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Application of *Bifidobacterium animalis* subsp. *lactis* as Starter Culture for Fermentation of Baltic Herring (*Clupea harengus membras*) Mince

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ABSTRACT

Fish is a well-known source of proteins, minerals, fat-soluble vitamins, antioxidants and other bio-active ingredients. Fish and its processing by-products are relatively cheap raw materials however still rarely are used to create food products with increased nutritive value, functional foods or feed supplements. Application of certain strains of probiotic bifidobacteria for fermentation of non-dairy substrates is a great challenge for industry-targeted research and industry with further offering to the market of functional food products. Our study is the first report on application of bifidobacteria for fermentation of fish. It was shown that a new fish-based functional food product can be obtained after fermentation of Baltic herring mince, supplemented with carbohydrates and NaCl, with a single-strain probiotic culture *Bifidobacterium animalis* subsp. *lactis* Bb12. Evaluation of FT-IR spectroscopy data indicated changes of proteins and the composition of total carbohydrates in fermented Baltic herring samples compared to the control. Glucose and sucrose ensured quick acidification and the decrease of pH, to achieve the cell count of *B. lactis* Bb12 up to 10^8 CFU g^{-1}, thus meeting requirements of the viable cell-count of probiotic bacteria in functional food products. However the highest cell counts of probiotic bacteria and acidification were reached using fish mince supplemented with sucrose (2%) and NaCl (1%). The obtained fermented baltic herring paste or its concentrate-after freeze-drying, still with high content of viable probiotic cells (10^7-10^8 CFU g^{-1}), can be used as functional food product as well as food and feed ingredients.

Key words: Fermented fish, baltic herring, *Bifidobacterium*, probiotic, food and feed supplements

INTRODUCTION

Fermentation of raw materials by their indigenous microflora usually is applied for production of fermented foods. However, in this case many risks must be taken into account like insufficient safety of the obtained products and unsteady fermentation process due to the instability of a multi-strain starter culture. In industrial production of fermented foods spontaneous fermentation

and its related risks should be minimized. Therefore are used microbiologically definite starter cultures that ensure faster and more controlled fermentation process and final products with constantly desired quality. Starter cultures are extensively studied in respect to physiological properties of certain strains and their mutual relationship with the raw-material and growth environment (Faid *et al*., 1997; Sahlin, 1999; Dai *et al*., 2013).

Fish is well-known as an excellent source of proteins, minerals, fat-soluble vitamins (A, D, E) and antioxidants. Fish lipids are a valuable source of polyunsaturated fatty acids, especially omega-3. Fish e.g., Baltic herring (*Clupea harengus membras* (L.)) and its by-products is a relatively cheap raw-material, with an unexploited potential to increase its nutritional value (De Arruda *et al*., 2007). Up to 18-20 million t of raw-material from fish industry every year are wasted worldwide (Elvevoll, 2004). Fish origin raw-materials could be used for development and industrial production of foods with added nutritional value, functional foods, biomedical products and animal feedstuffs (Dragnes and Elvevoll, 2003; Elvevoll, 2004).

Mainly Lactic Acid Bacteria (LAB) are used to increased the nutritive value of fish by fermentation either with spontaneous or specific starter cultures (Leisner, 1992; Gelman *et al*., 2000; Koffi-Nevry *et al*., 2011) thus obtained products are with highly improved sensoric properties and are microbiologically safe during an extended shelf-life (Leroy and De Vuyst, 2004).

It has been widely reported that a number of metabolites, such as lactic and acetic acids produced by heterofermentative LAB, hydrogen peroxide and bacterocins, produced during fermentation process, exhibit antimicrobial properties thereby improving the safety of fermented fish (Baishya and Deka, 2009). It is reported, that the inoculation of fish raw-materials with *Lactobacillus sakei* 2a significantly inhibited the growth of spoilage microorganisms maintaining good microbiological quality (Santo *et al*., 2003) as well as the antimicrobial properties of LAB against potentially, pathogenic microorganisms (Ostergaard *et al*., 1998; Schnurer and Magnusson, 2005). It is suggested, that the growth of heterofermentative LAB producing both lactic and acetic acids is important for the inhibition of competing spoilage and pathogenic bacteria caused by fast decrease of pH in the beginning of fermentation process (Adams and Hall, 1988). Nevertheless probiotic bifidobacteria, also ensure the maintenance of fermentation process and definite organoleptic properties of the final product (Leroy and De Vuyst, 2004).

