

## THE APPLICATION OF AUTOCHTHONOUS POTENTIAL OF PROBIOTIC *LACTOBACILLUS PLANTARUM* 564 IN FISH OIL FORTIFIED YOGHURT PRODUCTION

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**Abstract** - The objective of this work was to investigate the survival of autochthonous, potentially probiotic bacteria *Lactobacillus plantarum* 564, and the influence of long-chain polyunsaturated fatty acid omega-3 (omega-3 PUFA) fish oil fortification on the sensory quality of yoghurt. Three variants of yoghurt were produced using starter cultures of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* (Chr. Hansen, Denmark), and the potentially probiotic *Lactobacillus plantarum* 564 (Culture Collection of the Department for Industrial Microbiology, Faculty of Agriculture, University of Belgrade) as follows: (1) without omega-3 PUFA; (2) with 100 mg/l omega-3 PUFA; and (3) with 200mg/l omega-3 PUFA. The survival of potential probiotic *Lb. plantarum* 564, the changes of starter bacteria counts, changes of pH values, as well as sensory evaluation, were examined during 3 weeks of yoghurt storage. Cells of *Lb. plantarum* 564 were maintained at  $>10^8$  cfug<sup>-1</sup>. Starter bacteria counts were  $>10^7$  cfug<sup>-1</sup> for streptococci and  $>10^6$  cfug<sup>-1</sup> for lactobacilli. The changes of pH were within normal pH of fermented milks. Sensory evaluation showed that all variants of yoghurt produced with *Lb. plantarum* 564 and 2 concentrations of omega-3 polyunsaturated fatty acids had a high sensory quality (above 90% of maximal quality), and which did not change significantly throughout the examined storage period. Although the sensory quality of the control sample was evaluated as better, the experimental samples fortified with fish oil were also characterized with very acceptable sensory properties. Results of high viability of potential probiotic *Lb. plantarum* 564, as well as very acceptable yoghurt sensory properties, indicate that this strain can be successfully used in the production of yoghurt fortified with PUFA omega-3 fish oil as a new functional dairy product.

**Key words:** Autochthonous potential probiotic, yoghurt, fish oil; omega-3 PUFA, sensory quality

### INTRODUCTION

The term functional food refers to biologically active food that positively affects human health. Recently, the world has seen an upward trend in the production and use of functional foods, as well as research on the benefits of these products to consumer health. Products with probiotic bacteria, including dairy products and especially fermented milks such as yoghurt, are one of the most popular functional foods. By definition, probiotics are “live microorganisms, which

upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition” (Guarner et al., 1998). It is recommended that products with probiotics should contain at least  $10^7$  live microorganisms per g or ml (Ishibashi et al., 1993) in order to achieve their positive effects on consumer health. According to the literature, the health benefits for consumers attributed to probiotic bacteria can be categorized as either nutritional or therapeutic. Nutritional benefits include their role in enhancing the bioavailability of calcium, zinc, iron, manganese, copper, and phos-

phorus (Annuk et al., 1999) and an increase of the digestibility of protein and synthesis of vitamins in yoghurt (Barbara et al., 2000). The therapeutic benefits of probiotics include treatments of conditions such as gastrointestinal disorders, hypercholesterolemia and lactose intolerance, suppression of procarcinogenic enzymes, inhibitory effects on Ehrlich ascites tumor cells, immunomodulation, and treatment of food-related allergies (Begley et al., 2005).

In recent years, the probiotic activity of lactic acid bacteria (LAB) has been in the forefront and an increasing number of food supplements as well as pharmaceutical preparations are being promoted with health claims based on several characteristics of certain strains of LAB, particularly from the genera *Lactobacillus* and *Bifidobacterium* (McFarland et al., 1997; Kaur et al., 2002). The different LAB strains isolated from traditional fermented foods constitute a reservoir of unexplored potential in biotechnology. Thus for example, the autochthonous white brined cheeses differ in the presence of a wide range of LAB strains, with different metabolic properties (Radulović et al. 2011; Seratlić et al., 2013). Considering the fast-growing interest in the application of probiotic strains in different foods, including dairy products, it is believed that it is possible to isolate some strains with potential probiotic ability from among the autochthonous strains. Nowadays the criteria for the selection of LAB strains from traditional products are extensive and include technological and biochemical criteria, as well as investigations of their probiotic ability (Radulović et al., 2010; Petrović et al 2012).

