

The naturally derived “Stoletnik” dairy product as a model for nutraceutical traditional food

Hr. Chomakov¹, I. Pantchev^{1,2}, G. Rakleova¹, Z. Nikolov³, Zh. Dimitrov³, M. Yordanova⁴, A. Atanassov^{1*}

¹ Joint Genomic Center Ltd., 8 Dragan Tzankov Blvd., 1164 Sofia, Bulgaria

² Department of Biochemistry, Faculty of Biology, Sofia University, 8 Dragan Tzankov Blvd., 1164 Sofia, Bulgaria

³ LB Bulgaricum, 14 Malashevska str., 1225 Sofia, Bulgaria

⁴ Rodopa Milk, 4770 Smilyan, Smolyan, Bulgaria

Received October 25, 2017; Revised November 20, 2017

Current tendency for improving quality of live led, among all, to a demand for novel food products with beneficial effect on human health. The key requirement is that these products are either of natural origin or produced through organic farming and with as little processing as possible. Major sources for such products are the traditional regional recipes – ethnic foods. These recipes are an extract of a long-term experience (decades or even centuries) and reflect local biodiversity as well. Sporadic research on their composition and properties revealed significant beneficial effects on human health, sometimes in unexpected areas. These preliminary studies place the traditional foods as a promising new source for high quality healthy food products. In this paper we made an attempt to outline the beneficial properties of a traditional dairy product “Stoletnik” using the latest scientific knowledge in the field. Its traditional recipe was successfully converted into an industry-grade technology and was implemented in a small-scale production.

Keywords: Lactobacillus, Functional food, Nutraceuticals, Yogurt, Cheese, Curd, Dairy product

INTRODUCTION

The extensive advance during the last three decades in the fields of biochemistry, molecular biology, genetics and human physiology brought to existence a new class of food products called ‘functional foods’. A significant part of this newly formed class consists of local or ethnical food products – a direct result of culture interchange on a global scale.

There is no single definition of the term ‘functional food’. Currently adopted definition, debated at the 9th International Conference on "Functional Foods and Chronic Diseases: Science and Practice" at the University of Nevada, Las Vegas on March 15-17, 2011 states that: "Functional Food is a natural or processed food that contains known biologically-active compounds which, when in defined quantitative and qualitative amounts, provides a clinically proven and documented health benefit, and thus, an important source in the prevention, management and treatment of chronic diseases of the modern age" [1].

The medicinal values of certain food ingredients allow such products to be qualified as nutraceuticals or food medicines [2]. The term covers a wide range of plants (vegetables, fruits, spices) as examples of nutraceuticals. These products have found their

place not only in food industry but also in the pharmaceutical production.

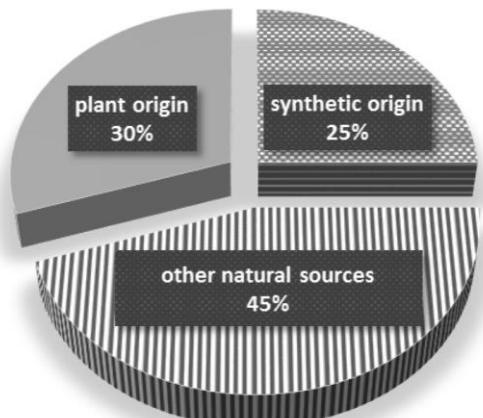


Figure 1. Ratio of natural and synthetic sources in the new chemical compounds [3].

Since 1981 to 2010 two thirds of the active agents in the new chemicals originate from natural sources (Figure 1) [3].

Origins of the “Stoletnik” dairy product

“Stoletnik” (“Centenarian”) is a contemporary dairy product fulfilling the definition of functional food. It took nearly 30 years for its development by the late Prof. Hristo Chomakov on the basis of a traditional recipe from Rhodope Mountains region in Bulgaria. During the last five years the thorough description of the product resulted into small-scale industrial production by a dairy company located in the same region – “Rodopa Milk”.

* To whom all correspondence should be sent:
E-mail: atanassov@jgc-bg.org

"Stoletnik" is among the best examples of a food product, combining Bulgarian biodiversity – lactic acid bacteria as probiotics and plant additives as prebiotics. In 2017 "Stoletnik" was recognized as a Utility Model by the Patent Office of the Republic of Bulgaria (2693 from 13.09.2017).

