LACTIC ACID BACTERIA: EFFECT ON THE QUALITY AND SAFETY OF FISHERY PRODUCTS

NATAŠA PAVLIĆEVIĆ[1], VESNA ĐORĐEVIĆ[2], MIRJANA DIMITRIJEVIĆ[3], MARIJA BOŠKOVIĆ[3], RADMILA MARKOVIĆ[3], MILAN Ž. BALTIĆ[3]
[1]*Veterinary Speciality Institute "Subotica", Segedinski put 88, 24000 Subotica, Serbia*
[2]*Institute of meat hygiene and technology, Kaćanskog 13, 11000 Belgrade, Serbia*
[3]*Faculty of Veterinary Medicine, Bulevar Oslobođenja 18, 11000 Belgrade, Serbia*

MLEČNO KISLEINSKE BAKTERIJE – UTICAJ NA KVALITET I BEZBEDNOST PROIZVODA OD RIBE

Apstrakt

Prisustvo mlečno kiselinskih bakterija (MKB) u mesu ribe dugo vremena bilo je zanemarivano s obzirom na njihov nizak broj u mesu ribe, što je uslovljeno specifičnostima ribljeg mesa. Međutim, promena navika u ishrani ljudi u poslednjoj dekadi prošlog veka, kao i sve veći zahtevi potrošača po pitanju kvaliteta hrane koju konzumiraju doveli su do toga da se industrija hrane sve više razvija u pravcu proširenja asortimana proizvoda. To se naročito odnosi na povećanu proizvodnju "ready-to-eat" hrane, kao što su blago konzervisani proizvodi od ribe (BKPR). Za ove proizvode, karakteristično je da se u procesu proizvodnje dodaju određene količine sastojaka, kao što su šećer i so, što dovodi do promena u svojstvenim karakteristikama mesa ribe (npr. snižava se a_w vrednost mesa). Ovakve promene u mesu ribedovode do inhibicije rasta mikroflore koja je odgovorna za nastanak kvara mesa ribe i proizvoda od ribe, istovremeno stimulišući rast mikroflore, kao što su mlečno kiselinske bakterije. Takođe, i najčešći načini pakovanja ovih proizvoda, a to su vakuumiranje i pakovanje u modifikovanoj atmosferi, favorizuju rast mlečno kiselinskih bakterija, a inhibiraju rast mikroflore karakteristične za meso ribe. Samim tim, poslednjih godina, raste i interesovanje za ispitivanje značaja prisustva mlečno kiselinskih bakterija i njihovog efekta na kvalitet i bezbednost ovih proizvoda od ribe, pakovane na različite načine. Proces u kojem se u hranu dodaju veće količine odabranih mikroorganizamau cilju inhibicije nepoželjnih mikroorganizama naziva se biokonzervacija. MKB su upravo mikroorganizmi od izbora za ovu tehnologiju, s obzirom na širok spektar i veliki broj inhibitornih komponenti koje nastaju kao posledica njihovog metabolizma. Sa aspekta mikrobiološke bezbednosti blago konzervisanih proizvoda od ribe, najveći rizik predstavlja prisustvo Listeria monocytogenes. Za pojedine sojeve mlečno kiselinskih bakterija utvrđeno je da veoma uspešno inhibišu L. monocytogenes u ovim

proizvodima. Međutim, za iste je utvrđeno i da nemaju nikakvog uticaja na odlaganje pojave prvih znakova kvara, tj da nemaju uticaja na održivost ovih proizvoda. Takođe, primena mlečno kisleinskih bakterija u blago konzervisanim proizvodima od ribe ograničena je iz razloga što BKPR nisu fermentisani proizvodi, te bi primena MKB-a kao protektiva mogla da dovede do promene organoleptičkih svojstava i nutritive vrednosti krajnjeg proizvoda koji bi, na taj način, bio neprihvatljiv za potrošača. Podaci iz literature upućuju na to da je jednostavnije kontrolisati rast patogenih mikroorgaizma primenom pojedinih sojeva mlečno kiselinskih bakterija, tj. osigurati bezbednost proizvoda, nego poboljšati kvalitet BKPR. Stoga su brojna istraživanja usmerena kako bi se pronašli oni sojevi koji bi imali zaštitno delovanje u smislu mikrobiološke bezbednosti, zatim pozitivan uticaj na kvalitet, odnosno održivost, a da pri tome ne bi menjali organoleptičke osobine i nutritivna svojstva, od čega bi nesumnjive koristi imali kako potrošači, tako i proizvođači. Poslednjih godina sprovode se istraživanja u cilju primene mlečno kisleinskih bakterija kao probiotika za živu ribu, koja su dala pozitivne i ohrabrujuće rezultate. Nauka o primeni probiotika u akvakulturi je na samom početku i za cilj ima proizvodnju hrane za ribe koja ima pozitivan efekat na zdravlje ribe.

