Preparation of Bioactive Kefir with Added Flaxseed (\textit{Linum usitatissimum} L.) Extract

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Abstract

Flaxseed (\textit{Linum usitatissimum} L.) is an important food, oil, and fiber crop of the family Linaceae. Although flaxseed has been consumed as a food ingredient for several centuries, its nutritional benefits have not yet been completely established. Flaxseed is a good source of lignans, nonstarch polysaccharides, and high-quality proteins. Hence, in this study, we aimed to prepare a bioactive kefir containing flaxseed and to examine the physicochemical characteristics of kefir containing different concentrations of flaxseed. We investigated the pH and sensory evaluation of bioactive Kefir containing different concentrations of flaxseed. We investigated the pH, total anthocyanins (TAs), and sensory evaluation of bioactive Kefir containing different concentrations of flaxseed. The pH of this bioactive kefir decreased, whereas the TA content increased with increasing incubation time: however, these parameters were not affected by the amount of added flaxseed. As the addition rate of flaxseed increased, the scores for overall acceptability, texture, color, flavor, and taste in sensory evaluations were generally the same as or lower than the control. There were no significant differences in overall acceptability, texture, color, flavor, and taste between the control and treated groups. Therefore, further studies are needed to develop methods for production of health-improving kefir as a dietary supplement based on the functional properties of flaxseed.

Keywords

flaxseed (\textit{Linum usitatissimum} L.), lignans, kefir, sensory evaluation, dietary

Introduction

Kefir was a self-carbonated alcoholic fermented milk, originating from the Caucasian Mountains (Garrote et al., 1997), and could be made from any type of processed or raw milk such as cows’, goats’ or sheep’s milk (Cais-Sokolińska et al., 2008). Kefir was made through fermentation of milk using kefir grains and was a traditional beverage from Central Asia and Eastern European.

Recently, kefir has increasingly popularity around the world as new beneficial beverage (Jeong et al., 2017). The main difference between kefir and other fermented milks was the starter culture. According to the bacteria types, kefir grains contained \textit{Lactobacillus}, \textit{Lactococcus}, \textit{Leuconostoc} and \textit{Acetobacter} with two major bacteria \textit{Streptococcus thermophilus} and \textit{Lactococcus lactis} consisting about 53-65% of the total microflora. Also kefir included \textit{Kluyveromyces}, \textit{Saccharomyces}, \textit{Candida} and \textit{Torulopsis} genus which could help to digest lactose so as to reduce lactose intolerance (Turani et al., 2014). Until now, various benefits for kefir in nutrition and health have been reported through previous studies. Now, dietary fiber from new sources to use in food industry as a source of prebiotic has been investigated by many scientists. In general, non-digestible polysaccharides
such as galacto-oligosaccharides, fructooligo-saccharides and cyclodextrins were recognized as prebiotic substances (Patel and Goyal, 2012). It could selectively stimulate the growth and activity of the gastrointestinal micro-flora, simultaneously (Lin et al., 2011) Among them, flaxseed (*Linum usitatissimum* L.) was the seed from the plant and could be consumed as food. Health benefits claims of flaxseed were related to its components such as lignans, α-linolenic acid, and soluble dietary fiber or mucilage/gum. According to various previous researches, the consumption of flaxseed showed several health benefits to prevent colon cancer and to reduce the risk of cardiovascular disease.

In general, flaxseeds were the richest dietary source of lignan precursors (Fig. 1). Lignans were polyphenols found in plants and Lignan precursors were found in a wide variety of plant-based foods, including seeds, whole grains, legumes, fruit, and vegetables (Borriello et al., 1985; Rickard et al., 1996). When consumed, lignan precursors were converted to the enterolignans, enterodiol and enterolactone, by bacteria that normally colonized the human intestine. Enterodiol and enterolactone had weak estrogenic activity but also could exert biological effects through nonestrogenic mechanisms (Kaur and Gupta, 2002; Westcott and Muir, 2003). Lignan-rich foods were part of a healthful dietary pattern, but further studies on the role of lignans in the prevention of hormone-associated cancers, osteoporosis, and cardiovascular disease would be needed (Fig. 2).

Besides, the heterogenic polysaccharide known as flaxseed mucilage/gum was the soluble fiber components that constituted approximately 6 to 8% of the whole seed on a dry weight basis. When flaxseed was fermented in vitro, it was resulted in producing the high amounts of acetate and

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**Fig. 1.** Various chemical structure of lignin precursors in flaxseed (*Linum usitatissimum* L.).
Source: Rickard et al., (1996).

