https://doi.org/10.17221/411/2017-CJFS

Influence of Flaxseed Components on Fermented Dairy Product Properties

Kristina BIALASOVÁ[1], Irena NĚMEČKOVÁ[2], Jan KYSELKA[1], Jiří ŠTĚTINA[1], Kateřina SOLICHOVÁ[1] and Šárka HORÁČKOVÁ[1]

[1]Department of Dairy, Fat and Cosmetics, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic;
[2]Dairy Research Institute Ltd., Prague, Czech Republic
**Corresponding author: sarka.horackova@vscht.cz*

Abstract

Bialasová K., Němečková I., Kyselka J., Štětina J., Solichová K., Horáčková Š. (2018): Influence of flaxseed components on fermented dairy product properties. Czech J. Food Sci., 36: 51–56.

The addition of flaxseed meal and flaxseed oil on the growth and viability of *Lactobacillus acidophilus* CCDM 151 and yoghurt culture CCDM 21 during cold storage in fermented milk was tested. It was found that the oil addition in the amount of 0.6% w/w in milk did not influence the growth and acid production of *Lactobacillus acidophilus* CCDM 151, while the acidification activity of yoghurt culture was slightly lower compared to pure milk and connected with lower growth of *Streptococcus thermophilus*. On the contrary the addition of meal in amount of 7.6% w/w into milk stimulated the growth and acid production of *Lactobacillus acidophilus* CCDM 151. The viability of both tested cultures during one month storage of fermented milks at 5 ± 1°C was not influenced by the oil supplementation but the addition of meal decreased their viability significantly. The unusual volatile compounds acetone and butane-2-on were detected by SPME-GC in yoghurt with meal. Unlike oil, the addition of flaxseed meal increased the yoghurt firmness and influenced negatively yoghurt taste and flavour.

Keywords: flaxseed meal; flaxseed oil; *Lactobacillus acidophilus*; textural analysis; yoghurt culture

Currently, both researchers and consumers are paying great attention to so-called 'functional foods' containing components such as probiotic microorganisms, prebiotics and their combination (synbiotics), fibre, essential fatty acids, etc. Generally the increased consumption of fibre can help to control weight and serum cholesterol levels, reduce blood pressure and improve intestinal functions. Moreover certain types of fibre, mostly oligosaccharides, have a prebiotic effect defined as a selective stimulation of one or more beneficial bacteria in the colon (Gibsson 2004). Fibre can also protect probiotic microorganisms during food processing and storage as well as improve their stability both in products and the intestinal tract (Saarela *et al.* 2006) as these bacteria are often sensitive to low pH, processing or storage temperature and other factors (Tripathi & Giri 2014). Water soluble and insoluble fibres (galactooligosaccharides, fructooligosaccharides, lactose derivatives, inulin and polydextrose) have been suggested as potential probiotic protectants (Charalampopoulos *et al.* 2002). Food fortification by flax seed components has been proven to have many health benefits (Mercier *et al.* 2014). Flaxseed is an important source of ω-3-fatty acids, especially α-linolenic acid 50–65%, plant lignans,

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QJ1510274, and by the Ministry of Education, Youth and Sports of the Czech Republic, specific university research No. 20-SVV/2017.

https://doi.org/10.17221/411/2017-CJFS

soluble/insoluble fibre, cyclic peptides and various minerals (Bustamante *et al*. 2015). Omega-3-fatty acids are considered to be associated with blood lipids improvements, may reduce the risk of cardiovascular disease, osteoporosis, diabetes or gastrointestinal disease (Bloedon & Szapáry 2004; Shim *et al*. 2014). However, the effect of flaxseed components on lactic acid bacteria has not yet been studied in much detail. The aim of this study was to test the influence of addition of flaxseed meal (a source of fibre and lignans) and flaxseed oil (an important source of ω-3-fatty acids) to milk on the growth, acidification properties and storage stability of *Lactobacillus acidophilus* CCDM 151 and yoghurt culture CCDM 21 and to evaluate the texture and sensory properties of subsequent fermented products.

MATERIALS AND METHODS

Microorganisms. *Lactobacillus acidophilus* CCDM 151 and Yoghurt culture CCDM 21 (Culture Collection of Dairy Microorganisms, Laktoflora®; Milcom, Czech Republic) were used in this work.