During the last 15 years there is increasing industrial interest in incorporating long chain omega-3 polyunsaturated fatty acids (omega-3 PUFA) into foods and dietary supplements. This is because there is a growing body of evidence that these omega-3 PUFA have a number of health-beneficial effects as well as a nutritional role (Ruxton et al., 2004). Fish oil is a predominant dietary source of omega-3 PUFA, which consists mainly of eicosapentaenoic acid (EPA C20:5) and docosahexaenoic acid (DHA C22:6).

The beneficial health effects of omega-3 PUFA intake are well demonstrated for the prevention of hypertension, cardiovascular diseases, type 2 diabetes, rheumatoid arthritis, Crohn's disease and cancer, as well as in improving the development and functioning of the brain, retina and testis (Banning, 2005; Connor, 2000; Simopoulos et al., 1999). Nowadays dietary recommendations suggest that the consumption of omega-3 PUFA should be increased.

The European Academy of Nutritional Sciences (EANS) has recommended a minimal average intake of 0.2 g omega-3 PUFA (EPA plus DHA) per person per day (Ruxton et al., 2004; Tautwein, 2001). The dietary guidelines formulated by the UK Joint Health Claims Initiative (2004) suggested an intake of at least 0.45 g of EPA + DHA per person per day. The average omega-3 PUFA content in the Western-style diet is 0.15 g per day, and the ratio of omega-6 to omega-3 acids is usually 10 to 1 or even higher, which is caused by an elevated consumption of vegetable fats and oils and other products containing these fats as well as low consumption of fish (Sanders, 2000). Due to the lower consumption of oily fish in many developed countries where a Western-style diet is predominant, the average fish intake is currently far below the recommended 2-3 fish servings per week (Sanders, 2000). Hence, alternative ways to ensure a proper omega-3 PUFA intake are needed. Without radical changes in eating habits, an innovative way to elevate the omega-3 PUFA intake is by consumption of various foods fortified with fish oil (Kolanowski and Laufenberg, 2006).

At present, functional foods containing omega-3 lipids are one of the fastest growing food product categories in the US and Europe (Jacobsen, 2008), and there are already a few omega-3 acid-enriched products on the market, including dairy products.

However, it is also known that fortification with omega 3 PUFA may significantly affect the odor and taste of enriched foods (Jacobsen, 2008). Hence, a major challenge in relation to the use of omega-3 PUFA in food application is their susceptibility to

lipid oxidation, which gives rise to the formation of undesirable “fishy” flavors.

A prerequisite for the successful development of foods enriched with omega-3 PUFA is that oxidation of the lipids is prevented. Due to the high susceptibility of fish oil to oxidation, fortified food products should be processed, packed, stored and distributed in conditions eliminating all factors promoting the oxidation of PUFA, especially access of oxygen, UV light, elevated temperature and humidity, as well as metal ion content of the product – Fe, Cu and Mn (Jacobsen, 1999). It appears that the most suitable foods for fortification with omega-3 PUFA are those that are frequently consumed, stored for a short time at low temperature in packages with no permeability of air and light. Good examples of such foods are fermented dairy products whose shelf life is relatively short (Kolanowski and Weibrodt, 2007).

Several studies have shown that yoghurt enriched with omega-3 PUFA in contrast to omega-3 enriched milk, can be an especially good vehicle for fish oil enrichment, since the products are resistant to oxidation (Nielsen et al., 2009). However, it is very important to determine the correct content of omega-3 PUFA which can be added without deterioration of the sensory properties.

