Lactic acid bacteria in traditional dry sausage production

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Pregledni rad

ABSTRACT

Spontaneously fermented dry sausages that are produced locally are considered to be recognized and appreciated traditional products. They are characterized by specific sensory properties and represent a source of valuable nutrients. However, a multiple choice of raw materials and additives, as well as varying production conditions affect variable microbiological and sensory quality of traditional sausages. The inherently present, indigenous population of lactic acid bacteria (LAB) plays a key role in ensuring the health and safety of such products. Indigenous populations of LAB that are selected and applied as starter cultures could not only reduce risks caused by microbial contamination during slaughtering or hunting and during the processing of meat, but could also favourably affect the organoleptic properties of the end product. The aim of this paper was therefore to demonstrate the specificity of lactic acid bacteria and the possibility of their application in the fermentation of traditional dry sausages that are produced from the meat of domestic or wild animals.

Key words: *lactic acid bacteria, fermented sausages, domestic or wild animals*

INTRODUCTION

Dry fermented sausages produced from the meat of domestic or wild animals hold an important place in Croatian tradition, where they are not only characterized by distinctive aroma and flavour, but by extended shelf life as well. Such sausages are mainly produced by local artisans that follow traditional recipes, what today, when we witness a trend of increased demand for food that is labelled authentic or traditional, further increases their value (Trichopoulou et al., 2007). A positive attitude of customers towards traditional products is the result of considering traditional food a part of local or regional cultural heritage, and considering hunting a recreational activity that forms a part of personal identity. Traditional fermented sausages are produced from different types of meat, predominately pork. However, the production of sausages from the meat of wild game, such as boar and deer, is gaining in popularity. Different sources of raw materials, seasonality and varying production conditions, as well as a multiple choice of additives often result in products with questionable sensory (organoleptic) and microbiological properties, and highly varied content and quality (Kovačević et al., 2009; Kos et al., 2015). Since spontaneously fermented traditional sausages are produced without adding starter cultures, their organoleptic properties and microbiological stability depend on the metabolic activity of the naturally occurring microbiota, mainly lactic acid bacteria (LAB) and coagulase-negative cocci, as well as meat enzymes. Lactic acid bacteria are a claid of heterogeneous Gram-positive, coagulase-negative bacteria, with species within the genera *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Enterococcus* and *Streptococcus* being prevalent during the ripening of traditional sausages (Lund and Baird - Parker, 2000). LAB are widespread in nature and can often be isolated from human and animal saliva and faeces that could along with other environmental microorganisms and microorganisms on the equipment contaminate meat mixture during the production of sausages. To increase recognizability, encourage competitiveness and ensure microbiological safety of spontaneously fermented sausages, it is necessary to control the entire production process. Strains of lactic acid bacteria (LAB) that are selected and applied as starter cultures do not only ensure both microbiologi-

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cal quality and stability of the end product in modern production, but often exert a positive effect on the sensory properties of the finished product (Cenci – Goga et al., 2012; Frece et al., 2014). The aim of this paper was therefore to demonstrate the specific role of LAB in such sausages, as well as the possibility of using LAB as a starter, bioprotective or probiotic culture, all based on a detailed review of available literature.

Primary microbiota of animal carcasses

Primary carcass microbiota depends on a number of factors, such as the type of microorganisms on the skin of animal or in gastrointestinal tract and muscle tissue, as well as the conditions present at slaughter or kill and the manner of further meat processing (Gill, 2007; Kegalj et al., 2012). Contaminating microbiota includes technologically significant microorganisms, as well as spoilage microorganisms and pathogenic bacteria. When meat is not immediately consumed or in some way preserved, due contamination is closely followed by a decrease in quality and represents danger to human health. Raw meat macrobiota mainly consists of Gram-negative, mostly psychrotrophic, bacteria. Predominant genera include *Pseudomonas, Acinetobacter* and *Moraxella*, as well as *Brochothrixthermosphacta*, *enterobacteria*, *staphylococci, micrococci*, spore-forming bacteria, LAB and yeasts (Lawrie, 1998). The research by Deutz et al. (2006) on the impact of manner in which wild game is killed on carcass microbiota showed that animals that were shot in the chest had a total aerobic bacteria count of 4.6 CFU/cm^2, while animals that were shot in the abdomen had a total aerobic bacteria count of 5.6 CFU/cm^2. In a similar study, Avagnina et al. (2012) point out that, in comparison with animals shot in other body parts, animals that were shot in the abdomen had higher aerobic bacteria count.

