Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XIV, Issue 1 - 2015, pag. 7 - 14

YOGURTS ENRICHED WITH PEA PROTEIN

***Zapryana DENKOVA[1], Emil FILIPOV[1], Bogdan GORANOV[1], Iliyan DOBREV[1], Velichka YANAKIEVA[1]**

[1] *University of Food Technology, Department of Microbiology,*
zdenkova@abv.bg
*Corresponding author
Received 19th February 2015, accepted 23rd March 2015

Abstract: *With the increasing cost of animal breeding, vegetable proteins seem to be a good alternative to this problem. The ability of three yogurt starters with probiotic strain Lactobacillus delbrueckii ssp. bulgaricus to develop in cow's milk, enriched with pea protein, was examined. The three starters developed in cow's milk, enriched with pea protein, the concentration of the active cells reaching 10^{13} - 10^{14} cfu/cm^3. Yogurts containing different percentages of pea protein (0-10%) with the selected starters were prepared. The yogurt obtained with Starter M and Starter S using cow's milk, enriched with 2 – 4% pea protein had the best organoleptic properties so they were stored at 4 ± 2°C for 25 days. There were no significant changes in the concentration of viable cells and the titratable acidity. The obtained yogurts enriched with pea protein are new dietary probiotic foods. Along with the inherent prebiotic ingredients of pea, significant amount of useful microflora (10^{13}-10^{14}cfu/cm^3) enters the human gastrointestinal tract, which is necessary to restore and maintain the balance of the gastrointestinal microflora, which is essential for human health.*

Keywords: *Lactobacillus, pea protein, pea milk, starter, yogurt*

1. Introduction

The widespread use of canned foods in modern man's diet, the massive use of antibiotics and other antimicrobial agents, the permanent stress and poverty, the deteriorating environmental situation led to deterioration of health. All these factors have direct effects on the balance of the gastrointestinal microflora. To restore and maintain the balance of the microflora in the stomach and intestines beneficial lactobacilli should be taken in with food.

The consumption of lactic acid products introduces live beneficial bacteria necessary for the normalization of microflora in the gastrointestinal tract. This equilibrium is influenced by various factors of the medium (physical, chemical, biological). Its violation leads to disruption of the functions not only of the digestive system, but also of the related organs and systems.

The restoration and maintainance of the balance of the microflora in the gastrointestinal tract is achieved through introduction of beneficial microorganisms (lactobacilli and bifidobacteria) by consumption of concentrates of viable cells called probiotics or functional foods. Moreover, in order to carry out their inherent preventive role, the concentration of viable cells of lactobacilli and bifidobacteria should exceed 10^8 cfu/g/cm^3.

Generally, probiotic bacteria are included in the composition of yoghurts and other fermented yogurt beverages [1-4]. Nowadays, non-dairy probiotic preparations imported into beverages or applied in the form of tablets, capsules,