It is known that fermented milks containing probiotics, including yoghurts, are the most widespread functional food products, and they can present a solid base for the addition of other bioactive supplements. However, to date there is little literature data about the possibility of making probiotic yoghurt with added omega-3 PUFA, or of their potential influence on the viability of probiotics.

Hence, the first objective of this study was to examine the viability of autochthonous potential probiotic bacteria *Lactobacillus plantarum* 564 in yoghurt fortified with fish oil. The second objective was to investigate the influence of fish oil fortification on the sensory properties of yoghurt during 21 days of storage.

## MATERIALS AND METHODS

### *Materials*

For production of the experimental yoghurt, starter culture mixes of *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus* (Chr. Hansen, Denmark), potential probiotic *Lb. plantarum* 564 and purified fish oil were used. The potential probiotic strain, *Lb. plantarum* 564, was isolated from traditionally made white brined cheese (Radulović et al., 2010). This strain belongs to the collection of the Department for Industrial Microbiology, Faculty of Agriculture, University of Belgrade. The strain was cultured in MRS broth (Merck, Darmstadt, Germany) at 37°C. Purified fish oil contained ca. 300 g kg<sup>-1</sup> omega-3 PUFA (DSM nutritional products, Basel, Switzerland).

### *Yoghurt production*

Milk was heat-treated at 92-94°C for 10 min. After the milk was cooled to 42°C, it was inoculated with yoghurt starter culture mix (1mL/100mL) and *Lb. plantarum* 564 (in conc. 10<sup>8</sup> cfu ml<sup>-1</sup>) and divided into three equal parts: a control variant was produced without omega-3 PUFA, one test variant was produced with 100 mg/l omega-3 PUFA, and a second test variant was produced with 200 mg/l omega-3 PUFA. The mixtures were incubated at 40°C until a pH around 4.5 was reached. After fermentation, the yoghurt was put into 100-ml plastic bottles and stored at 4°C for 21 days.

### *Determination of viability of strains in yoghurt*

Ten ml of each yoghurt sample was weighted aseptically, transferred to sterile saline diluents containing 1% peptone and homogenized for 2 min using a Stomacher 400 (Seward, London, UK). Appropriate dilutions of the samples were prepared using the same diluents and plated in duplicate on different growth media. Cell enumeration of *Lb. delbrueckii* subsp. *bulgaricus* and *Lb. plantarum* 564 were carried out on MRS agar (Merck, Darmstadt, Germany) after incubation under anaerobic conditions for 48 h

at 42°C and 37°C, respectively. Cell enumeration of *Streptococcus thermophilus* was carried out on M17 agar (Merck, Darmstadt, Germany) for 48 h at 42°C. The survival of potential probiotic *Lb. plantarum* 564 and changes in the starter bacteria counts were examined during 3 weeks of yoghurt storage. Sampling was carried out on storage days 1, 7, 14 and 21. Microbiological data were transformed into logarithms of the number of colony-forming units (cfug<sup>-1</sup>).

#### Determination of yoghurt pH

The pH of the yoghurt samples was measured by a pH-meter Hanna, HI 83141 (Hanna Instruments USA). The presented results are the mean values of three measurements.

#### Sensory analysis

The sensory evaluation of yoghurt was conducted after 1, 7, 14 and 21 days of storage. Panel members evaluated the yoghurt for appearance, color, texture and consistency, odor and taste using a 5-point scale, with 1 being the worst and 5 the best quality. Depending on the importance of attributes, they were multiplied by 3, 1, 4, 2 and 10, respectively. The total sensory quality (100) was expressed as a percentage of the maximum quality.

## RESULTS AND DISCUSSION

#### Starter and probiotic bacteria counts

The changes in the starter bacteria counts in yoghurts during 21 days of storage are shown in Fig. 1.

