Table 5. Class means and standard deviations (in brackets) of the meat quality traits used in discriminant analyses

	Discriminant analysis 1				Discriminant analysis 2			
	pH_{45}	pH_{24}	CIE-L*	W.H.C	pH_{45}	pH_{24}	CIE-L*	W.H.C
Exudative meat	6.06 (0.291)	5.59[a] (0.140)	46.76 (4.219)	8.65[a] (1.261)	6.01[a] (0.288)	5.59 (0.154)	46.91 (4.293)	8.85[a] (1.138)
Non-exudative meat	6.12 (0.261)	5.67[b] (0.198)	46.82 (5.465)	8.01[b] (1.515)	6.13[b] (0.265)	5.65 (0.182)	46.74 (5.143)	8.19[b] (1.485)

Numbers in columns with different exponents are statistically different ($p<0.05$)

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BACTERIOCINOGENIC STARTER CULTURES VS. *LISTERIA MONOCYTOGENES*

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SUMMARY

In this paper the use of bacteriocinogenic lactic acid bacteria and bacteriocins in food is presented, in particular regarding their antilisterial activity. Results of most studies are affirmative to the standardisation of the use of bacteriocinogenic starter cultures, since they enhance

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the safety of products due to more intensive reduction of pathogen. The majority of data on positive influence of bacteriocinogenic starters are related to studies performed on fermented sausages, but antilisterial effects has been also described in raw vacuum packaged poultry meat, cooked cured meat products and fish.

Key words: *Listeria monocytogenes, bacteriocinogenic starter cultures*

INTRODUCTION

The addition of lactic acid bacteria cultures to different types of food either slows down or prevents the growth of spoilage bacteria (hetero-fermentative lactobacilli, clostridiae, *Leuconostoc* spp.) and pathogenic microorganisms. This action is result of more intensive acidification and synthesis of antimicrobial products such as organic acids (lactic), CO_2, hydrogen peroxide or bacteriocins (Zdolec et al., 2005a). In recent years bacteriocins have been frequently mentioned as potential substitutes for some chemical preservatives in the production of the so-called minimally processed foods for which there is an increasing demand on the market. Today, the researches of bacteriocins are being directed towards both the molecular bases of their synthesis and optimisation of extraction and stabilisation procedures. It is well known that the activity of bacteriocins in food depends on numerous factors: type of food (solid, liquid etc.), food composition, presence of inhibitors (enzymes), technological conditions of food production etc. Use of bacteriocins as peculiar preservatives in food production has still been restricted to bacteriocin nisin (E234) in dairy industry. Bacteriocins are applied indirectly in meat products, i.e. by introduction of starter cultures that synthesise bacteriocins (Zdolec et al., 2007a). Their inhibitory action on individual pathogenic microorganisms in meat products has been confirmed, primarily on the bacterium *Lis-*

Table 1. Occurrence of foodborne listeriosis in Europe in the period 1990-2002 (De Valk et al., 2005)

Year	Country	Number of cases	Transmission	Incriminated food
1992	France	279	Foodborne	Pork tongue in gel
1992	Spain	24	Foodborne	Unknown
1992	Norway	6	Foodborne	Cold roast-meat
1993	France	38	Foodborne	Rillettes (pork)
1993	Italy	18 (gastroenteritis)	Foodborne	Salad with rice
1994-95	Sweden	9	Foodborne	Fish salad
1995	France	36	Foodborne	Cheeses (raw cow's milk)
1995	Island	5	Unknown	Unknown
1996	Denmark	3 (gastroenteritis)	Unknown	Unknown
1997	France	14	Foodborne	Cheeses (raw cow's milk)
1997	Finland	5	Foodborne	Cold-smoked Californian trout
1997	Italy	1566 (gastroenteritis)	Foodborne	Salad with maize
1998-99	Finland	25	Foodborne	Butter
1999	England & Wales	2	Foodborne	Cheese/salad/ sandwich
1999	France	3	Foodborne	Cheeses (raw cow's milk)
1999	France	10	Foodborne	Paste
1999-2000	Finland	10	Foodborne	Vacuum-packed fish products
2000	France	32	Foodborne	Pork tongue in gel
2000	Portugal	1	Foodborne	Cheese
2000	Spain	15	Foodborne	Unknown
2001	Belgium	1+2 (gastroenteritis)	Foodborne	Ice-cream
2002	France	11	Foodborne	Sausage paste

teria monocytogenes (Foegeding et al., 1992; De Martinis and Franco, 1998; Nissen and Holck, 1998; Lahti et al., 2001; Liserre et al., 2002; Hugas et al., 2002; Työppönen et al., 2003; Hadžiosmanović et al., 2005; Zdolec et al., 2006; Zdolec et al., 2007b).

