Innovative Romanian Food Biotechnology
Vol. 7, Issue of September, 2010
Received April 15, 2010 / Accepted September 10, 2010

RESEARCH ARTICLE

ATTEMPTS TO OBTAIN A NEW SYMBIOTIC PRODUCT BASED ON SOY MILK

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Abstract

Four different species of Lactobacillus (Lb. plantarum, Lb. brevis, Lb. johnsonii and Lb. delbrueckii ssp. bulgaricus) were used in an attempt to obtain a symbiotic product based on soy-milk. Lactic fermentation process was used to generate a product with enhanced acceptability for the consumer, while inulin was used as prebiotic compound. Inulin concentrations ranging from 1% to 9% were tested as probiotic bacteria supporters and 5% proved to be the most suitable. Also, it was found that the fermented product exhibited sensorial qualities and rheological behavior similar to yoghurt.

Keywords: soy milk, inulin, lactic acid bacteria, Lactobacillus, symbiotic product, yogurt-like product

Introduction

Soy milk contains protein of biological value, which is equivalent to that of milk or egg protein, but without the cholesterol, and a low level of saturated fatty acids. Increasingly, more studies have revealed the protective role of soy foods in a wide range of health conditions including cardiovascular diseases, cancer and osteoporosis (Messina and Setchell, 1994). Protein with high biological value, lecithin and unsaturated fats, non-metabolizable carbohydrates (stachyose and raffinose) and biologically active compounds like isoflavons, phytoestrogens (genistein and daidzein), give to soy milk the ability to prevent especially the diseases of the digestive tube (Tham *et al.*, 1998). It is scientifically proven that soy milk has anti-tumor activity (i.e. hormone modulated breast and prostate cancers) (Steinmetz and Potter, 1991), reduces the risk of cardiovascular diseases by reducing serum levels of low density lipoproteins (LDL) and triglycerides, reduces blood pressure and increases flexibility and permeability of blood vessels (Messina *et al*, 2003).

Soy milk has proven antiradical activity, through the isoflavones, which acts as an effective reducing tool of oxidative degradation of DNA, preventing premature aging and the emergence of diseases like Alzheimer's (Hsieh *et al.*, 2009).

Many soy products have limited human use in the Western hemisphere due to undesirable off-flavours (Favaro Trindade, 2001). Soy milk and other soy beverages are often characterized as having unbalanced ''beany'' flavors and chalky

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mouthfeel. Therefore, formulation changes that enhance the overall flavor and textural characteristics of soy beverages may be necessary to further increase soy consumption (Potter *et al*., 2007).

Fermented soymilk is the most recently developed of all traditional soy food and it can be regarded as a symbiotic product even without any prebiotics enrichment according to previous reports. Oligosaccharides from soybeans have proven themselves to be prebiotics (Chow, 2002; Cheng *et al*., 2005). In addition was shown that fermented soymilk have numerous advantages over non-fermented one (Chow, 2002). The fermentation may reduce flatulence, destroy undesirable pathogens (that cause real health problems in Third World countries), improve product flavor and reduce beany flavor, give new textures, and, when un-pasteurized, protect those who have eaten it from intestinal infections, and help replenish the intestinal flora (Trindade *et al*., 2001).

Fermented soy milk products may provide economic and nutritional benefits, because they can be prepared at higher protein levels at comparable or lower cost than regular fermented milk products (Karleskind *et al*., 1991). Soy-milk-based yogurts, namely, sogurt, have emerged as a popular alternative to traditional dairy-based yogurts due to their reduced level of cholesterol and saturated fat and because they are free of lactose (Pyo and Song, 2009). In this respect, the use of soy-milk adapted strains of lactobacilli and streptococci is also recommended (Gavin *et al*., 1990).

Inulin is a β-fructosaccharide of vegetal origin. In significant quantities is found in chicory, kale, onion and sea grass. Inulin has multiple physiological actions. It has prophylactic action in colon cancer or gastrointestinal infections by stimulating the activity of bifidobacteria (Flamm *et al*., 2001; Lou *et al*., 2009). It has been suggested that the beneficial effect of inulin could be due to the ability of bifidobacteria to change the colonic environment by inhibiting detrimental bacteria via the formation of bacteriocins, the successful competition for substrates or adhesion sites on the gut epithelium, and stimulation of the immune system (Miller-Catchpole, 1989; Gibson *et al*., 1995).