Initial lactobacilli and streptococci counts were above 10<sup>8</sup> cfug<sup>-1</sup> and there were no significant differences between the yoghurt variants (average numbers are shown). The initial level of streptococci was high and slightly decreased between days 7 and 14 of storage. However, their number was still high and above 10<sup>6</sup> cfug<sup>-1</sup> (6.80 log cfug<sup>-1</sup>) at the end of the storage period. The level of lactobacilli was also high at the beginning, but during storage a more pronounced decrease in their numbers was found.

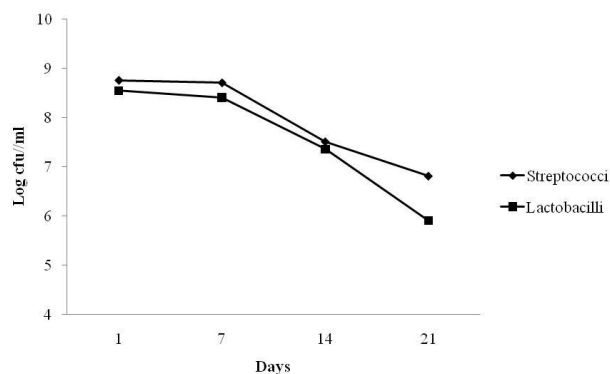


Fig. 1. The changes in yoghurt starter bacteria counts during yoghurt storage.

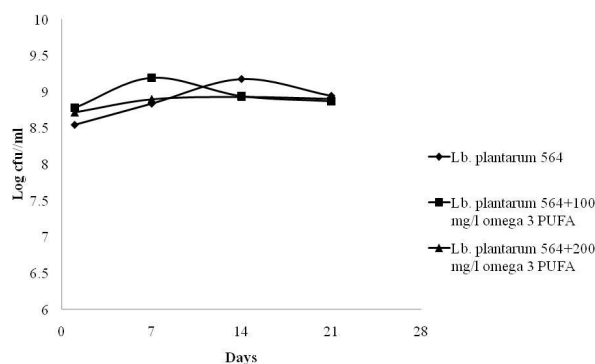


Fig. 2. Viability of the potentially probiotic strain *Lb. plantarum* 564 during yoghurt storage.

At the end of the storage time, the number of lactobacilli was slightly under 10<sup>6</sup> cfug<sup>-1</sup> (5.90 log cfug<sup>-1</sup>). Similar results were found by other authors. Kneifel et al. (1993) reported that the counts of lactobacilli in fresh yoghurts varied between 5.5 x 10<sup>7</sup> and 6.5 x 10<sup>8</sup> cfu ml<sup>-1</sup>, and the counts of streptococci varied from 3.5 x 10<sup>7</sup> to 1.2 x 10<sup>9</sup> cfu ml<sup>-1</sup>. The authors reported that about 80% of the yoghurts had higher counts of cocci than rods, and that the stability the microflora during storage time differed markedly among the cultures. Dave and Shah (1996) also reported that viable counts of starter culture bacteria in yoghurt increased and then declined during a 28-day refrigerated storage study. They stated that *S. thermophilus* was more stable (app. 10<sup>7</sup> cfug<sup>-1</sup>), while *Lb. delbrueckii* ssp. *bulgaricus* lost viability rapidly to < 10<sup>5</sup> cfu/g after about 20 days.

Andino (2011) showed that the addition of microencapsulated salmon and menhaden oils had no effect on the LAB counts of yoghurts during 4 weeks of storage. However, the author found that viable counts of LAB decreased (from about 8.7 log cfu/g to 6.3) during the storage study of all yoghurts, which indicates certain instability of the starter cultures during shelf life.

