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Meat spoilage during distribution

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Abstract

Meat spoilage during distribution can be considered as an ecological phenomenon that encompasses the changes of the available substrata (e.g., low molecular compounds), during the prevailing of a particular microbial association, the so-called specific spoilage organisms (SSO). In fact, spoilage of meat depends on an even smaller fraction of SSO, called ephemeral spoilage organisms (ESO). These ESO are the consequence of factors that dynamically persist or imposed during, e.g., processing, transportation and storage in the market. Meanwhile spoilage is a subjective judgment by the consumer, which may be influenced by cultural and economic considerations and background as well as by the sensory acuity of the individual and the intensity of the change. Indeed, when spoilage progresses, most consumers would agree that gross discoloration, strong off-odors, and the development of slime would constitute the main qualitative criteria for meat rejection.

On the other hand, meat industry needs rapid analytical methods or tools for quantification of these indicators to determine the type of processing needed for their raw material and to predict remaining shelf life of their products. The need of an objective evaluation of meat spoilage is of great importance. The use of metabolomics as a potential tool for the evaluation of meat spoilage can be of great importance. The microbial association of meat should be monitored in parallel with the estimation of changes occurring in the production and/or assimilation of certain compounds would allow us to evaluate spoilage found or produced during the storage of meat under different temperatures as well as packaging conditions.

Keywords: Meat spoilage; Quantitative microbiology; Chemometrics; Bioinformatics; Predictive modelling; Chill chain; ANN; Machine learning; Meat chemistry; Metabolomics

1. Introduction

The scientific attention on meat microbiology, increased when large amounts of meat started being shipped long distances (e.g., from Australia to the UK) and continued in the 1950s with the growth of supermarkets. Meat spoilage is not always evident and consumers would agree that gross discoloration, strong off-odors, and the development of slime would constitute the main qualitative criteria for meat rejection. In general, spoilage is a subjective judgment by the consumer, which may be influenced by cultural and economic considerations and background as well as by the

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sensory acuity of the individual and the intensity of the change.

At the same time and at the dawn of the 21st century in an environment of momentous technological progress and evolution of consumer lifestyles the European food industry is called to meet to seemingly contradictory market demands. While, in general, new technologies are rapidly being accepted and absorbed, consumers expectations of food (meat) products is relatively ambivalent. They seek food products of upgraded sensory quality and increased functional and nutritional properties combined with a traditional, and wholesome image, as well as guaranteed safety but yet less processing, and fewer additives or "technological" interventions. At the same time they expect extended shelf life and high convenience in preparation and use. To meet market demands producers and regulators concentrate on the development and application of structured quality and safety assurance systems based on thorough risk analysis and prevention through controlling monitoring, and recording of critical parameters through the entire life cycle of products. These systems include the primary production and ideally extend to the consumer's table. Systematic management of meat product safety *via* HACCP includes raw material selection, control of conditions during processing and distribution (Koutsoumanis, Taoukis, & Nychas, 2003; Koutsoumanis & Taoukis, 2005; Sofos, 2005). The latter is the weaker link of the system. Conditions during transportation and at the retail level are out of manufacturer's direct control and often deviate from specifications. Temperature control is completely lacking from the store to domestic storage and until the time of preparation and consumption. Some quantitative evidence is available from studies and surveys at distribution, retail and domestic level to illustrate the magnitude of the problem. In South European countries 30% of refrigerated foods were kept above 10 °C in retail cabinets and household refrigerators and even in North Europe 5% were above 13 °C in retail and 21% above 10 °C in households (Kennedy et al., 2005).

A modern quality and safety assurance system should focus on prevention rather than inspection, through monitoring, recording and controlling of critical parameters during the entire meat's life cycle that includes the post-processing phase and extends to the time of use by the final consumer (Koutsoumanis et al., 2003; Maurice, 1994). Increasing attention is given on the role and the logistics of transport, storage and handling. (Broekmeulen, 2001; Browne & Allen, 1998; Dubelaar, Chow, & Larson, 2001; Ross, 1996; Tijkens, Koster, & Jonker, 2001). The risk potential, the shelf life and final quality of chilled products processed and packed under good manufacturing practices and good hygiene practices (GMPs and GHPs) are determined by the applied temperature conditions in the chilled distribution chain. Since in practice significant deviations from specified conditions often occur, temperature monitoring and recording should be a prerequisite for food chain control (Wells & Singh, 1989).

