Acta Alimentaria 51 (2022) 2, 218–227
DOI: 10.1556/066.2021.00253

AKADÉMIAI KIADÓ

Development and characterisation of functional cultured buttermilk fortified with flaxseed

I. Mahmoudi*, O. Ben Moussa, M. Chouaibi, A. Telmoudi, W. Boukari and M. Hassouna

Department of Food Technology, Research Unity 'Bio-Preservation and Valorization of Agricultural Products UR13-AGRO 02', 58 Avenue Alain Savary, 1003 El Khadhra, Tunis, Tunisia

ORIGINAL RESEARCH PAPER

Received: December 6, 2021 • Accepted: March 8, 2022
Published online: April 22, 2022

ABSTRACT

Cultured buttermilk is a dairy beverage with a high nutritive value. In the current study, functional cultured buttermilk was formulated using probiotic *Lactobacillus plantarum* and flaxseed fortification to improve the potential health benefits. The cultured buttermilk samples were analysed for pH, lactic acidity, colour, phase separation, viscosity, microbiology and sensory properties. The results showed non-significant changes in acidity and pH. However, flaxseed fortification decreased phase separation and increased viscosity of buttermilks. In addition, a significant difference in colour attributes was revealed between samples. Sensory characteristics of cultured buttermilks were acceptable to produce a functional food.

KEYWORDS

buttermilk, probiotic, flaxseed, functional food

1. INTRODUCTION

Functional foods have a potentially positive effect on human health beyond basic nutrition due to their bioactive components (Fardet, 2015). Milk and dairy products have been an important part of the human diet from ancient times in many parts of the world (Zhao et al., 2018).

* Corresponding author. Tel.: +216 22 233426; E-mail: imenmahmoudi15@yahoo.fr

Buttermilk is a low-cost by-product, obtained during butter manufacturing (Barukčić et al., 2019). This liquid phase contains most of the water-soluble components of the cream (Zhao et al., 2018). Buttermilk has only recently gained attention as a potential source of functional ingredients due to the presence of microorganisms beneficial to health. Currently, this co-product is gaining more and more attention due to its unique structure, characteristics, and promising applications due to its appreciable content of phospholipids (amphiphilic molecules) from the membrane of the milk fat globules (Kumar et al., 2015). The production of buttermilk products would have a great economic impact (Mudgil et al., 2016). Also, Conway et al. (2014) reported that buttermilk induced reduction in plasma levels of total cholesterol and TGs in individuals. Cultured buttermilk is a fermented dairy product with therapeutic value. The consumption of cultured buttermilk in the diet helps in digestion, boosting the immune system, and reducing serum cholesterol levels (Nirgude et al., 2013). However, cultured buttermilk is naturally deficient in nutritive components and dietary fibre.

Linseed (*Linum usitatissimum*), as prebiotic, is a fibre and food crop that is cultivated in cold areas of the world. The protein content of flaxseed is high in arginine (approximately 56–70%), and contains soluble fibre (20%) and insoluble fibre (9%). This plant contains a number of essential fatty acids, including linoleic (ω6), α-linolenic, eicosapentaenoic, and docosahexaenoic acids (Kaur et al., 2018). All these fatty acids are known to significantly reduce cardiovascular problems. Flaxseed contains other biologically active substances such as bioactive peptides, soluble fibre, lignan, vitamins (predominantly γ-tocopherol), and minerals (magnesium, calcium, and phosphorus, etc.) (Sarabandi et al., 2019).

To the best of our knowledge, none of the above studies provided a probiotic fermented buttermilk product with flaxseed.

Therefore, the current study attempted to make a complete nutritive beverage containing dietary fibre, to evaluate flaxseed fortified buttermilk as a carrier of the probiotic bacteria, and to characterise it in terms of physicochemical, rheological, microbiological, and sensory attributes.

2. MATERIALS AND METHODS

2.1. Materials

Buttermilk was obtained from the Central Dairy of the North (Tunisia). Flaxseed powder was purchased from a local market in Tunis, Tunisia (Table 1). Commercial yogurt starter culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (YFL901) was purchased from CHR HANSEN, France. The probiotic cells of *Lactobacillus plantarum* M63 were activated according to Mahmoudi et al. (2021).

