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PREDOMINANT MICROFLORA OF VACUUM-PACKED FRANKFURTERS

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The aim of the investigation was to determine microbial contamination and to isolate predominant bacteria of vacuum packed frankfurters, being in trade circulation, during storage at different temperatures. The frankfurters were stored at 4°C, 8°C, and 15°C. The total count of microorganisms with regard to psychrotrophic bacteria and lactic acid bacteria (LAB) was determined. Predominant colonies were isolated for further characterization. In addition, pH values were measured over the storage time. The initial microbial quality of frankfurters was satisfactory. The largest increase in the number of microorganisms was observed during the first week of storage at all temperatures. Three species (*Leuconostoc mesenteroides* ssp. *mesenteroides, Lactobacillus fermentum* and *Weisella viridescens)* dominated in the spoiled product. Sensory changes in the frankfurters were not linked with bacterial number but with the kind of spoiling flora.

INTRODUCTION

Meat and meat products are a good medium for bacterial growth. The type of microbial contamination introduced by the processing of meat and physicochemical factors (temperature, pH, nutrients, water activity, composition of the atmospheres) applied during storage play a key role in the activity and growth of microorganisms in meat products [Leistner, 1992]. The significance of stringent hygiene and temperature to the product's quality has often been reported. In addition, to prevent product's deterioration it usually needs to be packed. Currently, vacuum-packaging and modified atmosphere packaging are generally in use. Vacuum packaging inhibits the microbiological growth and delays the development of spoilage due to a slow proliferation of bacteria capable of tolerating anaerobic conditions [Jeremiach, 2001]. Lactic acid bacteria (LAB) are the major bacterial group associated with the spoilage of vacuum-packed meat products [Lyhs *et al.,* 1999; Samelis *et al.,* 2000 a,b; Sakala *et al.,* 2002; Jones, 2004]. LAB is a broad group of microaerophilic bacteria that can produce a variety of compounds antagonistic to the growth of other organisms (*e.g.* bacteriocins). Atypical streptobacteria belonging to the species *Lactobacillus sake* and *L. curvatus* have often been shown to form the most important spoilage population of vacuum-packed meat products. In addition, obligate heterofermentative lactobacilli, leuconostocs and *Carnobacterium* have been found in vacuum-packed sausages [von Holy *et al.,* 1991; Lyhs *et al.,* 1999; Samelis *et al.,* 2000 a,b; Sakala *et al.,* 2002; Jones, 2004]. The aim of our investigation was to determine microbial contamination and to isolate predominant bacteria of vacuum-packed frankfurters, being in trade circulation, during storage at different temperatures.

MATERIALS AND METHODS

Samples. Vacuum-packed frankfurters from one plant (from one production run) were taken from a trade refrigerator (2°C). The samples were stored at 4°C, 8°C and 15°C, up to expiry date declared by the producer. Before the storage (time zero) and every sixth day, the total count of microorganisms with regard to psychrotrophic bacteria and lactic acid bacteria (LAB) was determined (each time five probes from each temperature). Predominant colonies were isolated for further characterization. In addition, pH values were measured over the storage time.

pH assay. For all samples, pH was measured every sixth day. A sample (10 g) was blended with 100 g of 0.1 mol/L KCl solution. Precision digital pH meter OP-208/1 (Radelkis, Hungary) was used for the measurements.

Sensory assessment. The samples were assessed in terms of colour, odour and appearance of exudation using three descriptors: (i) Colour: (1) fresh, light pink; (2) moderately altered; (3) markedly altered, grayish to greenish; (ii) Odour: (1) typical of the product; (2) milky, sour; (3) repulsive, typical of spoilage beginning, and (iii) Exudation: (1) absent; (2) small amount, lightly opalescent, (3) markedly turbid, dense to sticky. Sensory evaluation was carried out by a panel of six assessors.

Bacterial enumeration. The aerobic plate count and psychotrophic bacteria were determined on Plate Count Agar (PCA, Merck), incubated at 30°C for 72 h and at 6°C for 6 days, respectively. Lactic acid bacteria were determined on

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TABLE 1. Change of pH and microbial quality of frankfurters during storage.