lyophilized preparations are gaining increasing popularity [5, 6].
Soy milk is a good substrate for the development of lactobacilli and bifidobacteria with probiotic properties. By culturing of symbiotic combinations or single strains of lactobacilli, streptococci, Denkova *et al.*, 2001 [7] obtained fermented soy foods, such as soy yogurt, soy acidophilic yogurt (*Lactobacillus acidophilus*) and soy bifid yogurt (*Bifidobacterium bifidum*), and soy yogurt beverages (acidophilic-bifid beverage). The concentration of active cells exceeds 10^9cfu/cm^3 and the storage life of soy fermented foods is more than 20 days in a refrigerator (4±2ºC). The authors found that the aroma substances , such as lactic, acetic, tartaric and malic acid, acetaldehyde, diacetyl, generated by the metabolism of lactobacilli and bifidobacteria removed soy off- flavor and soy fermented foods receive consumers‘ acceptance.
Pea beans contain small amounts of carbohydrates, dietary fiber, lipids, saturated fatty acids, but the predominat substances are starch and valuable vegetable protein, vitamins (β-carotene, vitamins A, E, H, PP, B vitamins, vitamin C (in a raw state)), mineral elements such as iron, zinc, copper, manganese, aluminum, boron, molybdenum, fluorine, vanadium, nickel, titanium, silicon, lead, selenium, zirconium, cobalt, chromium, potassium, phosphorus, sulfur, chlorine, calcium, magnesium, sodium, i.e., possesses rich mineral composition. Carbohydrates are less in quantity, while starch is more in mature pea beans.
All substances in pea beans have beneficial impact on the health of the body. Peas are useful in anemia and obesity, improves the condition of the liver, kidneys, cardiovascular system. Green peas exhibit antiseptic activity.
The cellulose in the pea beans helps to purify the stomach and intestines of slag; nicotinic acid (vitamin PP) maintains the level of cholesterol and reduces the risk of oncologic diseases; thiamine (vitamin B_1) improves the functioning of the brain; vitamin H (biotin) exhibits antioxidant properties, regulates the level of blood sugar and stimulates the activity of the digestive and nervous system.
The reduction of the amount of cow's milk as well as the increasing cost of animal breeding leads to seeking new ways to develop functional foods. Proteins of plant origin offer a good alternative for solving this problem. There has been considerable success in replacing part of cow's milk with soy milk in the production of a number of products currently available on the market [8]. Inspired by this success, researchers continue exploring the possibilities for substitution of cow‘s milk with other vegetable proteins. Currently, another vegetable attracts the attention of researchers - *Pisum sativum* [9]. Several processes for the extraction of the gliadin and glutenin (main pea proteins) from the whole pea beans can be used. One of them is water-vapor extraction of the pea beans to obtain pea milk rich in bioactive substances. But the most commonly used method is the grinding of the pea beans and concentrating the protein by drying to obtain an isolate which is subsequently resuspended to obtain „pea-milk“.
Pea protein isolate has a number of beneficial functional qualities such as good solubility in water, stability at high temperatures, good foaming capacity and high oil in water emulsifying power [10] and good stability in terms of shearing and retorting [11, 12]. Then pea milk is mixed with the skimmed cow's milk at different ratios, and digested by microorganisms as a new raw material/substrate.
From an economic perspective pea protein costs are almost half as much as milk

protein and 25% less than soy protein. In addition, the price of pea protein is relatively stable and resistant to fluctuations [12].

Bacteria from the genus *Lactobacillus* are be able to use fractions of the pea protein hydrolysates deleterious for other bacteria as substrates independently from the state of their existence. Therefore, the consumption of foods enriched with pea protein may beneficially affect the human organism [13].

The purpose of the present research is to explore the possibilities of enriching yogurt foods with pea protein and obtaining of fermented probiotic foods.

2. Materials and methods

Strains and media

Three yogurt starters containing different probiotic strain of *Lactobacillus delbrueckii* ssp. *bulgaricus* were used in the present research: Starter M, Starter D and Starter S.

The used media were

- Skim milk with titratable acidity of 16-18ºT;
- LAPTg10-broth;
- LAPTg10-agar;
- Medium for detection of molds and yeasts in milk and dairy products at 25°C according to ISO 6611/2004;
- Baird Parker agar base (*Staphylococcus aureus*);
- Medium for detection and isolation of *Salmonella* sp. by horizontal process according to BS EN ISO 6579/2003;
- Chromocult TBX-agar (*E. coli*);
- PCA agar (medium for the determination of TBA (total bacterial abundance) according to BS ISO 6610:2002;
- Pea milk.

Preparation of pea milk

Raw peas (*Pisum sativum* L.) were ground to flour in the laboratory mill.

Samples (35 g) of ground pea flour were extracted with 140 ml of 50 mM Tris–HCl (pH 8.8) for 1 h at 4 °C with constant agitation and subsequently centrifuged (20 000 g, 20 min). The extraction was repeated twice. The supernatant, containing albumins and globulins, was dialysed at 4 °C for 48 h against distilled water and later on lyophilised [13, 14]. Pea isolate, containing 81.34% pea protein, was obtained and was used to prepare 10% pea milk.

Determination of the titratable acidity

Ten cm^3 of each sample were mixed with 20 cm^3 of distilled water. The titratable acidity was determined by titration of each sample with 0.1 N NaOH using phenolphthalein as an indicator until the appearance of a pale pink colour persisting over 1 min. One Torner degree (°T) corresponds to 1 cm^3 0.1 N NaOH, needed for the neutralisation of an equivalent amount of organic acid, contained in 100 cm^3 of the cultural medium [15].

