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The microbiological condition of minced pork prepared at retail stores in Athens, Greece

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ABSTRACT

Minced pork samples ($n=150$) obtained from butchers' shops and supermarkets in Greece, during summer ($n=75$) and winter ($n=75$), were subjected to microbiological analysis. Microbial counts (log CFU/g) for the parameters tested were: total viable count (TVC), 6.8 ± 1.0; *Pseudomonas* spp., 6.4 ± 1.2; *Brochothrix thermosphacta*, 5.9 ± 1.1; lactic acid bacteria, 5.3 ± 1.0; yeasts and moulds, 4.6 ± 0.7; hydrogen sulfide (H_2S)-producing bacteria, 4.3 ± 1.3; *Enterobacteriaceae*, 3.6 ± 1.2; total coliforms, 2.9 ± 1.1; *Escherichia coli*, 1.4 ± 0.7; *Staphylococcus* spp., 4.3 ± 1.0; *S. aureus*, 2.4 ± 0.9, and *Listeria* spp., 1.4 ± 0.6. The highest correlations were between TVC and pseudomonads, *B. thermosphacta* and H_2S-producing bacteria, while the lowest were between total coliforms and all other groups of microorganisms except *Enterobacteriaceae*. The type of retail outlet and the seasonality of sampling did not have any significant effects ($p>0.05$) on minced pork meat quality. Interrelationships between (i) meat quality and shelf life, (ii) hygienic conditions during mince preparation and (iii) personnel hygiene were revealed.

1. Introduction

Mince is a potentially hazardous meat product with a very short shelf life. Studies on the microbiological quality of mince have shown the marked influence of storage temperature and packaging atmosphere (Emswiler, Pierson, & Kotula, 1976; von Holy & Holzapfel, 1988), as well as the effect of the type of retail outlet sampled and the season at which mince was analysed (Kammenou, Metaxopoulos, & Drosinos, 2003; Nychas, Robinson, & Board, 1991; Rao & Ramesh, 1988) on the microbial populations recorded.

Pork is the most widely consumed meat in the European Union (EU) (Verbeke, Pérez-Cueto, & Grunert, 2011). In a quantitative large-scale consumer survey conducted in five European countries (*Q-PorkChains*, 6th Framework Programme integrated project), minced pork meat had one of the highest market penetration levels among 30 specific pork products, with a percentage of over 90% (Verbeke, Pérez-Cueto, de Barcellos, Krystallis, & Grunert, 2010). Although, certain efforts have been made to evaluate microbiological quality and safety of mince in Greece (Kammenou et al., 2003; Papadopoulou, Panagou, Tassou, & Nychas, 2011), the microbiological condition of minced pork prepared at retail stores in Athens has not been studied to an extent adequate enough to correlate parameters of interest regarding meat quality, hygienic conditions during mince preparation and personnel hygiene. Thus, the objective of the present work was to investigate the microbial association of minced pork meat, as affected by the type of retail outlet and season, in order to evaluate its microbiological quality and hygiene. To this end, distribution of microbial populations was determined and multivariate analysis performed.

2. Materials and methods

2.1. Mince preparation

Pre-packaged, vacuum or modified atmosphere sealed, mince is not preferred and it is rarely used by Greek consumers. Common preparation practice of mince in the Greek meat retail market is the original selection by the consumer of the cuts to be used for mincing. Meat cuts are stored aerobically at refrigerating temperatures (2–4 °C). Preparation of freshly produced mince takes place with the physical presence of the consumer and it is a statutory obligation for meat retailers. After preparation, mince is packed with a kraft-type wrapping paper.

2.2. Minced pork samples

One hundred and fifty samples (150) of minced pork prepared upon request from whole meat cuts (shoulder or thigh) in different retail outlets (butchers' shops, $n=127$ and supermarkets, $n=23$) within the metropolitan area of Athens, Greece, were purchased

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over a ten-month period consisting of two different seasons. Half of the samples (butchers' shops, $n=66$ and supermarkets, $n=9$) were obtained during summer (May to September, 2009), while the rest (butchers' shops, $n=61$ and supermarkets, $n=14$) were collected during winter (November to March, 2010). The samples were transported under refrigeration in polystyrene boxes or isothermal bags. Immediately upon arrival at the laboratory, samples were stored at chill temperature (4 ± 2 °C) and drawn separately in random sequence for analysis. In any case, the analysis started within two hours after arrival at the laboratory.