2. Factors affecting spoilage

Spoilage of meat can be considered as an ecological phenomenon that encompasses the changes of the available substrata (e.g., low molecular compounds) during the proliferation of bacteria that consist the microbial association of the stored meat. The prevailing of a particular microbial association, of meat depends on factors that persist during processing, transportation and storage in the market. It is well established that in any food ecosystem includes five categories of ecological determinants (e.g., intrinsic, processing, extrinsic, implicit, and the emergent effects). These influence the establishment of the particular microbial association and determine the rate of attainment of a climax population so called (by the) "Ephemeral/specific spoilage micro-organisms $-E(S)SO$", i.e., those which are able to adopt various ecological strategies (Koutsoumanis & Nychas, 2000; Nychas, Marshall, & Sofos, 2007). These ecological strategies, developed by the ESO, are the consequence of environmental determinants (e.g., stresses, destructive or enrichment disturbance of the ecosystem, the availability of energy or oxygen competitors), and allow them to proliferate in all available niches. In fact, all of the determinants mentioned above constitute a virtual ecological niche or in other words-an *n*-dimensional hypervolume or hyperspace cloud (HSC) in which an organism influence in (micro) space and time (Boddy & Wimpenny, 1992). Indeed the ecosystem approach is pertinent in an analysis of changes occurring in fresh meat during the distribution chain. Therefore, in practice, scientists and technologists involved in meat industries attempt to control (e.g., temperature) or modify some or all of the parameters noted above in order either to extend the shelf life of meat or to create new products with acceptable shelf life.

In this communication, emphasis will be given to Implicit (intrinsic biotic parameters) as well as to Extrinsic factors.

2.1. Implicit [intrinsic biotic parameters] factors

2.1.1. The specific role of ephemeral spoilage organisms (ESO)

The microbiological quality of meat depends on the physiological status of the animal at slaughter, the spread of contamination during slaughter and processing, the temperature and other conditions of storage and distribution (Table 1). In fact, some of the microorganisms originate from the animal's intestinal tract as well as from the environment with which the animal had contact at some time before or during slaughter (Koutsoumanis & Sofos, 2004). This is evident with studies on the origin of the contaminants showing an association of work surfaces with the presence of *Enterobacteriaceae* on meats. Other psychrotrophic bacteria are recovered from hides and work surfaces within an abattoir as well as from carcasses and butchered meat at all stages of processing (Gill, 2005; Koutsoumanis, Geornaras, & Sofos, in press, chap. 52).

Table 1
Genera of bacteria commonly found on meats and poultry

Microorganisms	Gram reaction	Fresh	Processed
Achromobacter	−	X[a]	
Acinetobacter	−	XX[a]	X
Aeromonas	−	XX	X
Alcaligenes	−	X	
Alteromonas	−	X	X
Arthrobacter	±	X	X
Bacillus	+	X	X
Brochothrix	+	X	X
Campylobacter	−	X	
Carnobacterium	+	X	
Chromobacterium	−	X	
Citrobacter	−	X	
Clostridium	+	X	
Corynebactenum	+	X	X
Enterobacter	−	X	X
Enterococcus	+	XX	X
Escherichia	−	X	
Flavobacterium	−	X	
Hafnia	−	X	X
Janthinobacterium	−		X
Klebsiella	−	X	
Kluyvera	−	X	
Kocuria	+	X	X
Kurthia	+	X	
Lactobacillus	+	X	XX
Lactococcus	+	X	
Leuconostoc	+	X	X
Listeria	+	X	X
Microbacterium	+	X	X
Micrococcus	+	X	X
Moraxella	−	XX	
Paenibacillus	+	X	X
Pantoea	−	X	
Proteus	−	X	
Providencia	−	X	X
Pseudomonas	−	XX	X
Shewanella	−	X	X
Staphylococcus	+	X	X
Streptococcus	+	X	X
Vibrio	−	X	
Weissella	+	X	X
Yersinia	−	X	

Based on Nychas et al. (2007).
[a] X = known to occur, XX = most frequently isolated.

As mentioned above a vast number of studies in meat microbiology have established that spoilage is caused only by the fraction of the initial microbial association that dominates (Nychas et al., 2007). Although similar genera have been reported in the literature as dominating in this fraction, the findings at the species level are diverse (Nychas et al., 2007). The domince of various species (ephemeral spoilage) with a the genus is also of importance. This concept has contributed significantly to our understanding, of meat foods spoilage. The range of microbial taxa found in meat under various storage conditions is given in Table 1. A consortium of bacteria, commonly dominated by *Pseudomonas* spp., is in most cases responsible for spoilage of meat stored aerobically at different temperatures (−1 to 25 °C; Koutsoumanis et al., in press, chap. 52; Koutsoumanis, Stamatiou, Skandamis, & Nychas, 2006; Stanbridge & Davis, 1998) (Table 2). It is now well established that under aerobic storage three species of *Pseudomonas*, *Ps. fragi*, *Ps. fluorescens* and *Ps. lundensis* are the most important. The population of pseudomonads to the arbitrary level of 10^{7-8} CFU/g, has been attributed to slime and off-odors formation (Tables 3 and 4). However, in practice both these characteristics become evident when the pseudomonads have exhausted the glucose and lactate present in meat and begin to metabolise nitrogenous compounds such as amino acids (Table 5).

Cold-tolerant *Enterobacteriaceae* (e.g., *Hafnia alvei, Serratia liquefaciens, Enterobacter agglomerans*) also occur on chilled meat stored aerobically (Nychas, Drosinos, & Board, 1998) but in terms of numbers they do not contribute to the microbial associations. Although rarely, if ever, contributing significantly to the spoilage flora on meat and meat products, *Enterobacteriaceae* have been considered as indicators of food safety. With ground beef, *Pantoea agglomerans*, *Escherichia coli*, and *Serratia liquefaciens* were the major representatives of this family.

