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The spoilage microflora of cured, cooked turkey breasts prepared commercially with or without smoking

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Abstract

Lactobacillus sakei subsp. *carnosus* was predominant in the spoilage flora of sliced, vacuum-packed, smoked, oven-cooked turkey breast fillets which developed mild, sour spoilage flavors after 4 weeks storage at 4°C. In contrast, *Leuconostoc mesenteroides* subsp. *mesenteroides* predominated in the spoilage flora of sliced, vacuum-packed, unsmoked, boiled turkey breast fillets from the same plant which were also stored at 4°C. The spoilage flora of the unsmoked breasts grew faster than that of the smoked breasts and was more diverse. *Lactobacillus sakei*, *Weissella viridescens* and an atypical group of leuconostoc-like bacteria were also members of the unsmoked turkey breasts flora. Consequently, the unsmoked breasts spoiled after 2 weeks at 4°C: the packs swelled and the meat developed strong sour odors and flavors and abundant slime. Except for the unidentified leuconostocs, which apparently survived boiling of the unsmoked turkey, all the spoilage organisms contaminated the meats during the slicing and vacuum packaging operations. From their biochemical reactions and cellular fatty acid profiles, the atypical leuconostocs were more closely related to *Leuconostoc carnosum* than *W. viridescens*. Carnobacteria and *Brochothrix thermosphacta* were present in relatively large numbers on the raw turkey, but were not numerous in the spoilage flora of the cooked, vacuum-packed meat products.

Keywords: Processed poultry; *Lactobacillus sakei*; *Leuconostoc mesenteroides*; Meat processing hygiene

1. Introduction

Cured turkey breast fillet is a popular, low-fat ($<3\%$) product of the Greek meat industry. Two different fillet types are manufactured in many Greek meat plants: (i) unsmoked fillets which are steam-

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cooked or boiled; and (ii) fillets smoked and cooked in ovens. The cooked products are either sliced and packaged under vacuum at the processing plant, or are sliced at the time of purchase from retail outlets. The sliced, vacuum-packed turkey products spoil relatively rapidly. With the unsmoked fillets, spoilage is faster and appears as souring, ropiness and/or swelling of the pack. Since the production of processed poultry is steadily increasing in Greece, the rapid spoilage of these products has become a major problem of the meat industry, especially with respect to unsmoked turkey fillets.

Numerous studies, reviewed recently (Borch et al., 1996; Korkeala and Bjorkroth, 1997), have shown that the type of spoilage occurring in sliced, cured turkey is very common in vacuum-packed processed meats and is associated with lactic acid bacteria (LAB). The rate and severity of sour spoilage, however, varies between different cooked meat products or meat processing plants. This is because the LAB differ in their ability to predominate and eventually cause spoilage in cooked meats (Kitchell and Shaw, 1975; Morishita and Shiromizu, 1986; Korkeala and Makela, 1989; Makela et al., 1992; Dykes et al., 1994; Bjorkroth and Korkeala, 1996; Bjorkroth et al., 1998; Samelis et al., 1998a), depending on the meat plant environment, the product type, the processing method, the packaging conditions and the storage temperature (Borch et al., 1996; Samelis et al., 2000).

Most of the published work on the identification of spoilage LAB in cooked meats has been focused on pork and red meat products (Borch et al., 1996), particularly emulsion sausages made from mixtures of pork, beef and fat (Korkeala and Bjorkroth, 1997). In contrast, few studies have dealt with spoilage of heat-processed poultry, especially that of whole-poultry products (Mead, 1983; Yang and Ray, 1994). However, given the strong selective effect of the product composition and the cooking method on the dominant LAB species in cooked meats (Samelis et al., 2000), more information on the microbiology of cured turkey breasts is needed to take measures to increase their shelf-life. In this study, the microbial quality, the sources of contamination and the changes in numbers and types of spoilage organisms during processing of the two types of cured turkey fillets, i.e. unsmoked and smoked, were investigated. The main objective of this study was to explain, in microbiological terms, the different patterns of spoilage observed for the two types of turkey product.

2. Materials and methods

2.1. Processing of turkey breast fillets

Samples were manufactured at a local meat plant according to standard practices. Two batches of turkey breast fillets, one prepared commercially with smoke and the other without smoke, were analyzed. Batches were 1 week apart and used different stocks of raw materials. The meat was frozen skinless turkey breasts purchased as ready-to-process from suppliers abroad. The frozen meat blocks were taken from their original packs and allowed to thaw at 20°C for 18 h. The meat temperature after thawing was kept at 0–2°C.