Cultivation, determination of cell count and pH. For the inoculation of tested media, overnight cultures (1% v/v inoculum) cultivated in skimmed UHT milk (Madeta, Czech republic) at 37°C in 5% (v/v) CO_2 atmosphere for *L. acidophilus* CCDM 151 and aerobically at 30°C for yoghurt culture were used. The number of *L. acidophilus* CCDM 151 cells was determined according to ISO 20128:2006 and the number of yoghurt culture cells according to ISO 7889:2003. The pH values were measured using a pH meter 3020 (Jenway, UK) provided with a combined electrode.

Flaxseed components. Flaxseed meal (Organic Brown Flax Fibre 300–500 µm; Functional Whole Food, New Zealand) and flaxseed oil (Functional Whole Food, New Zealand) were used in this study. Flaxseed meal was characterised as follows: proteins 31.5 w/w% (Kjeldahl method), lipids 20.6 w/w% (Soxhlet extraction), water 10.3 w/w% (halogen moisture analyser Mettler Toledo HR73, Switzerland), insoluble fibre 38 w/w% and soluble fibre 7 w/w% (manufacturer's specification). Fatty acid composition was determined according to AOCS Official Methods Ce 1f-96 (2002). Analysis was performed on an Agilent 6890N Gas Chromatograph (Agilent Technologies, USA) and SP™ 2560 capillary column (Supelco, USA) 0.25 mm × 100 m; film thickness 0.2 µm was used. The average composition was: palmitic acid 5.4%, stearic acid 4.9%, oleic acid 20.1%, linoleic acid 15.1% and linolenic acid 54.5%.

Cultivation of strains. The effect of the addition of flaxseed meal in the ratio of 7.6 g to 92.4 g of skimmed UHT milk (milk + meal) and flaxseed oil in the ratio of 0.6 g with 0.06 g soy lecithin (Mogador, Czech Republic) to 99.34 g of skimmed UHT milk (milk + oil) on the growth of selected dairy cultures was tested compared to the growth in pure skimmed UHT milk (milk). Flaxseed oil was first stirred (stirrer RZR 2021; Heidolph, Germany) with pre-heated (60°C) milk and lecithin at 200 *g* for 5 min and further homogenised (T-25 basic Ultra-Turrax®; IKA, Germany) at 13 500 *g* for 4 min followed by another stirring (200 *g*, 10 min). All media used were heat treated at 90°C for 10 min, inoculated after cooling either by 1% (v/v) *L. acidophilus* CCDM 151 or 0.1% (v/v) yoghurt culture CCDM 21 to get a starter concentration 10^6 CFU/g and cultivated at the appropriate temperature for 16 hours. Samples were analysed after fermentation and after 14 and 28 days storage at 5 ± 1°C. The results are the means from two independent fermentations, both parallel samples were analysed twice (n = 4).

HPLC analysis of organic acids. Before the analysis the samples with flax meal were diluted in 1 : 10 ratio with distilled water. To a 250 µl sample, 1.6 ml of ethanol was added, allowed to stay for 30 minutes. The mixture was then centrifuged (13 000 *g*, 10 min and 4°C) and filtered through a 0.22 µm membrane prior to injection of 20 µl into the chromatographic system. Separation was performed using HPLC system (Agilent 1260 Infinity; USA) with precolumn 50 × 8 mm and polymer column IEX H, 250 × 8 mm (both Watrex, Czech Republic) connected to UV/VIS detector (210 nm). Aqueous solution of sulfuric acid (9 mmol/l) was used as mobile phase at 0.6 ml/min flow rate, the column temperature was 60°C.

Analysis of volatile compounds. Volatile compounds were isolated from the sample headspace by solid phase microextraction (SPME) and determined by gas chromatograph Agilent Technologies 7890 (USA) coupled with mass spectrometry detector 5975C (Agilent Technologies, USA). Five grams of sample were tempered in a glass vial at 40°C for 30 min and then conditioned SPME fibre was exposed to the sample headspace for 1 min. The analyses were performed on HP5 column (Agilent Technologies, USA), particle size 0.32 mm × 30 m, 0.25 µm; with temperature mode: initial temperature

https://doi.org/10.17221/411/2017-CJFS

40°C for 7 min followed by an increase of 5°C/min up to 240°C. Helium was used as a mobile phase with 0.9 ml/min flow rate.