**Fig. 2.** Various beneficent effects of dietary lignan in flaxseed (*Linum usitatissimum* L.).
Source: Kaur and Gupta (2002), Westcott and Muir (2003).
propionate (short-chain fatty acids, SCFA) (Fodie et al., 2009). Also, when flaxseed mucilage used as food additives, it could capture free radicals to have anti-tumor and anti-
oxidant properties and could oxidize proteins, lipid or DNA to prevent cancers, simultaneously (Gutiérrez et al., 2010). Therefore, the goal of this study was to make the bioactive Kefir added with flaxseed (Linum usitatissimum L.) with upgrading the quality of organoleptic properties. In this experiment, among various physicochemical characteristics of bioactive kefir produced, TA & pH and sensory evaluation of the bioactive kefir added with flaxseed (Linum usitatissimum L.) were analyzed.

Materials and Methods

1. Crude materials extracted from flaxseed (Linum usitatissimum L.)
Flaxseed (Linum usitatissimum L.) was provided from Center for One Health, College of Veterinary Medicine, Konkuk University in Seoul, Korea. After cleaning and drying, the dried flaxseed (Linum usitatissimum L.) was macerated in 100% sterilized distilled water for 48 hours with occasionally stirring at 25°C. Then, the soluble ingredients were filtrated through 0.45 μm Millipore™ and stored at −20°C before use.

2. The preparation of bioactive Kefir added with flaxseed (Linum usitatissimum L.)
Kefir grains was obtained from Center for One Health, College of Veterinary Medicine, Konkuk University in Seoul, Korea. And kefir grain was used to ferment milk for pro-

| Flaxseed (Linum usitatissimum L.) | Cleaning and drying |
|-----------------------------------|---------------------|
| The dried flaxseed (Linum usitatissimum L.) was macerated in 100% sterilized distilled water for 48 hours with occasionally stirring at 25°C. | The soluble ingredients were filtrated through 0.45μm Millipore™ |
| The filtered soluble ingredients were stored at −20°C before use. | Heat treated cow milk
| Cooling at room temperature (about 25°C) | Inoculation of Kefir grain (about 2% to 3%) |
| Addition of (0% as control, 1%, 2% and 3%) | Fermentation at room temperature (about 25°C) for 24 hours |
| Storage at 4°C | |

Fig. 3. The flow chart for preparing crude ingredients extracted from flaxseed (Linum usitatissimum L.).

Producing bioactive kefir (Fig. 4). Bioactive Kefir samples were added with different concentration rates of flaxseed (Linum usitatissimum L.) (0% as control, 1%, 2%, and 3%). And then the bioactive kefir samples were stored at 4°C.

3. The pH & titratable acid of the bioactive Kefir added with flaxseed (Linum usitatissimum L.)
The pH of the homogenized yoghurt was determined using a digital pH meter (Orion Star A211, USA) and titratable acid (TA) was determined by titration with 0.1 N NaOH. Bioactive kefir sample (3 g) was transferred into an Erlenmeyer flask containing 27 mL of dH2O. Three to five drops of 0.1% phenolphthalein as pH indicator were added. The yoghurt mixture was then titrated with 0.1N NaOH with continuous stirring until a stable pink color was achieved. The amount of acid produced during fermentation was calculated as follows:

$$TTA \text{ (% Lactic acid)} = \frac{\text{Dilution factor} \times V \text{ NaOH} \times 0.1 \text{ N NaOH Factor} \times 0.009}{\text{Weight of sample (g)}} \times 100$$

Where $V_{\text{NaOH}}$ is the volume (mL) of 0.1 N NaOH required to neutralize the acid: a dilution factor of 10 was used.

4. The sensory evaluation of the bioactive Kefir added with flaxseed (Linum usitatissimum L.)
The sensory evaluation were carried out by 10 trained panelists between 20 and 50 years of age. The samples were coded with three digit numbers and randomly served at 7
to 10°C in plastic cups (10 mL). All assessors completed a test assessment form to compare the five sensory attributes (appearance, flavor, taste, and overall acceptability) by using a five-point hedonic scale (1, extremely poor; 2, poor; 3, fair; 4, good; 5, excellent).