Table 1. Composition of buttermilk and flaxseed

Materials	Parameters (%)						
	Protein	Fat	Ash	Lactose	Carbohydrates	Total solids	Moisture
Buttermilk	2.5±0.04	2.7±0.1	–	4.2±0.03	–	14.25±0.01	85.75±0.01
Flaxseed	18±0.03	40.8 ± 0.04	3.33±0.03	–	1.5±0.05	94.1± 0.02	5.9±0.02

–: not identified.

2.2. Preparation of cultured buttermilks

The butter was manufactured at the Central Dairy of the North (Tunisia). The milk was pasteurized at 95 °C for 5 min. Then, the cream was churned.

The fermented buttermilk was manufactured according to Central Dairy of the North (Tunisia) (Fig. 1). The buttermilk was heated at 95 °C for 5 min. Then, flaxseed powder was added at 3 and 5% (w/100 mL) (Ardabilchi et al., 2019). Also, sugar syrup and strawberry flavour were added. After cooling to inoculation temperature (44 °C), starter cultures were used (1.5 g L^{-1} ≈ 10^8 CFU mL^{-1}). Next, the buttermilk was divided into six equal batches as follows: 1) a control (BMC), 2) inoculated with the probiotic *L. plantarum* M63 (BMP), 3) fortified with flaxseed (3%) (BMF1), 4) fortified with flaxseed (5%) (BMF2), 5) inoculated with *L. plantarum* and fortified with flaxseed (3%) (BMPF1), and 6) inoculated with *L. plantarum* and fortified with flaxseed (5%) (BMPF2). Three replicates of each batch were prepared.

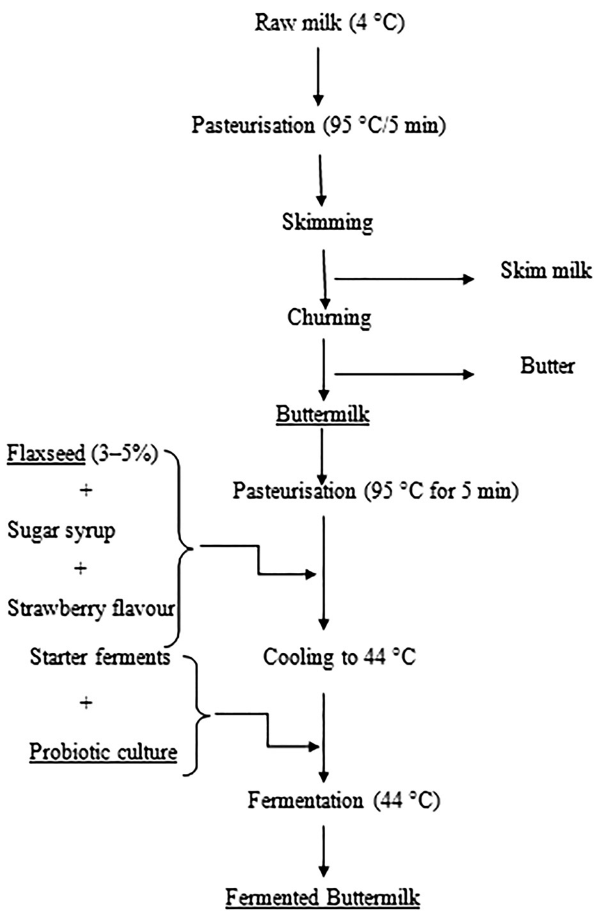

Fig. 1. Buttermilk making process

After mixing, each sample was distributed into sterilised and coded glass bottles under aseptic conditions. After that, the fermentation was made in an oven at 44 °C. The final pH of buttermilk fermentation should reach 4.5.

2.3. Physicochemical analyses

The pH of each sample was measured using a microprocessor pH-meter BT-500 (Boeco, Hamburg, Germany). Lactic acidity changes were measured as reported by AOAC (1990) (Method 947.05). The total protein content was determined by the Kjeldahl method (AOAC 1990, Method 984.13). The total solids content was determined using an air oven (Memmert, UL-60, Germany) (AOAC, 1990, Method 990.20).

