5*, 661–667.
Kullisaar, T., Songisepp, E., Mikelsaar, M., Zilmer, K., Vihalemm, T., & Zilmer, M. (2003). Antioxidative probiotic fermented goats' milk decreases oxidative stress-mediated atherogenicity in human. *British Journal of Nutrition, 90*, 449–456.
Lai, K. K., Lorca, G. L., & González, C. F. (2009). Biochemical properties of two cinnamoyl esterases purified from a *Lactobacillus johnsonii* strain isolated from diabetic resistant rats' (BB-DR) stool samples. *Applied and Environmental Microbiology, 76*, 5018–5024.
Meyer, H. L. (2007). Les probiotiques dans le monde. *Revue Laitière Française, 676*, 20–23.
Okawa, H., Ohsihi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry, 95*, 351–358.
Oliszewski, R., Medina, R. B., González, S. N., & Pérez Chaia, A. B. (2007). Esterase activities of indigenous lactic acid bacteria from Argentinean goats' milk and cheeses. *Food Chemistry, 101*, 1446–1450.
Ong, L., Henriksson, A., & Shah, N. P. (2006). Development of probiotic cheddar cheese containing *Lactobacillus acidophilus*, *L. casei*, *L. paracasei* and *Bifidobacterium* spp. and the influence of these bacteria on proteolytic patterns and production of organic acid. *International Dairy Journal, 16*, 446–456.
Ozer, B. H., & Kirmaci, H. A. (2010). Functional milks and dairy beverages. *International Journal of Dairy Technology, 63*, 1–15.
Phillips, M., Kailasapathy, K., & Tran, L. (2006). Viability of commercial probiotic cultures (*L. acidophilus*, *Bifidobacterium* sp., *L. casei*, *L. paracasei* and *L. rhamnosus*) in Cheddar cheese. *International Journal of Food Microbiology, 108*, 276–280.
Pripp, A. H., Sorensen, R., Stepanek, L., & Sorhaug, T. (2006). Relationship between proteolysis and angiotensin-I-converting enzyme inhibition in different cheeses. *LWT – Food Science and Technology, 39*, 677–683.
Ranadheera, R. D. C. S., Baines, S. K., & Adams, M. C. (2010). Importance of food in probiotic efficacy. *Food Research International, 43*, 1–7.
Saxelin, M., Tynkkynen, S., Mattila-Sandholm, T., & de Vos, W. (2005). Probiotic and other functional microbes: From markets to mechanisms. *Current Opinion in Biotechnology, 16*, 1–8.
Settanni, L., & Moschetti, G. (2010). Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits. *Food Microbiology, 27*, 691–697.
Songisepp, E., Kullisaar, T., Hütt, P., Elias, P., Brilene, T., Zilmer, M., & Mikelsaar, M. (2004). A new probiotic cheese with antioxidative and antimicrobial activity. *Journal of Dairy Science, 87*, 2017–2023.
Srinivasan, M., Sudheer, A., & Menon, V. (2007). Ferulic acid: Therapeutic potential through its antioxidant property. *Journal of Clinical Biochemistry and Nutrition, 40*, 92–100.
Stanton, C., Gardiner, G., Lynch, P. B., Collins, J. K., Fitzgerald, G., & Ross, R. P. (1998). Probiotic cheese. *International Dairy Journal, 8*, 491–496.
Van Nieuwenhove, C. P., Gauffin Cano, P., Pérez-Chaia, A. P., & González, S. N. (2011). Effect of functional buffalo cheese on fatty acid profile and oxidative status of liver and intestine of mice. *Journal of Medicinal Food, 14*, 420–427.
Vinderola, C. G., Mocchiutti, P., & Reinheimer, J. A. (2002). Interactions among lactic acid starter and probiotic bacteria used for fermented dairy products. *Journal of Dairy Science, 85*, 721–729.
Vitaglione, P., Napolitano, A., & Fogliano, V. (2008). Cereal dietary fibre: A natural functional ingredient to deliver phenolic compounds into the gut. *Trends in Food Science & Technology, 19*, 451–463.
Wang, X., Geng, X., Egashira, Y., & Sanada, H. (2004). Purification and characterization of a feruloyl esterase from the intestinal bacterium *Lactobacillus acidophilus*. *Applied and Environmental Microbiology, 70*, 2367–2372.