Scientific Research Publishing

Food and Nutrition Sciences, 2021, 12, 670-680
https://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

Effect of Flaxseed on Bile Tolerances of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus*

Meghana Theegala[1], René Adalberto Chigüila Arévalo[1], Valerie Viana[1], Douglas Olson[1,2], Kayanush Aryana[1,2*]

[1]School of Animal Sciences, Louisiana State University, Baton Rouge, USA
[2]School of Nutrition and Food Sciences, Louisiana State University Agricultural Center, Baton Rouge, USA
Email: *KAryana@agcenter.LSU.edu

How to cite this paper: Theegala, M., Arévalo, R.A.C., Viana, V., Olson, D. and Aryana, K. (2021) Effect of Flaxseed on Bile Tolerances of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus*. *Food and Nutrition Sciences*, **12**, 670-680.
https://doi.org/10.4236/fns.2021.127050

Received: May 20, 2021
Accepted: July 10, 2021
Published: July 13, 2021

Abstract

Consumption of flaxseed provides health benefits. Bile tolerance allows survival of probiotics in the intestinal tract. The objective was to determine whether or not flaxseed enhances bile tolerance of *Lactobacillus acidophilus* (*L. acidophilus*) LA-K, *Lactobacillus delbruekii* ssp. *bulgaricus* (*L. bulgaricus*) LB-12, and *Streptococcus salivarius* ssp. *thermophilus* (*S. thermophilus*) ST-M5. Control and experimental (62 g flaxseed/L) broths containing 0.3% oxgall were prepared for each culture, sterilized, cooled, inoculated, and plated for 8 h. Growth of each microorganism in both the control and experimental broths was evaluated by the slope of the regression line of its log count versus time after inoculation. Flaxseed significantly enhanced growth of *L. acidophilus* but not *L. bulgaricus* and *S. thermophilus* over 8 h compared to its corresponding control. Therefore, flaxseed improved the bile tolerance of *L. acidophilus* but not of *S. thermophilus* and *L. bulgaricus*.

Keywords

Flaxseed, Bile tolerance, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, Probiotic

1. Introduction

The plant *Linum usitatissimum* L., commonly known as flax plant, has been grown in Europe and Asia for as long as 10,000 years [1]. Five main nutritional components of flaxseed are fiber, protein, lignans, oil and vitamins as determined by gas chromatography and high-performance liquid chromatography

(HPLC) techniques [2]. Flaxseed meal is rich in bioactive compounds and their extracts exhibit strong anti-radical activity [3]. Flaxseed has the highest amount of the plant lignan secoisolariciresinol diglucoside (SDG) [4]. Whole flaxseed contains 6.1 - 13.3 mg SDG/g flaxseed [5]. Bacteria in the colon convert the SDG into mammalian lignans including enterodiol and enterolactone [6], which are responsible for anti-estrogenic and weak estrogenic effects in mammalian tissue [7]. Multiple studies have shown that flaxseed consumption leads to improvements in metabolism of glucose or lipids or both [4] [8] [9] [10] [11] [12]. Supplementing biscuits with flaxseed and sidr leaf has been shown to provide various health benefits including renal-protective and immune-enhancing properties [13].

An early definition of the term "probiotic" deals with one species of protozoa that produces growth-promoting factors that benefits the growth of another species [14]. A more recent and commonly used definition of probiotics is "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [15]. Species of *Lactobacillus* and *Bifidobacteria* are the most common types of probiotics, but other types of probiotics also exist. *L. acidophilus* (first described by Ernst Moro in 1900) is a well-known probiotic that is commonly added to yogurt. *L. bulgaricus* (first described by Stamen Grigoroff in 1905) and *S. thermophilus* (first described by Sigurd Orla-Jensen in 1919) are used as yogurt starters [16] as they produce lactic acid and have a symbiotic relationship with each other. These yogurt starters have also been referred to as probiotics [17] [18]. Some studies have shown that yogurt bacteria can survive transit through the human gastrointestinal tract since these organisms can be recovered in feces [19] [20]. A summary of health benefits provided by probiotics has been given by Fijan [21].