Table 2
Spoilage association dominating on fresh meat stored at 0–4 °C under different gas atmospheres

Gas composition	Meat and poultry
Air	*Pseudomonas* spp.
>50% CO_2 with O_2	*Brochothrix thermosphacta*
50% CO_2	*Enterobacteriaceae*, lactic acid bacteria
<50% CO_2 with O_2	*B. thermosphacta*, lactic acid bacteria
100% CO_2	Lactic acid bacteria
Vacuum packaged	*Pseudomonas* spp., *B. thermosphacta*, *Sh. putrefaciens*

Based on Nychas et al. (2007) and on unpublished data (Koutsoumanis et al., 2007).

Table 3
Common defects in meat products and causal bacteria

Defect	Meat product	Bacteria
Slime	Meats	*Pseudomonas*, *Lactobacillus*, *Enterococcus*, *Weissella*, *Brochothrix*
H_2O_2 greening	Meats	*Weisella*, *Leuconostoc*, *Enterococcus*, *Lactobacillus*
H_2S greening	Vacuum packaged meats	*Shewanella*
H_2S production	Cured meats	*Vibrio*, *Enterobacteriaceae*
Sulfide odor	Vacuum packaged meats	*Clostridium*, *Hafnia*
Cabbage odor	Bacon	*Providencia*
Putrefaction	Ham	*Enterobacteriaceae*, *Proteus*
Bone taint	Whole meats	*Clostridium*, *Enterococcus*
Souring	Ham	Lactic acid bacteria, *Enterococcus*, *Micrococcus*, *Bacillus*, *Clostridium*

Based on Nychas et al. (2007); Skandamis and Nychas (2002), Nychas et al. (2006).

Table 4
Factors and precursors affecting the production of odours end-products of Gram-negative bacteria (e.g., *Pseudomonas* spp., *Shewanella putrefaciens*, *Moraxella*)

End-product	Meat/meat model	Factors	Precursors
Sulfur compounds			
Sulfides	+	Temperature and substrate (glucose) limitation	Cysteine, cystine, methionine
Dimethylsulfide	+		Methanethiol, methionine
Dimethyldisulfite	+		Methionine
Methyl mercaptan	+		nad
Methanethiol	+		Methionine
Hydrogen sulfide	±[a]	High pH	Cystine, cysteine
Dimethyltrisulfide	+	nad[b]	Methionine, methanothiol
Esters			
Methyl esters (acetate)	+	Glucose (l)[c]	nad
Ethyl esters (acetate)	+	Glucose (l)	nad
Ketones	+	nad	nad
Aromatic hydrocarbons	+		
Aliphatic hydrocarbons	+		
Aldehydes			
2-Methylbutanal	+	nad	*iso*-Leucine
Alcohols			
Methanol	+	nad	nad
Ethanol	+	nad	nad
2-Methylpropanol	+	nad	Valine
2-Methylbutanol	+	nad	*iso*-Leucine
Other compounds			
Ammonia	+	Glucose (l)	Amino acids

Modified from Nychas et al. (2007).
[a] Production only by *Shewanella putrefaciens.*
[b] nad = no available data.
[c] (l) low concentration of glucose.

Table 5
Order (1 = first) of substrate utilization during growth of major muscle spoilage bacteria[a]

Substrate	Aerobic					Anaerobic[b]				
	A	B	C	D	E	A	B	C	D	E
Glucose/glucose-6-Ph	1	1	1	1	1	1	1	1	1	1
Lactate	2	2		2						
Pyruvate	3	3				2[c]				
Gluconate/Gl-6-Ph	4	4				2[c]				
Proprionate		5								
Formate							1[c]			
Ethanol		6								
Acetate		7				2[c]				
Amino acids	5	8	2	3		2[c]	1[c]		2	2
Ribose			3							
Glycerol			4							

Modified from Nychas et al. (2007).
[a] A: *Pseudomonas* spp.; B: *Shewanella putrefaciens;* C: *Brochothrix thermosphacta;* D: *Enterobacter* spp.; E; lactic acid bacteria.
[b] Under oxygen limitation and/or CO_2 inhibition.
[c] No specific order is given.

Brochothrix thermosphacta and lactic acid bacteria have been detected in the aerobic spoilage flora of chilled meat but they are not considered to be important in spoilage except possibly for lamb (Holzapfel, 1998). These organisms have been isolated from beef carcasses during boning, dressing and chilling. Moreover, lairage slurry, cattle hair, rumen contents, walls of slaughter houses, the hands of workers, air in the chill room, neck and skin of the animal as well as the cut muscle surfaces have been shown to be contaminated with this organism. Both lactic acid bacteria and *Br. thermosphacta* are of the main, if not the most important, cause of spoilage, which can be recognized as souring rather than putrefaction (Table 3). This type of spoilage is one of the two distinct situations related to spoilage that are possible in meat and is commonly associated with meat packed under vacuum or modified atmospheres as the result of competition between facultatively anaerobic Gram-positive flora. The second situation is where competition is between Gram-negative floras. The physiological attributes of the organisms in the latter case, under the imposed ecological determinants, are shown in Tables 6 and 7.