Inulin prevents the occurrence of cardiovascular diseases by reducing hepatic lipogenesis and lipemia. In high sugars diet, it reduces the risk of constipation stimulating peristalsis (inulin ingestion improved constipation in 9 of 10 subjects), increases the bioavailability of calcium (calcium fixation in bone) with the incidence in preventing osteoporosis. Inulin cannot be digested except through bacterial activity; they can alter the composition of human gut flora by a specific fermentation which results in a community predominated by bifidobacteria (Hidaka *et al.,* 1986)

Regarding its sensorial properties, inulin, has a slightly sweet taste that can improve the repulsive taste of soy milk. Inulin can be used to replace fat in liquid, aqueous food, improving their palatability.

The inulin adding in soy milk, which does not contain lactose or other fermentable sugars like mammals milk, is an option to make it suitable for fermentation by lactic acid bacteria cultures. Thus, it is possible both to make soy milk acceptable for consumers (eliminating any remaining traces of beany flavor) and to obtain a symbiotic product.

Taking into consideration the above mentioned properties of soy milk and inulin, it was decided to ferment them in order to obtain a symbiotic product with a consistency resembling to yoghurt.

Materials and methods

Materials

The soy milk used in this study is a sterilized product –**Soy Beverage Inedit** from *Dr. Oetker Company, Romania* – obtained from selected ingredients and certified as organic. According to the information given on the product label, it contains 1.8% proteins, 0.5 % sugars and 1, 5 % lipids.

As inulin supplement in soy milk was used *Fibruline instant,* a product that contains 95% inulin and 5% monosaccharides and disaccharides and is commerciallised in Romania by the company *Enzyme & Derivates S.A. Romania*.

Microorganisms

Lactobacillus plantarum-13GAL, *Lactobacillus brevis*-16 GAL, *Lactobacillus jhonsonii*- La1, *Lactobacillus delbruekii ssp bulgaricus*-LDB lactic acid bacteria strains were used in the experiments. Lactobacilli strains were isolated from cereal and pure cultures were freeze-dried after using polyethylene glycol like cryoprotectant medium. Each strain was reactivated on MRS broth (*Scharlau Chemie S.A., Spain*). Each soy milk variant was inoculated with lactic bacteria developed in overnight cultures in order to ensure 10^6cfu · g^{-1}.

Inulin supplemented soy milk based symbiotic product preparation

Five inulin supplemented soy milk variants (1%, 3%, 5%, 7%, 9%) were used for each LAB strain, along with a no-inulin supplemented soy milk as reference, in order to establish the effect of inulin addition on lactic acid bacteria growth and acid production. Volumes of 100 cm^3 soy milk were introduced in 200 ml Erlenmayer flasks, inoculated with lactobacilli and incubated at 37^{o}C for 24 hours.

Acidity and post acidity measuring

Soy milk acidity was measured at different time, for 24 hours starting from 0 hours. Titrable acidity was determined with 0.1 N NaOH solution and expressed in grams of lactic acid per 100 cm^3 of fermented product.

Fermented soy milk samples were stored at 5^{o}C, 15^{o}C and 20^{o}C and post-acidity was assayed after 1 week and 2 weeks respectively. Temperatures of 15^{o}C and 20^{o}C were chosen in order to test the stability of fermented product above the refrigeration domain.

Viability of microorganisms

Lactobacilli viability was assessed by plate counting. Appropriate serial dilutions were inoculated on MRS agar and incubated at 37^{o}C, for 48 h, under anaerobic conditions (2.5 l Oxoid AnaeroJar used with the AnaeroGen sachet).