Microbiological criteria for foodstuffs are defined within Commission Regulation (EC) No. 2073/2005 (EC 2005) and refer only to the hygienic criteria for processing domestic animal carcasses, i.e. standards for processing game meat are not included. In recent years research with the aim of defining the limits of acceptability related to the microbial diversity and quantity of certain species in wild game meat was conducted. Paulsen (2011) suggests that microbiological standards of game meat should be similar to those that apply to domestic animals, that is, the total aerobic bacteria count should not exceed 6 log CFU/cm^2, and the total E. coli count should not exceed 2 log CFU/cm^2.

The importance of lactic acid bacteria in traditional dry sausage production

The LAB count at the beginning of sausage fermentation usually amounts to 3.2 to 5.3 log CFU/g (Drosinos et al., 2005; Fontana et al., 2005; Comi et al., 2005), but in the first days of fermentation increases to 7 - 9 log CFU/g (Comi et al., 2005) and remains constant during ripening (Cocolin et al., 2001). Within a claid of LAB in sausages, the most common is *Lactobacillus*. Various studies have shown that the most frequently isolated species include *Lactobacillus sakei, Lactobacillus plantarum* and *Lactobacillus curvatus* (Andrighetto et al., 2001; Fontana et al., 2005;Rantsiou et al., 2005; Kozačinski et al., 2008; Lebert et al., 2007). The research conducted by Papamanoli et al. (2003) showed that 90% of LAB isolates that were isolated from naturally fermented dry sausages belonged to the genera Lactobacillus.

In the production of sausages, the main role of LAB is the acidification of mixture realized through the production of organic acids (mainly lactic acid) that occurs in order to inhibit the growth and production of toxins by undesirable microorganisms. Because due to decrease in pH value caused by the growth of LAB population in the first phase of sausage production various chemical, physical and microbiological reactions take place, the acidification plays a central role not only in the control of undesirable microorganisms, but also in the development of flavour, colour and texture of sausages during fermentation.

Water activity (a_w), i.e. water that can be used for metabolic pathways of present microorganisms, and pH value are considered limiting factors for the growth of microorganisms. Fresh meat has a pH value favourable for the growth of microorganisms (pH 5.6 - 6.0) and a relatively high water activity (0.98 - 0.99), while fermented dry sausages are characterized by a pH value of between 5.2 and 5.8, and a water activity value of between 0.85 - 0.91 at the end of ripening period (Vignolo et al., 2010). Since LAB, *staphylococci* and *micrococci* are not sensitive to low pH values, after a week of fermentation they represent the predominant bacterial flora.

The growth and survival of pathogens, such as *Salmonella*, *Yersiniaenterocolitica* and *Campylobacter* is in fermented meat products largely suppressed due to the combination of low pH value and produced lactic acid, as well as the low aw value. *Listeria monocytogenes* has a slightly higher survival rate. However, in dry sausages it usually disappears during the third week of ripening (Työppönen et al., 2003). The production of neurotoxins and the growth of *Clostridium botulinum* can be controlled by adding nitrites and maintaining low pH and a_w values (Peck and Stringer, 2005). *Staphylococcus aureus* and *Escherichia coli* pathogens represent species that are most resistant to conditions during the fermentation and ripening of sausages, what at the same time represents the greatest potential risk. However, such risk in fermented meat products is not very high (Erkkilä et al., 2000).

According to the conclusions of the American Food and Drug Administration Panel of Experts on food security and stability (USFDA, 2001), the pH value of 4.6 inhibits the growth of spore-forming pathogens, whereas such value for the vegetative pathogens is lower and amounts to 4.2. The growth of most pathogenic bacteria is limited when water activity value amounts to 0.86 (USFDA, 2001). Sole exception is Staphylococcus aureus, which grows when water activity value reaches 0.83 and can produce toxins when water activity value amounts to 0.88 (NSW Food Authority, 2008).