In general, the metabolic activity of the ephemeral microbial association, which prevails in a meat ecosystem under certain aerobic conditions or generally introduced during processing, leads to the manifestation of changes or spoilage of meat. These changes or spoilage are related to the (i) type, composition and population of the microbial association and, (ii) the type and the availability of energy substrates in meat. Indeed the type and the extent of spoilage is governed by the availability of low-molecular weight compounds (e.g., glucose, lactate) existing in meat (Nychas & Skandamis, 2005; Nychas et al., 1998). By the end of this phase changes and subsequently overt spoilage is due to catabolism of nitrogenous compounds and amino acids as well as secondary metabolic reactions.

2.1.2. Spoilage; microbes or indigenous enzymes' responsibility?

The post-mortem glycolysis, caused by indigenous enzymes, ceases after the death of the animal when ultimate pH reaches a value of 5.4–5.5. Afterwards, the contribution of meat indigenous enzymes in its spoilage is negligible compared to the microbial action of the microbial flora (Nychas & Tassou, 1997; Tsigarida & Nychas, 2001). Indeed, it needs to be noted that the indigenous proteolytic and lipolytic enzymes may not even be enough to affect meat conditioning (ageing). In this case, enzymes, or other chemicals, or mechanical means are applied to play an artificial role in meat tenderization (Koohmaraie, 1994; Lawrence, Dikeman, Hunt, Kastner, & Johnson, 2003a). As far as the role of proteolysis is

Table 6
End products formed by *Brochothrix thermosphacta* in model meat system or in naturally spoiled meat (M) poultry (P)[a]

End-product	M/P	Factors	Precursors
Aerobically			
Acetoin	+	Glucose (h), pH (h/l), T (h/l)	Glucose (mj), alanine (mn), diacetyl
Acetic acid	+	Glucose (h), pH (h/l), T (h/l)	Glucose (mj), alanine (mn)
L-Lactic acid	(np)	T (h), pH (h), O_2 (l)	Glucose
Formic acid	+	T (h), pH (h),	Glucose
Ethanol	+	T (h), glucose	nad
CO_2	+	Nad	Glucose
iso-Butyric acid	+	Glucose (l), T (l), pH (h)	Valine, leucine
iso-Yaleric acid	+	Glucose (l), T (l), pH (h)	Valine, leucine
2- Methylbutyric	+	Glucose (l), pH (h)	*iso*-Leucine
3- Methylbutanol	+	Glucose (h), pH (l)	nad
2,3-Butanediol	+	Glucose (h), T (h/l)	Diacetyl
Diacetyl	+	Nad	nad
2-Methylpropanol	+	Glucose (h)	Valine
L-Lactic acid	+	Glucose (h), pH (h),T (ns)	Glucose
Acetic acid	+	O_2(h), glucose (l)	Glucose
Ethanol	+	T (h), pH (h)	nad
Formic	+	T (h), pH (h)	nad

Factors and precursors of these end products are also presented.
Modified form Nychas et al. (2007).
[a] (h), High pH, concentration of glucose, or storage temperature; (l), low pH, concentration of glucose or storage temperature; (h/l), contradictory results; (ns), not significant factor; (mj), major contribution; (mn), minor contribution; (np), no production under strictly aerobic conditions; nd, not-determined; nt, not-tested; na, not analyzed; nad, no available data; *T*, temperature.

Table 7
Factors and precursors affecting the maximum formation of end-products of lactic acid bacteria (*Lactobacillus* sp., *Leuconostoc* sp., *Carnobacterium* sp.) in meat model system or naturally spoiled meat

End-product	Homofermentative	Heterofermentative
L-Lactic acid	+	+
D-Lactic acid	+	+
Acetic acid	+	+
Acetoin/diacetyl	+	nad
Hydrogen peroxide	+	−
Formic acid	+	+
Ethanol	+	+

Modified form Nychas et al. (2007).
nad = not available data.

concerned, Nychas and Tassou (1997) showed clearly that autolysis (e.g., indigenous proteolytic enzymes) did not contribute in spoilage. In their study the role of the ephemeral spoilage groups of the final microbial association, was found to govern the spoilage pattern. These patterns, which were evident even during the earliest stages of storage regardless of microbial populations (Schmitt & Schmidt-Lorenz, 1992a, 1992b), could not be attributed to the indigenous proteolytic meat enzymes (autolysis), but to the microbial proteolytic activity (Nychas & Tassou, 1997). These changes in the proteolytic profile, were evident even if lactic acid bacteria, e.g., weak proteolytic in comparison to *Pseudomonas* spp., (Law & Kolstad, 1983) were the ephemeral spoilage bacteria prevailing at the end of storage. It is well known that the proteolytic activity of pseudomonads can lead to their penetration into meat (Gill & Penney, 1977; Gupta & Nagamohini, 1992). In such case, the proteolytic bacteria may gain an ecological advantage through penetration because they then have access to a new niche with newly available resources (e.g., nutrients) for exploitation, which would not be accessible or available to the non- or less proteolytic bacteria.