Rhelogical measurments

Fermented soy milk samples were gently stirred before starting the rheological analysis. In order to determine rheological parameters it was used an AR 2000 Rheometer and the protocol developed by Hassan and coworkers (2003). This protocol uses two flow steps in order to obtain the flow curves. For the flow step 1 the shear rate was varied continuous from 0.1000 to 100.0 s^{-1} and the shear stress was recorded at increasing shear rates (upward flow curve). For the the flow step 2 the shear rate was varied continuous from 100.0 to 0.100 and the shear stress was recorded at decreasing shear rates (downward flow curve). The flow steps were applied in linear mode. The temperature was maintained at 4^{0}C during the measurements.

There was also performed a frequency sweep on the samples at frequencies ranging from 0.1000 to 10.00 Hz (Rao 1999). The temperature was continuous maintained at 4^0 C and the applied strain was 5.00 in logaritmic mode. The *Power low* model by TA Data Analysis has been applied, in order to achieve value of rheological parameters. The elastic modulus ($G^{'}$), the viscous modulus ($G^{''}$) were recorded as functions of frequency.

Sensory evaluation

Sensory analysis was conducted by a 10 member panel represented by students who graduated a Sensory Analysis course (Faculty of Food Science and Engineering, University *Dunarea de Jos* Galati) and have abilities for sensorial analysis. The samples were served at 8±2^{0}C in plastic cups labeled with three digit codes. To rinse their mouths between samples the panelists used water. Crackers were supplied as needed to aid in removing beany flavor between tasting.

The most relevant attributes for soy milk fermented product, which are presented in Table 1, were established based on the opinion of Fávaro Trindade and co-workers (2001). Evaluation used a partition scale that rates the intensity of a particular stimulus by attributing it a value on a five point scale (Table 2) (Meligaard, Civille, *et al* 1999).

Table1. *Rated sensorial characteristcs and atributes of symbiotic product*

Sensorial characteristic	Atribute	
	positive	negative
Visual texture	Lack of syneresis	Syneresis
Texture and consistency in the mouth	Consistent Firm Creamy	Chalky Airy Greasy
Taste	Sweet Acid	Soy Bitter Oily Beany

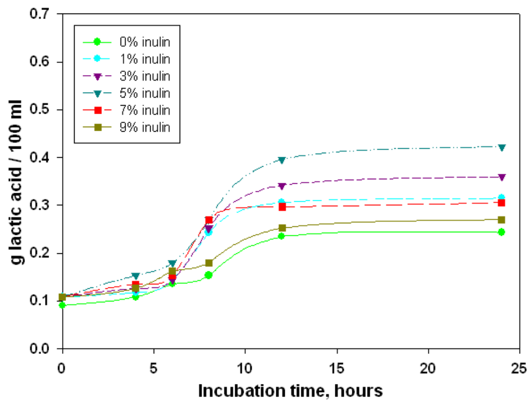
Table 2. *Five point scale for sensory for positive attributes assessment*

Number	***Significance***
0	**None**
1	**Very poor**
2	**Poor**
3	**Moderate**
4	**Strong**
5	**Very strong**

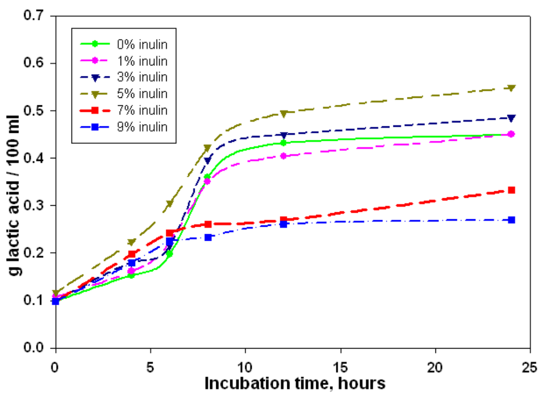
Results and discussion

Acidity dynamics for lactic acid bacteria cultures

Behavior of cultures used in this study showed that their development is dependent of inulin concentration added on the soy milk. However the LAB strains exhibited different yields in terms of inulin metabolism. Increasing of inulin concentration has a stimulating effect of LAB cultures but just from 1% to 5%. Concentrations above 5% inulin generated a slight inhibition of all tested LAB. In samples without inulin, cultures' growth was weak or was absent because soy milk contains low amounts of carbohydrates, which are insufficiently to sustain the LAB growth (Figure 1).



