Lactic acid bacteria and their influence on the shelf life of heat-treated meat products

Josef Kameník[1], Marta Dušková[1], Ondrej Šedo[2], Zbyněk Zdráhal[2], Ines Lačanin[1]

[1]Faculty of Veterinary Hygiene and Ecology
University of Veterinary and Pharmaceutical Sciences Brno
[2]Faculty of Science
Masaryk University
Brno, Czech Republic

Abstract

The aim of the study was to analyse lactic acid bacteria (LAB) in frankfurters with a finely comminuted batter after their packaging in a modified atmosphere (N_2/CO_2: 70/30) and subsequent storage for 21 days at 2 °C. Microbiological tests of the production plant environment for the presence of LAB and potential product contamination were also performed. Species determination of isolated bacterial strains was performed by the MALDI-TOF MS method. LAB could not be isolated either from the surface or from the product immediately after packaging. The growth of LAB did, however, occur during two weeks of storage. After 21 days the population of LAB exceeded values of 10^6 colony-forming units (CFU) cm^{-2} on the surface of the product and 5 x 10^5 CFU·g^{-1} in the product. The species *Leuconostoc carnosum, Lactococcus lactis* and *Lactobacillus curvatus* were isolated from the product surface, and the species *Lactococcus garviae, Leuconostoc carnosum* and *Pediococcus pentosaceus* from frankfurters after three weeks. The results of swabs from the environment indicated a source of product contamination by LAB. Eleven species of LAB were isolated from the working environment.

Lactobacillus spp., Lactococcus spp., Leuconostoc spp., frankfurters, MALDI-TOF MS

Introduction

The ability of bacteria to adapt to the conditions of the external environment is a key factor that decides on their survival and their chances of further growth. Psychrotrophic lactic acid bacteria are well adapted to the meat product environment that is characterised by microaerophilic conditions, refrigeration temperatures and a low a_w value (< 0.98) and by the presence of sodium chloride and nitrite (Samelis et al. 2000). Certain species of the genus *Lactobacillus*, such as *L. sakei* and *L. curvatus*, make up the dominant microflora in dry fermented sausages (Bonomo et al. 2008; Cocolin et al. 2009). They play an unambiguously positive role in these products by releasing lactic acid, active aromatic substances and compounds with antimicrobial properties.

There is, on the other hand, a large group of heat-treated meat products in which the presence of microorganisms and their metabolic activity is undesirable as they cause spoilage and limit the shelf life of the meat products. Psychrotrophic LAB also make a crucial contribution to these negative effects (Slongo et al. 2009; Audenaert et al. 2010). Their presence is often associated with secondary contamination during packaging or slicing as they cannot survive heat treatment (Franz and Von Holy 1996). There are, however, also thermo-resistant species that are not destroyed by high temperatures (70 °C as a rule) and that are capable of continued multiplication under suitable conditions (Milbourne 1983; Comi and Iacumin 2012). The microbial population of frankfurters is represented most frequently by the LAB species *Lactobacillus sakei*, *Lactobacillus curvatus, Leuconostoc gelidum, Leuconostoc carnosum, Leuconostoc mesenteroides,*

Address for correspondence:
MVDr. Josef Kameník, CSc., MBA
Department of Meat Hygiene and Technology
Faculty of Veterinary Hygiene and Ecology
University of Veterinary and Pharmaceutical Sciences Brno
Palackého tř. 1/3, 612 42 Brno, Czech Republic

Phone: +420 541 562 008
E-mail: kamenikj@vfu.cz
www.maso-international.cz

Carnobacterium piscicola, Carnobacterium divergens and *Weissella viridescens*, and perhaps by another gram-positive bacteria *Brochothrix thermosphacta* (Iacumin et al. 2014).

The aim of this study was to determine the population of LAB in a heat-treated product of the frankfurter type during the course of storage within the product's shelf life and to analyse the production plant environment in relation to possible sources of contamination of meat products during their production.

Materials and Methods

A product in the heat-treated meat product group – frankfurters with a finely comminuted batter (pork meat, mechanically separated poultry meat, skin emulsion, ice) – was made by producer A in accordance with its own internal standard and stuffed in collagen casings. A proportion of the products were treated with preparation X (producer deliberately not stated) based on liquid smoke following heat treatment (temperature 70 °C/10 min) and cooling to 4 °C. The rest of the production batch was not treated. Both groups were packed in a modified atmosphere (N_2/CO_2: 70/30), weight of one package 500 g, and stored at 2 °C. Sampling and microbiological analysis focusing on the presence and quantity of LAB were performed immediately after packing and again on days 7, 14 and 21. Swabs were also taken from the production plant environment (batter filling, heat treatment, cooling and packaging). The swabs were placed in a sterile MRS broth (Oxoid, UK) and transported to the laboratory at a temperature of 2 ± 2 °C where they were tested for the presence of LAB. Microbiological testing was performed in accordance with ISO 7218 (2008) and ISO 6887-1 (1999). The MRS agar (Oxoid, UK) was used for determination of LAB. Microaerophilic cultivation was performed at 30 °C for a period of 72 hours. Isolates with a negative oxidase and catalase reaction were identified by the MALDI-TOF MS method (Dušková et al. 2012). Bacterial strains were prepared for analysis according to a standard protocol (Freiwald and Sauer 2009). Analysis was performed on an UltraFlex III device and mass spectra processed with the use of BioTyper software (Bruker Daltonics).