For phase separation study, 25 mL buttermilk was filled into graduated measuring cylinders (glass) and let it stand for 72 h at 25 °C. The cultured buttermilks were observed for phase separation after storage of 12, 24, 48, and 72 h. The aqueous phase in cultured buttermilk was separated from the serum phase. This phase was expressed as mL/25 mL of cultured buttermilks.

2.4. Colour evaluation

The colorimetric parameters L^* (lightness), a^* (redness), and b^* (yellowness) of fermented buttermilks were determined using a colorimeter (Minolta Chroma Meter CR-300, Tokyo, Japan) according to AOAC 1990 (Method 960.44).

2.5. Viscosity

The apparent viscosity was measured using a viscometer (Rheomat RM-180, Germany) according to AOAC (1990, Method 962.09).

2.6. Bacterial cell counts

S. thermophilus colonies were enumerated on M17 agar (Biokar Diagnostics, France) after incubation at 44 °C for 48 h. *Lactobacillus bulgaricus* numbers were determined on MRS agar after incubation at 37 °C for 48 h. The number of *L. plantarum* was determined according to Mahmoudi et al. (2021).

2.7. Sensory evaluation

The sensory evaluation of cultured buttermilks (at 4 °C) was performed one day after production with 30 panellists (10 teachers and 20 students). Each sample was presented with 50 mL in a plastic cup and labelled with a 3-digit-code. Sensory analysis of appearance and texture, colour, sweet taste, acidity, flaxseed flavour, aroma, bitterness, creaminess, and overall acceptability was performed using 7-point hedonic scales. Sensory properties were explained beforehand to the panellists (Tomic et al., 2017).

2.8. Statistical analysis

All experiments were repeated three times. Experimental data were analysed using SPSS 20.0. Analyses of significant difference and variance were performed by one-way ANOVA ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Physicochemical properties of cultured buttermilks

Lactic acidity of control, probiotic, and flaxseed powder fortified cultured buttermilk was fixed at 0.75% as presented in Table 2. Lactic acidity of cultured buttermilk showed an increasing trend with the increase of flaxseed powder level ($P > 0.05$). pH of buttermilk samples ranged from 4.25 ± 0.1 to 4.28 ± 0.1 ($P > 0.05$). These findings are in line with the study of Mudgil et al. (2016), who showed non-significant changes in the pH of control and *Aloe vera* juice fortified buttermilk.

Total solids contents in cultured buttermilks were different ($P < 0.05$) (Table 2). The highest content was observed in BMF2 sample (22.4 ± 0.1%). This can be explained by the high content of total solids in flaxseed. The BMPF2 sample had the highest protein level of 16.5 ± 0.3% ($P < 0.05$).

3.2. Phase separation

During storage, the appearance of whey on the surface of fermented products is considered a product defect due to the separation of whey from the protein network (Mudgil et al., 2016). Throughout the storage study of 72 h at 25 °C, phase separation in control sample was significantly ($P < 0.05$) higher than in flaxseed fortified and probiotic buttermilk samples. Until 12 h of storage, probiotic (BMP), fortified flaxseed (BMF1; BMF2), and flaxseed fortified probiotic (BMPF1; BMPF2) samples showed significant phase separation compared to control (1.1 ± 0.1 mL/25 mL) (Table 2). After 72 h of storage at 4 °C, control as well as other cultured buttermilk samples showed a marked increase in phase separation with 7.0 ± 0.1 mL/25 mL. The fortification of flaxseed significantly decreased phase separation in all cultured buttermilk samples, which could be attributed to the gelling property (mucilage and fibres) of flaxseed. Indeed, they interact with the constituents of milk (mainly proteins), which allows the compactness of the protein network, immobilisation of a large amount of free water and less susceptibility to water release (Ardabilchi et al., 2019). Similarly, Delikanli and Ozcan (2017) reported the gelling capacity of the *A. vera* to interact with milk proteins, preventing free movement of water.

3.3. Colour

Colour is a major factor in food choice by consumers (Amjadi et al., 2019). Colour parameters (L^*, a^*, b^*) of buttermilk samples are shown in Table 3. The statistical analyses indicated that there were significant differences among samples ($P < 0.05$). The L^* values of cultured buttermilks decreased with the increase of flaxseed content and inoculation with *L. plantarum*. However, the b^* values exhibited an increasing trend in samples with the increase in flaxseed level. Thus, the highest and the lowest values of b^* were attributed to the BMPF2 and BMC samples, respectively. This finding can be related to the high carotenoid content of flaxseed (Bekhit et al., 2018). Moreover, Ardabilchi et al. (2019) reported that the colour of yogurt may be due to the original darker colour of flaxseed.