The viability of potential probiotic *Lb. plantarum* 564 in three variants of yoghurt made without and with different concentrations of omega-3 PUFA is shown in Fig. 2. In all yoghurt variants, the initial counts of *Lb. plantarum* 564 bacteria were above  $10^8$  cfu $^{-1}$ . During the whole storage period, a high rate of probiotic cells was found. These results are especially important to achieve positive therapeutic effects of probiotics on consumer health. It was stated that a minimal concentration of probiotic  $10^7$  cfu/g or ml of food should be present at the moment of intake to assure a favorable impact on consumer health (De Vuyst, 2000). No significant differences between the three variants of yoghurt were found. However, it is important to emphasize that in literature there are little data regarding the relation between probiotics and omega-3 PUFA. Song et al. (2011) investigated the sensory properties and viability of probiotics (*Bifidobacterium longum*, 8 log CFU/g) in chocolate ice cream supplemented with omega-3 PUFA. They found that the viability of the probiotics was higher in samples with omega-3 fatty acids during a 4-week storage period at  $-30^{\circ}\text{C}$ . It is believed that probiotics can enhance the absorption of omega-3, thereby increasing their tissue levels. Kastel et al. (2007) found that PUFA-potentiated probiotics (*Lb. casei* ssp. *paracasei*) positively affected the adhesion of lactobacilli, pH and level of organic acids in the digestive tract of germ-free piglets. This research means that taking omega-3 PUFA and probiotics together could maximize the health benefits of each supplement. It is important that there is no negative correlation between probiotics and omega-3 PUFA. Some studies have reported that they can even be microencapsulated together and in this way, it is possible to create a multifunctional supplement.

### Changes in pH

The changes in pH of the yoghurt variants are shown in Fig. 3. As can be seen, the pHs of all samples were within normal pH of fermented milks. In accordance with the starter bacteria count, which was very similar among the samples, these results were expected.

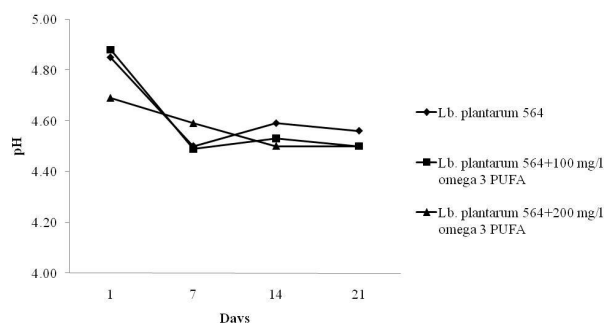


Fig. 3. Changes in pH during yoghurt storage.

### The sensory evaluation

The results of sensory analysis of the yoghurt made with probiotics and with or without omega 3 PUFA during 21 days of storage are shown in Table 1.

All three variants of yoghurt and their sensory parameters were evaluated with very high scores and can be described as products with very acceptable sensory properties. The control yoghurt samples made with potential probiotic *Lb. plantarum* 564 were evaluated with the highest scores compared to the samples produced with the addition of different contents of omega 3 PUFA. Higher scores of the control samples were found throughout the storage period. However, yoghurts fortified with omega-3 PUFA were also scored with percentages of maximal quality above 90% and described as an acceptable product. The sensory parameters, such as appearance, color and consistency, were very similar between the samples. A small difference in smell and taste was found between the control and experimental samples. These results were expected because it is known that the addition of fish oil may have a big influence on