Ingredients of the "Stoletnik" dairy product – brief description of their properties.

All "Stoletnik" ingredients are of natural origin. The main ingredients of "Stoletnik" are yogurt, white brined cheese, curd (1:1:1) along with small amounts of onions, ground red pepper and unrefined sunflower oil. Since this product relies on fermentation, another important component is its microflora. The microflora consists of viable and active cells of probiotic bacteria — *Lactobacillus delbrueckii* ssp. *bulgaricus* (*Lb. bulgaricus*), *Streptococcus thermophilus* (*Str. thermophiles*), *Lactobacillus casei* (*Lb. casei*), *Lactobacillus plantarum* (*Lb. plantarum*) at 10^8 cfu.g⁻¹.

Bulgarian yoghurt is a biologically transformed dairy product resulting from the activity of *Lb. bulgaricus* and *Str. thermophilus*. The product contains not only a large number of lactic acid bacteria in the order of 10^8 cfu.g⁻¹, but also a rich assortment of biologically active components. Recently it was found that viable cells of *Lb. bulgaricus* possess high cytotoxic activity against the cancer cell line HT-29, which reaches 81%, best expressed after 48 hours of fermentation [4]. Cytotoxic activity is also possessed by the peptides derived from the milk proteins, hydrolysed by *Lb. bulgaricus*. This cytotoxic activity varies between 0.96% and 41.25% in the case of the curd protein lactoferricin. Also, during lactic acid fermentation, *Lb. bulgaricus* hydrolyses x-casein, liberating a peptide with strong anti-oxidative properties [5]. In that way Bulgarian yogurt protects the individual from the every-day oxidative stress, closely associated with diseases such as cancer, atherosclerosis and diabetes [6].

The decomposition of viable cells of *Lb. bulgaricus* and *Sir. thermophilus* in the intestinal tract stimulates the activity of B-cells and the synthesis of IL-6, resulting in increased secretion of dimeric IgA. The production of IgA offers protection to the mucus layer of the intestinal tract, which is the most important health-promoting effect of Bulgarian yogurt. During lactic acid fermentation proteins are hydrolyzed into fragments, which strongly stimulate the growth of bifidobacteria [7]. The unhydrolysed milk sugar in yoghurt is another important bifidogenic factor [8]. Bifidobacteria, in turn, enhance the synthesis of anti-viral IgA and decrease the risk factors of carcinogenesis.

Ripe white brined cheese is the second basic ingredient of the product. White brined cheese is a fermented dairy product with high nutritional value determined by the components of milk and the metabolites formed during the ripening performed by lactic acid bacteria. White brined cheese has a number of health-beneficial effects on the organism. This product is a source of biologically active peptides with anti-hypertensive and anti-oxidative activity, as well as probiotic bacteria *Lb. casei* and *Lb. plantarum*. The product contains anti-carcinogenic compounds and a large quantity of biologically available calcium. Data demonstrating the stimulating effect of *Lb. plantarum* on the synthesis of a biologically active component TRAIL are of special interest with respect to the anti-tumor properties of white brined cheese [9]. TRAIL is a chromium-containing ligand and is endogenously synthesized as a membrane protein or as soluble protein in the lymphocytes [10]. TRAIL plays an essential role in the suppression of carcinogenesis since insufficient expression enhances the malignant character of tumors in mice [11]. *Lb. plantarum* induces TRAIL synthesis and increases the activity against cancer cells through induction of endogenous TRAIL synthesis by immunity cells. The synthesis of the TRAIL component stimulated by *Lb. plantarum* elevates the activity of the natural killer cells, which effectively attack malignant tumor cells both *in-vitro* and *in-vivo* [9]. The same study reveals a notable biological property of *Lb. plantarum* — the induction of TRAIL synthesis is not strain-specific, which is otherwise the case for the most of the activities of probiotic bacteria. The other representative of probiotic bacteria, *Lb. casei*, also has anti-tumor activity *in-vitro* and *in-vivo* [12].