Storage temperature	Days	pH	Total count of microorganisms*	Psychrotrophs*	LAB*
			log (cfu/g)		
4°C	0	6.25±0.21	1.48±0.12	1.48±0.05	1.30±0.11
	6	6.27±0.15	5.75±0.69	5.40±0.27	5.08±0.47
	12	6.13±0.17	6.48±0.55	5.98±0.39	6.38±0.66
	18	6.02±0.19	6.78±0.61	6.76±0.51	6.77±0.38
8°C	6	6.15±0.22	6.25±0.41	5.58±0.44	6.04±0.52
	12	5.42±0.07	7.08±0.66	6.52±0.43	7.04±0.37
	18	5.01±0.05	7.26±0.37	6.66±0.53	7.18±0.47
15°C	6	6.17±0.09	6.57±0.42	5.83±0.21	5.73±0.22
	12	5.44±0.17	6.89±0.37	5.88±0.42	6.49±0.41
	18	4.86±0.11	7.04±0.62	6.36±0.34	6.94±0.33

*mean±SD

de Man, Rogosa, Sharpe (MRS, Merck) agar, incubated at 30°C for 72 h under anaerobic conditions (Gas-Pack System, BBL, Becton Dickinson, CO_2 envelope). The lowest detection limit of the above enumeration techniques was monitored at 10 cfu/g.

Bacterial identification. The number and types of colonial morphology present on each plate were then determined using Bergey's manual criteria. Morphology types representing more than 10% of the total population were collected (five colonies from each plate). Gram staining, oxidase and catalase tests were performed using standard procedures [Trojanowska *et al.*, 2001]. Oxidation or fermentation of glucose was examined in Hugh Leifson (HL) medium (BTL, Poland) after incubation for 72 h. Predominant Gram-positive, catalase and oxidase negative bacilli and coccobacilli were identified to a species level using API 50CHL tests (Bio Mérieux, France). Identification of an organism was recorded as uncertain when the probability of identification was less than 60%.

Statistical analysis. Students *t*-test was used to evaluate data distribution. The statistical analyses (means, SD, correlations, Student *t*-test) were carried out using Origin 6.1 software.

RESULTS AND DISCUSSION

Microbiological findings for vacuum-packed frankfurters stored for 18 days at different temperatures are presented in Table 1. The initial microbial quality of the product was satisfactory. Frankfurters contained *ca.* 10^1-10^2 cfu/g aerobic bacteria. Similar numbers of colonies were also observed after the incubation on PCA plates at 6°C, which indicates that some psychrotrophs were predominant flora at the start of the storage. The average pH was 6.25. This level of post-process contamination points to good hygienic standards at the processing plant. This is very important as a high contamination at this stage shortens the product's shelf-life, irrespective of the storage conditions [Korkeala & Bjorkroth, 1997]. The storage of frankfurters at 4°C, 8°C and 15°C caused an increase in the population numbers of all investigated groups. The largest increase in bacterial numbers (from 4 to 5 log) was observed during the first week of storage at all temperatures. After 18 days of storage, the total count of microorganism present in frankfurters stored at 4°C was at a level of 10^6–10^7 cfu/g and it was about half log-cycle lower than in products stored at other temperatures. The microflora of the product stored at this temperature was predominated by (*ca.* 100%) psychrotrophic LAB. The selection toward this group was observed after the first storage week. It was observed that after 6 days of storage gram (+), oxidase (-), and catalase (-) organisms made up *ca.* 100% of isolated flora (Figure 1). During storage at this temperature, the differences between pH values of products were not observed ($p>0.20$). The presence of not only psychrotrophic LAB, but also strains more sensitive to cooling temperatures, was observed in the product stored at 8°C. Lactic acid bacteria were the predominating group of contaminating flora also in this case. After 18 storage days, LAB number increased