STARTER CULTURES AND *LISTERIA MONOCYTOGENES*

In addition to known, natural limiting factors for growth of pathogenic microorganisms in some food products (law pH and water activity, nitrites, NaCl etc.), the bio-conservation procedure is of significance in total protective activity (Leistner and Gorris, 1995). In that sense, the utmost attention is paid to the use of bacteriocinogenic lactic acid bacteria in fermented food products because of their antagonistic action on the bacterium *L. monocytogenes*.

L. monocytogenes is a ubiquitous bacterium, permanently present in the environment, which has been isolated from a large number of various foods and production facilities (Živković et al., 1998; Kozačinski and Hadžiosmanović, 2001; Thévenot et al., 2005a, Colak et al., 2007). According to the report of De Valk et al. (2005), the number of foodborne listeriosis in Europe is not negligible (Table 1).

In spite of scarce literature data on this disease related to the consumption of fermented meat products, the resistance of disease-causing microorganisms to low pH, low water activity or higher salt concentrations should not be neglected. However, as reported by Thévenot et al. (2005a), in case of contamination of fermented sausages, the population of *L. monocytogenes* is usually reduced during ripening, primarily due to drying process. In our studies of traditionally fermented sausages, manufactured without the addition of starter cultures, the reduction of *L. monocytogenes* count below the limit of detection was also recorded till the end of ripening period (day 28) (Zdolec et al., 2005b; 2007c). Furthermore, the importance of the origin of *L. monocytogenes*, i.e. its adaptability to substrate that significantly influences the growth in the filling of fermented sausages was pointed out (Thévenot et al., 2005b). According to these authors, the level of contamination with the bacterium *L. monocytogenes* in the course of manufacturing process depends on the strain used ($P<0.001$), and it is the result of drying and not of fermentation process. The strains of *L. monocytogenes*, well adapted to substrate (isolated from sausages), showed a higher level of resistance (reduction 1.5 $\log_{10}$) compared to non-adapted strains (reduction 3 $\log_{10}$) till day 35 of ripening period. According to Hugas et al. (1997), the efficiency of bacteriocinogenic starter cultures in the control of *L. monocytogenes* largely depends on substrate in which they are used and on the establishment of optimum conditions in which they can exhibit their optimum activity.

It is incontestable that the level of microbial contamination in sausage fillings is the result of implementation of good hygienic practice in the course of slaughter of animals, slaughtering processing, cutting, filling preparation (cutting in small pieces, curing), in other words of the entire technological process of production. In addition, the conditions of ripening (micro- and macroclimate) and the use of starter cultures significantly influence the survival and growth of bacteria of the genus *Listeria* in fermented sausages. As reported by Encinas et al. (1999), *Listeria* species in «chorizo» sausages were not detected in samples collected from an industrial facility and produced with the addition of starter cultures, sorbates and ripening under controlled conditions at higher temperatures. On the contrary, in sausages produced in an artisan facility without the addition of starter cultures and under controlled conditions at lower temperatures, the count of *Listeria* spp. was about 3.5 log CFU/g, 1.17 log CFU/g respectively in sausages produced without starter cultures under macroclimate conditions. Monitoring of the production during 32 days in the first case (without starter, controlled conditions) revealed inhibited growth of *Listeria* spp., with their number in the finished product reduced by 0.5 log, whilst in sausages produced without starter cultures under external climate conditions their number exceeded the initial number.

As reported by Foegeding et al. (1992), the use of bacteriocinogenic starter culture *Pediococcus acidilactici* caused a 10-fold reduction in the population of *L. monocytogenes* during fermentation of sausages (smoking sections, 48 hours at 38°C) in comparison with sausages inoculated with non-bacteriocinogenic strain of the same culture. Survival of *L. monocytogenes* in the filling was conditioned also by pH value of filling. Thus, it was found that at pH < 4.9 *L. monocytogenes* was completely eliminated from the filling during ripening. However, also in sausages with a higher pH value there was a higher elimination of *L. monocytogenes* during ripening in the presence of bacteriocinogenic strain *Pc. Acidilactici*, what was attributed to direct activity of in situ-produced pediocin.