For the unsmoked fillet type, thawed breasts were mechanically pumped with 50% brine containing salt, nitrates, nitrites, sodium ascorbate, sodium polyphosphate, flavorings and sucrose, pH 7.0. The temperature of the brine was 1–4°C. After brining, the meat was transferred to a tumbling machine and 'massaged' two times in succession, for 30 min each, at 6–7°C under vacuum. The resting periods between tumbling and moulding were 8 and 12 h, respectively. The tumbled meat was then filled into plastic casings under vacuum, shaped into square 5 kg moulds in stainless-steel containers and cooked by steam to a core temperature of 68–69°C for 4 h. The product was held at this core temperature for 2 min.

For the smoked fillet type, brining and tumbling were carried out on the same processing line. The meat was pumped with 35% brine, which contained different amounts of the additives listed above. After tumbling, the meat was filled into cylindrical 3 kg nets placed in cloth packs and cooked in an oven to a core temperature of 72–73°C for 5 h. In the beginning, smoke was applied at 45–50°C to enhance the desired aroma and color formation, then heating was continued until the final core temperature was reached.

After the heat treatment, both turkey fillet types were cooled to 10°C by water and/or air in an intensive cooler for 8–10 h. The finished products were stored at 2°C, after which they were either sold whole in their original packs, or sliced and vacuum-

packed in the factory cutting room. Sliced fillets were packed in polyacrylamide/polyethylene film (Wolf, Walsrode, Germany) having the following properties: the film under the product was 120 μm thick and had a permeability at 20°C, <24 cm^3 $O_2/m^2/24$ h/atm, not permeable to N_2, while that over the product was 75 μm thick and had a permeability at 20°C, <35 cm^3 $O_2/m^2/24$ h/atm, <5 cm^3 $N_2/m^2/24$ h/atm. The water vapour transmission rate of both films was <1.5 g/m^2/24 h at 20°C and 100% relative humidity.

2.2. Sampling procedure along the processing line

Samples were withdrawn at different stages along the processing line as follows. For both fillet types, the sampling points prior to heat treatment were: (i) after thawing, (ii) after brining, (iii) after first tumbling, (iv) before moulding. Five samples of meat weighing about 100 g each were randomly taken from each sampling point using sterile knives and transported to the laboratory in sterile beakers under refrigeration.

After heating, two whole turkey moulds, one unsmoked and the other smoked, were collected at random and sampled for microbiological analysis, as described below. The remainder was cut aseptically into pieces weighing about 0.5 kg each and immediately vacuum-packed in the laboratory. These 0.5 kg pieces were stored in a cold room at 4°C together with an appropriate number of samples of sliced turkey fillets in vacuum packs from the same product batches, prepared in the factory cutting room. Two samples, one sliced and one whole, were removed from storage at 0, 3, 6, 12, 18, 24 and 30 days and analyzed microbiologically.

2.3. Microbiological sampling and analysis

A composite 25 g sample of raw turkey meat was weighed into sterile stomacher bags by aseptically removing 5 g portions from each one of the five 100 g samples taken from each sampling point at a pre-cooking stage. For vacuum-packed samples, 25 g was also taken aseptically after the removal of the plastic film. For all samples, 225 ml of sterile 0.1% (w/v) peptone water was added and homogenized in a stomacher (Lab Blender, Seward, London, UK) for 1 min at room temperature. Serial decimal dilutions were prepared in 0.1% peptone water and duplicate 1 ml or 0.1 ml samples of appropriate dilutions poured or spread on non-selective and selective agar plates.

Aerobic mesophilic bacteria were determined on Plate Count Agar (Oxoid, Unipath, Basingstoke, UK), incubated at 30°C for 72 h; lactic acid bacteria on de Man, Rogosa, Sharpe (MRS) Agar (Oxoid), incubated at 30°C for 72 h under anaerobic conditions (Gas-Pack System, BBL, Becton Dickinson, Cockeysville, MD, USA); *Brochothrix thermosphacta* on Streptomycin Thallus Acetate Agar (STAA), incubated at 25°C for 48 h; pseudomonads on Cetrimide–Fucidin–Cephaloridine (CFC) medium (Oxoid), incubated at 25°C for 48 h; enterobacteria on Violet Red Bile Dextrose Agar (VRBD, Merck, Darmstadt, Germany), overlayed with 5 ml of the same medium and incubated at 37°C for 24 h; enterococci on Slanetz and Bartley Agar (Oxoid), incubated at 37°C for 48 h; Micrococcaceae on Kranep Agar (Merck), incubated at 30°C for 72 h; staphylococci on Baird–Parker Agar (Merck), incubated at 37°C for 48 h; aerobic sporeformers on Casein Soya Tryptone Agar (Merck), inoculated after inactivation of vegetative cells by heating in a water bath at 80°C for 10 min and incubated at 30°C for 72 h; sulfite-reducing clostridia in tubes containing 20 ml of melted (45°C) Sulfite–Polymyxin–Sulfadiazine Agar (SPS, Merck), inoculated with 10 ml from the first dilution (equal to 1 g of meat), cooled rapidly in ice-water and overlayed with 2 ml of sterile paraffin oil to exclude oxygen, and incubated at 37°C for 24 h; yeasts on Rose Bengal Chloramphenicol Agar (Oxoid), incubated at 25°C for 5 days.

