

Scientific research on Kefir

Extract taken from: Farnworth, E. R. (2006) Kefir – A Complex Probiotic. *Food Science and Technology Bulletin: Functional Foods, Volume 2, Issue 1*, (An online, peer-reviewed journal hosted on www.foodsciencecentral.com.)

Health benefits of kefir

Kefir has had a long history of being beneficial to health in Eastern European countries, where it is associated with general wellbeing. It is easily digested (Alm 1982c) and is often the first weaning food received by babies. Many of the studies regarding health benefits of kefir have been published in Russian and Eastern European journals and therefore are not easily accessible to Western science (Batinkov 1971; Ormisson and Soo 1976; Evenshtein 1978; Safonova *et al.* 1979; Ivanova *et al.* 1981; Sukhov *et al.* 1986; Besednova *et al.* 1997; Oleinichenko *et al.* 1999). However, the health benefits of kefir were demonstrated in Canada as early as 1932 (Rosell 1932).

Stimulation of the immune system

It has been proposed that stimulation of the immune system may be one mechanism whereby probiotic bacteria may exert many of their beneficial effects (De Simone *et al.* 1991; Gill 1998); this may be a direct effect of the bacteria themselves (Cross 2002). However, peptides formed during the fermentation process or during digestion have also been shown to be bioactive, and demonstrate a variety of physiological activities, including stimulation of the immune system in animal models (LeBlanc *et al.* 2002; Matar *et al.* 2003).

Thoreux and Schmucker (2001) fed kefir produced from grains to young (6 months) and old (26 months) rats and found an enhanced mucosal immune response in the young animals, as shown by a higher anti-cholera toxin (CT) IgA response compared to controls. Both young and old rats had significantly increased total non-specific IgG blood levels, and a decreased systemic IgG response to CT. Taken together, Thoreux and Schmucker concluded that kefir, like other probiotics, was exerting an adjuvant effect on the mucosal immune system, perhaps produced by bacterial cell wall components.

Inhibition of tumour growth

Shiomi *et al.* (1982) were the first to report the antitumour effects of a water-soluble polysaccharide (approximate molecular weight 1 000 000 Da) isolated from kefir grains. Whether given orally or intraperitoneally, the polysaccharide was able to inhibit the growth of Ehrlich carcinoma or Sarcoma 180 compared to control mice receiving no kefir-derived polysaccharide (Shiomi *et al.* 1982; Murofushi *et al.* 1983). The mechanism of action was not clear, since *in vitro* incubation of the two cancer cell lines with the polysaccharide showed low cytotoxicity during 42 hours of incubation. This group then went on to show that this water-soluble polysaccharide was able to reach the spleen and thymus of mice and, based on the response to thymus-dependent and thymus-independent antigens, concluded that oral immune enhancement was mediated through T-cell, but not B-cell activity. (Murofushi *et al.* 1986). More recently, a water soluble polysaccharide fraction from kefir grains was shown to inhibit pulmonary metastasis of Lewis lung carcinoma, whether the kefir-derived polysaccharide was given orally before or after tumour transplantation. Murofushi *et al.* (1983) also reported the antitumour effectiveness of kefir grain polysaccharides regardless of the time of administration, although they cautioned that larger doses may only be more effective if administered after establishment of the tumours. A water-insoluble fraction containing kefir grain microorganisms, rather than the water-soluble polysaccharide fraction, significantly inhibited metastasis of highly colonized B16 melanoma. (Furukawa *et al.* 1993; Furukawa *et al.* 2000). It was suggested that the water-soluble polysaccharide suppressed tumour growth by means of the lymphokine activated macrophage (Mφ) via the gut-associated lymphoid tissue, while the water-insoluble microorganism fraction acted through an increase of NK cell activity.

Feeding kefir itself (2 g/kg body weight by intubation) was more effective in inhibiting tumour (Lewis lung carcinoma) growth than yoghurt, when given for 9 days after tumour inoculation (Furukawa *et al.* 1990). It was also shown that mice receiving kefir had an improved delayed-type hypersensitivity response compared to tumour-bearing mice receiving no kefir, although the mean survival time was not affected (Furukawa *et al.* 1991). Kubo *et al.* (1992) also reported that feeding kefir (100–500 mg/kg body weight) inhibited the proliferation of Ehrlich ascites carcinoma. In addition, kefir,

from which the grains had been removed by filtration, were shown to kill or arrest the growth of fusiform cell sarcomas induced by 7,12-dimethylbenzanthracene in mice when the kefir was injected intraperitoneally (Cevikbas *et al.* 1994). Examination of tissue in kefir-treated mice showed a small amount of mitosis, some stromal connections and, in some cases, disappearance of tumour necrosis.