5. The statistical analysis

Two separate experiments with duplicate assays were performed. Data were expressed as means. Statistical analysis was performed using one-way analysis of variance (ANOVA: GraphPad Prism 5, USA) followed by Duncan’s post hoc test for mean comparison. Statistical significance was established as \(p<0.05\).

Results and Discussion

1. The pH and TA of bioactive Kefir added with 0 to 3% of flaxseed (Linum usitatissimum L.)

In general, TA is high depending on the decrease of pH. In this study, the pH was decreased to about 4.51 after the fermentation of kefir premix. The pH value of bioactive kefir added with flaxseed (Linum usitatissimum L.) (1%, 2%, and 3%) showed 4.50, 4.52, and 4.51, respectively. Also, the TA was increased to approximately 0.90% after the fermentation of bioactive kefir premix. The TA contents of bioactive kefir added with flaxseed (Linum usitatissimum L.) (1%, 2%, and 3%) showed 0.92%, 0.91%, 0.92%, respectively. Then, the pH and TA value between control kefir and kefir added with flaxseed (Linum usitatissimum L) (1%, 2%, and 3%) were similar, and there was not any significant difference of TA and pH between control group and treated group. In general, fermented dairy products were characterized by an acidic taste originating from the presence of lactic acid, a by-product of lactic fermentation. Therefore, titratable acidity (TA) and pH were commonly used as measurements of acidity to determine the quality of milk before and during the production of fermented dairy products (Ntsame Affane et al., 2009). Furthermore, the acidity of Kefir was very significant during production of kefir, because over or deficient production of acidity in kefir could have an influence to mask the buttery character and then to change the structure of the product (Vedamathu, 2006).

When compared to previous researches, the range of pH normally reported for Kefir samples was 3.5~4.5 and the range of TA varied between 0.50 and 1.50 g 100 mL\(^{-1}\) (Simova et al., 2002; Chen et al., 2012), and the appropriate range of pH for a commercially available yogurt was between 3.27 and 4.53, and the value of TA was in the range of 0.7% and 1.20% (Adolfsson et al., 2004).

Namely, the results of this present study showed a very similar trend with those of various previous studies (Simova et al., 2002; Ntsame Affane et al., 2011; Chen et al., 2012).

2. The sensory evaluation of bioactive kefir added with flaxseed (Linum usitatissimum L.)

The sensory evaluation of the bioactive kefir was evaluated by 10 trained panelists of ages 20 to 50 years, and the results are summarized in Fig. 5.

The bioactive kefir was prepared with flaxseed (Linum usitatissimum L.) at concentrations of 0, 1, 2, and 3%, respectively. The overall acceptability scores of bioactive kefir with flaxseed (Linum usitatissimum L.) (0% as control, 1%, 2%, and 3%) showed 4.4, 4.5, 4.4, and 4.4, respectively. The texture scores of bioactive kefir with flaxseed (Linum usitatissimum L.) (0% as control, 1%, 2%, and 3%) showed 4.7, 4.7, 4.6, and 4.6, respectively. The colorscores of bioactive kefir with flaxseed (Linum usitatissimum L.) (0% as control, 1%, 2%, and 3%) showed 4.8, 4.8, 4.7, and 4.5, respectively. The flavor scores of bioactive kefir with flaxseed (Linum usitatissimum L.) (0% as control, 1%, 2%, and 3%) showed 3.7, 4.0, 4.2, and 4.2, respectively. And the taste scores for bioactive kefir with flaxseed (Linum usitatissimum L.) (0% as control, 1%, 2%, and 3%) showed 4.3, 4.4, 4.2, and 4.4, respectively.

Based on the statistical analysis of the sensory evaluation, there was not any significant difference of overall acceptability, texture, color, flavor, and taste between control group and treated group. Hence, among the experimental group, the scores of all categories except flavor were the same or lower in flaxseed (Linum usitatissimum L.)-containing bioactive kefir with 1%, 2%, and 3% compared with the control group. Summarizing the results of this study, the addition of flaxseed (Linum usitatissimum L.) did not affect the sensory evaluation such as overall acceptability,
Fig. 5. Sensory evaluations of the bioactive Kefir added with 0% as control, 1%, 2%, and 3%, of flaxseed (Linum usitatissimum L.).