Results and Discussion

The results of tests for the presence of LAB in samples of fine frankfurters are shown in Table 1. LAB were practically undetectable immediately following production. After a week's storage, however, multiplication began to be evident on the surface, and after three weeks the population of LAB reached more than 1 x 10^6 CFU cm^{-2}. It did not prove possible to demonstrate any action of preparation X in inhibiting LAB on the surface of the frankfurters. Any surface microflora was most probably caused by contamination when the product was handled during packaging. The species *Leuconostoc carnosum, Lactococcus lactis* and *Lactobacillus curvatus* were isolated on the surface of the frankfurters after 21 days. *Leuconostoc mesenteroides* also occurred in the frankfurter samples after two weeks of storage. Any positive findings of LAB in the product can be regarded as thermo-resistant strains that managed to survive heat treatment. Although the first two tests did not detect LAB in the product, an increase in their number occurred after two weeks of storage. After 21 days, the population of LAB reached a level in excess of 5 x 10^5 CFU·g^{-1}. The species *Lactococcus garviae, Leuconostoc carnosum* and *Pediococcus pentosaceus* were detected in the centre of the frankfurters. Vasilopoulos et al. (2010) tested cooked hams prepared from pork meat. The number of bacteria fell beneath the limit of detection of < 2 log CFU g^{-1} after heat treatment at $t_{max.}$ 72 °C. The growth of surviving cells on MRS agar to $\log_{10}$ 6.65 ± 1.15 (min. 4.08; max. 7.54) occurred following storage of the hams at 7 °C for a period of four weeks. Almost half of the isolates were comprised of the species *Leuconostoc carnosum* (Vasilopoulos et al. 2010). Pothakos et al. (2014) also found the same species in heat-treated meat products.

Iacumin et al. (2014) investigated the population of LAB in frankfurters of Italian origin, which did not show signs of spoilage during storage at refrigeration temperatures, along with products that did show sensory deviations testifying to the spoilage process. A predominance of homofermentative lactobacilli, amounting to as much as 70% of the population of LAB (*Lb. sakei*), was isolated in frankfurters without sensory deviations.

The remaining 30% were representatives of the genus *Leuconostoc* (*Leuc. mesenteroides*, *Leuc. carnosum*). A predominance of heterofermentative LAB (30% *Leuc. mesenteroides*, 30% *Leuc. carnosum*, 5% *Weissella viridescens*, 5% *Carnobacterium divergens*, 5% *Enterococcus faecalis*) was, in contrast, seen in the products showing signs of spoilage. Just 5% was made up of strains of the species *Lb. sakei*, while 20% of isolates belonged to the species *Brochothrix thermosphacta* (Iacumin et al. 2014). More LAB were found in the product than on the surface after two weeks in the study presented here. This testifies to minimal contamination during packaging and, therefore, to the good level of environmental hygiene.

Table 1. Results of microbiological analysis for the presence of LAB in samples of fine frankfurters packed in a modified atmosphere and stored for 21 days at 2 °C

Analysed product	After packing	After 7 days	After 14 days	After 21 days
Untreated frankfurters/surface	< 2	6.9	< 2	4.1×10^6
Frankfurters with preparation/surface	< 2	6.5×10^3	4.5×10^1	1.6×10^6
Frankfurters	$< 5 \times 10^1$	$< 5 \times 10^1$	1.3×10^2	5.8×10^5

The number of LAB is given as colony-forming units (CFU) per 1 cm^2 for the surface and per 1 g for the product

Swabs were taken from the production plant environment to determine possible sources of contamination. The aim was to determine the species representation of LAB in the environment, not their quantitative determination. The results of this species identification are given in Table 2.

Table 2. Results of microbiological analysis for the presence of LAB in the production plant environment

Batter stuffing shop/worktables	Cooling chambers	Packing lines/handling tables
Lactobacillus curvatus/fructivorans	*Aerococcus viridans*	*Lactobacillus fructivorans/curvatus*
Leuconostoc citreum		*Leuconostoc mesenteroides*
*Lactobacillus garvieae**		*Pediococcus pentosaceus**
Lactobacillus brevis		*Lactococcus lactis*
Lactobacillus plantarum		*Lactobacillus curvatus**
*Lactobacillus fermentum**		*Lactobacillus fermentum*
Lactobacillus fructivorans/curvatus		

*isolate with BioTyper log (score) < 2.0 (probable identification at the genus level)

From Table 2 it is clear that the production plant environment may be a source of contamination with LAB for the products manufactured. In contrast to final products, however, 11 species of LAB were isolated from the environment, while only 6 species were isolated from the frankfurters (surface or product). A number of species evidently do not survive heat treatment and smoking. Practically all the species of LAB found in the frankfurters (with the exception of *Leuconostoc carnosum*) could also be demonstrated in the production plant environment.

Conclusions

LAB may make a significant contribution towards the spoilage of meat products as they are capable of surviving heat treatment and are also widely represented in the

production plant environment. Although practically none can be isolated immediately after heat treatment and subsequent packaging, they can multiplicate during the storage period to reach a level that may lead to product spoilage. The species isolated also included *Leuconostoc carnosum* which was not, however, confirmed in the environment.

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