There is no doubt that microbiological activity is by far the most important factor influencing the changes that cause spoilage in meat. However, it should be clarified that, it is the microbial activity (growth) per se, rather than the activity of microbial enzymes and as a consequence, it is the accumulation of metabolic by-products that characterizes food spoilage (Nychas et al., 2007). Thus, it is important in the context of meat spoilage to include interactions between microbial growth and their enzyme activity.

2.1.3. Chemistry of spoilage

It is well established that glucose, lactic acid, and certain amino acids followed by nucleotides, urea and water-soluble proteins (Table 5) are catabolized by almost all the bacteria of the meat microflora (Gill, 1986; McMeekin, 1982; Nychas et al., 2007). The former compounds are the essential energy sources for the massive growth of microcosm on the meat despite their negligible quantity in comparison to proteins. It is shown that actual concentration of these compounds can affect the type (e.g., saccharolytic, proteolytic), the rate of spoilage and, moreover, seems to be the principal precursor(s) of those microbial metabolite(s) that we perceive as spoilage (Koutsoumanis & Nychas, 1999; Nychas et al., 1998; Skandamis & Nychas, 2002; Tsigarida & Nychas, 2001).

2.1.3.1. Substrate(s) and meat ecosystems. There are 3 classes of substances that are used by the microbial association (i) compounds contributing in the glycolytic pathway (e.g., glucogen, glucose, glucose-6phosphate, lactate, etc.), (ii) metabolic products (e.g., gluconate, gluconate-6-phosphate, pyruvate, lactate, etc.) and (iii) nitrogen energy sources (e.g., aminoacids, proteins) (Gill, 1986; Nychas, Dillon, & Board, 1988, 1998).

Glucose has been found to be the precursor of many off-odors during meat storage (Nychas et al., 1998) while its limitation could cause a switch from a saccharolytic to an amino-acid degrading metabolism in at least some bacterial species (Tables 4 and 6). The changes of glucose and lactate (second in order carbon energy source) as well as their oxidative products (e.g., gluconate, gluconate – 6 ph) have been proposed for describing or predicting the degree of spoilage (Boers, Dijkmann, & Wijngaards, 1994; Nychas et al., 1988; Seymour, Cole, & Coote, 1994). This is evident especially with meat stored under aerobic conditions where pseudomonads are the major contributors of spoilage. The sequential catabolism of D-glucose and L- and D-lactic with D-glucose used preferentially to lactate, while the oxidation of glucose and glucose 6-phosphate *via* the extracellular pathway caused a transient accumulation of D-gluconate and an increase in the concentration of 6-phosphogluconate. Finally, it was shown that under aerobic conditions the sum of the free amino acids, and the water-soluble proteins increased during storage and it corresponded well with colony counts. Nychas and Arkoudelos (1990) and Nychas and Tassou (1997) showed that this increase occurred in meat samples with a relatively high concentration of glucose. Moreover, the increase of free amino acid increase under aerobic conditions was higher than that occurring under modified atmosphere conditions. These observations could be of importance commercially since spoilage is most frequently associated only with post-glucose utilization of amino acids by pseudomonads (Gill, 1986).

2.1.3.2. Interaction of ESO and communication in meat ecosystem. Pseudomonas sp., *Brochothrix thermosphacta,* Lactic acid bacteria and *Shewanella putrefaciens* are considered to be the main spoilage bacteria of low and high pH raw meat, stored in chill temperature aerobically or vacuum/map conditions (Garcia-Lopez, Prieto, & Otero, 1998; Stanbridge & Davis, 1998). It heeds to be stressed that the final composition of microbial flora eventually characterizes the type of spoilage (Nychas et al., 1998). In other words, spoilage is the outcome of the imposed environmental conditions and the microbial interaction (Nychas et al., 1998; Tsigarida, Boziaris, & Nychas, 2003). This concept has not been fully exploited in meat microbiology. The nutrient contribution may be related to positive (synergistic/syntrophic) or competition for nutrients/energy (e.g., under excess, limitation or starvation), metabiosis (production of a favorable environment), cell-to-cell communication (e.g., quorum sensing) could also affect the physiological attributes of the organisms under the imposed ecological determinants (Nychas, Skandamis, Koutsoumanis, & Baranyi, 2006; Nychas et al., 2007). Indeed, Koutsoumanis and Nychas (1999) and Tsigarida and Nychas (2001) reported that the chemical changes occurring in naturally contaminated fish and meat significantly differed from those on sterile muscle tissue when it was individually inoculated with the Ephemeral spoilage organisms. Studies in co-culture model systems (Tsigarida & Nychas, 2006; Tsigarida et al., 2003) found to be helpful in simplifying the natural food ecosystem and permit to understand the mechanisms whereby development of potential ESO is affected by possible interactive behaviors and identify the responsible metabolite which may be further used as a unique chemical spoilage index.

This can be important in understanding spoilage, as it was found that there is an interaction between the abovementioned bacteria. Indeed, *Pseudomonas* sp. can outgrow *Sh. putrefaciens* due to the ability of the former either to produce siderophores (Gram & Dalgaard, 2002) or to use glucose in faster rate than the latter (Tsigarida et al., 2003). This interaction can be the major factor governing the development of spoilage flora (Nychas et al., 2007).