For experimental purposes, the lowest detection limit of the above enumeration techniques was monitored at 10^2 cfu g^{-1}, except for sulfite-reducing clostridia where the respective limit was absence g^{-1}. If the population on a selective medium was above 10^5 cfu g^{-1} at a given processing stage, it was considered significant for the product quality or shelf-life and, therefore, 10 randomly selected colonies were isolated from the highest dilution plates for further characterization. As LAB were expected to dominate in sliced fillets stored in vacuum, random MRS colonies were additionally selected from processing stages when counts were below 10^5 cfu g^{-1} in an attempt to determine the contamination source.

For all the other microbes, if their populations

were below 10^5 cfu g^{-1}, only the selectivity of the growth medium was checked by carrying out the following rapid tests on about 10% of the colonies, according to Harrigan and McCance (1976): the oxidase reaction was tested for colonies grown on STAA and CFC agars and only the negative and positive ones were enumerated, respectively; growth of Micrococcaceae on Kranep agar was confirmed by phase-contrast microscopy and the catalase test; staphylococcal colonies showing lecithinase activity on Baird–Parker's medium were streaked on Nutrient agar and rapidly tested for agglutination by the Staphyslide test (BioMerieux, Marcy l'Etoile, France); random enterococcal isolates were streaked on Kanamycin Aesculin Azide agar (Merck) to observe aesculin hydrolysis.

2.4. pH assay

For all samples the pH was measured by blending 10 g of sample with 90 ml of distilled water. A composite 10 g sample was prepared (about 2 g taken from every 100 g sample of raw turkey meat) for measuring pH before cooking. A Knick 646 digital pH meter (Knick, Elektronische Messgerate, Berlin, Germany) was used for the measurement.

2.5. Identification of lactic acid bacteria

A total of 180 colonies isolated from MRS agar plates at different processing stages were characterized. Strains were grown in MRS broth at 30°C, checked for purity by streaking on MRS agar and maintained in MRS broth plus 20% glycerol (Merck) at −20°C. Plates with pure cultures were used to test rapidly for cell morphology by phase-contrast microscopy, Gram reaction by the KOH method, and catalase formation by dropping a 3% H_2O_2 solution (Merck) directly onto each plate. Gram-positive and catalase-negative strains were further identified by a limited number of tests: gas production from glucose, arginine hydrolysis, growth at 10 and 45°C, growth in 8 and 10% NaCl, slime formation and fermentation of the following sugars (Merck or Sigma–Aldrich, UK): L-arabinose, cellobiose, galactose, inulin, lactose, maltose, mannitol, melibiose, raffinose, ribose, sucrose, sorbitol, trehalose and xylose. After grouping, one to five representative isolates from each group were tested for lactic acid configuration. The methods used for the tests were as described by Samelis et al. (1994). To confirm the identification of certain LAB groups, the presence of major differentiating fatty acids, such as cyclopropane fatty acids with 19 carbon atoms (C19 cycl) and C20:1, in their cellular lipid fraction was tested as described by Samelis et al. (1998b).

3. Results

3.1. Microbial quality of tumbled turkey prior to cooking

After thawing, the turkey breasts contained about 10^5 cfu g^{-1} of aerobic mesophilic bacteria, $<10^3$ cfu g^{-1} of enterobacteria, enterococci, aerobic sporeformers and staphylococci, and <10 cfu g^{-1} of sulfite-reducing clostridia (Table 1). Pseudomonads were predominant in both batches. Micrococcaceae in batch I and *Br. thermosphacta* were present in relatively high numbers, whilst LAB were minor at this stage. Overall, the raw turkey used to make unsmoked fillets (batch I) was of slightly lower microbial quality than that used to make smoked fillets (batch II) (Table 1).

After brining, some microbial populations increased, more so in batch II than in batch I (Table 1), suggesting that contamination was occasional. Since the fresh brine contained $<10^3$ aerobic mesophilic bacteria ml^{-1} (data not shown) and variations in the microbial load of different breasts were reduced by analyzing five samples, the different contamination levels between batches I and II indicated that hygienic conditions could vary during brining.