3.4. Viscosity

Viscosity can be affected by the processing methods, type of starter cultures, heat treatment, and formulation composition (Jaster et al., 2018). The viscosity values of cultured buttermilks are

Table 2. Physicochemical properties of fermented buttermilks

Samples	Parameters (%)							
	pH	Acidity	Total protein	Total solids	Liberated lactoserum (mL/25 mL)			
					12 h	24 h	48 h	72 h
BMC	4.25 ± 0.1^{a}	0.75 ± 0.1^{a}	2.3 ± 0.2^{a}	15.7 ± 0.2^{a}	1.1 ± 0.1^{a}	1.5 ± 0.2^{a}	6.25 ± 0.6^{a}	7.5 ± 0.1^{a}
BMP	4.3 ± 0.1^{a}	0.75 ± 0.1^{a}	2.24 ± 0.4^{a}	15.7 ± 0.1^{a}	0.8 ± 0.1^{b}	1.3 ± 0.5^{a}	6.1 ± 0.8^{a}	7.2 ± 0.3^{a}
BMF1	4.3 ± 0.1^{a}	0.7 ± 0.1^{a}	8.85 ± 0.1^{b}	20.2 ± 0.1^{b}	0.8 ± 0.1^{b}	0.9 ± 0.3^{b}	5.7 ± 0.1^{b}	6.3 ± 0.3^{b}
BMF2	4.3 ± 0.1^{a}	0.75 ± 0.1^{a}	16 ± 0.1^{c}	22.4 ± 0.1^{b}	0.62 ± 0.1^{c}	0.72 ± 0.3^{b}	5.7 ± 0.1^{b}	6 ± 0.2^{b}
BMPF1	4.25 ± 0.1^{a}	0.75 ± 0.1^{a}	15.9 ± 0.2^{c}	20.4 ± 0.2^{b}	0.75 ± 0.2^{b}	0.9 ± 0.4^{b}	5.6 ± 0.4^{b}	6 ± 0.6^{b}
BMPF2	4.25 ± 0.1^{a}	0.75^{a}	16.5 ± 0.3^{c}	21.9 ± 0.2^{b}	0.62 ± 0.3^{c}	0.7 ± 0.3^{b}	5.62 ± 0.6^{b}	5.9 ± 0.1^{b}

$^{a,\ b}$: Mean values ±SD (n=3) within a column with different superscript letters and within a row with different superscript letters are significantly different ($P < 0.05$). BMC: buttermilk control; BMP: buttermilk with probiotics; BMF1, BMF2: buttermilks with 3 and 5% flaxseed, respectively; BMPF1, BMPF2: buttermilks with probiotics and 3 and 5% flaxseed, respectively.

Table 3. Colour parameters of fermented buttermilks

Samples	Colour parameters		
	L*	a*	b*
BMC	80±0.9[a]	−4.03±0.3[a]	5.67±0.55[a]
BMP	73.05±0.7[b]	−4.27±0.5[a]	5.69±0.3[a]
BMF1	68.89±4.5[c]	−1.22±0.2[b]	11.89±1.24[b]
BMF2	59.94±0.67[e]	−1.75±0.4[c]	13.38±0.6[c]
BMPF1	68.74±0.87[c]	−1.21±0.8[b]	11.88±1[b]
BMPF2	59.11±1.2[e]	−1.79±0.47[c]	13.33±0.7[c]

[a, b]: Mean values ±SD (n=3) within a column with different superscript letters and within a row with different superscript letters are significantly different ($P < 0.05$). BMC: buttermilk control; BMP: buttermilk with probiotic; BMF1, BMF2: buttermilks with 3 and 5% flaxseed, respectively; BMPF1, BMPF2: buttermilks with probiotics and 3–5% flaxseed, respectively.