So far the examples related to positive responses (synergistic/syntrophic) of bacteria in the food sector, was evident mainly with the transformation of a substratum to edible food (e.g., yoghurt, sausages, olives, etc.). On the other hand, competition for nutrients (e.g., under excess, limitation or starvation), oxygen or hydrogen sources (in aerobic or anaerobic ecosystems respectively), production of substances, i.e., bacteriocins, acids, volatile compounds (e.g., diacetyl), which can restrict growth, can be considered as negative response (antagonistic/competitive interaction) of synergisms (Drosinos, Lampropoulou, Mitre, & Nychas, 1997; Pin, García de Fernando, Gonzalo, & Ordóñez, 2002; Tsigarida & Nychas, 2006).

Another example of the interactive properties of Gram-negative bacteria spoiling foods is their ability to produce chemical communication signals, acylated homoserine lactones (AHLs). It was recently shown that these AHLs compounds can be found in wide range of foods (fish, meat and vegetable products; Smith, Fratamico, & Novak, 2004) and the concentration increases as growth of Gram-negative bacteria takes place. The role of AHLs in (muscle) food spoilage is currently unknown, but several phenotypes (pectinolytic, lipolytic, proteolytic and chitinolytic activities) potentially involved in spoilage of different foods have been linked to AHL regulation in several bacteria (Gram & Dalgaard, 2002). AHLs can be extracted from meat fillets and minced meat at point of spoilage and are produced by several important raw meat spoilage bacteria (Nychas et al., 2007). Elucidation of the role of AHLs in muscle food spoilage will be an important area for future research.

2.2. Extrinsic factors

2.2.1. Effect of temperature

Temperature seems to be the most important factor that influences the spoilage as we all as the safety of meat (Koutsoumanis & Taoukis, 2005). Indeed modern lifestyle and the evolution of consumer requirements over the past decade have led to significant increase of demand for fresh (raw) meat.

The mass consumption of fresh meat and meat products, as well as the new consumer patterns, i.e., reduced cooking times for minimal quality loss, microwave cooking, have accentuated the need for constant and systematic control of the temperature handling of raw meat products, throughout their distribution in the chill chain, from the point of production to their final consumption. Several studies have been recently carried out to assess the importance of low temperature handling of these meat products, focusing on the effect of temperature fluctuations or temperature abuses during handling on product quality (Koutsoumanis and Taoukis, 2005; Koutsoumanis et al., in press, chap. 52, 2006; McMeekin et al., 2006).

Thus an important aspect of meat fresh (raw) distribution and consumption is the effective monitoring of time/temperature conditions that affect both safety and overall quality of meat. It is generally recognized by the European industry, retailers, food authorities and even consumers that several stages of the actual chill chain, such as transfer points or storage rooms, are found to be the weakest link in chilled perishable food management. Meat products, unless appropriately packaged, transported and stored, spoil in a relatively short time.

2.2.2. Meat chill chain

The meat chill chain starts with two maim steps; the primary and secondary chilling. Both steps are important for microbiological stability, eating quality and production yield (Koutsoumanis & Taoukis, 2005).

In particular, the primary chilling is the process of cooling meat carcasses after slaughter from body to refrigeration temperatures. During primary chilling rapid growth of both pathogenic and spoilage microorganisms may occur. Although the European Union legislation requires a maximum final meat temperature of 7 °C before transport or cutting there is not any published information related to limits on chilling time. The rapid reduction of temperature on the carcass surface can prevent microbial growth and extent product shelf life. It is clear that rapid chilling offers a number of other advantages in product quality and production economics.

After primary chilling, any following handling such as cutting, mincing, etc., will increase the temperature of meat, thus the secondary chilling is required to reduce temperature below 7 °C. Secondary chilling is also of great importance in the case of pre-cooked meat products. The temperature of meat after the cooking process should be rapidly reduced from ≈60 to 5 °C, to prevent or reduce growth of pathogens that have been survived the heat process or re-contaminate the product. In addition, rapid cooling of cooked meat products is important for avoiding quality problems caused by the overcooking that occurs during slow cooling.

Among the technologies used to chill meat and meat products before transportation are; (i) air chilling (ii) immersion chilling (iii) spray chilling and (iv) vacuum cooling.

The effectiveness of air chilling applications depends on a number of factors including air temperature and velocity, relative humidity, weight and fat cover of the products, and product loading, while the immersion chilling is probably the least expensive method and provides very rapid cooling with no risk of freezing.

Spray chilling is an alternative method to immersion chilling which has been increasingly used especially in the USA (Allen, Hunt, Luchiari Filho, Danler, & Golls, 1987; Johnson, Doyle, & Cassens, 1988), and is based on combination of sprays and air during the initial stage of the chilling cycle and the use of air only for the rest of the chilling period. Finally, vacuum cooling is a rapid batch process whereby moist products containing free water are cooled by evaporation of moisture under vacuum (Mellor, 1980). The main advantage of this technology is that the rapid cooling under vacuum can significantly reduce bacterial counts of phychrophiles and mesophiles after being stored for several days (McDonald, Sun, & Kenny, 2000). Among the disadvantages of vacuum cooling, the most important is the large weight loss of the meats.