The LAB and bifidobacteria usually, do not possess strong proteolytic properties. However, some strains of *Lactobacillus sake*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus curvatus* and *Lactobacillus pentsus* can ensure the hydrolysis of muscle proteins resulting in release of free amino acids caused by proteolytic action of endogenous microbial enzymes (Candogan *et al*., 2009; Fadda *et al*., 2010; Castellano *et al*., 2012). Besides, the enzymatic capability of *Bifidobacterium animalis* subsp. *lactis* to hydrolyze milk proteins also has been reported (Janer *et al*., 2005). Proteolysis is one of the most important biochemical processes of fish or meat fermentations. It influences the final texture and flavour by producing the low-molecular-weight compounds e.g. peptides, amino acids and their derivatives. Nie *et al*. (2014) showed remarkably higher concentrations of free amino acids in fish sausages fermented with *Lactobacillus plantarum* ZI49. Fish are rich in proteins and lipids and therefore applicable for fermentation with starter cultures utilizing these as energy sources due to the action of proteases and lipases. Hydrolysis of fish-lipids results in increased concentrations of valuable polyunsaturated fatty acids and glycerol or other alcohols participating in formation of unique sensory properties of fermented product (Nie *et al*., 2014).

Bifidobacterium animalis subsp. *lactis* Bb12 has a long history of research confirming its probiotic properties and beneficial influence on human health (Jungersen *et al*., 2014). Consequently, application of a certain accepted probiotic strain of bifidobacteria for fermentation of non-dairy substrates is a great challenge for industry-targeted research and commercial production of these healthy products (Mattila-Sandholm *et al*., 2002; Semjonovs *et al*., 2014).

To our knowledge up to date there are no reports on studies of fermentation of fish particularly Baltic herring by bifidobacteria. Baltic herring (*Clupea harengus membras*) is widespread and industrially fished in the Baltic Sea and gulf of Riga (Jauja and Zilinska, 2012). Relatively large stocks, quite limited food application, beyond sprats and some other local fish preserves and high dietary value comprise Baltic herring as a promising raw-material for development of new probiotics containing functional foods.

MATERIALS AND METHODS

Preparation of the starter culture: Probiotic strain *Bifidobacterium animalis* subsp. *lactis* Bb12 (*B. lactis* Bb12) (Chris. Hansen A/S, Denmark) was obtained from the Collection of Microorganisms of the Institute of Microbiology and Biotechnology, University of Latvia. The strain was subcultured twice in Man-Rogosa-Sharpe (MRS) broth at 37°C for 24 h. The cells were harvested by centrifugation (5000 rpm, 5 min), washed twice with saline solution (0.85% NaCl) and finally resuspended in the same solution at a concentration of 10^{10} CFU mL^{-1}. The cell suspension of *B. lactis* Bb12 was then used as starter culture for fermentations inoculated to an initial level of 10^7 CFU g^{-1}.

Preparation of Baltic herring mince for fermentation: Fresh Baltic herring (*Clupea harengus membras*) was purchased from the local market (Riga, Latvia). Each fish was washed in cold water, fins removed, beheaded, gutted, washed again and drayed with a cotton diaper. Obtained fish carcasses were minced. Fish mince was mixed with salt, glucose and sucrose or their combinations (1-4%) and divided in batches. After preparation batches were transferred into flasks and sterilized by heating for 10 min at 121°C. Fermentations were performed in 100 mL flasks at 35°C for 48 h. The initial pH of the sterilized mince before fermentation was 6.56±0.3.

After fermentation 10 g of samples were freeze-dried with VirTis BenchTop 6K (SP Industries Inc., USA) to 92±2% of dry matter.