Bile tolerance refers to an *in vitro* test that measures the ability of a bacterial strain to survive in a media that contains bile salts with similarities to conditions within the small intestines to give an indication of the likelihood that a bacterial strain would survive in the host. It is an important selection criteria for probiotics for incorporation into foods and beverages. Sherman [22] mentioned the tolerance for bile in the enterococcus division. In an early attempt in classification, Wheater [23] classified various lactobacilli cultures into *L. acidophilus*, *L. bulgaricus*, and a third intermediate group based on many tests including tolerance to bile salt (sodium tauroglycocholate). Lankaputhra and Shah [24] have shown that survival of *L. acidophilus* in 1.0% and 1.5% bile varied substantially among different strains. Bile tolerance and metabolism of bile salts have recently been reviewed by Ayyash [25].

There have been many recent attempts to improve bile tolerance including addition of various ingredients and application of various processing techniques. However, it does not appear that there are any reports examining if bile tolerances of free *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus* can be improved by addition of flaxseed. Some studies [26] [27] have shown that growth of *L.*

acidophilus, *L. bulgaricus*, and *S. thermophilus* can be improved by addition of flaxseed. It was hypothesized that the bile tolerances of *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus* could potentially be improved by addition of flaxseed. With hopes of also utilizing health benefits provided by flaxseed, the objective of this study was to determine if flaxseed can enhance bile tolerance of *L. acidophilus* LA-K, *L. bulgaricus* LB-12 and *S. thermophilus* ST-M5.

2. Materials and Methods

2.1. Materials

The following supplies were obtained for this project. *L. acidophilus* LA-K (a pure culture strain commonly added as a probiotic to yogurt), *L. bulgaricus* LB-12 (a pure culture strain commonly used as a starter culture in yogurt), and *S. thermophilus* ST-M5 (another pure culture strain commonly used as a starter culture in yogurt) were obtained from Chr. Hansen (Milwaukee, WI). Broths that were used included BD Difco Lactobacilli MRS broth (Becton, Dickinson and Company, Sparks, MD) and Oxoid M17 broth (Oxoid Ltd., Basingstoke, Hampshire, England). Oxgall was obtained from United States Biological (Swampscott, MA) and sodium thioglycolate (mercaptoacetic acid, sodium salt) was obtained from Acros Organics (Geel, Belgium). Flaxseed that was used in the MRS and M17 broths was obtained from a local grocery store. Agar (Acros Organics, Geel, Belgium) was used in the MRS broth and M17 broth to form MRS agar and M17 agar, respectively. Lactose (Oxoid Ltd., Basingstoke, Hampshire, England) was used in both the M17 broth and agar. BD Bacto peptone (Becton, Dickinson and Company, Sparks, MD) was used for making dilutions.

2.2. Determination of Bile Tolerance

Bile tolerance was determined according to Pereira & Gibson [28] with modifications. A control (no flaxseed) broth and experimental (61.85 g flaxseed/L) broth were prepared for each culture. For *L. acidophilus* LA-K and *L. bulgaricus* LB-12, MRS broths [29] were prepared by mixing 55 g of MRS broth, 3 g of oxgall, 2 g of sodium thioglycolate, and 1 L of distilled water. For *S. thermophilus* ST-M5, 37.25 g of M17 broth [30], 3 g oxgall, and 950 mL of water were mixed. Also, 50 mL of a 10% lactose solution for the M17 broth was prepared. The M17 broth and the lactose solution were autoclaved separately at 121˚C for 15 min. The lactose solution was then added aseptically to the M17 broth containing oxgall after autoclaving. All broths were tempered to 37˚C before inoculation.

After inoculating the broths with the cultures forming a 10^{-2} dilution, broth samples were taken hourly for 8 h for dilution with 0.1% Bacto peptone and pour plating. *L. acidophilus* LA-K was plated in MRS agar and incubated anaerobically at 37˚C for 48 h. *L. bulgaricus* LB-12 was plated in MRS agar and incubated anaerobically at 37˚C for 72 h. *S. thermophilus* ST-M5 was plated in M17 agar containing 5% of a 10% (w/w) lactose solution and incubated aerobically at 37˚C for 24 h. The change in counts (reported as log CFUs/mL) over 8 h for the experimental broths was compared to the corresponding change in counts of the control (no flaxseed) for each culture. Three replicates were performed for both *L. acidophilus* LA-K and *L. bulgaricus* LB-12 while four replicates were performed for *S. thermophilus* ST-M5.