2.3. Microbiological analysis

A representative analytical unit of 25 g, taken randomly from different parts of each sample (500 g of mince), was transferred aseptically into a sterile stomacher bag (Seward Medical, London, UK) and 225 ml of sterile maximum recovery diluent (0.1% w/v peptone and 0.85% w/v sodium chloride) (MRD, Biolife, 401691, Milan, Italy) were added. The content was homogenized at room temperature in a Stomacher 400-laboratory blender (Seward Medical), for 1 min at normal speed and for 30 s at high speed. Serial decimal dilutions in test tubes containing sterile MRD (Biolife) were prepared.

For the enumeration of total viable count (TVC), yeasts and moulds, *Pseudomonas* spp., *Brochothrix thermosphacta*, *Listeria* spp., *Staphylococcus* spp. and *S. aureus*, 0.1 ml of the appropriate dilution was spread in duplicate on plate count agar (Merck, 1.05463, Darmstadt, Germany), chloramphenicol glucose yeast extract agar (CGYE agar, Biolife, 401289), cetrimide fucidin cephaloridine agar (CFC agar, Biolife, 401960) with CFC pseudomonas supplement (Biolife, 4240075), streptomycin thallous acetate actidione agar (STAA agar, Biolife, 402079) with STAA supplement (Biolife, 4240052), PALCAM agar (Biolife, 401604) with Listeria PALCAM supplement (Biolife, 4240042) and Baird-Parker agar (BP agar, Biolife, 401116) with egg yolk tellurite emulsion 20% (Biolife, 423700), respectively. Especially for the enumeration of *Listeria* spp., 1 ml of the originally homogenized content was also spread in duplicate on three plates of PALCAM agar (Biolife) (0.33 ml on each plate), reducing in this case the level of detection (LOD) to 10 CFU/g or 1 log CFU/g. The Petri dishes were then incubated at 25 °C for 48 h (CFC and STAA), 72 h (plate count agar) or 5 days (CGYE), at 30 °C for 48 h (PALCAM) and at 37 °C for 48 h (BP). For the enumeration of *Enterobacteriaceae*, total coliforms and *Escherichia coli*, lactic acid bacteria (LAB), and hydrogen sulfide (H_2S)-producing bacteria, 1 ml of the appropriate dilution was poured in duplicate into 10 ml of molten (45 °C) violet red bile glucose agar (VRBGA, Biolife, 402188), Chromocult® (Merck, 1.10426), de Man, Rogosa and Sharpe agar (MRS agar with tween® 80, Biolife, 401728) and iron agar (IA, medium prepared from basic ingredients in the laboratory) (Gram, Trolle, & Huss, 1987), respectively. After setting, a 10 ml overlay of molten medium was added. Similarly, the Petri dishes were incubated at 25 °C for 48 h (IA), at 30 °C for 72 h (MRS) and at 37 °C for 24 h (VRBGA and Chromocult®). Three serial decimal dilutions were plated for every medium described.

All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium. For enterobacteria on VRBGA, only the large colonies with purple haloes were counted (Mossel, Eelderink, Koopmans, & van Rossem, 1979). The selectivity of the growth media was also checked routinely by carrying out rapid tests (e.g., Gram staining and microscopic examination, and catalase reaction) on about 10% of the colonies grown on countable plates, according to Harrigan (1998).

2.4. Measurement of pH

The pH of each sample was measured after the end of microbiological analysis, according to ISO 2917 reference method (Anonymous, 1999) by using a digital pH-meter (WTW, pH 526, Weilheim, Germany).

2.5. Statistical analysis of data

The microbial counts were converted to log CFU/g and multivariate analysis was performed. Converted counts were subjected to analysis of covariance (ANCOVA) using the software SPSS v15.0 (SPSS, Inc., Chicago, IL, USA). Independent variables included retail outlet and season of sample's purchase, as well as retail outlet-season interactions. A probability value of less than 0.05 ($p<0.05$) was defined as statistically significant. Distributions for populations of microorganisms with mean, standard deviation and 95% confidence interval were calculated using the software @Risk 4.5 (Palisade Corp., Ithaca, NY, USA). The chi-square test statistic was used to determine if the microbiological data embedded in the @Risk 4.5 software followed normal distribution. Principal component analysis (PCA) and Pearson correlation analysis were also performed with SPSS v15.0.