Lahti and colleagues (2001) have used bacteriocinogenic starter cultures *Pc. acidilactici PA-2* and *Lb. bavaricus MI-401* in combination with *Staphylococcus xylosus DD-34* and non-bacteriocinogenic starter culture *Lb. curvatus Lb3* with *Staphylococcus carnosus MIII* in fermented sausages inoculated with different concentrations of *L. monocytogenes* (5.05-5.41; 2.92-3.35 log CFU/g). In sausages with a lower level of contamination *L. monocytogenes* was not found in the finished product (49 days of ripen-

ing). On the other hand, the finding of *L. monocytogenes* at the end of ripening period was attributed to its higher initial count. Use of bacteriocinogenic cultures, however, resulted in evidently higher reduction of pathogens in the filling (< 2 log CFU/g after 21 days of ripening).

Työppönen and colleagues (2003) have compared the efficiency of commercial starter culture *Pc. pentosaceus* RM2000 and probiotic protective cultures *Lb. rhamnosus* E-97800, *Lb. rhamnosus* LC-705 and *Lb. plantarum* ALCO1 in the inhibition of *L. monocytogenes* in North European type dry sausages. In samples with pH 5.0-5.2, *L. monocytogenes* was eliminated from the filling till day 21 of ripening (of 28), irrespectively of the type of used starter culture. Lower pH values (4.7 – 4.9), however, led to more rapid elimination of pathogens in case of addition of protective cultures (day 7) than in case of sausages produced with commercial starter culture *Pc. pentosaceus* RM2000 (day 28). Differences in the level of inhibition of pathogens were attributed to the impact of numerous factors, including pH value, on the activity of antimicrobial substances (bacteriocins and other inhibitors of low molecular weight) in the filling.

Dicks and colleagues (2004) have investigated the antilisterial activity of bacteriocinogenic strains *Lb. plantarum* 423 and *Lb. curvatus* DF126 in ostrich meat salami. A rapid drop in the number of *L. monocytogenes* was recorded within 9 days of ripening (from the initial 7 log CFU/g to 4.7 log CFU/g), but the initial value (7 log CFU/g) was reached till the end of ripening period (day 22). The authors concluded that such «recovery» of Listeria was due to the loss of activity of bacteriocins and development of resistance to the bacteriocins used.

Benkerroum and colleagues (2003) have investigated the effect of bacteriocinogenic and non-bacteriocinogenic strain *Lc. lactis* subsp. *lactis* M on *L. monocytogenes* ATCC 7644 in fermented «marguez» sausages. The addition of either of types of strain resulted in reduced number of *L. monocytogenes* in the course of fermentation (24 hours at 30°C). However, the bacterium multiplication went on unobstructed in control sausage produced without the addition of *Lc. lactis* subsp. *lactis* M. The addition of bacteriocinogenic culture reduced the population of *L. monocytogenes* by 2.7 log, by 1.6 log respectively, in case of use of non-bacteriocinogenic culture. Aim of the next study performed by Benkerroum et al. (2005) was monitoring of growth of *L. monocytogenes* in dry-fermented sausages. The sausages were produced either without the addition of starter cultures or with the use of commercial starter culture (*S. xylosus* SL-25, *S. carnosus* LS-25, non-bacteriocinogenic *Lb. curvatus* LS-25) and bacteriocinogenic strains *Lc. lactis* subsp. *lactis* LMG21206 and *Lb. curvatus* LBPE. Increase in the initial number of pathogens (2-3 log CFU/g) was not recorded during ripening period (22 days) in any group of tested sausages. In both the control sausages (without starter culture) and sausages with added non-bacteriocinogenic starter culture there was no significant reduction in Listeria count till day 19 of ripening. On the other hand, the addition of *Lb. curvatus* LBPE reduced the number of *L. monocytogenes* below the limit of detection after only 4 hours of fermentation, and isolation was impossible after 8 days even with enrichment method. *L. monocytogenes* was eliminated from the filling after 15 days of ripening in sausages produced with *Lc. lactis* subsp. *lactis* LMG21206. Consequently, the authors recommended the use of the above-mentioned bacteriocinogenic starter cultures in fermented sausages for control of *L. monocytogenes*.