2.3. Statistical Analysis

The log plate count data were analyzed as a two factor (presence versus absence of flaxseed and time (hour) after inoculation) factorial experiment in a randomized block design using PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC). Slopes of regression lines of log counts as a function of time represent the net growth or decline of the bacteria over time, and a comparison of the slopes of the regression lines between the flaxseed-containing broth and the control would provide information about the effect of the presence of flaxseed on the growth of these bacteria in the presence of bile. Significant differences in slopes of regression lines were tested using a random coefficient model by examining the significance of the flaxseed (presence versus absence) and time (hours after inoculation) interaction term in the analysis of variance. Significance was set at $\alpha = 0.05$.

3. Results

3.1. *Lactobacillus acidophilus*

Figure 1 presents the log counts of *L. acidophilus* LA-K in MRS broths containing 0.3% oxgall and 0.2% sodium thioglycolate either in the presence or absence of flaxseed during an 8-h incubation. Although not statistically significant, log counts of *L. acidophilus* LA-K in the presence of flaxseed tended to be higher than in the absence of flaxseed. The slope of the regression line in the graph of log counts of *L. acidophilus* versus hours in the control broth was negative (−0.04109) and significant ($P = 0.0124$) (Table 1) indicating decreasing counts over time. Conversely, the slope of this type of graph for the flaxseed-containing

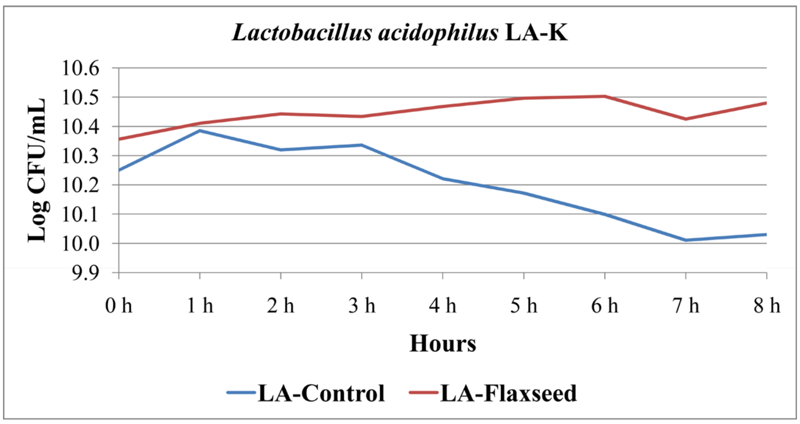

Figure 1. Log counts of *L. acidophilus* LA-K in MRS broths containing 0.3% oxgall and 0.2% sodium thioglycolate either in the presence or absence of flaxseed during an 8-h incubation.

broth was 0.01403, but not significant (*P* = 0.2030).

For *L. acidophilus*, the flaxseed (its presence versus its absence) and hour (after inoculation of *L. acidophilus*) factors were not significant in the analysis of variance (Table 2). However, the flaxseed and hour interaction was significant (*P* = 0.0147) indicating that the rate of change of log counts of *L. acidophilus* over time (hours) was significantly different for the flaxseed containing broth compared to the control broth. Therefore, the bile tolerance of *L. acidophilus* LA-K in MRS broth was significantly enhanced by flaxseed.

3.2. *Lactobacillus bulgaricus*

The log counts of *L. bulgaricus* LB-12 in MRS broths containing 0.3% oxgall and

Table 1. Parameter estimates for intercept and slope and corresponding *P* value for log counts of *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus* enumerated hourly over 8 h in presence or absence of flaxseed.