3. Results

3.1. Microbial populations

Distribution for TVC in minced pork meat was 6.8 ± 1.0 log CFU/g, with a range of 4.9 to 8.7 log CFU/g. With respect to retail outlet, average TVCs were 6.7 ± 1.0 and 7.2 ± 0.7 log CFU/g for butchers' shops and supermarkets, respectively. The distributions for the spoilage flora of minced pork and for enterobacteria, total coliforms, *E. coli*, *Listeria* spp., *Staphylococcus* spp. and *S. aureus* are given in Table 1.

Chi-square test statistic showed that all sets of converted microbial counts were normally distributed ($p>0.05$). *Pseudomonas* spp., *B. thermosphacta* and LAB were the three major components of the microbial association. *Listeria* spp. and *E. coli*, on the other hand, were the least predominant organisms of the association, followed by *S. aureus*, total coliforms and *Enterobacteriaceae*. In 105 out of 150 samples (70%) tested, *E. coli* populations were below the LOD of 10 CFU/g.

3.2. Multivariate analysis

ANCOVA showed no significant effects ($p>0.05$) of the type of retail store sampled and the season at which the samples were analysed. Pearson correlation coefficients (r) presented for the different groups of microorganisms detected in minced pork meat (Table 2) indicated that the highest correlations were between TVC and pseudomonads ($r=0.90$), *B. thermosphacta* ($r=0.86$) and H_2S-producing bacteria ($r=0.71$), while the lowest were between total coliforms and all other groups of microorganisms except *Enterobacteriaceae*.

Three principal components (PCs), accounted for 79.8% of the total variance, were selected during application of PCA. The 50.3% of the total variance was explained by PC1, 17.4% by PC2 and 12.1% by PC3. Based on PCA, the microbiological meat quality of the retail outlets sampled was mapped on a graph (Fig. 1). During PCA, loadings above 0.400 were considered significant, which meant that there was significant and preserved relationship between the tested variable and the corresponding PC. The first component (PC1) was related to the potential spoilage flora presented in Table 1 (plus TVC), the second (PC2) related to enterobacteria and total coliforms, and finally the third component (PC3) related to *Staphylococcus* spp.

4. Discussion

The composition of fresh minced meat spoilage flora at chill temperatures is decisively determined by the relative numbers of the initial psychrotrophic microorganisms and their growth rates at such temperatures. On aerobically stored meat from which mince is produced, *Pseudomonas* spp. have a marked advantage in growth rate at refrigerating temperatures compared to other genera and form a major component of the final spoilage flora (Gill & Newton, 1977, 1978). Indeed, pseudomonads were dominant at the outset

Table 1
Distributions for the spoilage flora and for hygiene indicator microorganisms of minced pork prepared from aerobically stored meat cuts at the time of purchase.

Microorganism(s)[a]	Sample prevalence (%)	Mean log number (log CFU/g)	Standard deviation (log CFU/g)	95% confidence interval (log CFU/g)
Pseudomonas spp.	100	6.4	1.2	4.1–8.8
Brochothrix thermosphacta	100	5.9	1.1	3.8–8.0
Lactic acid bacteria	100	5.3	1.0	3.4–7.3
Yeasts and moulds	100	4.6	0.7	3.3–5.9
H_2S-producing bacteria	100	4.3	1.3	1.6–6.9
Enterobacteriaceae	98	3.6	1.2	1.4–5.9
Total coliforms	87	2.9	1.1	0.8–5.0
Escherichia coli	30	1.4	0.7	1.1–2.8
Listeria spp.	36	1.4	0.6	0.3–2.6
Staphylococcus spp.	79	4.3	1.0	2.3–6.2
Staphylococcus aureus	20	2.4	0.9	0.6–4.2

[a] The pH distribution for all samples analysed ($n = 150$) was 5.9 ± 0.2, with 95% confidence interval from 5.5 to 6.3.

as expected, followed by *B. thermosphacta* and LAB, since these bacteria are mainly responsible for the reduced shelf life and spoilage of minced meat (Drosinos & Board, 1995a, 1995b; Koutsoumanis, Stamatiou, Drosinos, & Nychas, 2008).