During tumbling, pseudomonads and enterobacteria remained constant or decreased, unlike Gram-positive bacteria which tended to increase, mainly LAB (Table 1). Presumably, curing salts in combination with vacuum and reduced oxygen tension retarded growth of the Gram-negative bacteria. Besides, nitrite and low temperature did not enable the few sulfite-reducing clostridia and staphylococci to increase. It should be stressed that a reduced oxygen tension and a temperature not much above 6°C were maintained during the resting periods of the tumbling process, when the product was transferred as large stacked meat masses into open but deep, stainless steel containers and held at 10–12°C.

Table 1
Microbiological (log cfu g^{-1}) and pH changes[a] of raw turkey breast fillets along the processing line and prior to cooking

Processing stage	After thawing		After brining		After first tumbling		Before moulding	
	Batch I	Batch II	Batch I	Batch II	Batch I	Batch II	Batch I	Batch II
Aerobic mesophilic bacteria	5.22	4.91	5.32	5.26	5.43	5.36	5.88	5.63
Lactic acid bacteria	3.85	3.78	4.11	3.98	4.86	5.20	5.55	5.51
Pseudomonads	4.95	4.62	4.26	4.81	4.46	3.56	4.32	3.16
Enterobacteria	2.86	2.28	3.26	3.74	3.00	3.30	3.12	3.04
Brochothrix thermosphacta	4.18	4.08	4.35	4.84	4.73	4.95	4.89	4.33
Enterococci	<2.00	2.00	2.30	3.11	3.32	3.48	4.26	3.61
Micrococcaceae	4.32	<2.00	4.08	4.08	4.52	4.28	4.71	4.34
Staphylococci	2.18	<2.00	2.40	2.75	2.83	<2.00	2.75	<2.00
Aerobic sporeformers	<2.00	<2.00	<2.00	2.00	<2.00	<2.00	<2.00	<2.00
Sulfite-reducing clostridia	0.78	0.48	0.60	0.30	0.30	0	0	0
Yeasts	4.48	2.30	3.48	3.11	4.11	3.30	3.75	3.40
pH	5.8	5.9	6.5	6.4	6.4	6.2	6.3	6.2

[a] Each microbiological number is from one composite 25 g sample taken from five raw meat samples. Each pH value is from one composite 10 g sample taken from the corresponding five meat samples. Each sample was analyzed in duplicate (coefficient of variation $<5\%$).

As a result, LAB dominated while the initially predominant pseudomonads comprised a minor part of the meat microflora prior to moulding (Table 1).

3.2. Microbial quality of turkey fillets post-cooking

After heating and cooling, both whole fillet types contained less than 10^2 cfu g^{-1} of LAB, Micrococcaceae, enterococci, *Br. thermosphacta*, pseudomonads, enterobacteria, aerobic sporeformers and staphylococci, and were free of sulphite-reducing clostridia (results not shown). Aerobic mesophilic counts were 2.04 and 2.86 log cfu g^{-1} for the unsmoked and smoked fillets, respectively.

During subsequent storage of whole, vacuum-packed fillets at 4°C, no actual microbial growth was observed in the smoked product and, hence, no isolations were made: microbial counts remained $<10^3$ cfu g^{-1} after 30 days of storage. This was also observed in whole, unsmoked fillets until the 24th day of storage. After 30 days, however, 1.7×10^7 cfu g^{-1} of bacteria grew as white, pin-point colonies on PCA plates. Similar counts were also grown on MRS, suggesting that some LAB survived heating and grew in the product after recovery from heat injury.

In sliced, vacuum-packed fillets stored at 4°C, all selective counts except those of LAB remained constant and below 10^3 cfu g^{-1}, while sulphite-reducing clostridia were absent. Immediately after packaging (day 0), the sliced fillets contained 1.3–3.0×10^3 cfu g^{-1} of mesophilic bacteria, most of

Table 2
Changes[a] in numbers (log cfu g^{-1}) of mesophilic aerobic count (MAC) and lactic acid bacteria (LAB), and pH values during storage of vacuum-packed, sliced, cooked turkey breast fillets at 4°C

	Storage (days)						
	0	3	6	12	18	24	30
Unsmoked fillets (Batch I)							
MAC	3.11	4.38	6.74	8.38	8.46	7.82	7.95
LAB	2.54	3.79	6.61	8.30	8.42	8.11	7.91
pH	6.4	6.4	6.3	5.8	5.7	5.4	5.3
Smoked fillets (Batch II)							
MAC	3.47	3.30	4.38	6.83	7.86	7.54	8.16
LAB	3.02	3.21	3.79	6.65	7.60	7.34	8.22
pH	6.3	6.3	6.3	6.2	6.0	5.9	5.7