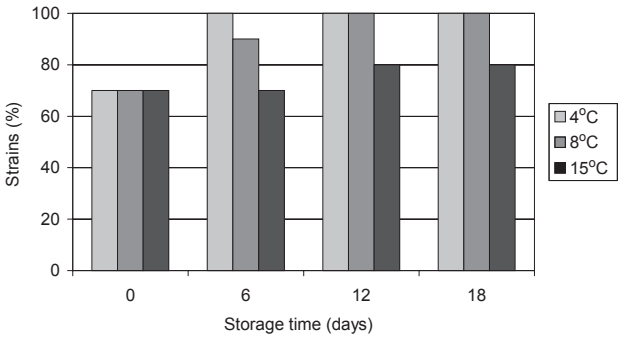

FIGURE 1. Percentage of gram (+), oxidase (-), and catalase (-) strains in the total count of microorganisms.

to the level of 10^7 cfu/g. Gram (+), oxidase(-), and catalase (-) bacteria constituted *ca.* 100% of isolated flora after 12 days of storage (Figure 1). During storage at 8°C, pH values decreased to 5.01 and were lower ($p<0.05$) than the initial pH values. In frankfurters stored at 15°C, a decrease in the content of LAB and psychrotrophic bacteria in total microflora was observed. The total count of microorganisms (7.04 log cfu/g) was higher ($p<0.01$) than the number of psychrotrophs (6.36 log cfu/g). By the end of storage (18 days) the isolated flora comprised *ca.* 80% gram(+), oxidase(-), and catalase (-) organisms (Figure 1). Also at this temperature, the pH of samples was observed to decrease ($p<0.01$). Both the growth and types of spoilage microflora were dependent on the storage temperatures. Gram-positive, catalase and oxidase negative rods were considered to be lactic acid bacteria (LAB). LAB form the major component of the microbial population on various types of vacuum-packaged meat products [Lyhs *et al.,* 1999; Samelis *et al.,* 2000a; Sakala *et al.,* 2002; Jones, 2004]. It is generally known that LAB can evoke sensory changes as well as changes in the physical appearance of spoiled vacuum-packaged sausages. The results of the sensory assessment are presented in Table 2. Initial samples examined immediately after purchase showed light pink colour, typical odour and taste. During storage at 4°C, sensory changes were not observed. In the case of frankfurters stored at 8°C and 15°C, changes were reported after six days, including sour aroma, taste and accumulation of slime and milky fluid. Spoilage LAB produce mostly lactic and acetic acid during logarithmic growth and especially at the stationary phase of growth [Korkeala & Bjorkroth, 1997]. Korkeala *et al.* [1989] found that taste and aroma scores decreased sharply from a constant level when LAB counts reached 1.4 x 10^7 cfu/g. In our study, the correlation between LAB count and pH values was not observed. The decrease in the pH of our samples as well as the accumulation of slime and milky fluid may, therefore, be attributed to the species of predominant LAB. There were no correlations between the sum of sensory descriptor values and aerobic plate (r=0.6117), psychrotrophs (r=0.5621) and LAB (r=0.5886) count. Sensory descriptor values were correlated with pH of products (r=-0.9415). These results are in line with the study of Korkeala *et al.* [1990]. They observed that samples may be deemed spoiled when the pH fell below 5.8 to 5.9.