Liu *et al.* (2002) studied the effects of soy milk and cows' milk fermented with kefir grains on the growth of tumours in mice, using freeze-dried kefir (produced from either soy or cows' milk) from which the grains had been removed following fermentation. Mice were injected with 0.2×10^8 Sarcoma 180 cells one week prior to the start of the feeding portion of the experiment. Tumour growth (volume) was estimated for up to 30 days, after which tumours were removed and weighed. Both soy milk kefir (-70.9%) and cows' milk kefir (-64.8%) significantly inhibited tumour growth, compared to mice in the positive control group. Microscopic examination of the tumours indicated that apoptosis may have been responsible for reduced tumour growth. Mice fed unfermented soy milk did not have reduced tumour volumes at day 30, and Liu *et al.* (2002) concluded that either the microorganisms themselves or any polysaccharides formed during fermentation by the kefir grains microflora were responsible for the antitumour response. Genistein itself has been shown to inhibit tumours (Murrill *et al.* 1996; Constantinou *et al.* 1996), although in this study genistein levels did not change during the fermentation process. Mice consuming kefir samples also had significantly increased levels of IgA in their small intestines compared to control animals, and it was proposed that the PP tissue was increasing IgA secretion into the intestine in response to food antigens.

Güven *et al.* (2003) proposed an alternative suggestion as to how kefir may protect tissues. They showed that mice exposed to carbon tetrachloride (a hepatotoxin to induce oxidative damage) and given kefir by gavage had decreased levels of liver and kidney malondialdehyde, indicating that kefir was acting as an antioxidant. Furthermore, their data showed that kefir was more effective than vitamin E (which is well known to have antioxidative properties) in protecting against oxidative damage.

Kefir and lactose intolerance

A proportion of the global population is unable to digest lactose (the major sugar found in milk), because of insufficient intestinal β -galactosidase (or lactase) activity (Alm 1982a). Research has shown, however, that lactose maldigestors are able to tolerate yoghurt, providing the number of live bacteria present in the yoghurt consumed is high enough (Pelletier *et al.* 2001). It is believed that the bacteria in the yoghurt matrix are protected by the buffering effect of the yoghurt. Bacterial cells remain viable, and the bacterial cell walls remain intact, and thus the β -galactosidase enzyme contained in the yoghurt-producing bacteria (*L. acidophilus*) is protected during transit through the stomach until it arrives at the upper gastrointestinal tract (Montes *et al.* 1995; De Vrese *et al.* 2001). It has also been shown that fermented milk products have a slower transit time than milk, which may further improve lactose digestion (Vesa *et al.* 1996; Labayen *et al.* 2001).

Some kefir grains have been shown to possess β -galactosidase activity which remains active when consumed (De Vrese *et al.* 1992). A recent study has shown that a commercial kefir produced using a starter culture containing six bacteria (but not *L. acidophilus*) and one yeast was equally as effective as yoghurt in reducing breath hydrogen in adult lactose maldigestors (Hertzler and Clancy 2003). Severity of flatulence in this group was also reduced when either yoghurt or kefir was consumed compared to milk.

De Vrese *et al.* (1992) showed that when pigs were fed kefir containing fresh grains, their plasma galactose concentrations rose significantly higher than pigs given kefir containing heated grains. The diet containing kefir and fresh grains had a β -galactosidase activity of 4.4 U/l, which was identified as being responsible for the hydrolysis of lactose in the intestine, thus yielding galactose that can be absorbed. Kefir itself contains no galactose (Alm 1982).

Antimicrobial properties of kefir

Garrote *et al.* (2000) tested the inhibitory activity of a supernatant of cows' milk fermented with kefir grains, against Gram-negative and Gram-positive bacteria. Gram-positive microorganisms were inhibited to a greater extent than Gram-negative microorganisms; moreover, both lactic and acetic acids were found in the supernatants. Garrote *et al.* (2000) showed that milk supplemented with lactic acid or lactic acid plus acetic acid at the concentrations found in the kefir supernatant also had inhibitory activity against *E. coli* 3. They concluded that organic acids produced during kefir fermentation could have important bacteriostatic properties even in the early stages of milk fermentation.