[a] Each sample was analyzed in duplicate (coefficient of variation $<5\%$).

which were LAB (Table 2). These results indicate a considerable level of contamination with LAB in the plant cutting room. LAB increased in all sliced samples during storage at 4°C, but the rate of this increase was remarkably faster in unsmoked fillets. The increase of LAB correlated well with the changes of the product pH (Table 2), as well as with the onset of spoilage, which was faster and more severe in unsmoked fillets. These packs swelled and the cured meat developed strong sour odors and abundant slime after 15 days. In contrast, smoked fillets did not swell, but only contained a small amount of drip and developed mild sour spoilage flavors during storage.

3.3. Characterization of the spoilage flora of vacuum-packed turkey fillets

The identification and percentage isolate distribution of 90 strains from samples of sliced turkey with more than 10^5 cfu LAB g^{-1} revealed differences in the predominant species in each fillet type (Table 3). *L. sakei* appeared to be predominant in smoked fillets, whereas *Lc. mesenteroides* subsp. *mesenteroides* appeared to dominate in unsmoked fillets. Several strains of *L. sakei*, *W. viridescens* and an atypical group of leuconostoc-like bacteria were also isolated from unsmoked fillets. The isolation frequency of *W. viridescens* was greater during the first 2–3 weeks, whilst *L. sakei* and the atypical leuconostocs were isolated by late storage at 4°C (Table 3).

It should be noted that isolates recovered from whole, unsmoked fillets after the third week of storage at 4°C were identical to the atypical leuconostoc-like bacteria reported in Table 3 (results not shown). This indicates that one important LAB type might survive the heating of whole, unsmoked fillets. This type could also grow and, thus, appear sporadically on the respective slices after 3–4 weeks of storage at 4°C (Table 3).

3.4. Characterization of the lactic flora of turkey breasts prior to cooking

Carnobacteria predominated on raw turkey breasts after thawing, with *C. maltaromicus* the species most frequently isolated (Table 4). However, while *C. maltaromicus* continued to predominate in batch I throughout the curing/tumbling process, in batch II carnobacteria were progressively replaced by strains of *L. sakei* and *Lc. mesenteroides*. The isolation of *Lc. mesenteroides* strains from both batches after tumbling is also noteworthy, and that atypical leuconostoc-like bacteria like those predominating in whole, unsmoked fillets after cooking were not isolated from raw tumbled turkey (Table 4).

3.5. Phenotypic differentiation of lactic isolates

The phenotypic criteria used to characterize the 180 LAB strains which in total were isolated during processing and storage of cured, cooked turkey breast fillets are presented in Tables 5 and 6. Initially, strains were divided into two major phenotypic groups based on their cell morphology: (i) 107 strains were clearly rod-shaped and, thus, could belong to the genera *Lactobacillus* or *Carnobacterium* (Table 5); members of *Carnobacterium* sp. were easily differentiated from other meat lactobacilli as they were slender, arginine-positive and L-lactate-producing rods, unable to grow in 8% salt;

Table 3
Percentage distribution of 90 strains of lactic acid bacteria isolated during storage of sliced turkey breast fillets at 4°C in vacuum packs. Ten isolates per sample were identified

	Smoked fillets				Unsmoked fillets				
Days of storage:	12	18	24	30	6	12	18	24	30
Lactobacillus sakei	90	90	100	100	10	–[a]	–	30	30
Leuconostoc mesenteroides	–	–	–	–	20	60	70	60	50
Leuconostoc carnosum	10	–	–	–	20	–	–	–	–
Weissella viridescens	–	10	–	–	50	40	30	–	–
Atypical leuconostoc-like bacteria	–	–	–	–	–	–	–	10	20

[a] < 10.

Table 4
Percentage distribution of 80 strains of lactic acid bacteria isolated during processing of turkey breast fillets prior to cooking. Ten isolates per sample were identified

	Processing stage							
	After thawing		After brining		After first tumbling		After moulding	
	Batch I	Batch II	Batch I	Batch II	Batch I	Batch II	Batch I	Batch II
Carnobacterium maltaromicus	70	50	50	40	50	10	70	20
Carnobacterium divergens	30	20	10	10	20	10	10	–[a]
Lactobacillus sakei	–	–	–	30	–	20	–	40
Lactobacillus curvatus	–	10	20	10	–	–	–	–
Lactobacillus coryniformis	–	–	–	10	–	–	–	–
Lactobacillus casei	–	–	–	–	–	10	–	–
Leuconostoc mesenteroides	–	–	–	–	20	50	10	40
Leuconostoc gelidum	–	–	10	–	10	–	10	–
Leuconostoc carnosum	–	10	–	–	–	–	–	–
Leuconostoc lactis	–	–	10	–	–	–	–	–
Weissella viridescens	–	10	–	–	–	–	–	–

[a] < 10.