Ključne reči: kvalitet, bezbednost, blago konzervisani proizvodi od ribe
Ključne reči: quality, safety, light preserved fish products

Lactic acid bacteria

Lactic acid bacteria are widespread in nature and can be found in many foods (dairy products, meat, fruit, vegetables, etc.), as well as in the intestinal tract of humans and animals. People have used the lactic acid bacteria for thousands of years in natural fermentation of milk, meat, vegetables and fruit. Acidification process as a result of the creation of lactic acid is one of the most desirable effects of lactic acid bacteria growth, with the inhibition of other microorganisms, including pathogenic microorganisms to humans. The presence of lactic acid bacteria in fish meat has been neglected for a long time due to their low number caused by the specific traits of the fish meat. However, changes in eating habits in the last decade of the last century, as well as the existing and growing consumer demand, have led to developing of the food industry in order to expand the range of products, particularly relating to increased production of ready-to-eat foods, such as light preserved fish products (LPFP).

For the production process of this type of product it is characteristic to add certain amounts of ingredients, such as sugar and salt, which leads to changes in the intrinsic properties of fish (e.g., lower a_w value of meat). These changes in the fish meat lead to inhibition of growth of microflora responsible for the occurrence of the spoilage of fish and fish products, while at the same time stimulate the growth of microflora, such as lactic acid bacteria (Leroi, 2010). Therefore, with the increase in production of fish products, especially light preserved products such as cold smoked fish, there is growing interest in testing for the presence of lactic acid bacteria and for their influence on the quality and safety of these fish products.

Lactic acid bacteria in fish meat and meat products

Microflora of live fish is a reflection of the microflora of the environment from which the fish is caught. Muscles of live fish are sterile. The bacteria are found on the skin, gills and alimentary tract (Baross and Liston, 1970), whose number and type depend on numerous factors such as the type of fish, water temperature and water salinity, the amount of soluble oxygen, the degree of pollution, diet, stress, etc. A typical microflora of the skin and gills of fish is generally represented with aerobes (Simidu et al., 1969). Research shows that the microflora isolated from the gastro intestinal tract of fish mostly (50 to 90%) consists of gram-negative bacteria (*Vibrio, Enterobacteriaceae, Acinetobactrer*) (Huber et al. (2004). Though atypical, it is generally accepted that lactic acid bacteria are a normal microflora of the intestinal tract of fish (Yang, 2007).

Fish meat has such properties which stimulate the growth of microorganisms. Despite the low content of carbohydrates (0.2-1.5%), fish meat is abundant in non-protein nitrogen compounds of low molecular weight, which are rapidly metabolized by bacteria. The high *post-mortem* pH of meat (around 6), and low content of carbohydrates favour the growth of gram-negative psychotropic bacteria present in the meat of fish, which, in the first few days, can reach a large number compared to the LAB (Leroi, 2010). Careless handling of raw material during primary processing and improper and unhygienic production can cause the contamination of fish with microorganisms that can be found in fish meat products (Gudbjornsdottir et al., 2010).