Textural analysis. Texture Analyser TA.XT plus (Stable Micro Systems Ltd., UK) was used to measure texture profile analysis. A cylindrical aluminium probe (P/20) was repeatedly punctured into sample to a defined depth (25 mm) at fixed speed 5 mm/s and sample temperature 5°C. Three textural characteristics, firmness, adhesiveness and cohesiveness, were evaluated (Bourne 2002). The results indicate the average of three determinations.

Sensory evaluation. Ten previously trained laboratory members were chosen as panellists. The samples were evaluated according to six attributes – appearance, flavour, taste, texture, overall rating and purchase intention. The rating of individual attributes was carried out using 0–10 scale (0 = unacceptable, 10 = excellent). Samples were fermented one day before evaluation and tempered to 15 ± 1°C. Each sample was coded by a random four digit number.

RESULTS AND DISCUSSION

First, the prepared media were cooled to fermentation temperature, 37°C for *L. acidophilus* CCDM 151 and to 30°C for yoghurt culture CCDM 21. Before fermentation, the average pH of milk was 6.60 ± 0.05; milk + oil 6.62 ± 0.01 and milk + meal 6.33 ± 0.04. Table 1 summarises the number of cells, pH changes and lactic and acetic acid concentration after 16 h cultivation. *L. acidophilus* CCDM 151 showed less growth activity in milk and in milk with oil. The pH values reached were only 5.51 ± 0.12 and 5.68 ± 0.06, respectively, and were not sufficient for fermented dairy products. On the contrary, the addition of flaxseed meal into milk stimulated its growth (by 0.8 log cycle) including acidification (pH 4.22 ± 0.14). Flaxseed components slightly decreased the number of *S. thermophilus* cells. Yoghurt culture produced a significantly higher amount of lactic acid and consequently pH reached after fermentation was around 4.42 ± 0.14 in all media tested. However, compared to milk, the decrease in pH was lower in milk with oil. Acetic acid, which can cause an undesirable off-flavour of fermented products, was not detected in media with *L. acidophilus* CCDM 151 and only in traces in media with yoghurt culture. The influence of plant components on growth and acidification ability has also been proved by other authors (Charalampopoulos *et al.* 2002). Sah *et al.* (2016) confirmed that milk supplementation by pineapple peel powder reduced fermentation time inoculated by *L. acidophilus* ATCC 4356 or *L. casei* ATCC 393, but in the study of Sendra *et al.* (2008) citrus fiber had inhibitory effect on *B. bifidum* CECT 870. Slow acidification of fermented products can bring the risk of potential contamination growth and therefore careful selection of culture, cultivation conditions and functional components is necessary for industrial applications.

Further, all prepared fermented products were stored at 5 ± 1°C for 1 month to investigate the influence of flaxseed components on cell viability. The cell number found after fermentation (1 day) and after 14, respectively 28 days storage is shown on Figure 1. No significant changes in pH were detected during the storage of fermented products (data not shown). Although the flaxseed meal promoted the growth of *L. acidophilus* CCDM 151 during fermentation, Figure 1 shows that there was a significant decrease in cell viability during storage compared to milk. Oil addition did not influence the cell viability. After 28 days of storage, the cell number

Table 1. The number of cells, pH changes and lactic and acetic acid concentrations

Culture	Medium	Cell count (log CFU/g)		Δ pH	Lactic acid (g/l)	Acetic acid (g/l)
L. acidophilus CCDM 151	milk	8.18 ± 0.14		1.07 ± 0.07	0.25 ± 0.02	nd
	milk & oil	8.19 ± 0.03		0.93 ± 0.05	0.17 ± 0.03	nd
	milk & meal	8.98 ± 0.20		2.11 ± 0.09	0.89 ± 0.07	nd
		L. delbrueckii ssp. *bulgaricus*	*S. thermophilus*			
Yoghurt culture CCDM 21	milk	8.23 ± 0.15	8.76 ± 0.32	2.21 ± 0.07	0.91 ± 0.06	traces
	milk & oil	8.32 ± 0.14	8.32 ± 0.14	1.59 ± 0.09	1.00 ± 0.12	traces
	milk & meal	8.20 ± 0.20	8.31 ± 0.23	1.87 ± 0.04	0.97 ± 0.11	traces