Table 5
Selected differentiating characteristics of rod-shaped lactic acid bacteria (lactobacilli) isolated during processing and storage of cured turkey breast fillets[a]

No. of isolates:	*Carnobacterium maltaromicus* 36	*Carnobacterium divergens* 11	*Lactobacillus sakei* 54	*Lactobacillus curvatus* 4	*Lactobacillus coryniformis* 1	*Lactobacillus casei* 1
Gas production	− /(+)	−	−	−	−	−
Arginine hydrolysis	+	+	+	−	−	−
Growth in 8% NaCl	−	−	+	+	(+)	+
Slime formation	−	−	47/54	−	−	−
Fermentation of						
Ribose	+	+	+	+	−	+
Mannitol	+	−	−	−	−	+
Inulin	24/36	−	NT	NT	NT	NT
Sorbitol	−	−	−	−	−	+
Raffinose	−	−	−	−	+	−
L-Arabinose	−	−	1/54	−	−	−
Maltose	+	+	2/54	+	+	+
Melibiose	9/36	−	+	−	−	−
Lactic acid configuration	L (5)[b]	L (2)	DL (5)	DL (1)	DL	L
Presence of cellular C19 cycl	− (5)	+ (2)	NT	NT	NT	NT

[a] +, Positive reaction; −, negative reaction; (+), weak positive reaction; 24/36, 24 out of 36 strains were positive; L or D, more than 90% of total lactic acid was of this type; DL, 11–89% of total lactic acid was D(+) type; NT, not tested.
[b] Number of representative strains examined in parentheses.

Table 6
Selected differentiating characteristics of heterofermentative, arginine-negative, coccoid lactic acid bacteria isolated during processing and storage of cured turkey breast fillets[a]

	Leuconostoc mesenteroides	*Leuconostoc carnosum*	*Leuconostoc gelidum*	*Leuconostoc lactis*	*Weissella viridescens*	Atypical leuconostoc-like bacteria
No. of isolates:	38	4	3	1	14	13
Slime formation	+	+	+	−	12/14	+
Fermentation of						
L-Arabinose	+	−	+	−	−	−
Cellobiose	+	+	−	−	−	−
Galactose	+	−	−	+	−	−
Maltose	+	+	+	+	+	−
Raffinose	+	−	+	+	−	−
Sucrose	+	+	+	+	2/14	+
Trehalose	+	+	+	−	−	−
Xylose	+	−	+	−	−	−
Lactic acid configuration	D (3)	D (1)	D (1)	D	DL (3)	DL (3)
Presence of cellular						
C19 cycl	+ (3)	+ (1)	+ (1)	NT	−[b] (10)	+ (10)
C20:1	− (3)	− (1)	− (1)	NT	+ (10)	− (10)

[a] For symbols, see Table 5.
[b] Strains possessed small amounts (< 1% of total fatty acid) or trace amounts of C19 cycl.

(ii) 73 strains were elongated cocci to short bacilli; when tested further, all 73 isolates produced gas from glucose, but not ammonia from arginine (Table 6). They were therefore referred to the genus *Leuconostoc* or the arginine-negative *Weissella* spp. (Collins et al., 1993). The combination of key biochemical characteristics used to identify our turkey isolates at species level or as atypical leuconostocs (Tables 5 and 6) will be discussed below.

4. Discussion

Scanty information is available on the spoilage LAB species in processed poultry, especially in whole-poultry products. *Lc. mesenteroides* subsp. *mesenteroides* was found to dominate with *L. fermentum* in sliced chicken breast (Beumer et al., 1996), and *Leuconostoc* spp. were prevalent in sliced turkey (Yang and Ray, 1994). In this study, *Lc. mesenteroides* subsp. *mesenteroides* predominated on slices of unsmoked, cured turkey breast fillets stored at 4°C in vacuum. In contrast, *L. sakei* predominated in sliced, smoked, cured turkey breast fillets from the same plant, which were also stored at 4°C in vacuum. To our knowledge, this is the first report stating the predominance of *L. sakei* in a smoked cured poultry meat. The spoilage flora of unsmoked fillets was more diverse, as it also included strains of *L. sakei*, *W. viridescens* and an unidentified group of leuconostoc-like bacteria (Table 3).