Determination of the concentration of viable cells

Appropriate serial dilutions in saline solution of the obtained yogurts were prepared and the spread plate method was applied. 0.1 cm^3 of the last three dilutions was used to inoculate LAPTg10-agar (for the enumeration of lactobacilli and streptococci) or the respective elective solid medium (for the enumeration of the specific microorganisms). The inoculated Petri dishes were incubated for 3 days at optimum temperature for the growth of the respective microorganisms until the appearance of countable single colonies (Frank & Yousef, 2004). The count of the colonies was then used to estimate the number of bacteria in the original sample.

Organoleptic assessment was performed in accordance with BDS 15612-83

Statistical analysis
Data from triplicate experiments were processed using MS Office Excel 2003 software, at level of significance of *$P<0.05$.*

3. Results and discussion

The ability of yogurt starters with probiotic strain *Lactobacillus delbrueckii* ssp. *bulgaricus* to develop in cow's milk, enriched with pea protein, was examined. Pea milk cannot be used for the preparation of foodstuffs alone, due to the high content of starch in it. Thus, extraction of pea protein which has good solubility and can be used in food production was required for the preparation of 10% pea milk. At that concentration of pea protein and below, pea cow's milk with increased density was obtained, but no layering of the milk during storage was detected. At concentrations of pea protein more than 10% there was clear layering of the pea milk during storage.
The microbiological status of the pea isolate (unflavored) was examined. The results of these experimental tests are shown in Table 1.

Table 1
Microbiological status of the pea isolate

TBA, cfu/g	Specific microorganisms, cfu/g			Molds and yeasts, cfu/g
	E.coli (TBX-agar)	*St.aureus*	*Salmonella* sp.	
$4.6x10^2$	<100	Not found	Not found	<100

Two groups of bacteria were found in the pea isolate – sporeforming and non-sporeforming bacteria. The presence of sporeforming bacteria proved that pasteurization is not an appropriate method for sterilization of the pea milk obtained using the pea isolate. Therefore the sterilization of the obtained pea milk is done at 121°C and 1 atm in order for the safety of the food products to be guarranteed.
Cow's milk containing 4% pea isolate was used for the primary screening for the starters able to grow in cow's milk enriched with pea protein. The inoculated milk substrates were incubated at 41 ± 1^oC for 4 – 5 hours until the coagulation of the yogurts.
The microbial purity of the cow's milk enriched with pea protein was determined (Table 2).

Table 2
Microbiological status of the cow's milk enriched with pea protein

TBA	Specific microorganisms, cfu/cm^3			Molds and yeasts, cfu/cm^3
	E.coli (TBX -agar)	*St. aureus*	*Salmonella* sp.	
<1	<10	Not found	Not found	<10

The three starters developed in cow's milk, enriched with pea protein, and produced lactic acid. The titratable acidity of the medium was within the range of 45-70°T. The concentration of the active cells reached 10^{13} - 10^{14} cfu/cm^3 (Fig. 1).

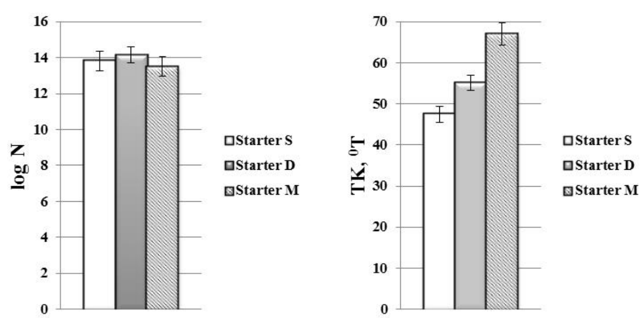


Fig. 1. Concentration of viable cells and titratable acidity of non-traditional yogurts, obtained with cow's milk, enriched with 4% pea protein. Incubation – 4 – 5 hours (until coagulation) at 41 ± 1^oC

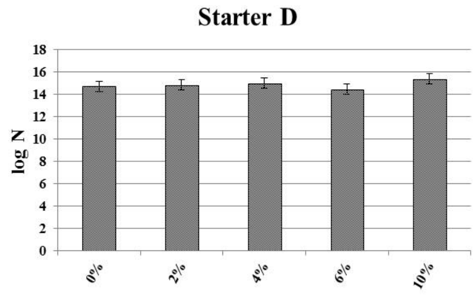
The ratio between *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* was from 1:1 to 1:3 which indicates that the yogurts bear a considerable amount of the probiotic strain *Lactobacillus delbrueckii* ssp. *bulgaricus* MZ2.