Bacteria	Control or Flaxseed	Parameter	Estimate	*P* value
L. acidophilus	Control	Intercept	10.3707	<0.0001
L. acidophilus	Control	Slope	−0.04109	0.0124
L. acidophilus	Flaxseed	Intercept	10.3853	<0.0001
L. acidophilus	Flaxseed	Slope	0.01403	0.2030
L. bulgaricus	Control	Intercept	9.1412	<0.0001
L. bulgaricus	Control	Slope	−0.02494	0.3127
L. bulgaricus	Flaxseed	Intercept	9.6777	<0.0001
L. bulgaricus	Flaxseed	Slope	0.01205	0.5945
S. thermophilus	Control	Intercept	10.2944	<0.0001
S. thermophilus	Control	Slope	−0.00275	0.8875
S. thermophilus	Flaxseed	Intercept	10.7818	<0.0001
S. thermophilus	Flaxseed	Slope	−0.01963	0.3272

Table 2. F values and P values for main effects (flaxseed and hour) and their interaction (flaxseed * hour) for log counts of *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus* enumerated hourly over 8 h in presence or absence of flaxseed.

Bacteria	Effect	F value	*P* value
L. acidophilus	Flaxseed	0.01	0.9213
L. acidophilus	Hour	4.29	0.1100
L. acidophilus	Flaxseed * Hour	17.80	0.0147
L. bulgaricus	Flaxseed	13.65	0.0201
L. bulgaricus	Hour	0.18	0.6913
L. bulgaricus	Flaxseed * Hour	1.49	0.2851
S. thermophilus	Flaxseed	10.96	0.0164
S. thermophilus	Hour	0.74	0.4253
S. thermophilus	Flaxseed * Hour	0.42	0.5423

0.2% sodium thioglycolate either in the presence or absence of flaxseed during an 8-h incubation are shown in **Figure 2**. The slopes of the regression lines in the graph for log count of *L. bulgaricus* over time were not significantly different from 0 for both the broth containing flaxseed and the control broth (**Table 1**).

For *L. bulgaricus*, presence or absence of flaxseed was significant ($P = 0.0201$) (**Table 2**), and log *L. bulgaricus* counts in presence of flaxseed were significantly higher than in its absence. However, hour was not significant. The flaxseed and hour interaction was not significant, indicating no significant differences in the rate of change of log counts of *L. bulgaricus* over time (hours) for the broth containing flaxseed versus for the control broth. Therefore, the bile tolerance of *L. bulgaricus* LB-12 in MRS broth was not significantly enhanced by flaxseed.

3.3. *Streptococcus thermophilus*

Figure 3 presents log counts of *S. thermophilus* ST-M5 in M17 broths containing 0.5% lactose and 0.3% oxgall either in the presence or absence of flaxseed during an 8-h incubation. Similar results were obtained for *S. thermophilus* as

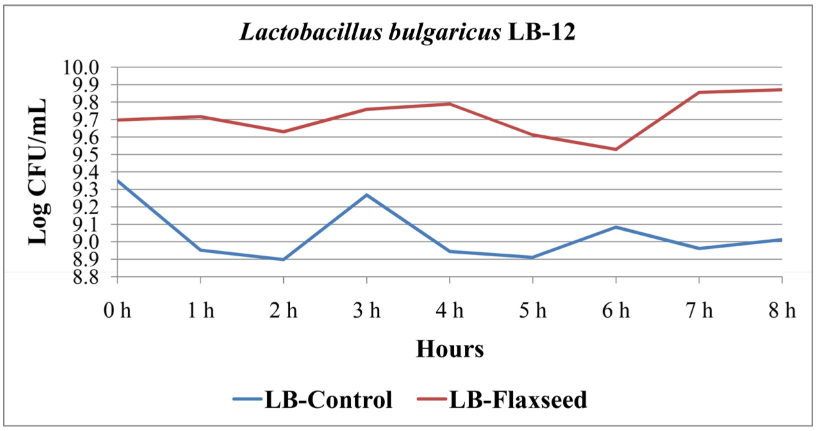

Figure 2. Log counts of *L. bulgaricus* LB-12 in MRS broths containing 0.3% oxgall and 0.2% sodium thioglycolate either in the presence or absence of flaxseed during an 8-h incubation.