Media and growth conditions: Man-Rogosa-Sharpe (MRS) growth medium was obtained from BD Difco (France). Composition of the MRS medium (g L^{-1}): 10.0 peptone, 8.0 beef extract, 5.0 sodium acetate, 4.0 yeast extract, 2.0 ammonium citrate, 2.0 KH_2PO_4, 1.0 Tween-80, 0.1 $MgSO_4 \times 7\ H_2O$, 0.05 $MnSO_4 \times 5\ H_2O$ and 20.0 glucose (De Man *et al*., 1960) was supplemented with 0.5 g L^{-1} of L-Cysteine hydrochloride hydrate (Sigma-Aldrich Co. LLC, USA) and used for maintenance, propagation and enumeration of *B. lactis* Bb12. After preparation, media were sterilized by heating (121°C, 10 min). pH of the medium was adjusted to 6.0±0.2 with HCl after sterilization.

Fish mince fermentations with *B. lactis* Bb12 were performed in 100 mL flasks at 35°C for 48 h.

Analytical methods: Fermentation samples (10 g) were homogenized in 100 mL of distilled water and the pH was measured with a digital pH meter (Mettler Toledo, Schwerzenbach, Switzerland). Using the same homogenate, lactic and acetic acids were detected.

The concentration of organic acids-lactic and acetic acids, were quantified by HPLC (Agilent 1100, HP, USA) with a refraction detector, column Shidex SH 1011, column temperature 50°C, mobile phase 0.01 M H_2SO_4 by flow 0.6 mL min^{-1}.

The total dry matter was determined gravimetrically as the residue after evaporation in oven at 105°C for16 h.

The FT-IR spectroscopy was used to evaluate the macromolecular composition of initial and fermented fish mince. The 10-20 mg of homogenized mince was suspended in 500 μL of distilled water and 5-10 μL of suspension used to record the FT-IR spectrum. The FT-IR spectra were recorded on a VERTEX 70 coupled with the HTS-XT microplate reader extension (Bruker Optik GmbH, Germany). Samples were dried on a 384 well silicon plate at T<50°C. Transmission spectra were registered over the range 4000-600 cm^{-1} and displayed as absorbance spectra. Spectra were acquired at a resolution of 4 cm^{-1} and 64 spectra were co-added. Each spectrum was baseline corrected by the rubber band method, CO_2 and H_2O bands were excluded. Each measurement is the average of at least three replicates. For data analyses were used spectra fitting in the absorption range 0.25-0.80, where the intensity of absorption band is directly proportional to the concentration. Vector normalization was used to compare mutually the spectra of samples. Data were processed using OPUS 6.5 software.

The samples (10 g) for *B. lactis* Bb12 viable cell-count (CFU g^{-1}) detection by the pour plated method were homogenized in 90 mL of sterile saline solution (0.85% NaCl) and the decimal dilutions were prepared. *Bifidobacterium lactis* Bb12 cell-count (CFU g^{-1}) was counted (Charteris *et al*., 1997) on agar-solidified MRS medium supplemented with L-Cysteine hydrochloride hydrate after 72 h at 37°C with anaerobic BD GasPak™ 150 System (Becton Dickinson and Company, USA).

Statistical analyses: The data presented are from at least three independent cultivations. All analytical measurements were repeated five times. The Student's t-test was employed to check the differences between means at a significance level $p<0.05$ using Statgraphics® Plus (Manugistics, Inc., US) and SPSS 11.0 for Windows (SPSS Inc. Ill., US).

RESULTS AND DISCUSSION

It has been reported, that during fermentation of foodstuffs the growth of spoilage microorganisms e.g. enterotoxigenic *Escherichia coli*, is limited due to their inactivation in acidic environment, if pH is 4 (Ndaw *et al*., 2008). The acidification can be achieved either using the starter cultures appropriate for fermentation of certain raw-materials or under the growth environment required for development of indigenous acidolactic microflora (Ross *et al*., 2002; Saithong *et al*., 2010).

Application of bifidobacteria for fermentation of milk products mainly in combinations with LAB starter cultures (Leroy and De Vuyst, 2004) is widely used for production of milk-based functional foods. There are few reports on application of bifidobacteria within a single-strain or mixed starter cultures for fermentation of plant origin raw-materials (Semjonovs *et al*., 2014). In order to achieve or enhance the desired sensoric properties of fermented product it can be supplemented with salt, spices and additional carbohydrates. The influence of these supplements on the sensoric properties and overall quality of the final product should be treated as an integral impact of particular added supplements and their influence on the metabolic pathways of microflora (both-indigenous and starter culture) involved in the fermentation process. It has been

reported that salt (Adams *et al*., 1987; Paludan-Muller, 1998) and carbohydrates (Adams *et al*., 1987; Muzaddadi and Mahanta, 2013) positively influence the final quality of fish products fermented by LAB.