Yogurts containing different percentage of pea protein (0-10%) with the selected starters were prepared. The fermentation process was conducted at 41±1°C for 4-17 часа until coagulation (Table 3, Fig. 2, Fig. 3, Fig. 4).

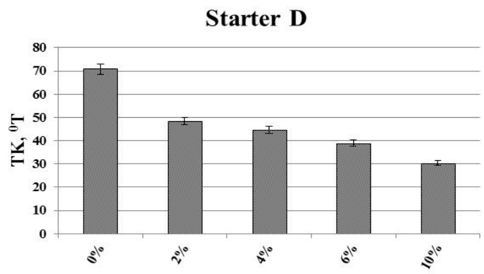
Table 3.

Organoleptic evaluation of the yogurts, obtained with cow's milk enriched with pea protein

Starter	**Pea isolate, %**	**Organoleptic evaluation**		**Microscopic pattern**
Starter M	0 %	Yogurt with distinct yogurt flavor; low density texture	Best yogurts - 2% and 4% pea isolate	Rich microscopic pattern; cocci:rods = 1:1
Starter M	2 %	Denser in texture, pleasant yogurt flavour, no pea off flavor		Rich microscopic pattern; cocci:rods = 1:1
Starter M	4 %	Dense in texture, soft yogurt flavour, no pea off flavor		Rich microscopic pattern; cocci:rods = 2:1
Starter M	6 %	Dense in texture, weak yogurt flavour, strong pea off flavor		Rich microscopic pattern; cocci:rods = 3:1
Starter M	10 %	Blunt, no yogurt flavour, pea off flavor		Poor microscopic pattern; cocci:rods = 1:1. Very long rods.
Starter S	0 %	Yogurt with typical yogurt flavour	Best yogurts - 2% and 4% pea isolate	Rich microscopic pattern; cocci:rods = 1:50
Starter S	2 %	Denser in texture, pleasant yogurt flavour, no pea off flavor		Rich microscopic pattern; cocci:rods = 1:1
Starter S	4 %	Denser in texture than the previous variant, distinct yogurt flavour, no pea off flavor		Rich microscopic pattern; cocci:rods = 2:1
Starter S	6 %	Denser in texture than the previous variant, very weak yogurt flavour, weak pea off flavor		Rich microscopic pattern; cocci:rods = 2:1 - 3:1
Starter S	10 %	Blunt, strong pea off flavor		Rich microscopic pattern; cocci:rods = 50:1 Long chains of cocci and rods
Starter D	0 %	Blunt, weak yogurt flavour		Poor microscopic pattern; cocci in doubles and chains
Starter D	2 %	Dense in texture, weak yogurt aroma, no pea off flavor		Poor microscopic pattern; cocci in doubles and chains, no rods
Starter D	4 %	Dense creamy texture, very weak yogurt flavour, no pea off flavor		Poor microscopic pattern; cocci in doubles and chains, no rods
Starter D	6 %	Dense creamy texture, weak rancid flavour		Poor microscopic pattern; cocci in doubles and chains, no rods
Starter D	10 %	Blunt, no yogurt flavour, pea off flavor		Poor microscopic pattern; too elongated cells, organized in long chains

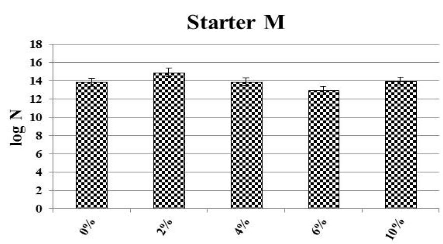


a)



b)

Fig. 2. Concentration of viable cells (a) and titratable acidity (b) of pea yogurt, obtained with Starter D. Incubation – 4 – 5 hours (until coagulation) at 41±1°C