Cheese is a rich source of biologically active peptides with various biological effects on the organism such as anti-oxidative, anti-microbial, anti-hypertensive, immunomodulating and anti-carcinogenic activities [13]. Special attention is drawn to the anti-oxidative properties of cheese with respect to the prevention and control of the oxidative stress on the organism. There is a strong correlation between the anti-oxidative activity and extent of cheese ripeness. The peptides formed during ripening are the anti-oxidative components in the cheese. *Lb. casei*, which is contained in the product, has the strongest anti-oxidative properties among all lactic acid bacteria and decreases and in particular cases eliminates the daily oxidative stress of the organism. At the same time *Lb. casei* reduces cholesterol levels, modulates the immune system and is a powerful antagonist of *Escherichia coli* [14]. The cheese is a rich source of biologically available calcium, which is absorbed better than calcium from

milk [15]. Calcium reduces the risk of colon cancer. The intake of substantial amounts of calcium with cheese and the presence in the product of potassium, magnesium and biologically active peptides has a very favorable effect on the lowering of blood pressure of the individual.

Cheese contains other anti-carcinogenic components such as conjugated linoleic acid (CLA), sphingolipids and biologically active peptides. Conjugated linoleic acid with its two biologically active forms cis-9 and trans-11 possesses well expressed anti-mutagenic properties. The two forms of CLA suppress carcinogenesis by modulating the proliferation of cells and the anti-oxidative mechanism [16]. The intake of linoleic acid results in lowering of serum cholesterol in blood, especially the low density cholesterol. At the same time the level of high density cholesterol is increased. The concentration of triglycerides in blood serum is lowered, which is related to reduction of the risk of cardio-vascular disease and heart attack. Conjugated linoleic acid in the organism can be increased by consuming dairy products including cheese with elevated content of CLA. In this respect milk obtained by animals grazing in mountain planes of over 1000 meters above sea level is of great interest. In the milk obtained from such animals the content of conjugated linoleic acid is two to three times higher than in the milk of animals grazing in lowlands. In France, Switzerland, Germany and Austria the milk of these animals is processed into yoghurt and different varieties of cheese. These products are offered to tourists, organized in so-called preventive vacations for two weeks in the respective region. The tourists consume these dairy products daily. In that way healthy nutrition is widely applied as preventive means against cardiovascular disease and cancer.

Sphingolipids are an interesting group of lipids related to cancer prevention. They express anti-carcinogenic properties upon consumption [17]. With respect to the anti-tumor properties of white brined cheese the application of a selected starter of *Lb. bulgaricus* and *Str. thermophilus* for the production of this product is of particular interest. Based on the cytotoxicity of *Lb. bulgaricus* on cancer cells we have enough evidence to suppose that cheese prepared with such a starter should possess enhanced anti-tumor properties compared to products from different lactic acid bacteria starters.

Curd and curd proteins are unique food ingredients. They possess not only high nutritional value, but also important biological properties with respect to improvement of health and protection of the organism from diseases [18]. Curd proteins comprise a-lactalbumin, 13-lactoglobulin,

lactoferrin, immunoglobulins, serum albumin, lactoferrin and other micro-components. These proteins are hydrolyzed by the proteases in the stomach and the pancreatic juices — mainly trypsin /chymotrypsin/ and the microorganisms. The hydrolysis results in the formation of biologically active peptides which play essential physiological role in the organism [19]. Curd proteins increase the pH of the medium to 6.2-6.5 and facilitate the survival of yogurt microflora — *Lb. bulgaricus* and *Str. thermophilus* in the presence of pepsin and low pH in the stomach (pH 1.5- 3.0). The matrix of curd proteins increases the tolerance of *Lb. bulgaricus* and *Str. thermophilus* to bile salts and strongly influences their survival in the intestinal tract. The digestion of 13-lactoglobulin by trypsin liberates a peptide with strong anti-hypertensive activity [19]. Among curd proteins α -lactalbumin is a potent anti-oxidant and a natural anti-tumor component which causes apoptosis of tumor cells. Also, α -lactalbumin stimulates the synthesis of mucin and strengthens the defense of the intestinal epithelium against pathogenic microorganisms.