Hydrogen peroxide is another metabolite produced by some bacteria as an antimicrobial compound. Yüksekdag *et al.* (2004a) showed that all 21 isolates of lactic acid bacteria from Turkish kefir produced hydrogen peroxide (0.04–0.19

ug/ml). In a later paper, they reported that 11 out of 21 strains of kefir lactococci produced hydrogen peroxide (Yüksekdağ *et al.* 2004b). All lactococci strains were effective in inhibiting growth of *Streptococcus aureus*, but were less effective against *E. coli* NRLL B-704 and *Pseudomonas aeruginosa*.

Behaviour of kefir bacteria in the gastrointestinal tract

One of the criteria for probiotic bacteria is that they should be able to withstand the harsh conditions of the gastrointestinal tract, including extreme pH conditions present in the stomach and the action of bile salts and digestive enzymes (Lee and Salminen 1995). It is also believed that one way in which probiotic bacteria could protect against pathogenic bacteria would be to compete with or displace pathogenic bacteria by adhering to intestinal epithelial cells. (Kirjavainen *et al.* 1998; Fujiwara *et al.* 2001; Gibson and Rastall 2003).

Kefir, because it is milk based, is able to buffer the pH of the stomach when ingested and thereby provide time for many of the bacteria to pass through to the upper small intestine (Farnworth *et al.* 2003).

Human studies of the effects of diet on intestinal microflora are limited to the analysis of faecal samples, although no detailed human study has been published in which kefir has been used. Marquina *et al.* (2002) used mice to study the effect of consuming kefir (source not defined) in a feeding study that lasted 7 months. They were able to show that the numbers of lactic acid bacteria in the mouse small and large intestines increased significantly. Streptococci increased by 1 log, while sulfite-reducing clostridia decreased by 2 logs.

Kefir and cholesterol metabolism

Positive effects of yoghurt consumption on cholesterol metabolism have been reported (Kießling *et al.* 2002; Xiao *et al.* 2003), although a review of the literature reveals that the results are at best moderate, and are often inconsistent (Taylor and Williams 1998; St-Onge *et al.* 2000; Pereira and Gibson 2002).

Several hypotheses have been proposed regarding the possible mechanism of action employed by bacteria to reduce cholesterol levels (St. Onge *et al.* 2002). Vujicic *et al.* (1992) showed that kefir grains from Yugoslavia, Hungary and the Caucase region were able to assimilate cholesterol in milk either incubated at 20°C for 24 h (reductions of up to 62%) or incubated and stored at 10°C for 48 h (reductions of up to 84%). These authors claimed that their results indicated that kefir grains had a cholesterol-degrading enzyme system. Similar results were reported for 27 lactic acid bacterial strains.

Conclusions

The microbiological and chemical composition of kefir indicates that it is a complex probiotic, as the large number of different bacteria and yeast found in it distinguishes it from other probiotic products. Since the yeasts and bacteria present in kefir grains have undergone a long association, the resultant microbial population exhibits many similar characteristics, making isolation and identification of individual species difficult. Many of these microorganisms are only now being identified by using advanced molecular biological techniques. The study of kefir is made more difficult, because it appears that many different sources of kefir grains exist that are being used to produce kefir.

The production of kefir depends on the synergistic interaction of the microflora in kefir grains. During the fermentation process, the yeasts and bacteria in kefir grains produce a variety of ingredients that give kefir its unique taste and texture. After fermentation, the finished kefir product contains many ingredients that are proving to be bioactive. At least one exopolysaccharide has been identified in kefir, although others may be present. Many bacteria found in kefir have been shown to have proteinase activity, and a large number of bioactive peptides has been found in kefir. Furthermore, there is evidence to show that kefir consumption not only affects digestion, but also influences metabolism and immune function in humans.

References

- Alm, L. 1982a. Effect of fermentation on lactose, glucose, and galactose content in milk and suitability of fermented milk products for lactose intolerant individuals. *Journal of Dairy Science* **65**: 346-352.
- Alm, L. 1982c. Effects of fermentation on curd size and digestibility of milk proteins *in vitro* of Swedish fermented milk products. *Journal of Dairy Science* **65**: 509-514.