2.2.3. Transportation

During meat marketing (transportation) route to the final user, for preparation and consumption, meat and meat products are stored in tracks, retail cabinets and home refrigerators. These points are of great concern for meat quality and safety.

Indeed industrial and or track's chambers have different characteristics and performances (Koutsoumanis & Taoukis, 2005). Size of the cabinets, initial temperature of the incoming meat, targeted temperature of storage, temperatures of the surroundings, mechanical characteristics (location of refrigeration machinery, compressors, ventilation, and insulation) and energy/cost matters are issues of first priority when considering cold store requirements. The management approach that is dominated in the meat market is related to the principle ''First In–First Out''. This management approach is also strictly adhered to in all stages of the chill chain, in most cases (but not always) through properly designed handling procedures in the chill storage rooms. The different points of transport, from cold storage to the retail outlet, and then to the consumer refrigerator, are critical points for the meat's overall quality and safety. In general, the vehicle must be provided with a good refrigerated system, while another weak point of the distribution is the transport period from the product purchase to

the consumer domestic refrigerator, a matter that has only limited reported in the literature with published data quantifying this parameter.

Temperature conditions within the retail cabinets play a significant role in the product final quality status, and there are several surveys that show a wide variation in product temperatures (Taoukis, Bili, & Gianakourou, 1998). However, since most of the data collected are indicative and do not describe conditions that dynamically changing and temperature distributions just show the "picture" of the situation at the time and place of the study (Taoukis et al., 1998).

The last but not the least part of the chill chain is related to refrigerated distribution. This stage is less studied probably due to difficulties in data collection, concerning temperature conditions in domestic refrigerators and freezers, consumer habits and approximate storage periods before consumption. However, when addressing the quality issue of chilled meat, from production to final consumption, in an integrated and structured way, such a period should be included in the evaluation of quality losses and safety risks in the chill chain.

3. Quantitative evaluation of spoilage

So far, sensory and microbiological analyses are most often used to evaluate the freshness, spoilage or safety of meat and meat products (European Commission, 2005). The disadvantages of sensory analysis, which is probably the most acceptable and appropriate method, is its reliance on highly trained panellists, which makes it costly and unattractive for routine analysis. On the other hand, microbiological analysis, either with traditional numbers (e.g., total viable) or with the use of molecular tools (PCR, RT-PCR, DGGE) are often misleading and scientists have shown that it is more meaningful to measure the responsible for spoilage microflora fraction (Nychas et al., 2007). Unfortunately, microbiological analyses are lengthy (traditional, conventional microbiology), costly and high-tech (molecular tools), and destructive to test products; therefore, efforts have been made to replace both microbiological and sensory analyses with biochemical changes occurring in muscle (e.g., various microbial metabolic products, termed as chemical spoilage indices – CSI), that could be used to assess meat spoilage (Huis in't Veld, 1996).

The philosophical concept of simplification proposed by Baranyi (McMeekin et al., 2006) and the introduction of bioinformatics and or databases such as ComBase and sym'preview as well as the application of predictive microbiology, which uses mathematic equations to describe the kinetics of microorganisms to determine the shelf life of various foods (Koutsoumanis & Nychas, 2000; Mataragas, Drosinos, Vaidanis, & Metaxopoulos, 2006), seems to be a new potential tool for the evaluation of spoilage/freshness. In specific cases, such as spoilage of fresh, cooked, cured meat product during the late stationary growth phase of the ESOs, a correction factor should be included in the developed spoilage model for reliable shelf life predictions Mataragas, Skandamis, Nychas, and Drosinos, 2007. Partial Least Square Regression (PLSR) could be used to investigate the correlation between ESOs growth, metabolic compounds and physicochemical measurements to predict with more accuracy the spoilage level of a product (Nychas & Skandamis, 2006; Olafsdottir, Lauzon, Martinsdottir, & Kristbergsson, 2006).

Recently, with the evolution of bioinformatics new approaches (e.g., principal components analyses – PCA, hierarchical cluster analyses – HCA, artificial neural networks), in parallel with mathematical models, have been applied to evaluate meat spoilage (Figs. 1 and 2; Nychas & Skandamis, 2006; Nychas et al., 2007). These approaches could provide rapidly information related to contribution of the ephemeral spoilage organism (Fig. 1) or to categorization of meat in regard to spoilage (Fig. 2). This step, e.g., HCA, is of great importance since so far mainly the microbiological methods have been used for the actual evaluation of meat spoilage despite the retrospective and the given limitation of their application.

It is well recognised that both meat industry and food (meat) scientists are seeking alternative techniques and/or instruments that will allow them to monitor, or to detect early signs of spoilage or even more to predict microbial spoilage. This prediction will permit for a more efficient management of these products.