Among of many beneficial factors of flaxseed, the flaxseed lignan SECO (secoisolariciresinol) and its diglucoside SDG (SecoisolariciresinolDiglucoside) were recognized as having various benefits for health. Especially, it had the antioxidant properties. In general, after ingestion, SDG was converted to enterolignans (enterodiol and enterolactone) by the intestinal microflora, and hence these metabolites (phytostrogens) were absorbed and could provide health benefits.

After feeding rats with specific doses of flaxseed, SDG decreased the systolic, diastolic and mean arterial pressure. SDG reduced the angiotensin I-induced rise in the arterial pressures and hence SDG was a potent Angiotensin-Converting Enzyme (ACE) inhibitor (Paschos et al., 2007; Prasad, 2009). And SDG supplementation could protect against the development of chronic diseases, cancer and diabetes on animal studies using various models of rat, mice and rabbit (Adolphe et al., 2010). Utilization of flaxseed for glycemic control would be associated to the decrease in risk of obesity and dyslipidemia, since these were risk factors for the development of diabetes and resistance to insulin (Bernachia et al., 2014).

Flaxseed supplementation resulted in the improvement in anthropometric measurements, blood pressure, and lipid profile in the experimental group, and hence flaxseed had the therapeutic potential in dyslipidemia (Katare et al., 2012). And Saxena and Katare (2014) also reported that body mass index (BMI) and body weight of the experimental group were significantly reduced as well as systolic and diastolic blood pressure, and a highly significant reduction in total cholesterol, triglycerides, low density lipoprotein-cholesterol (LDL-C) and low density lipoproteincholesterol (VLDL-C) levels were observed, simultaneously.

Lignans could protect against certain cancers, particularly hormone-sensitive cancers such as those of the breast, endometrium and prostate, by interfering with sex hormone metabolism (Bernachia et al., 2014). Flaxseed or pure lignans showed anticarcinogenic effects in many types of cancer, and flaxseed oil could inhibit the growth and development of tumors in the breast of laboratory animals (Lamblin et al., 2008). The influence of flaxseed lignans and oil components in reducing breast cancer risk and tumor growth was reviewed (Mason and Thompson, 2014).
Mechanisms included decreased cell proliferation and angiogenesis and increased apoptosis through modulation of estrogen metabolism and estrogen receptor and growth factor receptor signalling pathways. Also, flaxseed components were effective in the risk reduction and treatment of breast cancer (Mason and Thompson, 2014).

Lignans and other flaxseed compounds (ALA and fiber content) would be screened the efficacy in improving menopausal symptoms in women living with breast cancer and for potential impact on risk of breast cancer incidence or recurrence (Flower et al., 2013). They found that flax would be associated with decreased risk of breast cancer. Additionally, flax demonstrated anti-proliferative effects in breast tissue of women at risk of breast cancer, and also mortality risk could be reduced among those living with breast cancer (Flower et al., 2013).

Recently, there was scientifically proven that lignan and flaxseed oil reduced the growth of tamoxifen treated tumors by mechanisms involving signaling pathways, suggesting their potential use to aid in chemotherapies of some cancer types (Bernacchia et al., 2014). According to the effect of dietary flaxseed lignan or oil combined with tamoxifen treatment on tumor growth, SDG and flaxseed oil could reduce the growth of tamoxifen-treated tumors (Saggar et al., 2010). The dietary flaxseed could modestly lower serum levels of sex steroid hormones, especially in overweight/obese women (Sturgeon et al., 2008). Also, lignans could control the growth and differentiation of cultured human leukemic cells, possibly by interfering with DNA, RNA and/or protein synthesis (Cardoso et al., 2012). Since lignan cytotoxicity appeared to be low on normal immune cells, lignans could exert fungistatic, cytotoxic, antiviral activities and a hormonal modulation with causing a decrease in hot flashes which were characteristic of menopause (Xu et al., 2008). The clinical case study based on the impact of flaxseed supplementation (30 g/day) on hormonal levels in a 31-year-old woman with polycystic ovary syndrome was observed (Nowak et al., 2007). The flax consumption could alter circulating sex hormones and increased the urinary 2α-hydroxyestrone/16α-hydroxyestrone ratio associated with a lower risk of breast cancer. However, it is urgently needed for further research of flaxseed supplementation on various health benefits for human’s health.