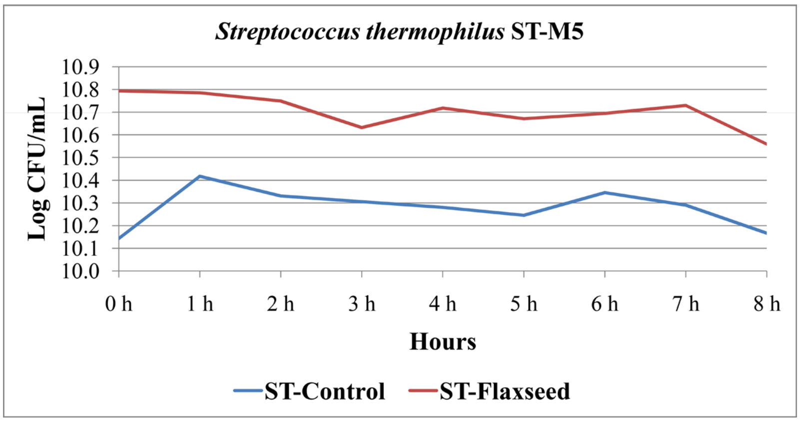

Figure 3. Log counts of *S. thermophilus* ST-M5 in M17 broths containing 0.5% lactose and 0.3% oxgall either in the presence or absence of flaxseed during an 8-h incubation.

for *L. bulgaricus*. The slopes of the regression lines in the graph for log count of *S. thermophilus* over time were also not significantly different from 0 for both the broth containing flaxseed and the control broth (**Table 1**).

Likewise, flaxseed was significant ($P = 0.0164$). Log counts of *S. thermophilus* ST-M5 in the presence of flaxseed were higher than in the absence of flaxseed. However, hour and the flaxseed and hour interaction were not significant (**Table 2**). Therefore, the bile tolerance of *S. thermophilus* ST-M5 in M17 broth was not significantly enhanced by flaxseed.

4. Discussion

Addition of various ingredients and application of various processing techniques have been shown to improve the bile tolerance of these microorganisms. Addition of up to 3% whey protein isolate improved the bile tolerance of pure cultures of *S. thermophilus* ST-M5 and *L. bulgaricus* LB-12 during 5 h of oxgall exposure [31]. Likewise, addition of 5% lactose improved the bile tolerance of pure cultures of *S. thermophilus* ST-M5 and *L. bulgaricus* LB-12 during 12 h of oxgall exposure [32]. Addition of inulin to yogurt milk improved the ability of *L. acidophilus* to tolerate bile salts compared to *L. acidophilus* in yogurt not containing inulin [33]. Encapsulating *L. bulgaricus* in alginate-milk microspheres allowed it to survive in 1% and 2% porcine bile salt solutions for 1 and 2 h while free *L. bulgaricus* did not survive [34]. Various low homogenization pressures ranging from 3.45 to 13.80 MPa improved bile tolerance of *S. thermophilus* ST-M5, and a homogenization pressure of 6.90 MPa improved bile tolerance of *L. acidophilus* LA-K during 10 h of exposure to 0.3% oxgall [35].

The effect of flaxseed on the growth of *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus* has also been investigated in other studies. Although the growth of *L. acidophilus* in MRS broth containing oxgall improved in the presence of flaxseed for 8 h in the present study, Bialasová *et al.* [36] found decreased growth of *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus* in the presence of flaxseed meal in milk during 28 d of storage. Differences between the present results and the results from Bialasová *et al.* [36] could be due to numerous factors including different strains of microorganisms, presence of bile in the present study, different time periods, and different growth media. However, Mihoubi *et al.* [26] found consistently increased *S. thermophilus* and *L. bulgaricus* counts in yogurt supplemented with 3% ground flaxseed compared with its control during the 3.5 h fermentation time and 28 d of storage. Likewise, Mousavi *et al.* [27] found higher *L. acidophilus* counts in stirred yogurt during 28 d of storage when supplemented with either 2% or 4% flaxseed. Smolová *et al.* [37] reported that they were able to keep *L. acidophilus* counts above 10^7 cfu/g in UHT skim milk containing various varieties and forms of flaxseed during 28 d of 4˚C to 6˚C storage. Bustamante *et al.* [38] found that *L. acidophilus* that was encapsulated with flaxseed mucilage either in the presence or absence of flaxseed soluble protein maintained greater relative viability for 6 h in MRS broth containing bovine bile.

It is important to note while the dosage of flaxseed was determined by SDG content, there is no proof the beneficial effect of flaxseed on *L. acidophilus* is strictly due to SDG. Further studies would have to be performed to determine which component of the flaxseed is responsible for this beneficial effect.