**Table 1.** The sensory evaluation of stored yoghurts.

Types	Days	Appearance	Color	Texture and consistency	Odour	Taste	% of Max. quality	Score
<i>Lb. plantarum</i> 564	1	5.00	5.00	4.90±0.2236	4.80±0.4472	4.85±0.2236	97.70	4.89
	7	5.00	5.00	4.90±0.2236	5.00	4.80±0.2739	97.60	4.88
	14	5.00	5.00	4.90±0.2236	4.80±0.4472	4.80±0.4472	97.20	4.86
	21	5.00	5.00	5.00	4.90±0.2236	4.80±0.4472	97.80	4.89
<i>Lb. plantarum</i> 564+100 mg/l omega 3 PUFA	1	5.00	5.00	4.90±0.2236	4.20±0.2739	4.20±0.2739	90.00	4.50
	7	4.80±0.4472	5.00	4.80±0.4472	4.80±0.4472	4.70±0.4472	95.20	4.76
	14	4.80±0.4472	5.00	4.80±0.4472	4.70±0.4472	4.47±0.3033	92.70	4.64
	21	5.00	5.00	4.98±0.0447	4.80±0.2739	4.65±0.4183	96.02	4.80
<i>Lb. plantarum</i> 564+200 mg/l omega 3 PUFA	1	5.00	5.00	4.90±0.2236	4.40±0.2236	4.60±0.2236	94.40	4.72
	7	4.80±0.4472	5.00	4.70±0.4472	4.70±0.4472	4.60±0.4183	93.60	4.68
	14	5.00	5.00	5.00	4.60±0.2236	4.60±0.2168	95.20	4.76
	21	5.00	5.00	5.00	4.70±0.4472	4.70±0.4472	96.40	4.82

the texture and/or taste of different foods enriched with omega-3 PUFA (Jacobsen, 2008, Martin-Diana et al., 2004.). Song et al. (2011) reported that panellists scored the presence of omega-3 PUFA in ice cream with a lower acceptability, but they indicated a willingness to purchase this kind of product as a source of omega-3 PUFA.

There are several studies describing the production of yoghurt containing fish oil without fishy odor and taste. Avramis and Jacobs (2008) reported that the addition of omega-3 PUFA after fermentation of the pre-mix did not produce significant off-flavor. Moreover, off-flavor formation could be avoided by pre-mixing the omega-3 PUFA with previously pasteurized or sterilized fruit puree. Akahoshi et al. (2000) described the production of yoghurt containing refined fish oil where the fishy odor was masked with sweeteners and by using an oxygen-blocking hermetic package. They said that regarding texture, omega-3 fortification yielded yoghurts with less firmness and higher syneresis. Similar results are those of Kolanowski and Laufenberg (2007), who found that enrichment of flavored yoghurt with up to 0.3% fish oil (0.15 % EPA and DHA) resulted in a product with acceptable sensory properties. In addition, Andino (2011) demonstrated that plain yoghurt could be

fortified with microencapsulated polyunsaturated fish oil and  $\alpha$ -tocopherol without significant deterioration of quality. In this study, the fatty acid composition and oxidative stability of products were not compromised and the majority of the characteristics of yoghurts remained unaffected.

In recent years, it has become evident that changes in consumer attitudes have led to a demand for more healthy foods. Chase et al. (2009) reported that the consumption of omega-3 products depends on the consumer's age, income, education and household composition. They also found that the presence of children in a home increases the purchasing frequency of omega-3 yoghurt.

In this study, one portion (250 ml) of yoghurt fortified with different concentrations of PUFA could provide 25-50 mg of omega-3 PUFA, which is 10-20% of RDA, significantly elevating the average level in nutrition (15% RDA omega-3 PUFA for prevention of coronary heart diseases per serving is recommended by the International Society for the Study of Fatty Acids and Lipids – ISSFAL, 1999. Rise et al. (2011) presented that the consumption of 2 yoghurts/day for 6 weeks, providing 64 mg EPA and 74 mg DHA, significantly increases blood omega-3

PUFA, and provides a useful approach to reaching the recommended intakes in populations. Dawczynski and Jahreis (2009) also showed that the daily consumption of omega-3 PUFA-enriched yoghurt for 5 weeks had a favourable effect on cardiovascular risk factors.

## CONCLUSION

The potentially probiotic *Lb. plantarum* 564 may be suitable for the production of yoghurt fortified with omega-3 PUFA. The level of survival of the probiotic was high during the storage period, which is necessary to achieve therapeutical effects. There were no significant differences in starter bacteria count.

Sensory evaluation showed that all samples differ with very acceptable and good sensory properties. The control yoghurt was evaluated as the best, but the samples produced with the addition of omega 3 PUFA were acceptable as regards sensory properties, and were without significant negative flavors.

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