Enterobacteriaceae, total coliforms and *E. coli* are considered as tentative indicators of the hygienic state of fresh meat or the hygienic conditions under which minced meat is handled. Besides, enterobacteria were highly correlated with total coliforms and to a lesser degree with *E. coli*. This last lower correlation suggests that each count gives different information in relation to hygienic practices. Indeed, enterobacteria are considered as a general indicator of the applied hygienic conditions, whereas *E. coli* is used as an indicator of possible fecal contamination. The same also applies for *Staphylococcus* spp., where the low correlation coefficients of staphylococci with all other members of the association suggest that each count gives different information as far as the hygienic practices are concerned. In that way, *Staphylococcus* spp. is used as an indicator of human contamination due to poor hygienic conditions during handling of the product.

TVC of mince is subject to large variation and at retail level it has been reported to range between 2.0 and 9.0 log CFU/g; with average or mean values mostly above 6.0 log CFU/g. In general, there is no difference in TVCs between retail beef and pork (i.e., steaks and minced meats) (Scriven & Singh, 1986). All categories of microorganisms detected in this work were correlated with TVC. The high correlation between counts of mesophiles and pseudomonads as well as the high incidence of *B. thermosphacta* and H_2S-producing bacteria, suggest that the shelf life of freshly produced mince might be short (e.g., 2–3 days).

The statistical analysis of data (ANCOVA) showed that the type of retail outlet and the seasonality of sampling did not have any significant effects ($p > 0.05$) on the microbiological quality of minced pork meat. These results are in accordance with the findings by Roberts, Britton, and Hudson (1980) and Hudson, Roberts, Crosland, and Casey (1986), even though significant differences ($p < 0.05$) in bacterial counts of mince have been identified between butchers' shops and supermarkets (Kammenou et al., 2003; Nychas et al., 1991), as well as for large processing plants producing minced meat (Hinton et al., 1998). Moreover, a seasonal effect on the microbiological quality of mince has been reported by Nychas et al. (1991). A probable explanation for the observed results on microbial counts would be the similar way in which mince is prepared upon request in both butchers' shops and supermarkets.

It is worthy to commend the present results in relation to European Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs (Anonymous, 2005, 2007). The average TVC values in the present work exceeded or equaled the food hygiene criterion for TVC in mince (≥ 6.7 log CFU/g) and thus the microbiological quality was judged as unsatisfactory (i.e., in total and for supermarkets) or marginally acceptable (i.e., butchers' shops). Considering the *E. coli* criterion (<1.7 log CFU/g) samples were of satisfactory quality. The food hygiene criterion for TVC and *E. coli* was exceeded in 56.0% (84) and 2.7% (4) of total samples (150) tested, respectively. These percentages were significantly higher compared to those found in a similar study by Paulsen, Smulders, Tichy, Aydin, and Höck (2011). According to the guidelines proposed by the Public Health Laboratory Service (PHLS) Advisory Committee for Food and Dairy Products, 5.3% (8), 4.8% (7) and 12.0% (18) of all minced pork samples analysed were of unsatisfactory microbiological quality due to *Listeria* spp. ($\geq 10^2$ CFU/g), *E. coli* ($\geq 10^2$ CFU/g) or *S. aureus* ($\geq 10^2$ - $<10^4$ CFU/g), respectively. Three samples (2.0%) were found to be of unacceptable microbiological quality due to high levels of *S. aureus* ($\geq 10^4$ CFU/g) (Gilbert et al., 2000).

5. Conclusions

It could be concluded that the distribution of populations in mince prepared from aerobically stored meat cuts demonstrates a classic synthesis of a microbial association, with dominance of pseudomonads.

Table 2
Correlation matrix[a] for the microflora of minced pork prepared from aerobically stored meat cuts at the time of purchase.