- Batinkov, E.L. 1971. Use of milk and kefir in peptic ulcer of the stomach and duodenum. *Voprosy Pitani* **30(4)**: 89-91. (in Russian)
- Besednova, N.N., Epshtein L.M., Gazha A.K., Borovskaia G.A., Besednov A.L., Rozhzhov I.V. and Smolina T.P. 1997. Therapeutic-prophylactic milk products with a new immunocorrector of natural origin. *Voprosy Pitani* **3**: 31-34. (in Russian - abstract only)
- Cevikbas, A., Yemni, E., Ezzedenn, F.W., Yardimici, T., Cevikbas, U. and Stohs, S.J. 1994. Antitumoural antibacterial and antifungal activities of kefir and kefir grain. *Phytotherapy Research* **8**: 78-82.
- Constantinou, A.I., Mehta, R.G., and Vaughan, A. 1996. Inhibition of N-methyl-N-nitrosourea-induced mammary tumors in rats by the soybean isoflavones. *Anticancer Research* **16**: 2617-2620.
- Cross, M.L. 2002. Microbes versus microbes: Immune signals generated by probiotic lactobacilli and their role in protection against microbial pathogens. *FEMS Immunology and Medical Microbiology* **34**: 245-253.
- De Simone, C., Rosati, E., Moretti, S., Bianchi, S.B. Vesely, R. and Jirillo, E. 1991. Probiotics and stimulation of the immune response. *European Journal of Clinical Nutrition* **45** (2, Suppl.): 32-34.
- De Vrese, M., Keller, B. and Barth, C.A. 1992. Enhancement of intestinal hydrolysis of lactose by microbial β -galactosidase (EC 3.2.1.23) of kefir. *British Journal of Nutrition* **67**: 67-75.
- De Vrese, M., Stegelmann, A., Richter, B., Fenselau, S., Laue, C. and Schrezenmeir, J. 2001. Probiotics-compensation for lactase insufficiency. *American Journal of Clinical Nutrition* **73**(2, Suppl.): 421S-429S.
- Evenshtein E. M. 1978. Use of kefir for stimulation of gastric secretion and acid-formation in patients with pulmonary tuberculosis. *Problemy Tuberkuleza* **2**: 82-84. (translated from Russian)
- Farnworth, E., Mainville, I. and Arcand, Y. 2003. Buffering capacity of milk products in an *in vitro* upper gastrointestinal tract model. Canadian Federation of Biological Societies, 46 Annual Meeting, Ottawa, June 12-14. Abstract # F065A.
- Fujiwara, S., Seto, Y., Kimura, A. and Hashiba, H. 2001. Intestinal transit of orally administered streptomycin-rifampicin-resistant variant of *Bifidobacterium longum* SBT2928: its long-term survival and effect on the intestinal microflora and metabolism. *Journal of Applied Microbiology* **90**: 43-52.
- Furukawa, N., Matsuoka, A. and Yamanaka, Y. 1990. Effects of orally administered yoghurt and kefir on tumor growth in mice. *Journal of the Japanese Society of Nutrition and Food Science* **43**: 450-453 (in Japanese - abstract only)
- Furukawa, N., Matsuoka, A., Takahashi, T. and Yamanaka, Y. 1991. Effects of fermented milk on the delayed-type hypersensitivity response and survival day in mice bearing Meth-A. *Animal Science Technology (Japan)* **62**: 579-585 (in Japanese - abstract only)
- Furukawa, N., Yokokawa, Y., Takahashi, T. and Yamanaka, Y. 1993. Effects of oral administration of water soluble fraction from kefir grains on glucose consumption and phagocytosis of peritoneal exudate cells in mice. *Animal Science and Technology (Japan)* **64**: 60-67. (in Japanese - abstract only)
- Furukawa, N., Matsuoka, A., Takahashi, T. and Yamanaka, Y. 2000. Anti-metastatic effect of kefir grain components on Lewis lung carcinoma and highly metastatic B16 melanoma in mice. *Journal of Agriculture Science Tokyo Nogyo Daigaku* **45**: 62-70, 2000.
- Garrote, G.L., Abraham, A.G. and De Antoni, G.L. 2000. Inhibitory power of kefir: the role of organic acids. *Journal of Food Protection* **63**: 364-369.
- Gibson, G.R. and Rastall, R.A. 2003. Gastrointestinal infections and the protective role of probiotics and prebiotics. *Food Science and Technology Bulletin: Functional Foods*. Available at <http://www.foodsciencecentral.com/fsc/ixid3664>.
- Gill H.S. 1998. Stimulation of the immune system by lactic cultures. *International Dairy Journal* **8**: 535-544.
- Güven, A., Güven, A. and Gülmez, M. 2003. The effect of kefir on the activities of GSH-Px, GST, CAT, GSH and LPO levels in carbon tetrachloride-induced mice tissues. *Journal of Veterinary Medicine B* **50**: 412-416.
- Hertzler, S.R. and Clancy, S.M. 2003. Kefir improves lactose digestion and tolerance in adults with lactose maldigestion. *Journal of the American Dietetic Association* **103**: 582-587.