Several studies on the effect of *Lb. sakei* I151, I154 and I155 on the population of *L. monocytogenes* have recently been performed. Different types of dry-fermented sausages in Central European and Mediterranean countries were analysed in the course of ripening - Hungarian salami, Bosnian sucuk, Sremska sausage and traditionally fermented sausages from NW Croatia (Čaklovica et al., 2006; Gasparik-Reichardt et al., 2006; Drosinos et al., 2006; Hadžiosmanović et al., 2006; Zdolec et al., 2007b). Results of the studies showed a significant contribution of protective cultures in the reduction of *L. monocytogenes* in the fillings during ripening. The addition of those cultures to Hungarian salami reduced the population of *L. monocytogenes* by about 2 log during 28-day-ripening period (from the initial 5.5 to 3.5 log CFU/g), and in the control sausage by about 1.5 log. In Bosnian sucuk, *L. monocytogenes* was completely eliminated from the filling during ripening (28 days) with the use of protective strains of *Lb. sakei* (reduction by about 5 log), what was not the case with control sausage (reduction from initial 4.85 to 3.83 log CFU/g). Similar results were recorded with Sremska sausage in which *L. monocytogenes* was not isolated from the finished product (day 28) that had been produced with the addition of protective strains (reduction of 4.0 – 4.5 log). On the other hand, this pathogen survived in the control sausage (< 2 log CFU/g). In our study the population of *L. monocytogenes* was reduced below the limit of detection already after 14 days with the use of strain *Lb. sakei* I155, day 28 of ripening respectively, in case of control samples and with the use of strains I151 and I154.

Individual species and strains of enterococci also manifest inhibitory activity against *L. monocytogenes owing to* bacteriocin synthesis. Use of enterocin CCM 4231 in fermented Hornad salami resulted in more intensive elimination of *L. monocytogenes* compared to control samples (Laukova et al., 1999). After 7 days of ripening, the population of *L. monocytogenes* in control sample amounted

to 10^7 CFU/g and in salami produced with enterocin it was only 10^4 CFU/g. Difference between test and control samples remained at the same level during the next 14 days. Use of enterocin AS-48 in the study performed by Ananou et al. (2005) showed dependence of the level of inhibition of *L. monocytogenes* on the initial concentration of enterocin.

There have been attempts to make good use of the activity of bacteriocins of lactic acid bacteria against *L. monocytogenes* also in the production of other meat products. Thus, sakacin P and sakacin P-producing *Lactobacillus sakei* were used in chicken cold cuts (Katla et al., 2002). After the inoculation of semi-purified bacteriocin and protective cultures, the products were vacuum-packed and stored at 4 and 10°C for 4 weeks. *Lb. sakei* induced the synthesis of bacteriocin under those conditions of storage and the added sakacin P was stable both at higher and lower concentrations. Bacteriostatic effect during 4 weeks of storage was recorded with the addition of 3.5 μg g^{-1} of sakacin. The addition of 12 μg g^{-1} caused the initial growth of *L. monocytogenes*, but of low intensity. After 4 weeks of storage, the number of *L. monocytogenes* in samples with lower concentration of sakacin P was by 2 log lower than in control samples (without sakacin). Bacteriocin was detected in samples also after 6 weeks of storage when a higher concentration of sakacin P was used. In the study performed by Mataragas et al. (2003) *Ln. mesenteroides* and *Lb. curvatus* L442 were used as potential protective cultures in the control of *L. monocytogenes* in cooked shoulder meat. Population of *L. monocytogenes* was reduced by about 1.5 log, and the addition of bacteriocin reduced it below the limit of detection (<100 CFU/g). Bacteriocinogenic strains of *Lb. sakei* and their purified bacteriocins (sakacin) efficaciously inhibited the growth of *L. monocytogenes* also in cooked ham (Bredholt et al., 1999; 2001) and fish (Katla et al., 2001).

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ZUSAMMENFASSUNG

BAKTERIOCINOGENE STARTER KULTUREN VS. LISTERIA MONOCYTOGENES

In dieser Arbeit sind Literaturangaben dargestellt, die sich auf die Anwendung der bakteriocinogenen Bakterien der Milchsäure und der Bakteriocine zwecks Verminderung der Bakterienzahl Listeria monocytogenes in Nahrung beziehen. Die Resultate der meisten Forschungen sind gegenüber Standardisierung der Anwendung von bakteriocinogenen Kulturen affirmativ, da sie die Sicherheit des Erzeugnisses durch die intensivere Reduktion von Pathogenen erhöhen. Die meisten Angaben über den positiven Einfluss von bakteriocinogenen Starter Kulturen bezieht sich auf die Untersuchung von fermentierten Würstchen, jedoch ist der antilisterialische Effekt beobachtet auch in frisch vakuumiertem Geflügelfleisch, gekochten gepökelten Fleischerzeugnissen und Fisch.

Schlüsselwörter: *Schweinefleisch, die Eigenschaften der Fleischqualität, Indikatoren, Diskriminantgruppen*

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