Therefore in our study salt, glucose and sucrose separately or in a mix were added to minced fish. Table 1 shows that addition of carbohydrate source to the Baltic herring mince significantly promoted the acidification of minced fish due to the increased production of organic acids. The cell counts of *B. lactic* Bb12 in samples with carbohydrates reached 10^8 CFU mL^{-1}, while decreased from 10^7 to $3.2\pm0.18\times10^4$ CFU g^{-1} in the sample with no additive. Moreover the value of pH increased in comparison with the initial (Table 1, 2). Despite the significantly weaker growth of *B. lactis* Bb12 and slow formation of organic acids, the sensoric properties of these samples were evaluated and recognized as unacceptable due to an unpleasant odor. Similarly, as it was reported for anchovy (*Stolephorus* sp.)-the increase of pH during fermentation assigned to the formation of ammonia indicating to the degradation of proteins (Anggo *et al*., 2015). Addition of salt (1-4%) to mince (Table 2) did not prevent the formation of ammonia during fermentation however it slightly promoted the growth and acidification of *B. lactis* Bb12. The relative salt tolerance of LAB and *B. lactis* Bb12 (Semjonovs *et al*., 2014) and positive influence of salt at low concentration on the lactic acid fermentation process and quality of the final product have been reported previously (Adams *et al*., 1987; Paludan-Muller, 1998).

Addition of glucose and sucrose to fish mince resulted in a significantly increased cell counts of *B. lactis* Bb12, production of organic acids, especially lactate and acetate and fast increase of pH-reaching the characteristic for fermented final product (Table 1). It is known that the primary role of starter cultures is to ferment the available carbohydrates and thereby decrease the pH. The combination of low pH and produced organic acids is the main factor for preservation in fermented fish products. Usually, pH should be below 5.0-4.5 in order to inhibit the pathogenic and spoilage bacteria (Owens and Mendoza, 1985; Ostergaard *et al*., 1998; Muzaddadi and Mahanta, 2013). As it is shown in Table 1, glucose and sucrose in concentrations of 2% exhibited more explicit impact on the growth of culture and formation of lactate and acetate if salt was not added. Though, bearing in mind that addition of salt to fermented fish products is required at least for improvement of

Table 1: Influence of carbon source on pH*, production of organic acids and cell count** in Baltic herring mince fermented by *Bifidobacterium animalis* subsp. *lactis* Bb12 for 48 h

Carbon source added (%)	pH±SE	Cell count (CFU g^{-1}±SE)	Lactate (mg mL^{-1}±SE)	Acetate (mg mL^{-1}±SE)	Propionate (mg mL^{-1}±SE)	Formate (mg mL^{-1}±SE)
Control (no supplement)	7.36±0.2	$3.2\pm0.18\times10^4$	0.42±0.06	0.92±0.08	0.12±0.02	0.11±0.01
Glucose, 1%	4.94±0.2	$4.0\pm0.21\times10^8$	5.66±0.28	2.63±0.12	0.66±0.03	0.56±0.03
Glucose, 2%	4.57±0.2	$5.8\pm0.18\times10^8$	12.06±0.58	5.78±0.23	0.71±0.04	0.28±0.01
Glucose, 4%	4.37±0.2	$3.3\pm0.16\times10^8$	13.87±0.64	7.16±0.33	0.70±0.03	0.18±0.01
Sucrose, 1%	5.01±0.2	$5.0\pm0.27\times10^8$	7.54±0.38	2.46±0.13	0.70±0.05	0.45±0.01
Sucrose, 2%	4.39±0.2	$3.5\pm0.17\times10^8$	11.14±0.54	5.24±0.23	0.67±0.03	0.32±0.01
Sucrose, 4%	3.91±0.2	$6.9\pm0.32\times10^8$	14.66±0.69	7.89±0.38	0.61±0.03	0.08±0.01

*Initial pH value was 6.56±0.3, **Initial cell count of *B. lactis* Bb12 was $2.2\pm0.23\times10^7$