In addition to the decrease of pH values, LAB contribute to the microbiological safety and the quality of meat products' sensory properties by synthesising antimicrobial compounds and compounds that are responsible for the flavour of finished product (Lücke, 2000; Talon et al., 2002). Hydrogen peroxide, which is alongside lactic acid produced by LAB, can also suppress the growth of other bacteria. Many LAB produce bacteriocins, i.e. proteins or peptides with antimicrobial effect against different bacterial strains (Cleveland et al., 2001). Compounds that were until now approved for commercial use in food production include nisin which is a by-product of *Lactococcus lactis* metabolism and which has not only been in use for many years but is also a licensed food additive in more than 45 countries (Settanni and Corsetti, 2008; Gupta et al., 2015), and bacteriocins (CclA, CbnBM1 and PisA) that are a by-product of *Carnobacterium maltaromaticum* UAL307 metabolism, namely *L. monocytogenes* inhibitors that are approved for use in processed meat in both US and Canada (Martin - Visscher, et al., 2011). The use of nisin in meat is not considered effective because the pH value of meat is greater than the pH value which is considered optimal for enzyme activity (Rayman et al., 1983) and because it interferes with meat compounds including phospholipids (De Vuyst and Vandamme, 1994) and glutathione (Rose et al. , 1999). An intensive work on finding new bacteriocins obtained from LAB, their purification, detailed characterization and possibilities of use in biopreservation of different foods and feedstuffs is currently under way (Parada et al., 2007; Nespolo and Brandelli, 2010). Unlike farming dependant methods, the development of modern methods such as the sequencing of new generations and metagenomic approaches for studying of microbial communities during fermentation have facilitated the efficient identification of strains that produce bacteriocins (O'Connor et al., 2015). In addition to LAB, coagulase-negative staphylococci (CNC) and bacteria of the genera Kocuria also participate in the ripening of fermented sausages and their importance is evident not only in their heightened activity in the reduction of nitrites and nitrates that leads to the development and stabilization of desirable red colour (Liepe, 1983), the decomposition of peroxide (Samelis et al., 1998), the limitation of lipid oxidation and the prevention of rancidity, but they also contribute to the development of the flavour caused by proteolysis and lipolysis in which esters and other aromatic compounds are formed (Cai et al., 1999).

It is well known that lipolysis holds a central role in the development of flavour. The release of free fatty acids in the breakdown of lipids is primarily caused by lipase found in meat (Kenneally et al., 1998; Galgano et al., 2003) and, to a lesser extent, by enzymatic activity of indigenous microflora. Alkenes, alkanes, alcohols, aldehydes, ketones and furans that represent the compounds responsible for the characteristic aroma and flavour of sausages are formed during the oxidative degradation of released fatty acids (Viallon et al., 1996; Chizzolini et al., 1998).. Not only do lactic acid bacteria display a low lipolytic activity, but previous research has shown that staphylococci are the most important for the breakdown of fat in sausages (Montel et al., 1998). The metabolism of meat proteins also plays an important role in the development of flavours. The content of meat proteins can vary depending on the type of animal and the feed it consumes. During the production of fermented sausages, meat protease and microbial enzymes cause the breakdown of meat proteins and the formation of small peptides and free amino acids that are considered precursors of due aromatic compounds (Aristoy and Toldrá, 1995; Sanz and Toldrá, 1997; Sanz et al., 1998). Meat protease, the cathepsin D-type enzyme in particular, is meanwhile responsible for the proteolysis and the formation of peptides (Hierro et al., 1999; Molly et al., 1997), whereas microbial enzymes hold greater significance in affecting oligopeptides that are formed during the later stages of ripening (Hughes et al., 2002). Although only to a small degree and depending on the strain, microbial proteolytic activity contributes to the initial breakdown of proteins (Kenneally et al., 1999; Molly et al., 1997; Sanz et al., 1999; Fadda et al., 2002). In fermented meat products proteolysis is largely attributed to bacteria within a claid of LAB, from which the mentioned proteolyticly important species include *Lactobacillus plantarum, Pediococcus pentosaceus* and *Lactobacillus acidophilus* (Toledano et al., 2011).

The potential of using LAB strains in the production of traditional dry sausages

According to Hammes et al. (1990) meat starter cultures are preparations that contain active or dormant microorganisms that develop the desired metabolic activity in meat and cause changes in the sensory properties of food. Most commercially available starter cultures