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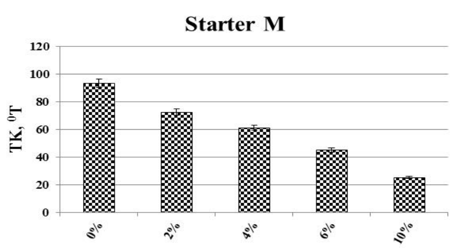
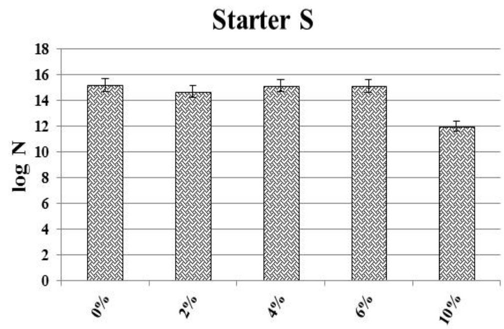
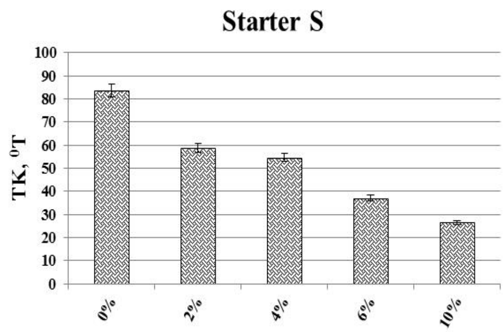


b)

Fig. 3. Concentration of viable cells (a) and titratable acidity (b) of pea yogurt, obtained with Starter M. Incubation – 4 – 5 hours (until coagulation) at 41±1°C



a)



b)

Fig. 4. Concentration of viable cells (a) and titratable acidity (b) of pea yogurt, obtained with Starter S. Incubation – 4 – 5 hours (until coagulation) at 41±1°C

The yogurt obtained with Starter M and Starter S using cow's milk, enriched with 2 – 4% pea protein had the best organoleptic properties.

Yogurts with the selected concentrations of pea protein for each starter (Starter M and Starter S) were prepared and stored at 4 ± 2°C for 25 days. There were no significant changes in the concentration of viable cells and the titratable acidity (Fig. 5, Table 4).

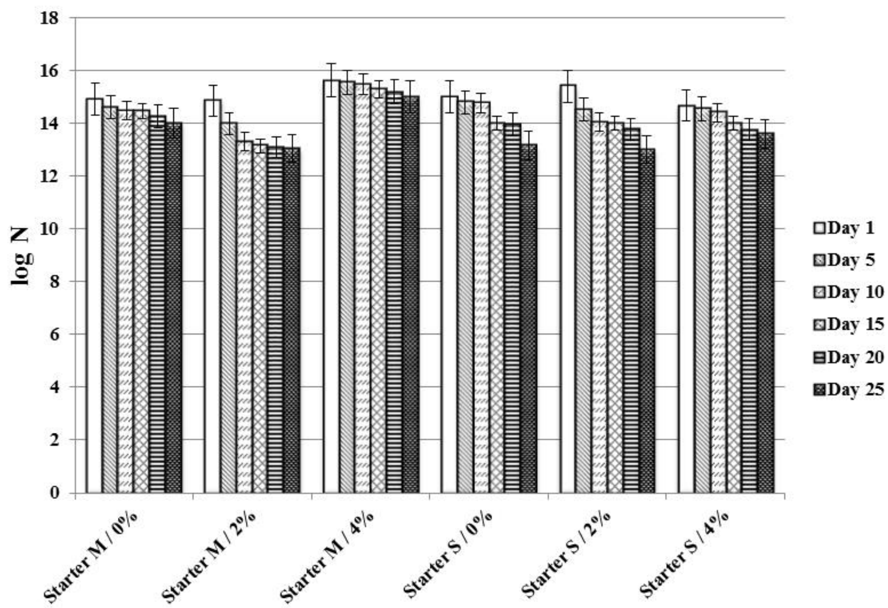


Fig. 5. Change in the concentration of viable cells of pea yogurts, obtained with Starter M and Starter S, during storage at 4±2^{O}C for 25 days.

Table 4.
Change in the titratable acidity of pea yogurts, obtained with Starter M and Starter S, during storage at 4±2^{O}C for 25 days

Starter	Pea isolate, %	Day 1	Day 5	Day 10	Day 15	Day 20	Day 25
Starter M	0 %	86	86,25	87.20	85.02	84.55	84.75
Starter M	2 %	68	67,55	67.45	61.60	66	65
Starter M	4 %	66	56,05	67.93	65.50	65.80	66.5
Starter S	0 %	82	65,08	72.28	77.90	83.13	82.18
Starter S	2 %	74	74,90	69.68	71.70	72.20	72.68
Starter S	4 %	60.5	60,15	60.73	60.7	60.33	61.75

The obtained yogurts enriched with pea protein are new dietary probiotic foods. Along with the inherent prebiotic ingredients of pea, significant amount of useful microflora (10^{13} - 10^{14}cfu/cm^3) enters the human gastrointestinal tract. It is necessary to restore and maintain the balance of microflora in the digestive tract, which is essential for human health.