Cultivation of *L. acidophilus* (CCDM 151) and yoghurt culture (CCDM 21) 16 h in tested media; nd – not detected

https://doi.org/10.17221/411/2017-CJFS

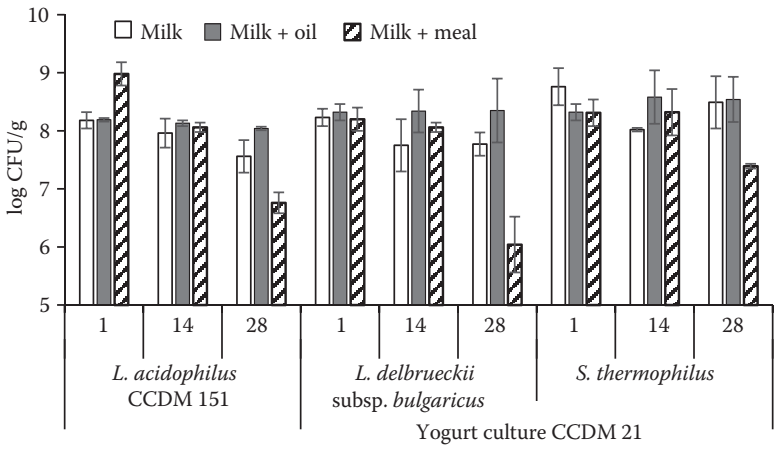

Figure 1. The number of cells after fermentation (1 day) and after 14 and 28 days storage at 5 ± 1°C in tested products

in fermented milk and oil-added milk was only by 0.61 and 0.65 log cycle CFU/g respectively lower than immediately after fermentation, whereas in milk with meal it was by 1.45 log cycle CFU/g. The same trend was observed for both microorganisms of yoghurt culture. The cell number in yoghurt with oil did not decrease during storage but the loss of cells in yoghurt with flaxseed meal was 0.98 log cycle CFU/g for *S. thermophilus* and 1.80 log cycle CFU/g for *L. delbrueckii* subsp. *bulgaricus*. There are very few studies documenting the effect of flaxseed components on growth or stability of lactic acid bacteria in milk and fermented milk. Vesterlund *et al.* (2012) demonstrated the increase of viability of probiotic bacteria *L. rhamnosus* GG during storage at 22°C for 14 months when milled flaxseeds were added to dried matrices. Also, Hadinezhad *et al.* (2013), who tested the influence of soluble flax fibre on the stability of kefir culture in combination with *L. acidophilus* B-4495 and *B. animalis* subsp. *lactis* 41405 during storage (28 days, 4°C), discovered its positive effect. In this study, the negative effect of flaxseed meal can be explained by high concentration of plant oil. Since the meal contains about 20% w/w of fat, the majority of it consists of long fatty acids. These acids were proved to have the antimicrobial and antifungal activities (Zheng *et al*. 2005; Calce *et al*. 2014). Likewise, other components of plant fibre, for example polyphenols, can influence the microorganisms' growth (Hervert-Hernández *et al*. 2009). Further, the microbial contamination of flaxseed meal, which was detected in heat treated media with meal, can act antagonistically against lactic acid bacteria. The raw meal contained 1.8 × 10^3 CFU/g of total microorganisms count; the heat treated medium (90°C, 10 min) less than 10 CFU/g. When this medium was cultivated (30°C, 16 h) without dairy culture addition the total count of microorganisms increased to 6.6 × 10^4 CFU/g and after storage (5 ± 1°C, 28 days) to 4.4 × 10^5 CFU/g. This suggests that the flax fibre used may exacerbate potential contamination in fermented products.

Furthermore, due to the off-flavour found (after bitter almonds), the comparison of volatile compounds of yoghurt and yoghurt with flaxseed meal was done using the SPME-GC method. An example of a chromatogram for yoghurt with meal is shown on Figure 2. Unlike yoghurt alone, these samples contained acetone and butane-2-one. Flax (*Linum usitatisimum*) and flaxseed meal contain cyanogenic glycosides (linamarin, linustatin, and neolinustatin) (Russo & Reggiani 2014) which can be degraded to cyanide hydrogen, ketones and aldehydes (Møller 2010).