Three species (*Leuconostoc mesenteroides* ssp. *mesenteroides, Lactobacillus fermentum* and *Weisella viridescens)* dominated in the spoiled product (Table 3). *Leuconostoc mesenteroides* ssp. *mesenteroides* was present in frankfurters irrespective of storage temperature. The participation of this species in the spoiled microflora decreased along with increasing storage temperature. *Weisella viridescens* species was identified only in the product stored at 4°C (*ca.* 21% of contaminating microflora). *Lactobacillus fermentum* was present in frankfurters stored at 8°C and 15°C. The bacterial flora was gradually selected towards this species along with increasing storage temperature. It is very important to know what species of LAB caused the spoilage of the product, as the spoilage potential of LAB is not only dependent on their growth rate, but also on the specific metabolic activity. The microorganism growth during storage results from the type of contamination introduced by the processing of meat and from the influence of the physicochemical factors applied during storage. The share of *Leuconostoc* sp. in the spoilage of vacuum-packed meat and meat-products is large. Leuconostocs have a pronounced ability to predominate, mainly *Lc. mesenteroides* and *Lc. carnosum,* in non-smoked whole--meats, such as ham and luncheon meat [Bjorkroth *et al.,* 1998; Samelis *et al.*, 1998, 2000a,b]. The prolific growth of gas- and slime-producing *Lc. mesenteroides* caused much swelling and ropines. It was observed in the case of frankfurters stored at 8°C and 15°C. *W. viridescens* was the first LAB species reported to survive pasteurization in cured meats. *W. viridescens* population was considered a major hygienic problem [Milbourne, 1983; Borch *et al.,* 1988]. *L. fermentum* was not reported as LAB species caused spoilage of the meat product. Only Beumer *et al.* [1996] found this species in sliced chicken breast.

TABLE 2. Mean values of sensory descriptors during the storage period.

Storage temperatures	Descriptor	Storage period (days)			
		0	6	12	18
4°C	colour	1	1	1	1
	odour	1	1	1	1
	exudation	1	1	1	1
	sum	3	3	3	3
8°C	colour	1	1	1	1
	odour	1	1	2	2
	exudation	1	2	2	2.5
	sum	3	4	5	5.5
15°C	colour	1	1	1	1
	odour	1	1	1.5	2
	exudation	1	2	2.5	3
	sum	3	4	5	6

TABLE 3. Percentage distribution of strains isolated from frankfurters after 18 days of storage at different temperatures.

Organism	Storage temperature		
	4°C	8°C	15°C
Leuconostoc mesenteroides ssp. *mesenteroides*	57	43	22
Weisella viridescens	21	-	-
Lactobacillus fermentum	-	36	43
Other LAB	22	21	15
Enterobacteriaceae	-	-	21

CONCLUSIONS

LAB were dominating microbial contaminants in vacuum-packed frankfurters, irrespective of storage temperature.

Leuconostoc mesenteroides ssp. *mesenteroides, Lactobacillus fermentum* and *Weisella viridescens* were found in the spoiled products. Quantitative composition of spoiling species was determined by storage temperature. Sensory changes in the frankfurters were not linked with bacterial number but with the kind of spoiling flora.

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MIKROFLORA PARÓWEK PAKOWANYCH PRÓŻNIOWO

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Celem badań było określenie stanu mikrobiologicznego oraz dominujących grup i gatunków mikroorganizmów w pakowanych próżniowo parówkach, dostępnych w obiegu handlowym, w zależności od temperatury przechowywania. Parówki przechowywano w temperaturach 4°C, 8°C i 15°C w czasie określonym terminem przydatności do spożycia. W odstępach sześciodniowych, metodami normatywnymi, określano ogólną liczbę drobnoustrojów z uwzględnieniem grupy bakterii psychrotrofowych oraz bakterii fermentacji mlekowej (LAB). Prowadzono również wstępną diagnostykę wyizolowanej mikroflory. Końcowy etap pracy obejmował określenie przynależności gatunkowej dominujących bakterii. Wyjściowy stan mikrobiologiczny parówek był zadowalający. Największy przyrost ilości drobnoustrojów, o cztery do pięciu cykli logarytmicznych, miał miejsce podczas pierwszych sześciu dni, niezależnie od temperatury przechowywania. W parówkach przechowywanych w 4°C w końcowym okresie przydatności do spożycia ogólna liczba drobnoustrojów znajdowała się na poziomie 10^6-10^7 jtk/g i była o około pół rzędu logarytmiczny niższa niż w pozostałych temperaturach. Niezależnie od temperatury przechowywania mikroflorę dominującą stanowiły LAB. W produkcie zepsutym dominowały trzy gatunki bakterii: *Leuconostoc mesenteroides* ssp. *mesenteroides, Lactobacillus fermentum, Weisella viridescens*. Procentowy udział poszczególnych gatunków w wyizolowanej mikroflorze zależał od temperatury przechowywania.