The predominant *L. sakei* isolated here did not display its well-established phenotypic heterogeneity, which resulted in taxonomic difficulties in many previous studies (Morishita and Shiromizu, 1986; Korkeala and Makela, 1989; Dykes et al., 1994; Samelis et al., 1994, 1995). All isolates of this species shared their phenotypic properties, and resembled *L. sakei* subsp. *carnosus* (Torriani et al., 1996), except for two strains isolated from raw turkey which differed in their reactions with arabinose and maltose (Table 5). Interestingly, the strains of *L. sakei* subsp. *carnosus* as well as those of *Lc. mesenteroides* subsp. *mesenteroides* from sliced turkey fillets were phenotypically identical (Tables 5 and 6) to the respective strains found to predominate in sliced cooked ham produced at the same plant previously (Samelis et al., 1998a). Presumably,

persistent phenotypes of *L. sakei* and *Lc. mesenteroides* are the most important post-contaminating LAB in this factory cutting room. These strains probably belong to specific genotypes that may colonize the plant. To verify this and the exact route of contamination, genotyping of random representatives of *L. sakei* and *Lc. mesenteroides* at different stages of processing of tumbled meats is required (Bjorkroth and Korkeala, 1996; Bjorkroth et al., 1998).

The differing ability of *L. sakei*, *Lc. mesenteroides* and other contaminating LAB to grow in cured turkey fillets prepared commercially with or without smoking was in accordance with our previous studies: *Lc. mesenteroides* outgrew *L. sakei* in sliced, vacuum-packed, cooked (unsmoked) ham manufactured in this plant and subsequently stored at 4 and 12°C (Samelis et al., 1998a). In a more recent, comparative study undertaken at two industrial meat plants (Samelis et al., 2000), *L. sakei* and *L. curvatus* predominated in smoked meats, such as pork loin, bacon, pariza, mortadella and frankfurters, whilst *Lc. mesenteroides* was predominant in unsmoked whole meats. Moreover, *W. viridescens* was isolated from smoked meats only, whereas *Lc. carnosum* and *Lc. citreum* occurred in unsmoked whole meats and emulsion sausages, respectively. These findings are in general agreement with previous workers. For example, the prevalence of homofermentative meat lactobacilli and the low to zero presence of *Leuconostoc* in vacuum-packed bacon or smoked pork loin is well documented (Kitchell and Shaw, 1975; Blickstad and Molin, 1983; Shaw and Harding, 1984). On the other hand, *Leuconostoc* and other heterofermentative LAB were commonly found to predominate in vacuum-packed, sliced cooked ham or luncheon meat (Shaw and Harding, 1989; Yang and Ray, 1994; Beumer et al., 1996; Holley, 1997; Bjorkroth et al., 1998).

Based on our findings, we conclude that the composition of the spoilage flora of sliced, vacuum-packed cured turkey was determined by the manufacturing method, particularly the heat process of each fillet type. The prevalence of *Lc. mesenteroides* in unsmoked turkey seemed to be due to the more friendly cured meat environment of this fillet type compared with the smoked product. Indeed, the injection of more brine, i.e. 50% instead of 35%, into the unsmoked meat suggested a higher water content and a_w value than for the smoked meat. This processing difference in combination with the absence of the antimicrobial effect of smoke might have stimulated the growth rate of LAB (Table 2) and selected a more diverse lactic flora (Table 3) by giving a better chance to a greater number of species to grow on the unsmoked meat. Recently, Samelis et al. (2000) observed a very good correlation between the LAB growth rate and type and important intrinsic (pH, moisture, brine concentration) and processing (smoking, cooking temperature, packaging atmosphere) factors. It was shown that the *L. sakei*/*curvatus* group had a great advantage for growth by increasing the 'hurdle effect' in vacuum-packed, cooked meats, i.e. decreasing the product pH and water content, increasing the brine concentration and applying smoke. In addition, hot smoking enhances the surface drying of smoked meats (Samelis et al., 2000). This may reduce a_w and increase the hurdle effect on the meat product surface, where LAB mainly grow (Korkeala and Lindroth, 1987; Holley, 1997).

Based on the preceding discussion, the different patterns of spoilage observed for the two types of turkey breast fillets were associated with the LAB species in each product, and their growth rate and metabolic activity during storage at 4°C. The prolific growth of gas- and slime-producing *Lc. mesenteroides* in unsmoked turkey caused much swelling and ropiness; this was also the case in cooked ham, especially when slices were stored at 12°C (Samelis et al., 1998a). In contrast, under the same packaging and chill storage conditions, homofermentative meat lactobacilli, mainly *L. sakei*, predominated and caused slower and milder sour spoilage in smoked turkey, confirming our previous observations for smoked pork loin, bacon and emulsion-type sausages (Samelis et al., 2000).