The addition of flaxseed meal to yoghurt, in a quantity consistent with the nutritional claim 'source of fibre' (Regulation 1924/2006), also significantly affected the yoghurt texture (Table 2) and sensory properties

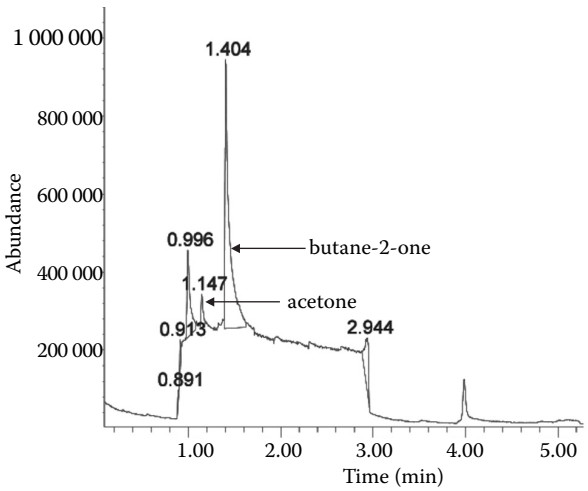

Figure 2. SPME-GC chromatogram of volatile compounds in yoghurt with flaxseed meal

https://doi.org/10.17221/411/2017-CJFS

Table 2. Textural properties of yoghurt and yoghurt with flaxseed meal

	Firmness (N)	Adhesiveness (N·s)	Cohesiveness	Firmness (N)	Adhesiveness (N·s)	Cohesiveness
	1 day			28 days		
Yoghurt	0.28 ± 0.02	0.41 ± 0.04	0.59 ± 0.01	0.27 ± 0.03	0.36 ± 0.07	0.62 ± 0.05
Yoghurt & meal	1.36 ± 0.08	0.56 ± 0.04	0.47 ± 0.02	1.60 ± 0.15	0.85 ± 0.12	0.59 ± 0.07

Samples after 16 h fermentation at 30°C and after 28 days storage at 5 ± 1°C

Table 3. Sensory evaluation and purchase intention of fermented milk products

Culture	Medium	Appearance	Aroma	Flavour	Consistency	Overall impression	Purchase interaction
L. acidophilus CCDM 151	milk	5.1 ± 0.99	3.4 ± 1.17	4.8 ± 0.63	2.9 ± 0.74	2.1 ± 0.74	2.6 ± 0.51
	milk & oil	5.2 ± 0.92	2.9 ± 0.88	2.1 ± 0.74	2.7 ± 0.67	2.0 ± 0.67	1.7 ± 0.48
	milk & meal	2.0 ± 0.82	1.8 ± 0.63	1.4 ± 0.52	1.8 ± 0.63	1.7 ± 0.82	1.4 ± 0.52
Yoghurt culture CCDM 21	milk	7.6 ± 0.84	8.0 ± 0.94	4.9 ± 0.74	7.9 ± 0.74	6.7 ± 0.82	5.6 ± 0.84
	milk & oil	7.5 ± 0.85	7.1 ± 0.99	4.1 ± 0.88	8.0 ± 0.67	6.2 ± 0.79	5.4 ± 0.70
	milk & meal	2.1 ± 0.87	3.0 ± 1.05	1.8 ± 0.63	4.1 ± 0.99	2.0 ± 0.67	1.7 ± 0.82

(Table 3). A claim that a food is a source of fibre, and any claim likely to have the same meaning for the consumer, may only be made where the product contains at least 3 g of fibre per 100 g or at least 1.5 g of fibre per 100 kcal (Regulation 1924/2006). In this work, the total content of fibre was 3.4 g in 100 g of product. The texture of the yoghurt was strongly dependent on milk supplementation. Flaxseed meal increased the firmness by around five times in yoghurt after fermentation and by around six times after 28 day storage due to a higher solid content and a high viscosity of flaxseed polysaccharides in milk (Velez-Ruiz *et al.* 2013). At the same time, although flax oil has a characteristic aroma, the yoghurt with oil addition was not evaluated negatively due to off-flavour (Table 3). The addition of flaxseed oil would be an important source of ω-3-fatty acids in consumers' diets. A claim that a food is a source of ω-3 fatty acids may be made where the product contains at least 0.3 g α-linolenic acid per 100 g (Regulation 1924/2006); in this case it was 0.33 g per 100 g. Milk and milk with oil fermented by *L. acidophilus* CCDM 151 reached a pH only 5.51 ± 0.12 and 5.68 ± 0.06 respectively after cultivation. This fact was judged by the panellists to be insufficient and therefore negative.

CONCLUSION

Flaxseed components may be a source of bioactive compounds that positively affect human health but can also negatively influence the sensory parameters of foods and the growth and stability of lactic acid bacteria in fermented foods. Interactions should be examined and an alternative solution could lie in a suitable combination of different types of lactic acid bacteria. Based on the results of this study, the addition of flaxseed oil to milk fermented by *L. acidophilus* CCDM 151 or yogurt culture CCDM 21 could be a promising option. Milk supplementation by oil did not influence the growth of dairy cultures and had a positive effect on their viability during storage.