- Ivanova, L.N., Bulatskaya, A.N. and Silaev, A.E. 1981. Industrial production of kefir for children. *Molochna i Apromyshlemast* 15-16 (DSA no. 106). (in Russian)
- Kiessling, G., Schneider, J. and Jahreis, G. 2002. Long-term consumption of fermented dairy products over 6 months increases HDL cholesterol. *European Journal of Clinical Nutrition* **56**: 843-849.
- Kirjavainen, P.V., Ouwehand, A.C., Isolauri, E. and Salminen, S.J. 1998. The ability of probiotic bacteria to bind to human intestinal mucus. *FEMS Microbiology Letters* **167**: 185-189.
- Kubo, M., Odani, T., Nakamura S., Tokumaru, S. and Matsuda, H. 1992. Pharmacological study on kefir - a fermented milk product in Caucasus. I. On antitumor activity (1). *Yakugaku Zasshi* **112**: 489-495.(in Japanese - abstract only)
- Labayen, I., Forga, L., Gonzalez, A., Lenoir-Wijnkoop, I. and Martinez, J.A. 2001. Relationship between lactose digestion, gastrointestinal transit time and symptoms in lactose malabsorbers after dairy consumption. *Alimentary Pharmacology and Therapeutics* **15**: 543-549.
- LeBlanc, J.G., Matar, C., Valdéz, J.C., LeBlanc, J. and Perdígón, G. 2002. Immunomodulating effects of peptidic fractions issued from milks fermented with *Lactobacillus helveticus*. *Journal of Dairy Science* **85**: 2733-2742.
- Lee, Y-K. and Salminen, S. 1995. The coming of age of probiotics. *Trends in Food Science and Technology* **6**: 241-245.
- Liu, J-R., Chen, M-J. and Lin, C-W. 2002. Characterization of polysaccharide and volatile compounds produced by kefir grains grown in soymilk. *Journal of Food Science* **67**: 104-108.
- Marquina, D., Santos, A., Corpas, I., Munoz, J., Zazo, J. and Pienado, J.M. 2002. Dietary influence of kefir on microbial activities in the mouse bowel. *Letters in Applied Microbiology* **35**: 136-140.
- Matar, C., LeBlanc, J.G., Martin, L. and Perdígón, G. 2003. Biologically active peptides released in fermented milk: role and functions. In: Farnworth, E.R., editor. *Handbook of fermented functional foods*: 177- 201. CRC Press, Boca Raton, USA.
- Montes, R.G., Bayless, T.M., Saavedra, J.M. and Perman, J.A. 1995. Effect of milks inoculated with *Lactobacillus acidophilus* or a yoghurt starter culture in lactose-maldigesting children. *Journal of Dairy Science* **78**:1657-1664.
- Murofushi, M., Shiomi, M. and Aibara, K. 1983. Effect of orally administered polysaccharide from kefir grain on delayed-type hypersensitivity and tumor growth in mice. *Japanese Journal of Medical Science and Biology* **36**: 49-53.
- Murofushi M., Mizuguchi J., Aibara K. and Matuhasi T. 1986. Immunopotentiative effect of polysaccharide from kefir grain, KGF-C, administered orally in mice. *Immunopharmacology* **12**: 29-35.
- Murrill, W.B., Brown, N.M., Zhang, J.X., Manzolillo, P.A., Barnes, S. and Lamartiniere, C.A. 1996. Prepubertal genistein exposure suppresses mammary cancer and enhances gland differentiation in rats. *Carcinogenesis* **17**: 1451-1457.
- Oleinichenko, E.V., Mitrokhin, S.D., Nonikov, V.E. and Minaev, V.I. 1999. Effectiveness of acipole in prevention of enteric dysbacteriosis due to antibacterial therapy. *Anitibiotiki i Khimioterapiya* **44**: 23-25. (in Russian - abstract only)
- Ormison, A.A. and Soo, T.R. 1976. Effect of lactic acid milk and kefir on the indicators of acid-base equilibrium of arterial blood in healthy young children and patients with acute pneumonia and acute bronchitis. *Pediatrics* **10**: 37-38. (translated from Russian)
- Pelletier, X., Laure-Boussuge, S. and Donazzolo, Y. 2001. Hydrogen excretion upon ingestion of dairy products in lactose-intolerant male subjects: importance of the live flora. *European Journal of Clinical Nutrition* **55**: 509-512.
- Pereira, D.I., and Gibson, G.R. 2002. Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. *Critical Reviews in Biochemistry and Molecular Biology* **37**:259-281.
- Rosell, J. M. 1932. Yoghurt and kefir in their relation to health and therapeutics. *Canadian Medical Association Journal*: 341-345.
- Safonova, T.Y., Yatsyk, G.V., Yurkov, Y.A. and Volkova, L.D. 1979. Effect of varying types of feeding on fatty acid composition of blood serum in premature infants. *Voprosy Pitani* **6**: 44-49.(in Russian, abstract only)
- Shiomi M., Sasaki K., Murofushi M. and Aibara K. 1982. Antitumor activity in mice of orally administered polysaccharide from kefir grain. *Japanese Journal of Medical Science and Biology* **35**: 75-80.