Table 2: Influence of salt (NaCl) on pH* production of organic acids and cell count** in Baltic herring mince fermented with *Bifidobacterium animalis* subsp. *lactis* Bb12 for 48 h

Salt (%)	pH±SE	Cell count (CFU g^{-1}±SE)	Lactate (mg mL^{-1}±SE)	Acetate (mg mL^{-1}±SE)	Propionate (mg mL^{-1}±SE)	Formate (mg mL^{-1}±SE)
Control (no salt added)	7.36±0.2	$3.2\pm0.18\times10^4$	0.42±0.06	0.92±0.08	0.12±0.02	0.11±0.01
1	7.48±0.2	$2.1\pm0.12\times10^4$	2.01±0.10	0.42±0.05	1.13±0.11	1.17±0.11
2	7.67±0.2	$2.4\pm0.08\times10^4$	2.70±0.12	0.75±0.07	1.16±0.08	1.66±0.12
4	7.65±0.2	$0.6\pm0.06\times10^4$	0.88±0.08	1.99±0.12	0.89±0.10	1.95±0.08

*Initial pH value was 6.56±0.3, **Initial cell count of *B. lactis* Bb12 was $2.2\pm0.23\times10^7$

organoleptic properties of the final product, the influence of salt on the growth of probiotic starter culture was assessed in the presence of glucose and sucrose (Table 3 and 4). The acidification rate in fish mince after 48 h was the lowest in the sample with 2% of sucrose and 1-2% of salt, followed by glucose with the same salt concentrations. No significant decrease of pH was observed for further fermentation after initial 48 h period, in fact further fermentation during 96 h resulted in a higher final pH of the product. In presence of both carbohydrates, 4% salt additive slightly suppressed the decrease of pH. As the optimal salt concentration should not inhibit the growth of bifidobacteria and improve flavour and texture of the product, the lowest salt concentration of 1% was optimal to obtain a lightly salted healthy product (Table 4). Table 4 shows that addition of 1% salt in the presence of glucose and sucrose significantly promoted the production of organic acids while in presence of sucrose the total acidity and concentration of acetic acid were higher.

Fourier transform infrared (FT-IR) spectroscopy has been successfully applied for analyses of various biological samples incl. food products and fish as well (Van de Voort, 1992; Hernandez-Martinez *et al*., 2014; Alamprese and Casiraghi, 2015). This method allows to estimate the composition of sample, monitor the macromolecular changes during processing, control the product quality, etc. In this study FT-IR spectroscopy was used to evaluate the composition changes of Baltic herring mince after fermentation with probiotic strain of bifidobacteria.

The FT-IR spectra of Baltic herring mince fermented with *B. lactis* Bb12 in presence of sucrose, glucose and NaCl in different variations are typical bio-sample spectra (Fig. 1). The main absorption bands present lipids in the region 3050-2800 cm^{-1} (CH_2 and CH_3 groups of fatty acids), proteins in the region at 17501-500 cm^{-1} (N-H and C = O vibrations, Amide I and Amide II), the mixed region at 1500-1200 cm^{-1} (vibrations originating from fatty acids, proteins and nucleic acids) and polysaccharides and carbohydrates at the region 1200-900 cm^{-1} (Naumann, 2000; Grube *et al*., 2002).

Figure 1 shows that the composition and content of lipids in Baltic herring mince was not changed by fermentation with bacteria Bb12 disregards of supplementation with sucrose, glucose or NaCl. In spectra of all fermented samples was seen the shift of Amide I band maximum to

Table 3: Influence of salt (NaCl) and carbohydrates on pH* of Baltic herring mince fermented with *Bifidobacterium animalis* subsp. *lactis* Bb12 for 96 h

	Time (h)		
Supplements (%)	24	48	96
Control (no supplement)	6.85±0.2	7.35±0.2	8.12±0.2
Glucose, 2%; salt, 1%	5.01±0.2	4.28±0.2	4.03±0.2
Glucose, 2%; salt, 2%	5.12±0.2	4.38±0.2	4.35±0.2
Glucose, 2%; salt, 4%	6.15±0.2	5.12±0.2	5.41±0.2
Sucrose, 2%; salt, 1%	5.25±0.2	4.25±0.2	4.33±0.2
Sucrose, 2%; salt, 2%	5.34±0.2	4.35±0.2	4.54±0.2
Sucrose, 2%; salt, 4%	6.05±0.2	5.40±0.2	5.60±0.2