shown in Fig. 2. As can be seen, a significant difference was found in viscosity values ($P < 0.05$). In fact, viscosity values of buttermilk samples significantly increased ($P < 0.05$) with increasing flaxseed content. The viscosity values are in the following order: 23.5 ± 0.31, 23.3 ± 0.8, 12.6 ± 1.6, 12.8 ± 0.44, 2.63 ± 0.7, and 2.41 ± 1.2 Pa s for BMPF2, BMF2, BMF1, BMPF1, and BMC samples, respectively. This behaviour can be attributed to the interaction of the fibre and protein of flaxseed with the water of buttermilk, which leads to a stronger three dimensional network of buttermilk. According to the previous studies, fibre and protein compounds can increase the water retention capacity, leading to the formation of viscous gels and consequently promoting higher viscosities (Ardabilchi et al., 2019). Also, we can report the symbiosis between probiotics and flaxseed as prebiotic, which enhance the viscosity of buttermilk (Fig. 2).

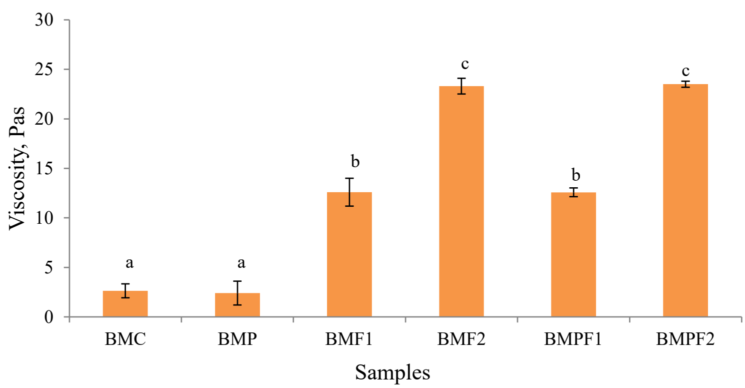

Fig. 2. Viscosity characteristics of fermented buttermilks
[a, b]: Mean values ± SD (n = 3) within a column with different superscript letters and within a row with different superscript letters are significantly different ($P < 0.05$). BMC: Buttermilk Control; BMP: Buttermilk with probiotics; BMF1, BMF2: Buttermilks with 3 and 5% flaxseed, respectively; BMPF1, BMPF2: Buttermilks with probiotics and 3–5% flaxseed, respectively

Table 4. Viable lactic acid bacteria counts in fermented buttermilks

Bacterial counts (log CFU mL^{-1})	Samples					
	BMC	BMP	BMF1	BMF2	BMPF1	BMPF2
L. plantarum	–	8.3±0.46^{a}	–	–	8.3±0.46^{a}	8.25±0.01^{a}
S. thermophilus	8.2±0.02^{a}	8.1±0.06^{a}	8.12±0.01^{a}	8.03±0.2^{a}	8.14±0.17^{a}	8.2±0.4^{a}
L. bulgaricus	8.1±0.08^{a}	8±0.08^{a}	8.22±0.08^{a}	8.16±0.08^{a}	8.11±0.08^{a}	8.15±0.08^{a}

–: not identified; ^{a,b}: mean values ±SD (n=3) within a column with different superscript letters and within a row with different superscript letters are significantly different (P < 0.05) BMC: buttermilk control; BMP: buttermilk with probiotic; BMF1, BMF2: buttermilks with 3–5% flaxseed, respectively; BMPF1, BMPF2: buttermilks with probiotics and 3 and 5% flaxseed, respectively.

3.5. Bacterial cell counts

The counts of probiotic bacteria *L. plantarum, L. bulgaricus*, and *S. thermophilus* are summarised in Table 4. The viable counts of *L. plantarum* M63 were 8.3 ± 0.46 and 8.25 ± 0.01 log CFU mL^{-1} in BMP and BMPF2 samples, respectively (P > 0.05). The probiotic counts were higher than the threshold number (10^7 CFU mL^{-1}) that is suggested to confer probiotic benefits (FAO/WHO, 2002). The viability of *L. plantarum* was also showed in cheese (Mahmoudi et al., 2021).

The counts of *L. bulgaricus* and *S. thermophilus* were similar in different buttermilk samples (P > 0.05). Thus, the buttermilk product could be considered as a fermented product beneficial to human health. The combination of *L. plantarum* and flaxseed represents a new option to add further value to dairy products.