a)



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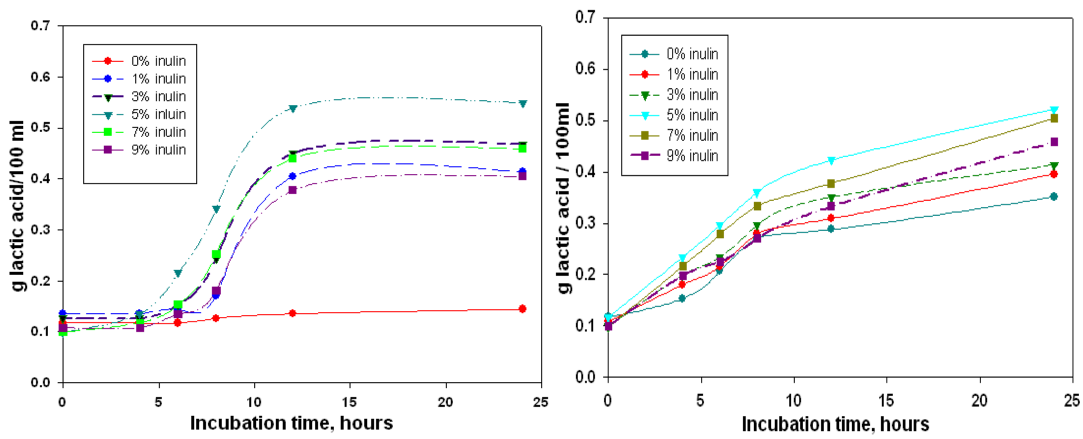

c) **d)**

Figure 1. *Influence of inulin concentration on the development of LAB cultures.*

a) Lactobacillus jonhsonii La1 strain;

b) Lactobacillus brevis 16 GAL strain;

c) Lactobacillus delbruekii ssp, bulgaricus LDB strain;

d) Lactobacillus plantarum 13 GAL strain.

Lactobacillus delbrueckii ssp bulgaricus – LDB strain exhibited the highest acidity value (e.g. 62 g lactic acid · 10^{-2} · cm^{-3}) after 10 hours of incubation at 37^{0}C in 5% supplemented soy milk samples. The other LAB strains exhibited acidity values close to those obtained for *Lactobacillus delbrueckii ssp bulgaricus,* after more than 15-20 hours. So, having in view that the strain of *Lactobacillus delbrueckii ssp bulgaricus* is faster than the other lactobacilli in producing lactic acid, it is recommended to be used for fermenting soy milk with added inulin.

Evaluation of post-acidification during fermented soy milk storage

Post acidification was evaluated just in soy milk samples supplemented with 5% inulin according with the results obtained in the previous experiment. At 5^{o}C the acidity level of the fermented product remained unchanged compared to the acidity existing at the beginning of storage. Just a slight post-acidification process occurred in fermented soy milk kept at 15 and 20^{o}C (Figure 2). This could be explained by the fact that metabolizable carbohydrates are not found in significant quantities in soy milk.

Rheological properties

Figure 3 shows the hysteresis curves obtained for the yogurt-like product made with each lactic acid bacteria culture, for the samples containing 5% inulin. The rheological tests, on fermented soy milk, were made after 24 hours of incubation at 37^{o}C. The shape of all flow curves indicated a thixotropic characteristic with yield stress. All the fermented soy milk samples shown approximately the same thixotropic behavior.

Acording with Hassan and coworkers (2003), a high $\mathbf{A_{up}}$[1] indicates an increasing of the structural breakdown of fermented soy milk during shearing. The A_{up} was lowest for the sample fermeted with *Lactobacillus plantarum*-13 GAL, indicating a low structural breakdown, which shows a good rheological stability. All samples exhibited characteristics typical for a weak viscoelastic gel, with G'> G" at all frequencies investigated (Table 3).