Slightly preserved fish products are characterized by the fact that these products are not or only slightly heat treated, with the salt content of less than 6% in the aqueous phase, with a_w value below 0.96, and with the pH greater than 5. Different types of marinated fish, fish in brine, cold smoked fish, etc. are classified into this group of products. In the production of these products the fresh fish is most commonly used. Technological process of production of LPFP features a few extra steps, which increase the possibility of cross contamination, and the characteristics of the production process are such that, on one side, they have impact on the reduction of the number of microorganisms, but not enough to completely eliminate them (Leroi et al., 1998 ; Gonzales-Rodriguez et al., 2002). These products are perishable; they are kept in a cool regime and they are usually packed in vacuum or in a mixture of gases, in order to prolong viability. Initial microflora depends on the hygiene in the production facility, but most often dominated by gram-negative bacteria that are typical for fresh fish. Adding of NaCl at a concentration of 5,5 - 6.5% in the aqueous phaselowers a_w value to 0.96. These values of the water activity in the product have an inhibitory effect on gram-negative bacteria, but the growth of microorganisms, such as LAB, is undisturbed, and the research results show that at the end of the storage periodLAB become the dominant microflora in these fish products (Truelstrup Hansen et al., 1995). Also, smoking as a stage in the production of cold smoked fish affects the final number of LAB in these products, because with the amount of smoke of 10 mg/kg the number LAB count increases. (Leroi and Joffraud, 2000). Packaging of fish and fish products in a vacuum or gas mixturesleads to major changes in the growth, composition and number of certain microorganisms that lead to spoilage. In the process of packaging of food in a vacuum microaerophilic environment is created, and carbon dioxide accumulates partly, thus inhibiting the growth of aerobic gram-negative bacteria and providing a better shelf life of meat (Soccol and Oetterer, 2003). In such circumstances, microflora is developing and lactic acid bacteria dominate during the entire period of storage of vacuum-packed products, up to the occurence of the product spoilage (Leroi et al., 1998; Truelstrup Hansen and Huss, 1998). However, the role of LAB, in the development of the spoilage of LPFP, where they constitute the dominant flora, is not fully understood.

The predominant microflora of cold smoked fish products packed in a mixture of gases is the one that is resistant to CO_2 (Siverstvik et al., 2002). Basically, the gram-negative bacteria are much more susceptible to the action of CO_2, and are also the most inhibited microorganisms in products packed in a mixture of gases (Jay et al., 2005). Gram-positive bacteria, such as lactic acid bacteria are not susceptible/sensitive to the action of carbon dioxide, and in fish products packed in a mixture of gases they become the dominant bacteria, in addition to being the species that has less potential to cause spoilage (McMillin, 2008). In the results of our tests, it was established that the dominant microflora of cold smoked trout packed in a mixture of gases formed during storage were LAB (approximately 7 log CFU / g after six weeks of storage), while in the samples of cold smoked vacuum packed trout somewhat lower lactobacilli count was recorded. Statistically significantly higher count of lactobacilli in cold smoked trout fillets packed in a mixture of gases can be explained by the fact that carbon dioxide exerted inhibiting effect on certain groups of microorganisms, especially on *enterobacteriaceae*, which contributed to the undisturbed growth of lactobacilli and to their higher count compared to vacuum packaged trout fillets (Kilibarda Nataša, 2010).

Bio-preservation and antagonism

The interaction between different bacterial species is a well-known phenomenon. Competition for substrate and antagonism are thought to be important in the selection of microflora in a particular ecological niche (Gram, 1993). Several kinds of interactions may occur in the food at the same time, leading to the final characteristics of the finished product. Lactic acid bacteria are mentioned as one of those responsible for the growth inhibition of pathogenic microorganisms. Acidification as a result of the creation of lactic acid is one of the best known mechanisms which lead to the inhibition of the undesirable microorganisms occurring as the result of LAB activity. It is known that lactic acid in its undissociated form can induce changes in the electrochemical proton gradient in the membranes of sensitive bacterial cells (Bower and Hiatela, 2010).

In the literature are also cited other mechanisms, such as the creation of inhibitory molecules, competition for substrate which could lead to the inhibition of pathogens in food. The process during which selected micro-organisms in large numbers are added into foods, in order to inhibit undesirable microorganisms, is called biopreservation (Leroi, 2010). LABs are microorganisms of choice for this technology, given the wide range and large number of inhibitory components that occur as a result of their metabolism (organic acids, hydrogen peroxide, diacetyl and bacteriocins). However, their application in fish products has not yet been practiced, partly because LPFP are not fermented products, and the application of LAB as protectionist could lead to changes in the organoleptic properties and nutritive value. Therefore, numerous studies have focused in recent years to identify those strains that could have a protective effect and in the same time do not modify the organoleptic and nutritional properties.