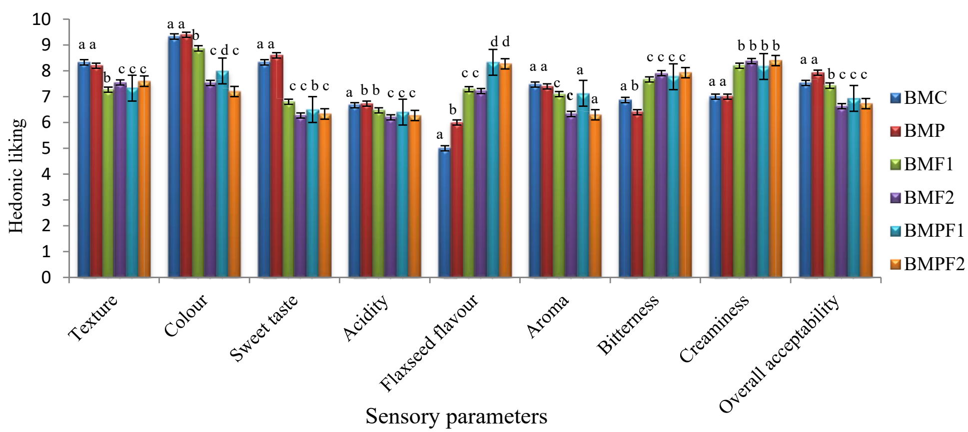

Fig. 3. Sensory analyses of fermented buttermilks

$^{a,\ b}$: Mean values ± SD (n = 3) within a column with different superscript letters and within a row with different superscript letters are significantly different (P < 0.05). BMC: buttermilk control; BMP: buttermilk with probiotics; BMF1, BMF2: buttermilks with 3–5% flaxseed, respectively; BMPF1, BMPF2: buttermilks with probiotics and 3 and 5% flaxseed, respectively

3.6. Sensory evaluation

Enrichment of buttermilks with fibre-containing ingredients improved water holding capacity and viscosity. Although similar trend was observed in this study, a significantly low texture index at a higher enrichment level (5%) was found, and the insoluble flaxseed powder particles can be hold responsible for the graininess and bad mouth-feel (Basiri et al., 2018). The higher scores for sweet taste were attributed to control (BMC) and probiotic (BMP) samples ($P < 0.05$) (Fig. 3). The cultured buttermilk samples containing flaxseed had a relatively lower acidity. Moreover, the fortified samples had the lowest preference in terms of colour, sweet taste, aroma, and overall acceptability from the panellist's perspective. The decrease in colour preference could be explained with increase in yellowness and reduction in whiteness and shininess as detected in the instrumental colour analysis. It was revealed that the addition of flaxseed reduced the overall acceptability ($P < 0.05$) due to its aroma and flavour, brownish-yellow colour, and graininess.

4. CONCLUSIONS

Fortification of probiotic buttermilk with flaxseed powder makes it a functional food as cultured buttermilk, which is naturally devoid of dietary fibre. Flaxseed fortification did not affect lactic acidity and pH and improved physicochemical characteristics, and viscosity of probiotic cultured buttermilk samples. In fact, it markedly reduced the phase separation, which a serious problem in reference to consumer acceptance. Therefore, probiotics and flaxseed were considered both suitable dietary agents and supplements for various products, which can help preventing most diseases owing to their high beneficial and nutritional properties.

ACKNOWLEDGMENTS

This study was sponsored by the «Tunisian Ministry of Higher Education and Scientific Research» from University of Carthage.

REFERENCES

Amjadi, S., Emaminia, S., Nazari, M., Davudian, S.H., Roufegarinejad, L., and Hamishehkar, H. (2019). Application of reinforced ZnO nanoparticle-incorporated gelatin bionanocomposite film with chitosan nanofiber for packaging of chicken fillet and cheese as food models. *Food and Bioprocess Technology*, 12: 1205–1219.

Aoac (1990). *Official methods of analysis*, 15th ed. Association of Official Analytical Chemists, Washington, DC USA, pp. 318–319.