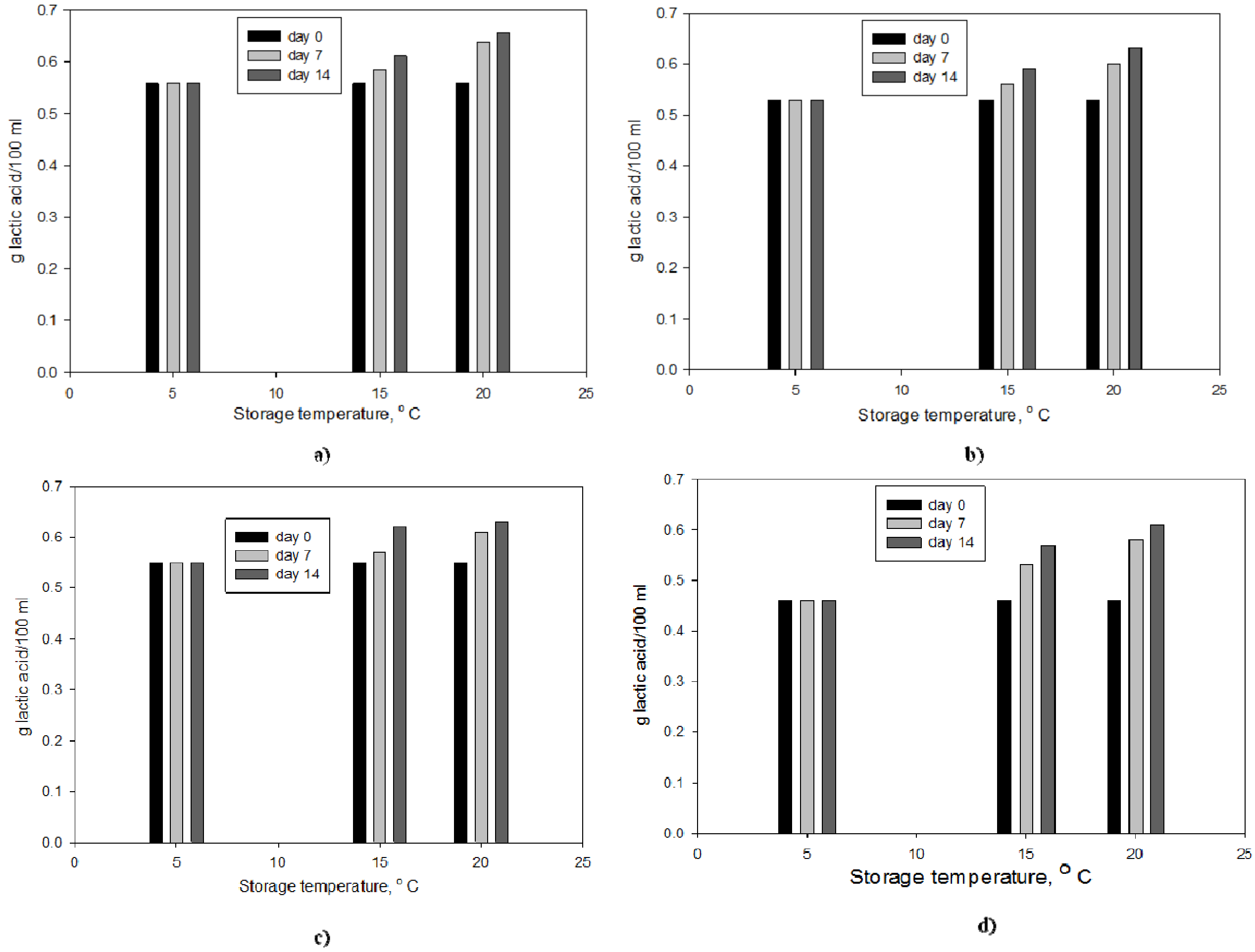

Figure 2*. Assayed post acidity during the fermented product storage.*

a) Lactobacillus delbrueki ssp, bulgaricus LDB strain;

b) Lactobacillus plantarum 13 GAL strain;

c) Lactobacillus brevis 16 GAL strain;

d) Lactobacillus jonhsonii La1 strain.