Safety and LAB

The greatest microbiological risk when it comes to LPFP is the *Clostridium botulinum* type E and *Listeriamonocytogenes*. The presence of *C. botulinum* in these products can be adequately controlled by the combination of parameters of production and storage (3.5% of salt in aqueous phase and storage temperature below 5^0C) (Heinitz and Johnson, 1998). However, *L. monocytogenes* can grow at low temperatures (0 C, at a pH of 4.5 and low a_w value). Though it can be found in the raw material, the presence of L. monocytogenes in the final product often is a result of cross-contamination during the production process, and, therefore, it is almost impossible in the production of LPFP to consistently have the final product without the presence *L. monocytogenes (Rorvick et al., 1995).* The characteristics of the technological production process are not sufficient to eliminate the presence of *L. monocytogenes* in the final product (Ribeiro Neunlist et al., 2005). For this reason, the use of protective microorganisms would have very important role in preventing *L. monocytogenes* in the final products of this type. In the literature, the LAB of genus *Carnobacterium* are often mentioned, which do not have the ability to create acid (aciduric bacteria non) and thus cause spoilage, and create bacteriocins which lead to inhibition of pathogenic microorganisms, primarily *L.monocytogenes*.

Sustainability/shelf life and LAB

Another important approach is that the use of protective cultures increases shelf life, or delays signs of spoilage. In the research, it was shown that bacteria of the genus *Carnobacterium* which have been shown to have an inhibitory action on the *L.monocytogenes* have an inhibitory effect on other microflora, and therefore do not delay the appearance of signs of spoilage in LPFP (Brillet et al., 2005). These data suggest that it is easier to control the growth of pathogenic cultures using the protective microorganismsthan to improve the quality of LPFP. The reason should be sought in the fact that the occurrence of spoilage of food is a result of a complex ecosystem, which consists of a variety of bacteria whose number and types vary from product to product. Although the interest in the application of bioprotectives aimed to improve the quality and safety of LPFPhas been present in the past twenty years, it is of great importance to perform further research in determination of those strains of LAB which would not change the organoleptic properties and nutritional value of the product, and would have positive effect on the quality and safety.

LAB – as probiotics

Probiotics are live microorganisms, mainly LAB, which when entered into the body in large quantities have a positive effect on human health. The use of probiotics is usually associated with dairy products. LPFP, although they contain a significant number of LAB, have never been considered as probiotics for human use, since they are not consumed in large amounts, as is the case with dairy products. In recent years research was carried out focused on the application of LAB as probiotics for live fish, which yielded positive results. Study on the application of probiotics in aquaculture is at the very beginning, and its aim is to produce fish feed that has a positive effect on the health of fish (Azad and Ai-Marzouk, 2008).

CONCLUSION

The modern consumer is looking for high-quality food which has maintained the sensory characteristics and the nutritional value of the raw materials from which it is produced, which has retained a natural appearance, taste and aroma, and is also safe for his/her health. LPFP packed in vacuum or mixture of gases largely meet these requirements, which is why their production is increasing from year to year. In these products, the environmental conditions are such as to favour the growth of LAB, which explains the great interest of the scientific community in the last decade, in order to investigate the influence of these microorganisms on the quality and safety of LPFP. It is necessary to examine in future research if LAB in products derives from fresh fish or their presence is a result of contamination during the manufacturing process, in order to better understand the way of contamination and therefore to control the contamination. The role of LAB in fish products is complex and depends on the type of fish, the characteristics of the production process, storage conditions, and on the bacterial species and strains and interaction between different bacterial species. The most important effect of their presence in LPFP is the impact on their safety, given the inhibitory effects on pathogenic microorganisms. Also, many studies are conducted to determine the effect of LAB on product quality in terms of extending its sustainability/shelf life, and therebythe presence and activity of LAB does not influence the organoleptic properties of the product. Similarly, research on the use of LAB as probiotics for live fish gave encouraging results, which will enable faster development of the market for LAB originating from fish.

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