5. Conclusion

The purpose of this study was to investigate the effect of flaxseed on the bile tolerance of *L. acidophilus* LA-K, *L. bulgaricus* LB-12 and *S. thermophilus* ST-M5. Whole flaxseed administered at 61.85 g/L of broth significantly increased the growth, and therefore bile tolerance, of *L. acidophilus* LA-K but not *L. bulgaricus* LB-12 and *S. thermophilus* ST-M5 in the presence of oxgall (3 g/L) between 0 to 8 h when compared to their control. Improving the *in vitro* bile tolerance of *L. acidophilus* would likely improve their survival in the host.

Acknowledgments

This research was supported in part by a Howard Hughes Medical Institute grant through the Undergraduate Biological Sciences Education Program to LSU.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Berglund, D.R. (2002) Flax: New Uses and Demands. In: Janick, J. and Whipkey, A., Eds., *Trends in New Crops and New Uses*, ASHS Press, Alexandria, 358-360.

[2] Herchi, W., Arráez-Román, D., Boukhchina, S., Kallel, H., Segura-Carretero, A. and Fernández-Gutierrez, A. (2012) A Review of the Methods Used in the Determination of Flaxseed Components. *African Journal of Biotechnology*, **11**, 724-731.

[3] Tavarini, S., De Leo, M., Matteo, R., Lazzeri, L., Braca, A. and Angelini, A. (2021) Flaxseed and Camelina Meals as Potential Sources of Health-Beneficial Compounds. *Plants*, **10**, 156. https://doi.org/10.3390/plants10010156

[4] Zhang, W., Wang, X.B., Liu, Y., Tian, H.M., Flickinger, B., Empie, M.W. and Sun, S.Z. (2008) Dietary Flaxseed Lignan Extract Lowers Plasma Cholesterol and Glucose Concentrations in Hypercholesterolaemic Subjects. *British Journal of Nutrition*, **99**, 1301-1309. https://doi.org/10.1017/S0007114507871649

[5] Johnsson, P., Kamal-Eldin, A., Lundgren, L.N. and Åman, P. (2000) HPLC Method for Analysis of Secoisolariciresinol Diglucoside in Flaxseeds. *Journal of Agricultural and Food Chemistry*, **48**, 5216-5219. https://doi.org/10.1021/jf0005871

[6] Westcott, N.D. and Muir, A.D. (1998) Process for Extracting Lignans from Flaxseed. Agriculture and Agri-Food Canada, Assignee. US Patent Number 5,705,618.

[7] Dixon, R.A. (2004) Phytoestrogens. *Annual Review of Plant Biology*, **55**, 225-261. https://doi.org/10.1146/annurev.arplant.55.031903.141729

[8] Cunnane, S.C., Ganguli, S., Menard, C., Liede, A.C., Hamadeh, M.J., Chen, Z.-Y., Wolever, T.M.S. and Jenkins, D.J.A. (1993) High-Linolenic Acid Flaxseed (*Linum usitatissimum*): Some Nutritional Properties in Humans. *British Journal of Nutrition*, **69**, 443-453. https://doi.org/10.1079/BJN19930046

[9] Pan, A., Sun, J., Chen, Y., Ye, X., Li, H., Yu, Z., Wang, Y., Gu, W., Zhang, X., Chen, X., Demark-Wahnefried, W., Liu, Y. and Lin, X. (2007) Effects of a Flaxseed-Derived Lignan Supplement in Type 2 Diabetic Patients: A Randomized, Double-Blind, Cross-Over Trial. *PLoS ONE*, **2**, e1148. https://doi.org/10.1371/journal.pone.0001148

[10] Yari, Z., Cheraghpour, M. and Hekmatdoost, A. (2021a) Flaxseed and/or Hesperidin Supplementation in Metabolic Syndrome: An Open-Labeled Randomized Controlled Trial. *European Journal of Nutrition*, **60**, 287-298. https://doi.org/10.1007/s00394-020-02246-9

[11] Yari, Z., Cheraghpour, M., Alavian, S.M., Hedayati, M., Eini-Zinab, H. and Hekmatdoost, A. (2021b) The Efficacy of Flaxseed and Hesperidin on Non-Alcoholic Fatty Liver Disease: An Open-Labeled Randomized Controlled Trial. *European Journal of Clinical Nutrition*, **75**, 99-111. https://doi.org/10.1038/s41430-020-0679-3