are mixtures of LAB, specifically the genera *Lactobacillus* and *Pediococcus* (Demeyer and Toldrá, 2004), and staphylococci and/or micrococci. Ammor and Mayo (2007) in their scientific review propose new criteria for the selection of LAB strains used as starter cultures in the production of dry sausages. Heterofermentative LAB are not suitable to be used as starters because they produce large amounts of CO_2 that may result in undesirable texture, and the concentration of acetic acid which may result in disruption of the organoleptic properties. Besides the homofermentative pathway of carbohydrate breakdown, the ability to quickly produce lactic acid in quantity sufficient to decrease the pH value to < 5.1, as well as the competitiveness of strain and natural raw meat microbiota, i.e. the capability to survive and maintain metabolic activity in anaerobic atmosphere, environment with high salt content (2 - 10%), low temperature (2 - 24 °C) and low pH value (4.2 - 6) are essential. The selected strain must have a good catalase activity, proteolytic and lipolytic activity, must reduce nitrates and nitrites, and demonstrate tolerance and synergy with other microbial components of due starter. It should not be pathogenic, nor exhibit toxic activity, to avoid the accumulation of biogenic amines, no amino decarboxylase activity should be present and in order to avoid horizontal gene transfer it should not contain genes responsible for antibiotic resistance.

Microbial diversity of traditional products represents the preferred source of wild strains that are prevalent in spontaneous fermentation and that, in comparison with commercial starters, have a higher metabolic activity, that can by forming or enhancing the aroma or by increasing the safety of food, contribute to the quality of products (Leroy et al., 2006). In their study, Frece et al. (2014) have compared the effects of indigenous strains isolated from traditional sausages and the effects of commercial starters on the quality of industrial sausages. Results of this research have not only shown that indigenous strains have the ability to survive even during industrial production, but, compared with commercial strains, they have also displayed better results in the development of sensory properties, stability and microbiological safety of sausages. In addition, bearing in mind that commercial starters are not equally efficient for all types of sausages, there is a need to select the appropriate strains that can maintain the necessary quantity and metabolic activity in certain mixture of meat and for certain method of fermentation. Strains isolated from the microbiota of fermented traditional sausages have therefore been proven to be most promising (Dalla Santa et al., 2014; Palavecino Prpich et al., 2015). However, due to considerable technological potential of their application, different approaches to the selection of appropriate strains are being considered. For example, Cenci - Goga et al. (2012) have shown that the application of selected lactococci and lactobacilli strains that were isolated from traditional cheeses and applied in the production of game meat sausages have led to the inhibition of growth of potential pathogens and improved sensory properties. In a similar study, Mrkonjić Fuka et al. (2015) have determined the technological characteristics of LAB strains that were isolated from Croatian traditional cheeses before they were applied as microbial cultures in the production of game meat sausages. The authors of this study point out that the aforementioned LAB strains have both proteolytic and antimicrobial potential, as well as the capability of acidification, that represents a prerequisite of their selection and application in a variety of fermented products. In addition to being used as starter cultures and due to their positive impact on health in terms of improving the microbial balance of the digestive tract of the host, various LAB strains, including lactobacilli in particular, have great potential to be used as probiotics. Even though until recently probiotics were primarily associated with dairy products, it has been shown that the sausage matrix represents a suitable medium for the transmission of probiotic strains (Klingberg and Budde, 2006; Rubio et al., 2014). Many studies have therefore focused on testing the survival skills of selected probiotic strains during fermentation, their functional characteristics and safety aspects (Ruiz - Moyano et al., 2010; Nogueira Ruiz et al., 2014). For example, Frece et al. (2010) have on an *in vivo* study conducted on mice shown that the strain *Lactobacillus plantarum* 1K isolated from Slavonian kulen could be used as a probiotic strain used to establish the disturbed balance of intestinal microflora. In addition, since these results have also demonstrated the reduction of opportunistic pathogen growth caused by the antimicrobial activity, the said strain also has the potential to be used as starter culture. Various other possibilities of producing high-quality, nutritionally balanced food are being investigated intensively. For example, Totosaus (2011) points out that the addition of vegetable fats and oils to cooked sausages represents a good way of increasing their nutritional value; Méndez - Zamora et al. (2015) have demonstrated the possibility of producing Frankfurter sausages by replacing the fat with fiber (inulin and pectin); Żochowski - Kujawska et al. (2013) have shown that the addition of vegetable juices containing proteolytic enzymes causes the softening of otherwise hard wild boar meat that is used in the production of dry sausages. This line of research highlights the need for the production of high-quality food by applying the latest scientific knowledge, wherein the use of LAB strains, in-

dependently or in combination with other (bio)technological solutions, shows great potential.

CONCLUSION

The modern requirements for high levels of food safety and quality, demand the complete control of production process. To this end, the focus of scientific interest was to explore the potential application of selected strains of lactic acid bacteria in the production of traditional sausages. Previous studies have demonstrated promising results, in the form of preventing the proliferation of potential pathogens and the improvement of organoleptic properties.

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