References

Bloedon L.T., Szapary P.O. (2004): Flaxseed and cardiovascular risk. Nutrition Reviews, 62: 18–27.

Bourne M.C. (2002): Food Texture and Viscosity. 2nd Ed. London, Academic Press: 182–186.

Bustamante M., Villarroel M., Rubilar M., Shene C. (2015): *Lactobacillus acidophilus* La-05 encapsulated by spray drying: Effect of mucilage and protein from flaxseed (*Linum usitatissimum*). LWT-Food Science and Technology, 62: 1162–1168.

Calce E., Mignogna E., Bugatti V., Galdiero M., Vittoria V., De Luca S. (2014): Pectin functionalized with natural fatty acids as antimicrobial agent. International Journal of Biological Macromolecules, 68: 28–32.

Charalampopoulos D., Wang R., Pandiella S.S., Webb C. (2002): Application of cereals and cereal components in functional foods: a review. International Journal of Food Microbiology, 79: 131–141.

https://doi.org/10.17221/411/2017-CJFS

Gibson G.R. (2004): Prebiotics. Best Practice & Research Clinical Gastroenterology, 2: 287–298.

Hadinezhad M., Duc C., Han N.F., Hosseinian F. (2013): Flaxseed soluble dietary fibre enhances lactic acid bacterial survival and growth in kefir and possesses high antioxidant capacity. Journal of Food Research, 2: 152–163.

Hervert-Hernández D., Pintado C., Rotger R., Goñi I. (2009): Stimulatory role of grape pomace polyphenols on *Lactobacillus acidophilus* growth. International Journal of Food Microbiology, 136: 119–122.

Mercier S., Villeneuve S., Moresoli Ch., Mondor M., Marcos B., Power K.A. (2014): Flaxseed-enriched cereal-based products. A review of the impact of processing conditions. Comprehensive Reviews in Food Science and Food Safety, 13: 400–412.

Møller B.L. (2010): Functional diversifications of cyanogenic glucosides. Current Opinion in Plant Biology, 13: 338–347.

Russo R., Reggiani R. (2014): Variation in the content of cyanogenic glycosides in flaxseed meal from twenty-one varieties. Food and Nutrition Sciences, 5: 1456–1462.

Saarela M., Virkajärvi I., Nohynek L., Vaari A., Mättö J. (2006): Fibres as carriers for *Lactobacillus rhamnosus* during freeze-drying and storage in apple juice and chocolate-coated breakfast cereals. International Journal of Food Microbiology, 112: 171–178.

Sah B.N.P., Vasiljevic T., McKechnie S., Donkor O.N. (2016): Physicochemical, textural and rheological properties of probiotic yogurt fortified with fibre-rich pineapple peel powder during refrigerated storage. LWT- Food Science and Technology, 65: 978–986.

Sendra E., Fayos P., Lario Y., Fernández-López J., Sayas-Barberá E., Pérez-Alvarez J.A. (2008): Incorporation of citrus fibers in fermented milk containing probiotic bacteria. Food Microbiology, 25: 13–21.

Shim Y.Y., Gui B., Arnison P.G., Wang Y., Reaney M.J.T. (2014): Flaxseed (*Linum usitatissimum* L.) bioactive compounds and peptide nomenclature: a review. Trends in Food Science & Technology, 38: 5–20.

Tripathi M.K., Giri S.K. (2014): Probiotic functional foods: Survival of probiotics during processing and storage. Journal of Functional Foods, 9: 225–241.

Velez-Ruiz J.F., Hernandez-Carranza P., Sosa-Morales M. (2013): Physicochemical and flow properties of low-fat yogurt fortified with calcium and fiber. Journal of Food Processing and Preservation, 37: 210–221.

Vesterlund S., Salminen K., Salminen S. (2012): Water activity in dry foods containing live probiotic bacteria should be carefully considered: A case study with *Lactobacillus rhamnosus* GG in flaxseed. International Journal of Food Microbiology, 80: 319–321.

Zheng Ch.J., Yoo J.-S., Lee T.-G., Choc H.-Y., Kim Y.-H., Kim W.-G. (2005): Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. LEBS Letters, 579: 5157–5162.

Received: 2017–11–09
Accepted after corrections: 2017–01–24
Published online: 2018–02–15