[12] Almehmadi, A., Lightowler, H., Chohan, M. and Clegg, M.E. (2021) The Effect of a Split Portion of Flaxseed on 24-h Blood Glucose Response. *European Journal of Nutrition*, **60**, 1363-1373. https://doi.org/10.1007/s00394-020-02333-x

[13] El Seedy, G.M., El-Shafey, E.S. and Elsherbiny, E.S. (2021) Fortification of Biscuit with Sidr Leaf and Flaxseed Mitigates Immunosuppression and Nephrotoxicity Induced by Cyclosporine A. *Journal of Food Biochemistry*, **45**, e13655. https://doi.org/10.1111/jfbc.13655

[14] Lilly, D.M. and Stillwell, R.H. (1965) Probiotics: Growth-Promoting Factors Produced by Microorganisms. *Science*, **147**, 747-748. https://doi.org/10.1126/science.147.3659.747

[15] FAO/WHO (2002) Guidelines for the Evaluation of Probiotics in Food. https://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf

[16] Aryana, K.J. and Olson, D.W. (2017) A 100-Year Review: Yogurt and Other Cultured Dairy Products. *Journal of Dairy Science*, **100**, 9987-10013. https://doi.org/10.3168/jds.2017-12981

[17] Uriot, O., Denis, S., Junjua, M., Roussel, Y., Dary-Mourot, A. and Blanquet-Diot, S. (2017) *Streptococcus thermophilus*: from Yogurt Starter to a New Promising Probiotic Candidate? *Journal of Functional Foods*, **37**, 74-89. https://doi.org/10.1016/j.jff.2017.07.038

[18] Wadhai, V.S. and Dhawas, V.K. (2011) Characterization and Study of *Lactobacillus bulgaricus* as Probiotic Bacteria. *Online International Interdisciplinary Research Journal*, **1**, 55-60.

[19] Mater, D.D.G., Bretigny, L., Firmesse, O., Flores, M.-J., Mogenet, A., Bresson, J.-L. and Corthier, G. (2005) *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* Survive Gastrointestinal Transit of Healthy Volunteers Consuming Yogurt. *FEMS Microbiology Letters*, **250**, 185-187. https://doi.org/10.1016/j.femsle.2005.07.006

[20] Elli, M., Callegari, M.L., Ferrari, S., Bessi, E., Cattivelli, D., Soldi, S., Morelli, L., Feuillerat, N.G. and Antoine, J.-M. (2006) Survival of Yogurt Bacteria in the Human Gut. *Applied and Environmental Microbiology*, **72**, 5113-5117. https://doi.org/10.1128/AEM.02950-05

[21] Fijan, S. (2014) Microorganisms with Claimed Probiotic Properties: An Overview of Recent Literature. *International Journal of Environmental Research and Public Health*, **11**, 4745-4767. https://doi.org/10.3390/ijerph110504745

[22] Sherman, J.M. (1937) The Streptococci. *Bacteriological Reviews*, **1**, 3-97. https://doi.org/10.1128/br.1.1.3-97.1937

[23] Wheater, D. (1955) The Characteristics of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*, *Journal of General Microbiology*, **12**, 123-132. https://doi.org/10.1099/00221287-12-1-123

[24] Lankaputhra, W.E.V. and Shah, N.P. (1995) Survival of *Lactobacillus acidophilus* and *Bifidobacterium* spp in the Presence of Acid and Bile Salts. *Cultured Dairy Products Journal*, **30**, 2-7.

[25] Ayyash, M.M., Abdalla, A.K., AlKalbani, N.S., Baig, M.A., Turner, M.S., Liu, S.-Q. and Shah, N.P. (2021) Invited Review: Characterization of New Probiotics from Dairy and Nondairy Products-Insights into Acid Tolerance, Bile Metabolism and Tolerance, and Adhesion Capability. *Journal of Dairy Science*. (In Press) https://doi.org/10.3168/jds.2021-20398

[26] Mihoubi, M., Amellal-Chibane, H., Mekimene, L., Noui, Y. and Halladj, F. (2017) Physicochemical, Microbial, and Sensory Properties of Yogurt Supplemented with Flaxseeds during Fermentation and Refrigerated Storage. *Mediterranean Journal of Nutrition and Metabolism*, **10**, 211-221. https://doi.org/10.3233/MNM-17151