Table 3. *Rheological parameters and relative loop areas associated with flow curves of symbiotic soybean milk product*

Parameter	**Cultures**			
	16 GAL	**13 GAL**	**LDB**	**La1**
Elastic modulus, G', at 1 Hz, [Pa]	5.739	5.181	5.045	5.559
Viscous modulus , G'' at 1 Hz, [Pa]	1.766	1.565	1.537	1.685
Consistency coefficient, K, [Pa · s^n]	0.301	0.269	0.264	0.288
Flow behavior index , n	0.042	0.042	0.044	0,041
A_{up}	831.85	581.21	691.91	788.32

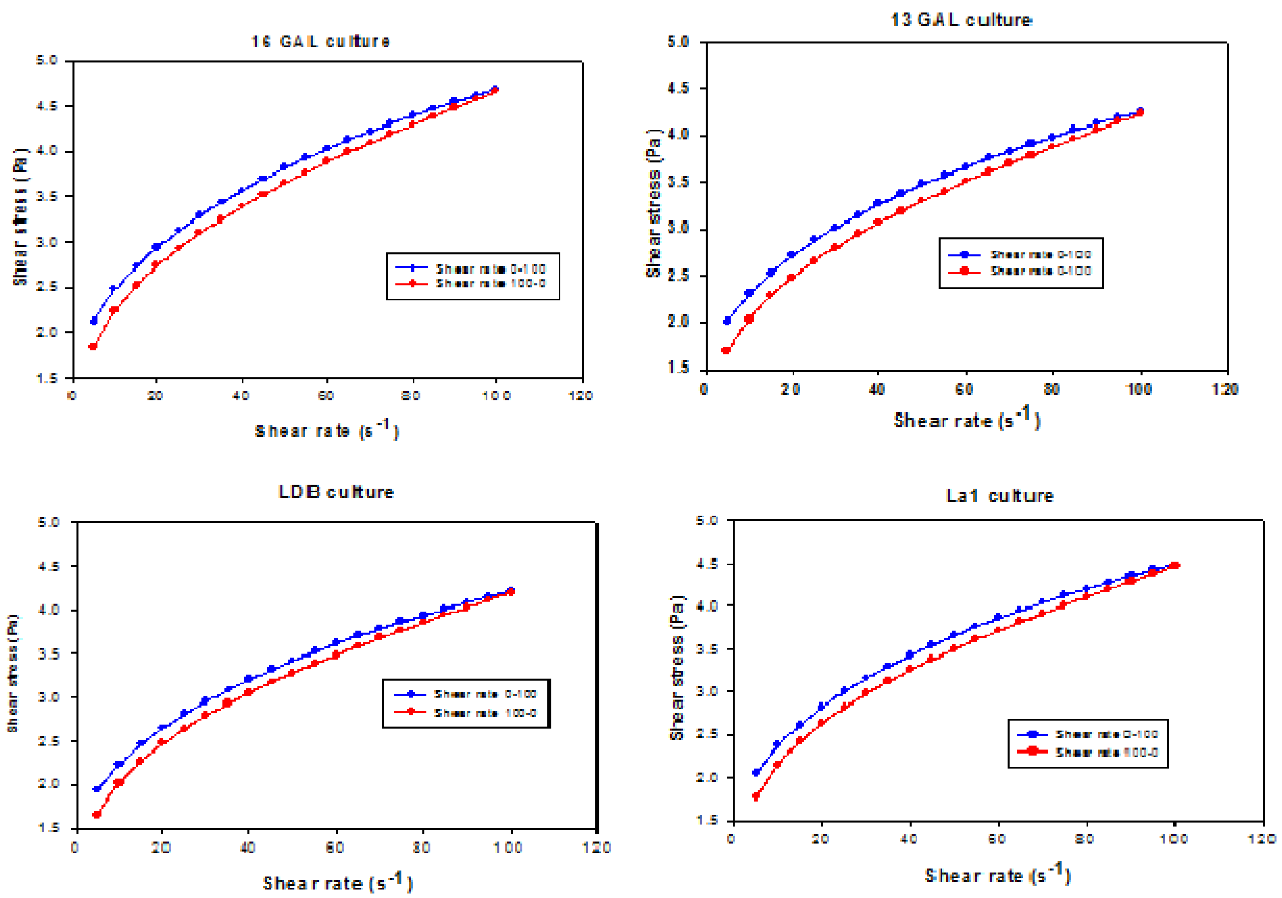

Figure 3. *Hysteresis curves of fermented soybean milk based product. Measurement runs were carried out at 4°C, for samples with 5% inulin only*