Microorganism(s)[b]	Total viable count	*Entero-bacteriaceae*	Total coliforms	Yeasts and moulds	Lactic acid bacteria	*Brochothrix thermosphacta*	*Pseudomonas* spp.	H_2S-producing bacteria	*Staphylococcus* spp.
Total viable count	1.00	0.61	0.43	0.64	0.57	0.86	0.90	0.71	0.22
Enterobacteriaceae	0.61	1.00	0.79	0.37	0.27	0.55	0.53	0.56	0.12
Total coliforms	0.43	0.79	1.00	0.11	0.07	0.09	0.20	0.02	0.08
Yeasts and moulds	0.64	0.37	0.11	1.00	0.44	0.64	0.71	0.58	0.18
Lactic acid bacteria	0.57	0.27	0.07	0.44	1.00	0.53	0.50	0.54	0.26
Brochothrix thermosphacta	0.86	0.55	0.09	0.64	0.53	1.00	0.86	0.73	0.08
Pseudomonas spp.	0.90	0.53	0.20	0.71	0.50	0.86	1.00	0.71	0.09
H_2S-producing bacteria	0.71	0.56	0.02	0.58	0.54	0.73	0.71	1.00	0.16
Staphylococcus spp.	0.22	0.12	0.08	0.18	0.26	0.08	0.09	0.16	1.00

[a] All correlations were significant ($p < 0.05$).
[b] *E. coli* and *S. aureus* were not included in the matrix due to insufficient data to perform the analysis, biasing the results.

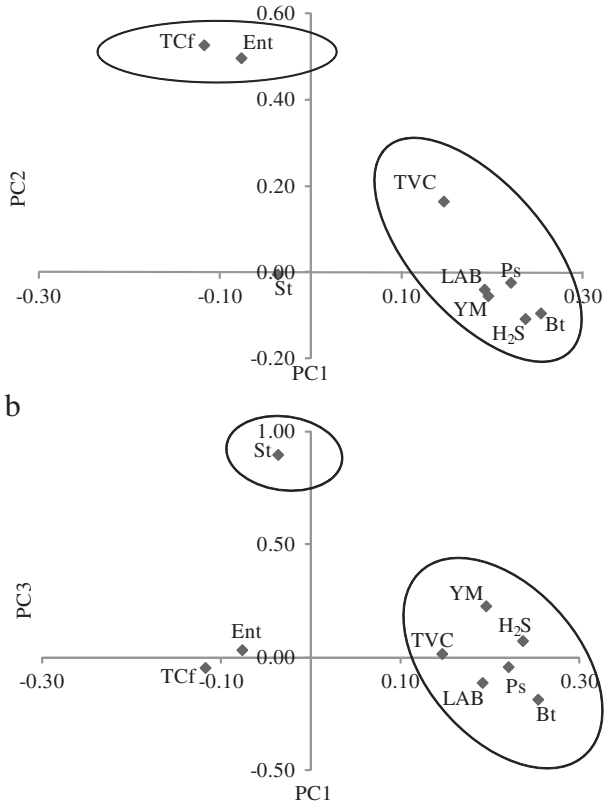

Fig. 1. Component coefficient score plot in rotated space with the significant variables to each extracted component (PC) according to the PCA. a) PC1 vs. PC2 and b) PC1 vs. PC3. TVC, total viable count; TCf, total coliforms; Ent, *Enterobacteriaceae*; LAB, lactic acid bacteria; Ps, *Pseudomonas* spp.; YM, yeasts and moulds; Bt, *B. thermosphacta*; H_2S, H_2S-producing bacteria; and St, *Staphylococcus* spp.

The three selected PCs revealed interrelationships between meat quality and shelf life (PC1), and hygienic conditions during preparation of minced meat with respect to retail outlet's equipment (PC2) and employees (PC3). Generally, a cumulative explained variance percentage above 50%, as in this case, is considered satisfactory for the extracted factors, with the first PC accounting for the major part of the total variance (Mataragas, Skandamis, Nychas, & Drosinos, 2007). Although pseudomonads, enterobacteria, total coliforms and *E. coli*, all characterise the microbiological quality of minced meat, they give a different kind of information; pseudomonads are related to shelf life, whilst the rest of the microbiological parameters are related to hygienic conditions during preparation of the product.

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