Conclusively, the bioactive kefir added with 0%, 1%, 2%, and 3% of flaxseed (Linum usitatissimum L.) showed the decrease of pH but the increase of TA. Especially, the bioactive kefir containing 1~3% concentration of flaxseed (Linum usitatissimum L.) received higher scores for overall acceptability, texture, color, flavor, and taste in the sensory evaluation. Hence, further studies are needed to product various bioactive kefir with improving the efficiency of flaxseed (Linum usitatissimum L.) through synergy with human health.

Disclaimer

The views expressed herein do not necessarily reflect those of the US Food and Drug Administration or the US Department of Health and Human Services.

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References

Adolfsson, O., Meydani, S. N. and Russell, R. M. 2004. Yogurt and gut function. Am. J. Clin. Nutr. 80:245-256.
Adolphe, J. L., Whiting, S. J., Juurlink, B. H. J., Thorpe, L. U. and Alcorn, J. 2010. Health effects with consumption of the flax lignan secoisolariciresinol diglucoside. Br. J. Nutr. 103:929-938.
Bernacchia, R., Preti, R. and Vinci, G. 2014. Chemical composition and health benefits of flaxseed. Austin Journal of Nutrition and Food Science, vol. 2, no. 8, id 1045, 9 pp.
Borrelli, R. C., Mennella, C., Barba, F., Russo, M., Russo, L., Krome, K., Erbersdobler, H. F., Faist, V. and Fogliano, V. 2003. Characterization of coloured compounds obtained by enzymatic extraction of bakery products. Food Chemical Toxicology, 41:1367-1374.
Borriello, S. P., Setchell, K. D. R., Axelson, M. and Lawson, A. M. 1985. Production and metabolism of lignans by the
human fecal flora. J. Appl. Bacteriol. 58:37–43.
Cais-Sokolińska, D., Romualda, Danków, R. and Pikul, J.
2008. Physicochemical and sensory characteristics of
sheep kefir during storage. Acta Sci. Pol., Technol. Alim.
7:63–73.
Cardoso, Cáraro, J. C., Inês, de Souzadantas, M., Rocha,
Espeschit, A. C., Duartemartino, H. S. and Rocha, Ri-
beiro, S. M. 2012. Flaxseed and human health: Reviewing
benefits and adverse effects. Food Research Interna-
tional, 28:203–230.
Chen, Y. P., Hsiao, P. J., Hong, W. S., Dai, T. Y. and Chen,
M. J. 2012. Lactobacillus kefiranofaciens M1 isolated
from milk kefir grains ameliorates experimental colitis
in vitro and in vivo. J. Dairy Sci. 95:63–74.
Flower, G., Fritz, H., Balneaves, L. G., Verma, S., Skidmore,
B., Fernandes, R., and Seely, D. 2013. Flax and breast
cancer: A systematic review. Integrative Cancer Thera-
pies 13:181–192.
Fodje, A. M. L., Chang, P. R. and Letterme, P. 2009. In vitro
bile acid binding and short chain fatty acid profile of
flax fiber and ethanol co-products. Journal of Medicinal
Food 12:1065–1073.
GANorkar, P. M. and Jain, R. K. 2014. Effect of flaxseed
incorporation on physical, sensorial, textural and chem-
ical attributes of cookies. International Food Research
Journal 21:1515–1521.
Garrote, G. L., Abraham, A. G. and De Antoni, G. L. 1997.
Preservation of Kefir grains, a comparative study. LWT-
Food Science and Technology 30:77–84.
Gutiérrez, C., Rubilar, M., Jara, C., Verdugo, M., Sineiro, J.
and Shene, C. 2010. Flaxseed and flaxseed cake as a
source of compounds for food industry. Journal of Soil
Science and Plant Nutrition 10:454–463.
Jeong, D., Kim, D. H., Kang, I. B., Kim, H., Song, K. Y.,
Kim, H. S. and Seo, K. H. 2017. Modulation of gut mi-
icrobiota and increase in fecal water content in mice
induced by administration of Lactobacillus kefiran-
ofaciens DN1. Food Funct. 8:680–686.
Katare, C., Saxena, S., Agrawal, S., Prasad, G. B. K. S. and
Bisen, P. S. 2012. Flax seed: A potential medicinal food.
J. Nutr. Food Sci. 2:120.
Kaur, N. and Gupta, A. K. 2002. Applications of inulin and