*Initial pH value was 6.56±0.3

Table 4: Influence of carbohydrate and /or salt on pH and content of organic acids in Baltic herring mince fermented with *Bifidobacterium animalis* subsp. *lactis* Bb12 for 48 h

	Lactate	Acetate	Propionate	Formate
Supplements added (%)	(mg mL^{-1}±SE)			
Control (no supplement)	3.05±0.20	1.66±0.18	1.28±0.06	0.56±0.03
Glucose, 2%	27.13±1.36	2.31±0.12	0.61±0.03	0.25±0.01
Glucose, 2%; salt, 1%	31.91±1.60	2.53±0.13	0.61±0.03	1.13±0.06
Sucrose, 2%	38.06±1.91	4.19±0.21	0.66±0.03	0.22±0.01
Sucrose, 2%; salt, 1%	40.57±2.03	5.40±0.27	0.60±0.03	0.18±0.01

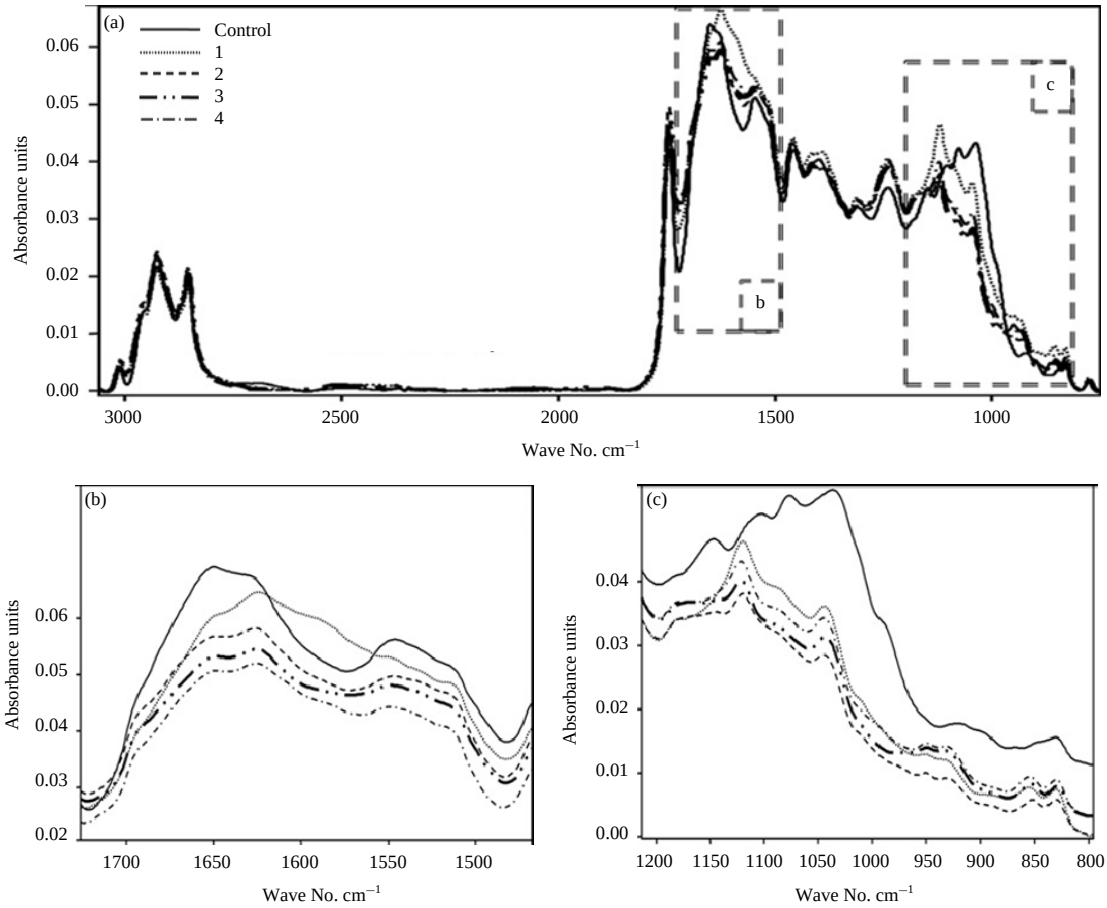

Fig. 1(a-c): FTIR spectra of Baltic herring mince fermented with *Bifidobacterium animalis* subsp. *lactis* Bb12 and amended with; 1: 2% glucose, 2: 2% glucose and 2% NaCl, 3: 2% sucrose, 4: 2% sucrose and 2% NaCl, (a) Full spectra, (b) Protein region and (c) Carbohydrate region