4. Conclusion

Yogurt starters able to grow in cow‘s milk enriched with pea protein were selected.
The optimal percentage of pea isolate in the milk substrate for the preparation of pea yogurts was determined to be 2% and 4%. The obtained pea yogurts had high concentration of viable cells (10^{13}-10^{14}cfu/cm^3) and moderate titratable acidity and the changes in these values during their 25-day storage are insignificant. Therefore they can be used as functional foods, providing the body with the necessary amount of useful microflora to carry out its inherent preventive effect. Moreover, these new products containing pea protein have a number of other beneficial effects on different systems of the human organism, thus ensuring its resistance to the negative factors of the

environment, including prevention of infections.

5. References

[1]. LAROIA S, MARTIN JH, Effect of pH on survival of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in frozen fermented dairy desserts. *Cultured Dairy Prod. J.* 26:13–21 (1991).
[2]. HAGEN M, NARVHUS JA, Production of ice cream containing probiotic bacteria. *Milchwissenschaft* 54:265–268 (1999).
[3]. DENKOVA R, YANAKIEVA V, DENKOVA Z, URSHEV Z, GORANOV B, SOTIROVA E, Identification and examination of some probiotic properties of *Lactobacillus plantarum* F3. *Food and Environment Safety* - Journal of Faculty of Food Engineering, Ştefan cel Mare University – Suceava 11 (4):22 – 29 (2012).
[4]. DENKOVA R, ILIEVA S, NIKOLOVA D, EVSTATIEVA Y, DENKOVA Z, YORDANOVA M, YANAKIEVA V, Antimicrobial activity of *Lactobacillus plantarum* X2 against pathogenic microorganisms. *Bulg. J. Agric. Sci.* 2 (19):108–111 (2013).
[5]. SCHREZENMEIR J, DE VRESE M, Probiotics, prebiotics, and synbioticsd approaching a definition. *Am.J. Clin. Nutr.*, 77, 361–364 (2001).
[6]. BERNI-CANANI R, CIRILLO P, TERRIN G, CESARANO L, SPAGNUOLO MI, DE VICENIO A, ALBANO F, PASSARIELLO A, DE MARCO G, MANGUSO F, GUARINO A, Probiotics for treatment of acute diarrhea in children: randomized clinical trail of five different preparations. *BMJ*, 335–340 (2007).
[7]. DENKOVA Z, MURGOV I, Soy fermented foods. Interobmen LTD, Plovdiv (BG) (2001).
[8]. CANABADY-ROCHELLE LS, MELLEMA M, Physical–chemical comparison of cow's milk proteins versus soy proteins in their calcium binding capacities. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 366 (1–3):110-112 (2010).
[9]. ADEBIYI AP, ALUKO RE, Functional properties of protein fractions obtained from commercial yellow field pea (*Pisum sativum* L.) seed protein isolate. *Food Chemistry* 128 (4):902-908 (2011).
[10]. DONSI F, SENATORE B, HUANG Q, FERRARI G, Development of novel pea proteinbased nanoemulsions for delivery of nutraceuticals. *Journal of Agricultural and Food Chemistry* 58(19):10653–10660 (2010).
[11]. SULLIVAN F, Strategic analysis of the European pea market (2010).
[12]. KENT RM, DOHERTY SB, Probiotic bacteria in infant formula and follow-up formula: Microencapsulation using milk and pea proteins to improve microbiological quality. A review. *Food Research International* 64:567–576 (2014).
[13]. ŚWIĄTECKA D, ŚWIĄTECKI A, KOSTYRA H, MARCINIAK-DARMOCHWAŁ K, KOSTYRA E, The impact of pea protein hydrolysates on bacterial physiological activity—An in vitro study. *International Journal of Food Microbiology* 140:263–270 (2010).
[14]. WEISS W, VOGELMEIER C, GÖRG A, Electrophoretic characterization of wheat grain allergens from different cultivars in bakers' asthma. *Electrophoresis* 14 (8):805–816 (1993).
[15]. DENKOVA Z, Development and application of probiotics. DSc Thesis. University of Food Technologies, Plovdiv, Bulgaria (BG) (2005).