Lactoferrin is another biologically active component of milk and curd with anti-carcinogenic properties [20, 21]. Under the hydrolyzing activity of pepsin in the intestinal tract [22] the peptide lactoferricine is formed, possessing strong anti-carcinogenic activity [23, 24]. Lactoferricine treatment of 5 weeks of duration substantially decreases proliferation of cancer cells; lactoferricine prolongs the S-phase of the cancer cell division cycle, resulting in decrease of tumor cell numbers. This prolongation of the cell division cycle lowers cancer risk as cancer development depends on the speed of cancer cell proliferation. When cancer develops, food components can slow down the growth of tumor cells. Possibly the decrease in the speed of cancer cell proliferation is the basic mechanism of lowering cancer risk by milk consumption. Lactoferricine has also strong anti-microbial activity and well expressed cytotoxic effect on cancer cells, which reaches 41%.

Curd protein plays an essential role in bone growth in the organism. Curd proteins stimulate the osteoblasts, which produce osteocalcin, the only one bone-specific protein [25]. Curd protein increases bone density in the organism and facilitates fast healing of surgery wounds. In this respect curd protein can serve as a potential therapeutic product in osteoporosis treatment or as local aid for fast treatment of fractures.

Ingredients of plant origin of the "Stoletnik" are fresh onions, ground dried red pepper and unrefined sunflower oil. These three ingredients provide phytosterols, inulin, compounds with phytoncide

activity, and a number of secondary metabolites. These components act synergically with the main ingredients and improve health-beneficiary properties of the product.

Phytosterols are naturally occurring plant sterols, which are structural analogues of cholesterol. They decrease cholesterol absorption in the intestinal tract, and especially influence the levels of low-density serum cholesterol [26].

The native inulin, contained in the product, stimulates the growth of health-beneficial intestinal microbiota — bifidobacteria, *Lb. casei*, *Lb. plantarum* and facilitates the survival of *Lb. bulgaricus* and *Str. thermophilus* in the intestinal tract. Inulin increases calcium absorption and stimulates the functioning of the immune system. Unlike other fermented milk products, in which the number of lactic acid bacteria decreases during storage, in "Stoletnik" the number of lactic acid bacteria increases and they are preserved in active state.

The presence of phytoncides serves as a biological means for preservation of the quality of the product and increasing the defense strength of the organism. The added ground red pepper is a rich source of potassium, which together with calcium, magnesium and biologically active peptides has very favorable influence on high blood pressure values in the organism. The question of the synergism of the separate food components in the product on the physiological processes in the organism remains open. Up to now it was found that mixing casein with curd proteins strongly increases antibody production in the organism. Possibly it is the synergism between the product ingredients that is responsible for its very good effect on the endothelial function of blood vessels.

The processing of the components is presented in Figure 2 as a simplified technological map.

All steps can be performed in standard dairy industry environment and do not require specialized equipment or unusual procedures. Final product can be stored for 96 h at 4-6°C.