oligofructose in health and nutrition. J. Biosci. 27:703–
714.
Lamblin, F., Hano, C. and Fliniaux, O. 2008. Interest of
lignans in prevention and treatment of cancers. Med.
Sci. 24:511–519.
Lin, B., Gong, J., Wang, Q., Cui, S., Yu, H. and Huang, B.
2011. In vitro assessment of the effects of dietary fibers
on microbial fermentation and communities from large
intestinal digesta of pigs. Food Hydrocol. 25:180–188.
Mason, J. K. and Thompson, L. U. 2014. Flaxseed and its
lignan and oil components: Can they play a role in
reducing the risk of and improving the treatment of
breast cancer? Applied Physiology, Nutrition, and Meta-
bolism 39:663–678.
Nowaka, D. A., Snydera, D. C., Brownb, A. J. and Demark-
Wahnefried, C. 2007. The effect of flaxseed supplementa-
tion on hormonal levels associated with polycystic
ovarian syndrome: A case study. Current Topics in Nu-
traceutical Research 5:177–182.
Ntsame, Affane, A. L., Fox, G. P., Sigge, G. O., Manley, M.
and Britz, T. J. 2009. Quantitative analysis of DL-lactic
acid and acetic acid in Kefir using near infrared reflec-
tance spectroscopy. Journal of Near Infrared Spectro-
scopy 175:255–264.
Ntsame, Affane, A. L., Fox, G. P., Sigge, G. O., Manley, M.
and Britz, T. J. 2011. Simultaneous prediction of acidity
parameters (pH and titratable acidity) in Kefir using
near infrared reflectance spectroscopy. International
Dairy Journal 21:896–900.
Paschos, G. K., Magkos, F., Panagiotakos, D. B., Votivea, V.
and Zampelas, A. 2007. Dietary supplementation with
flaxseed oil lowers blood pressure in dyslipidaemic pa-
patients. Eur. J. Clin. Nutr. 61:201–206.
Patel, S. and Goyal, A. 2012. The current trends and future
perspectives of prebiotics research: A review . 3 Biotech.
2:115–125.
Prasad, K. 2009. Flaxseed and cardiovascular health. J.
Cardiovasc Pharmacol. 54:369–377.
Rickard, S. E., Orcheson, L. J., Seidl, M. M., Luyengi, L.,
Fong, H. H. S. and Thompson, L. U. 1996. Dose–depen-
dent production of mammalian lignans in rats and in vitro
from the purified precursor secoisolariciresinol
diglycoside in flaxseed. J. Nutr. 126:2012–2019.
Saggar, J. K., Chen, J., Corey, P. and Thompson, L. U. 2010.
Dietary flaxseed lignan or oil combined with tamoxifen treatment affects MCF-7 tumor growth through estrogen receptor- and growth factor-signaling pathways. Molecular Nutrition & Food Research 54:415-425.
Saxena, S. and Katare, C. 2014. Evaluation of flaxseed formulation as a potential therapeutic agent in mitigation of dyslipidemia. Biomed J. 37:386-390.
Simova, E., Beshkova, D., Angelov, A., Hristozova, T. and Frengova, G., Spasov, Z. 2002. Lactic acid bacteria and yeasts in kefir grains and kefir made from them. J. Ind. Microbiol. Biotechnol. 28:1-6.
Sturgeon, S. R., Heersink, J. L., Volpe, S. L., Bertone-Johnson, E. R., Puleo, E. and Stanczyk, F. Z. 2008. Effect of dietary flaxseed on serum levels of estrogens and androgens in postmenopausal women. Nutr. Cancer 60:612-618.
Turan, I., Dedeli, Ö., Bor, S. and İliter, T. 2014. Effects of a kefir supplement on symptoms, colonic transit, and bowel satisfaction score in patients with chronic constipation: a pilot study. Turk. J. Gastroenterol. 25:650-656.
Vedamuthu, E. R. 2006. Other Fermented and culture-containing milks, in manufacturing yogurt and fermented milks (ed R. C. Chandan), Blackwell Publishing. Ames, Iowa, USA. doi:10.1002/9780470277812.ch19.
Westcott, N. D. and Muir, A. D. 2003. Flax seed lignan in disease prevention and health promotion phytochemistry reviews. Phytochemistry Reviews 2:401-417.
Xu, Y., Hall, C. and Wolf-Hall, C. 2008. Antifungal activity stability of flaxseed protein extract using response surface methodology. J. Food Sci. 73:9-14.