1625 cm^{-1} from 1650 cm^{-1} in control sample, moreover the shape of Amide II band also differed (Fig. 1a, b). The shifts of Amide bands in spectra of LAB in presence/interaction with phospholipids have been reported (Castellano *et al*., 2007).

The profile of carbohydrate region was different for control and fermented samples (Fig. 1c). The FT-IR spectrum of samples with additives shows broad absorption bands while pure substances or a mix of few components-separate peaks. The spectra of raw/control Baltic herring mince in the carbohydrate region showed a broad band while in fermented samples some well pronounced peaks. This indicates that during fermentation of minced Baltic herring with *B. lactis* Bb12 some carbohydrates where converted during the process of fermentation for culture growth and synthesis of metabolites.

As it is shown in Table 5 freeze-drying of Baltic herring minces fermented with *B. lactis* Bb12 secure a high survival of probiotics in dried product, which make them prospective for application in food and feed supplements. High survival of probiotic cells during freeze-drying can be attributed to the good protective properties of fermented fish mince itself, disregard of glucose, sucrose and

Table 5: Influence of carbohydrate and /or salt on the survival of *Bifidobacterium animalis* subsp. *lactis* Bb12 cells in fermented Baltic herring mince after freeze-drying

	Cell count (CFU g^{-1} dry weight±SE)	
Fermentation supplements (%)	Before freeze-drying	After freeze-drying
Control (no supplements)	$2.2\pm0.18\times10^{5}$	$8.2\pm0.26\times10^{4}$
Glucose, 2%	$1.1\pm0.05\times10^{8}$	$7.0\pm0.28\times10^{7}$
Glucose, 2%; salt, 1%	$0.9\pm0.04\times10^{8}$	$7.2\pm0.32\times10^{7}$
Sucrose, 2%	$1.5\pm0.09\times10^{8}$	$0.8\pm0.06\times10^{8}$
Sucrose, 2%, salt, 1%	$1.7\pm0.11\times10^{8}$	$1.2\pm0.08\times10^{8}$

salt in the fermented mince. Besides, significant protective impact of sucrose (2%) and salt (1%) additives was observed, which is in accordance with the previously reported cryoprotective influence of poly-sugars on the survival of LAB and bifidobacteria (Efiuvwevwere *et al*., 1999; Carcoba and Rodriguez, 2000; De Giulio *et al*., 2005; Ferreira *et al*., 2005; Duong *et al*., 2006). The obtained freeze-dried small-grained powder has a pleasant, typical fish, mild-sour aroma and a high content of viable *B. lactis* Bb12 cells (10^{7}-10^{8} CFU g^{-1}).

CONCLUSIONS

This study showed that Baltic herring mince can be fermented with a single-strain probiotic culture *Bifidobacterium animalis* subsp. *lactis* Bb12 in presence of additional carbohydrates and salt. FT-IR spectroscopy was shown to be a useful method for monitoring the changes of fish mash under variable fermentation environments. Addition of glucose and sucrose to fish mince is required for fast acidification and the decrease of pH caused by the growth of *B. lactis* Bb12 and reaching 10^{8} CFU g^{-1}, meeting the requirements for functional food product. It was shown that sucrose-2% and salt-1% in fish mince provided the best growth of probiotic bacteria and the acidification power. The obtained fermented Baltic herring paste with probiotics can be used as functional food product or dietary supplement for food and feed. Freeze-drying of Baltic herring paste results in a fish-based concentrate with high content of viable probiotic cells (10^{7}-10^{8} CFU g^{-1}) applicable for dietary use or as neutraceutic for food or feed supplementation.

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