Ardabilchi, M., Amjadi, S., Ardabilchi, M., Roufegarinejad, L., and Jafari, S.M. (2019). Fortification of yogurt with flaxseed powder and evaluation of its fatty acid profile, physicochemical, antioxidant, and sensory properties. *Powder Technology*, 359: 76–84.

Bekhit, A.E.D.A., Shavandi, A., Jodjaja, T., Birch, J., Teh, S., Mohamed Ahmed, I.A., Al-Juhaimi, F.Y., Saeedi, P., and Bekhit, A.A. (2018). Flaxseed: composition, detoxification, utilization, and opportunities. *Biocatalysis and Agricultural Biotechnology*, 13: 129–152.

Barukčić, I., Jakopovic, K.L., and Božanić, R. (2019). Valorisation of whey and buttermilk for production of functional beverages – an overview of current possibilities. *Food Technology and Biotechnology*, 57(4): 448–460.

Basiri, N., Haidary, S., Shekarforoush, S., and Niakousari, M. (2018). Flaxseed mucilage: a natural stabilizer in stirred yogurt. *Carbohydrate Polymers*, 187: 59–65.

Conway, V., Couture, M.D.P., Gauthier, S., Pouliot, Y., and Lamarche, B. (2014). Effect of buttermilk consumption on blood pressure in moderately hypercholesterolemic men and women. *Nutrition*, 30(1): 116–119.

Delikanli, B. and Ozcan, T. (2017). Improving the textural properties of yogurt fortified with milk proteins. *Journal of Food Processing and Preservation*, 41(5): e13101.

FAO/WHO (2002). *Guidelines for the evaluation of probiotics in food*. FAO/WHO, Food and Agriculture Organization of the United Nations/World Health Organization, London, https://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf.

Fardet, A. (2015). Complex foods versus functional foods, nutraceuticals and dietary supplements: differential health impact (Part 1). *Agro Food Industry HiTech Journal*, 26: 20–24.

Jaster, H., Arend, G.D., Rezzadori, K., Chaves, V.C., Reginatto, F.H., and Petrus, J.C.C. (2018). Enhancement of antioxidant activity and physicochemical properties of yogurt enriched with concentrated strawberry pulp obtained by block freeze concentration. *Food Research International*, 104: 119–125.

Kaur, R., Kaur, M., and Purewal, S.S. (2018). Effect of incorporation of flaxseed to wheat rusks: antioxidant, nutritional, sensory characteristics, and in vitro DNA damage protection activity. *Journal of Food Processing and Preservation*, 42(4): e13585.

Kumar, J., Gupta, V.K., Kumar, S., and Kumar, S. (2015). Effect of coagulants on the quality of chhana and rasogolla obtained from admixture of buffalo milk and buttermilk. *Journal of Food Science and Technology*, 52(3): 1736–1741.

Mahmoudi, I., Telmoudi, A., Chouaibi, M., and Hassouna, M. (2021). In vitro assessment of health-promoting benefits of sheep 'Testouri' cheese. *Acta Alimentaria*, 50: 54–64.

Mudgil, D., Barak, S., and Darji, P. (2016). Development and characterization of functional cultured buttermilk utilizing *Aloe vera* juice. *Food Bioscience*, 15: 105–109.

Nirgude, R., Binorkar, S.V., Parlikar, G.R., Kirte, M.C., and Savant, D.P. (2013). Therapeutic and nutritional values of takra (buttermilk). *International Research Journal of Pharmacy*, 4(2): 29–31.

Sarabandi, K., Jafari, S.M., Mohammadi, M., Akbarbaglu, Z., Pezeshki, A., and Khakbaz Heshmati, M. (2019). Production of reconstitutable nanoliposomes loaded with flaxseed protein hydrolysates: stability and characterization. *Food Hydrocolloids*, 96: 442–450.

Tomic, N., Dojnov, B., Miocinovic, J., Tomasevic, I., Smigic, N., Djekic, I., and Vujcic, Z. (2017). Enrichment of yoghurt with insoluble dietary fiber from triticale – a sensory perspective. *LWT – Foods Science and Technology*, 80: 59–66.

Zhao, Z., Feng, R., Rena, F., and Mao, X. (2018). Addition of buttermilk improves the flavour and volatile compound profiles of low-fat yogurt. *LWT – Food Science and Technology*, 98: 9–17.