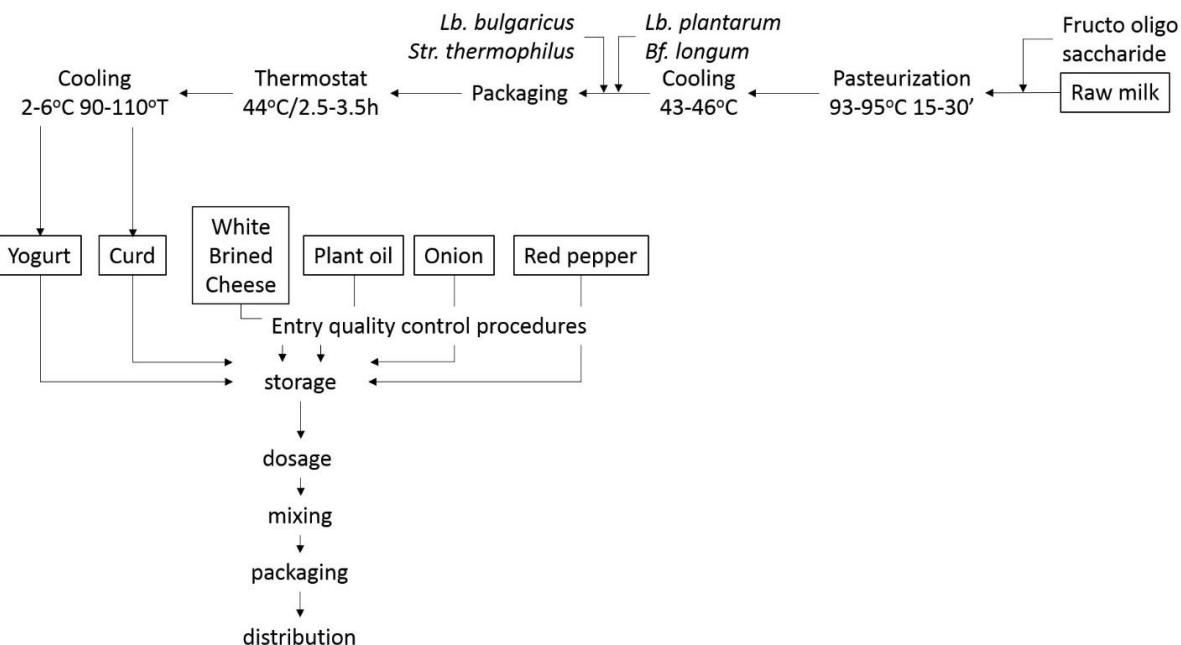


Figure 2. Simplified technological map of "Stoletnik" production.

CONCLUSION

The developed functional product "Stoletnik" contains a rich assortment of biologically active peptides with anti-hypertensive, anti-microbial, anti-tumor and immunomodulating properties. The product optimizes the functioning of the immune system; increases IgA secretion in the intestinal tract; protects the individual from infections; makes the organism more resistant to diseases; contains large quantities of anti-oxidants and protects the organism from every-day oxidative stress; improves

heart health; expresses high cytotoxicity to cancer cells. The product maintains the balance of intestinal microflora — a decisive condition for the health of the individual. Furthermore, the product facilitates the decrease of the total content of cholesterol, low-density cholesterol and triglycerides in blood serum; stimulates the cytotoxicity of immunocytes and activates the natural killer cells; improves bone density; enhances the healing of surgery wounds and fractures; improves the endothelial functions of the blood vessels. The product optimizes the physiological processes in the organism, improves

health, decreases the risk of diseases and ensures healthy aging.

“Stoletnik” is an example of successful conversion of a local home-made dairy product into an industrial technology. Present knowledge on its properties and the role of main ingredients is a good starting point for further research aiming at the development of novel variants with improved composition, properties and better preventive effect on human health. Current work on this topic will result in a set of modifications targeting different consumer groups.