Another processing factor of concern was the final core temperature of each fillet type. In most Greek plants, smoked meats (pork loin, turkey fillets, emulsion-type sausages, etc.) are oven-cooked until a core temperature of 72–74°C is reached. In contrast, unsmoked, whole meats, such as cooked ham, turkey breast, pork shoulder, etc., may be heated to core temperatures lower than 70°C for 1–2 min (Samelis and Metaxopoulos, 1999); this was also done in this study. Thus, bacterial heat survivors may more often arise in unsmoked than smoked tumbled meats, as

was the case with *Listeria* spp. and *Listeria monocytogenes* in another Greek plant (Samelis and Metaxopoulos, 1999). Here, the only organisms that apparently survived cooking of the unsmoked fillets were the atypical leuconostoc-like bacteria. In contrast, carnobacteria and *Br. thermosphacta* were not found in the vacuum-packed samples, although they were numerous during curing and tumbling of raw turkey breasts, as observed previously (Samelis et al., 1998a). Presumably, carnobacteria and *Br. thermosphacta*, which often predominate in vacuum/MAP-packed raw meat and poultry (Shaw and Harding, 1984; Borch et al., 1996), were either fully inactivated by heating or were unable to compete against the post-contaminating LAB. To our knowledge, there is only one early reference on the predominance of carnobacteria and *Br. thermosphacta* in the spoilage of smoked vacuum-packed ham from thigh turkey meat (Mead, 1983). In this study, the use of packaging film of low oxygen permeability might have contributed to the suppression of *Br. thermosphacta* in vacuum-packed turkey (Nielsen, 1983; Kotzekidou and Bloukas, 1996). On the other hand, carnobacteria are poor competitors in cured meats due to their low acid and salt tolerance (Shaw and Harding, 1984; Collins et al., 1987).

The atypical leuconostocs were not possible to identify at species level with the phenotypic criteria applied in the present study. Interestingly, similar strains were recovered from cooked, whole hams from the same plant (Samelis et al., 1998a). In that previous study, we reported that these atypical strains were likely to be uncommon, trehalose-negative variants of *Lc. carnosum*. In a more recent study, Bjorkroth et al. (1998) characterized *Lc. carnosum* as the specific spoilage LAB of vacuum-packed, sliced cooked ham and found that certain genotypes of this species were distributed throughout the ham processing line. This report is interesting to correlate with our findings. Based on their sugar fermentation pattern (Table 6, Samelis et al., 1998a), we maintain that atypical leuconostocs from Greek ham and turkey are closely related to *Lc. carnosum*, as the only key difference from this species (Shaw and Harding, 1989) is their inability to ferment trehalose. However, 15 ham isolates (not shown) and three representative strains from turkey (Table 6) were found to produce more than 25% L-lactic acid (Table 6). This is contrary to their classification for *Lc. carnosum*, or any other *Leuconostoc* spp., which produce solely D-lactate (Shaw and Harding, 1989; Stiles and Holzapfel, 1997). We therefore assumed a relation between our atypical strains and the only DL-lactate-producing and arginine-negative *Weissella*, i.e. *W. viridescens* (Collins et al., 1993). This was further supported by the fact that these strains could have survived cooking of ham (Samelis et al., 1998a) and turkey, which is consistent with the ability of *W. viridescens* to survive in cooked meats (Milbourne, 1983; Borch et al., 1988). However, unlike atypical leuconostocs (Table 6), *W. viridescens* ferments maltose (Collins et al., 1993). To clarify the relation between atypical isolates and *W. viridescens*, we checked for the presence of C19 cycl and C20:1 in the cellular lipids of 10 representative strains. This was done because *W. viridescens* can be differentiated from all other arginine-negative *Weissella* and *Leuconostoc* by its ability to form large amounts of cellular C20:1, while it contains zero to low amounts ($<1\%$ fatty acids) of C19 cycl (Samelis et al., 1998b). The results revealed high C19 cycl (17–32%) and zero C20:1 contents in all atypical strains (Table 6), indicating their closer phenotypic relation with *Leuconostoc* (Shaw and Harding, 1989; Samelis et al., 1998b). When the 10 typical *W. viridescens* isolates of this study were analyzed in parallel, all possessed more than 35% cellular C20:1 and traces of C19 cycl (Table 6), confirming the identification and reproducibility and reliability of the GC method (Samelis et al., 1998b). In conclusion, our atypical leuconostoc-like bacteria may either represent a new, arginine-negative but DL-lactate-producing *Weissella* species, or be wild *Lc. carnosum* strains which have crucially altered certain key biochemical properties. Molecular characterization is required to resolve the taxonomy of these meat isolates.

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