[27] Mousavi, M., Heshmati, A., Garmakhany, A.D., Vahidinia, A. and Taheri, M. (2019) Optimization of the Viability of *Lactobacillus acidophilus* and Physico-Chemical, Textural and Sensorial Characteristics of Flaxseed-Enriched Stirred Probiotic Yogurt by Using Response Surface Methodology. *LWT-Food Science and Technology*, **102**, 80-88. https://doi.org/10.1016/j.lwt.2018.12.023

[28] Pereira, D.I.A. and Gibson, G.R. (2002) Cholesterol Assimilation by Lactic Acid Bacteria and Bifidobacteria Isolated from the Human Gut. *Applied and Environmental Microbiology*, **68**, 4689-4693. https://doi.org/10.1128/AEM.68.9.4689-4693.2002

[29] de Man, J.C., Rogosa, M. and Sharpe, M.E. (1960) A Medium for the Cultivation of Lactobacilli. *Journal of Applied Bacteriology*, **23**, 130-135. https://doi.org/10.1111/j.1365-2672.1960.tb00188.x

[30] Terzaghi, B.E. and Sandine, W.E. (1975) Improved Medium for Lactic Streptococci and Their Bacteriophages. *Applied Microbiology*, **29**, 807-813. https://doi.org/10.1128/am.29.6.807-813.1975

[31] Vargas, L.A., Olson, D.W. and Aryana, K.J. (2015) Whey Protein Isolate Improves Acid and Bile Tolerances of *Streptococcus thermophilus* ST-M5 and *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12. *Journal of Dairy Science*, **98**, 2215-2221. https://doi.org/10.3168/jds.2014-8869

[32] Mena, B. and Aryana, K. (2018) Lactose Enhances Bile Tolerance of Yogurt Culture Bacteria. *Journal of Dairy Science*, **101**, 1957-1959. https://doi.org/10.3168/jds.2017-13919

[33] Iltar, R., Asci, A. and Kücükcetin, A. (2012) Viability and *in Vitro* Properties of *Lactobacillus acidophilus* Used in Yoghurt as Influenced by Inulin Addition. *Milchwissenschaft*, **67**, 142-146.

[34] Shi, L.-E., Li, Z.-H., Li, D.-T., Xu, M., Chen, H.-Y., Zhang, Z.-L. and Tang, Z.-X. (2013) Encapsulation of Probiotic *Lactobacillus bulgaricus* in Alginate-Milk Microspheres and Evaluation of the Survival in Simulated Gastrointestinal Conditions. *Journal of Food Engineering*, **117**, 99-104. https://doi.org/10.1016/j.jfoodeng.2013.02.012

[35] Muramalla, T. and Aryana, K.J. (2011) Some Low Homogenization Pressures Improve Certain Probiotic Characteristics of Yogurt Culture Bacteria and *L. acidophilus* LA-K. *Journal of Dairy Science*, **94**, 3725-3738. https://doi.org/10.3168/jds.2010-3737

[36] Bialasová, K., Němečková, I., Kyselka, J., Štětina, J., Solichová, K. and Horáčková, S. (2018) Influence of Flaxseed Components on Fermented Dairy Product Properties. *Czech Journal of Food Sciences*, **36**, 51-56. https://doi.org/10.17221/411/2017-CJFS

[37] Smolová, J., Němečková, I., Klimešová, M., Švandrlík, Z., Bjelková, M., Filip, V. and Kyselka, J. (2017) Flaxseed Varieties: Composition and Influence on the Growth of Probiotic Microorganisms in Milk. *Czech Journal of Food Sciences*, **35**, 18-23. https://doi.org/10.17221/356/2016-CJFS

[38] Bustamante, M., Villarroel, M., Rubilar, M. and Shene, C. (2015) *Lactobacillus acidophilus* La-05 Encapsulated by Spray Drying: Effect of Mucilage and Protein from Flaxseed (*Linum usitatissimum* L.). *LWT-Food Science and Technology*, **62**, 1162-1168. https://doi.org/10.1016/j.lwt.2015.02.017