Table 3 shows the rheological parameters of supplemented soy milk fermented using the four single lactic acid bacteria cultures. Different species of lactobacilli did not affect the flow behavior index, a measure of the departure from Newtonian flow. According with Hassan (1995), results were consistent with pseudoplastic flow (n < 1). All the samples exhibit approximately the same flow behavior index value, around 0.040 (Table 3).

Sensory evaluation

Sensory evaluation is an important step in global product quality. The data from sensory panel evaluation were summarized by using the central tendency $\bar{x}$.

$$\bar{x}=\frac{\left(\sum_{i=1}^{n} x_i\right)}{n}$$

The aim of sensory evaluation of the product was to show the fermentation product acceptability using sensory attributes which are important in its sensory profile. For this purpose it was created an ideal sensory profile over that was overlapped the profile gained through sensory evaluation by the 10 panelists. It was shown that the fermented product obtained has a close relative acceptability to the imagined ideal profile required. However future research on increasing the sensory acceptability of the product is required (Figure 4)

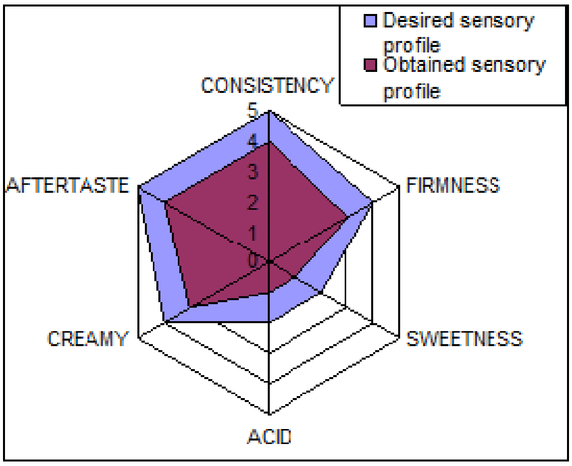

Figure 4. *SIB-: Lactobacillus plantarum (13GAL), SIE -Lactobacillus brevis (16 GAL), SIF-Lactobacillus johnsonii(La1), SIR-Lactobacillus delbrueckii bulgaricus (LDB)*

Conclusions

A yoghurt like symbiotic product was obtained by lactic fermentation of soy milk supplemented with inulin. Four species of *Lacobacillus* were tested as lactic fermentation agents in the experiments and *Lb. delbrueckii* proved to be the most suitable for carring on the fermentative process of soy milk.

It was proved that the addition of inulin has a positive influence on growth of lactic acid bacteria cultures up to amounts of 5%. Fermented symbiotic inulin supplemented soy milk can be considered a valuable product generated due to the cumulative effect of individual properties of inulin, soy milk and probiotic bacteria. However the aim of obtaining a product with a high acceptability has not been reached, it still remains a challenge in attempt to make soy milk a valuable product in terms of sensory properties. Future research concerning fermented soy milk can suggest using of other natural raw materials like muesli or fruits, which can mask the unpleasant soy taste.

This research proves that there are opportunities to develop a new trend and a market of functional foods including fermented soy milk that incorporates the health benefits and also a senses delight.

Aknowledgements

This research was supported by PN II CNCSIS ID-658, no. 315/1.10.2007 „Probiotic lactic acid bacteria for functional food on basis of germinated cereals" Project.

The work of Catalin Iancu was supported by Project SOP HRD - SIMBAD 6853, 1.5/S/15 - 01.10.2008".

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