REFERENCES

1. D.M. Martirosyan (ed.), Functional Foods and Chronic Diseases: Science and Practice. Food Science Publisher, 2011
2. V. Brower, *Nat. Biotechnol.*, **16**, 728 (1998).
3. D.J. Newman, G.M. Cragg, *J. Nat. Prod.*, **75**, 311 (2012).
4. C. Remillard, L. Ozimek, *Milchwiss.*, **63**, 424 (2008).
5. K. Igoshi, Y. Kondo, H Kobayashi, N. Rabata, H. Kawakami, *Milchwiss.*, **63**, 424 (2008).
6. M. Yakovlieva, T. Tacheva, S. Mihaylova, R. Tropcheva, K. Trifonova, *Benef. Microbes*, **6**, 505 (2015).
7. T. Vasiljevich, P. Shah, *Int. Dairy J.*, **18**, 456 (2008).
8. G. Schaafsma, *Int. Dairy J.*, **18**, 714 (2008).
9. M. Horinaka, T. Yoshida, A. Kishi, K. Akatani, T. Yasuda, J. Kouhara, M. Wakada, T. Sakai, *FEBS Lett.*, **584**, 577 (2010).
10. I. Monleon, M. J. Martínez-Lorenzo, L. Monteagudo, P. Lasierra, M. Taulés, M. Iturralde, A. Piñeiro, L. Larrad, M. A. Alava, J. Naval, A. Anel, *J. Immunol.*, **167**, 6736 (2001).
11. N. Zerafa, J. A. Westwood, E. Cretney, S. Mitchell, P. Waring, M. Iezzi, M.J. Smyth, *J. Immunol.*, **175**, 5586 (2005).
12. A. Takagi, T. Matsuzaki, M. Sato, K. Nomoto, M. Morotomi, T. Yokokura, *Med. Microbiol. Immunol.*, **188**, 111 (1999).
13. B. Walther, A. Schmid, R. Sieber, K. Wehrmüller, *Dairy Sci. Technol.*, **88**, 389 (2008).
14. W. Ylcheng, Z. Guo, Q. Zhang, L. Yan, Y. Ohen, X. C. Xiao-Minglin, W. Chen, H.-P. Zhang, *Int. J. Dairy Technol.*, **63**, 105(2010).
15. K. Kato, Y. Takada, H. Matsuyama, Y. Kawasaki, S. Aoe, H. Yano, Y. Toba, *Biosci. Biotechnol. Biochem.*, **66**, 2342 (2002).
16. M. A. Belury, *J. Nutr.*, **132**, 2995 (2002).
17. H. Vesper, E. M. Schmelz, M. N. Nikolova-Karakashian, B. L. Dillehay, D. V. Lynd, A. H. Merrill, *J. Nutr.*, **129**, 1239 (1999).
18. A. R. Madureira, C. I. Pereira, A. M. P. Gomes, M. E. Pintado, F. X. Malcata, *Food Res. Int.*, **40**, 1187 (2007).
19. A. R. Madureira, T. Tavares, A. M. Gomes, M. E. Pintado, F. X. Malcata, *J. Dairy Sci.*, **93**, 437 (2010).
20. N. van Belzen, *Sciences des Aliments*, **22**, 461 (2002).
21. S. Min, J. M. Krochta, *J. Food Sci.*, **70**, M87 (2005).
22. W. Bellamy, M. Takase, K. Yamauchi, H. Wakabayashi, K. Kawase, M. Tomita, *Biochim. Biophys. Acta*, **1121**, 130 (1992).
23. H. Wakabayashi, M. Takase, M. Tomita, *Curr. Pharm. Res.*, **9**, 1277 (2003).
24. J. S. Mader, A. Richardson, J. Salsman, D. Top, R. de Antueno, R. Duncan, D. W. Hoskin, *Exp. Cell Res.*, **313**, 2634 (2007).
25. R. Xu, *J. Dairy Sci.*, **92**, 3014 (2009).
26. S. B. Racette, X. Lin, M. Lefevre, C. A. Spearie, M. M. Most, L. Ma, R. E. Ostlund Jr., *Am. J. Clin. Nutr.*, **91**, 32 (2010).

ПРИРОДНИЯТ МЛЕЧЕН ПРОДУКТ „СТОЛЕТНИК“ КАТО МОДЕЛ ЗА ТРАДИЦИОННА ЛЕЧЕБНА ХРАНА

Хр. Чомаков¹, И. Панчев^{1,2}, Г. Раклеова¹, З. Николов³, Ж. Димитров³, М. Йорданова⁴, А. Атанасов^{1*}

¹ Съвместен Геномен Център ООД., бул. Драган Цанков 8, 1164 София, България

² Катедра по биохимия, Биологически факултет, СУ „Св. Климент Охридски“, 1164 София, България

³ LB Bulgaricum, ул. Малаешевска 14, 1225 София, България

⁴ Rodopa Milk, 4770 Смолян, България

Постъпила на 25 октомври, 2017 г.; коригирана на 20 ноември, 2017 г.

(Резюме)

Съвременната тенденция за подобряване качеството на живота доведе до необходимост от нови хранителни продукти с благоприятно влияние върху човешкото здраве. Основното изискване към тези продукти е да бъдат от естествен произход или да са произведени чрез органично земеделие и възможно малка обработка. Основните източници на такива продукти са традиционните местни рецепти – т. нар. етнохрани. Тези рецепти са плод на продължителен опит (десетилетия и дори столетия) и отразяват местното биоразнообразие. Спорадични изследвания върху състава и свойствата им са разкрили значителни благоприятни ефекти върху човешкото здраве, понякога в неочаквани области. Настоящите предварителни изследвания представят традиционните храни като обещаващ нов източник на висококачествени и здравословни хранителни продукти. В тази статия е направен опит за очертаване на благоприятните качества на традиционния млечен продукт „Столетник“ с използване на най-новите научни знания в тази област. Традиционната рецепта е развита успешно в промишлена технология, адаптирана за микро- и малки предприятия.