St-Onge, M.-P., Farnworth, E.R. and Jones, P.J.H. 2000. Fermented and non-fermented dairy product consumption: effects on cholesterol levels and metabolism. *American Journal of Clinical Nutrition* **71**: 674-681.

St-Onge, M.-P., Farnworth, E.R., Savard, T., Chabot, D., Mafu, A. and Jones, P.J.H. 2002. Kefir consumption does not alter plasma lipid levels or cholesterol fractional synthesis rates relative to milk in hyperlipidemic men. *BMC Complementary and Alternative Medicine*. Available at <http://www.biomedcentral.com/1472-6882/2/1/>

Sukhov, S.V., Kalamkarova, L.I., Li'chenko, L.A. and Zhangabylov, A.K. 1986. Microfloral changes in the small and large intestines of chronic enteritis patients on diet therapy including sour milk products. *Voprosy Pitani* **4**: 14-17. (in Russian, abstract only)

Taylor, G.R. and Williams, C.M. 1998. Effects of probiotics and prebiotics on blood lipids. *British Journal of Nutrition* **80**: S225-30.

Thoreux, K. and Schmucker, D.L. 2001. Kefir milk enhances intestinal immunity in young but not old rats. *Journal of Nutrition* **131**: 807-812.

Vesa, T.H., Marteau, P., Zidi, S., Briet, F., Pochart, P. and Rambaud, J.C. 1996. Digestion and tolerance of lactose from yoghurt and different semi-solid fermented dairy products containing *Lactobacillus acidophilus* and bifidobacteria in lactose maldigesters-is bacterial lactase important? *European Journal of Clinical Nutrition* **50**: 730-733.

Vujicic, I.F., Vulic, M. and Könyves, T. 1992. Assimilation of cholesterol in milk by kefir cultures. *Biotechnology Letters* **14**: 847-850.

Xiao, J.Z., Kondo, S., Takahashi, N., Miyaji, K., Oshida, K., Hiramatsu, A., Iwatsuki, K., Kokubo, S. and Hosono, A. 2003. Effects of milk products fermented by *Bifidobacterium longum* on blood lipids in rats and healthy adult male volunteers. *Journal of Dairy Science* **86**: 2452-2461.

Yüksekdağ, Z.N., Beyath, Y. and Aslim, B. 2004a. Metabolic activities of *Lactobacillus* spp. strains isolated from kefir. *Nahrung / Food* **48**: 218-220.

Yüksekdağ, Z.N., Beyatli, Y. and Aslim, B. 2004b. Determination of some characteristics coccoid forms of lactic acid bacteria isolated from Turkish kefir with natural probiotic. *Lebensmittel-Wissenschaft und-Technologie* **37**: 663-667.