3.1. Quantitative (meat) microbiology

In general, predictive or alternatively quantitative microbiology (McMeekin et al., 1997) involves knowledge of microbial growth responses to environmental factors expressed in quantitative terms by mathematical equations. Data and models can be stored in databases and used to interpret the effect of processing, distribution and storage conditions on microbial growth (McMeekin et al., 1997;

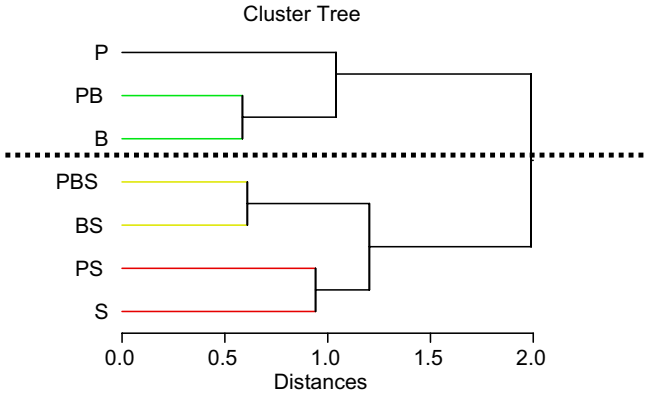

Fig. 1. Hierarchical cluster analysis based on data published from Tsigarida et al. (2003); Two major clusters were evident. The presence of *Shewanella putrefaciens* (S), distinct the type of spoilage in comparison with *Pseudomonads* (P) and *Br. thermosphacta* (B).

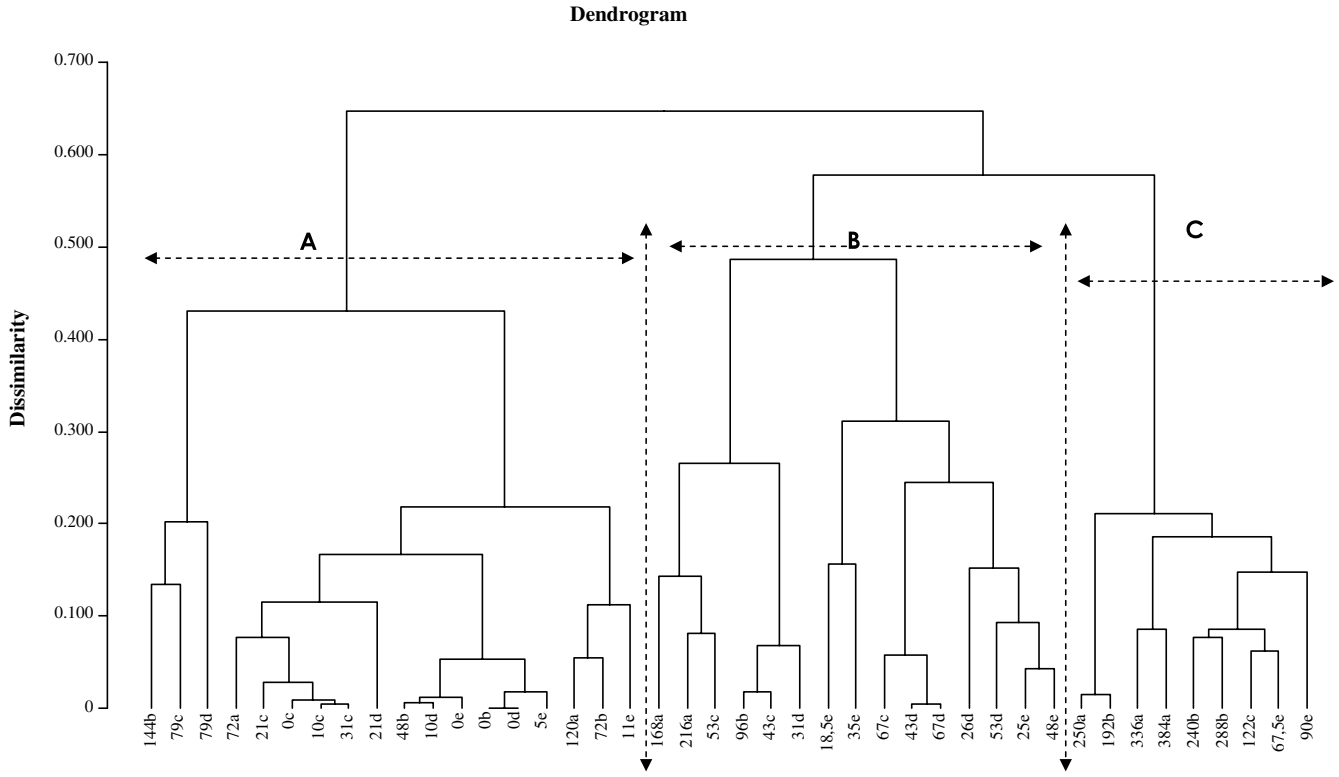

Fig. 2. The changes in the profile of 8 organic acids, of natural contaminated pork meat, as obtained during its aerobic storage at different temperatures (0, 5, 10, 15 °C), could be analysed with hierarchical cluster analysis and to further qualitatively categorized (A, fresh high/good quality; B good quality and C spoiled) (Nychas & Skandamis, 2006).

Van Impe & Bernaerts, 2000). This approach provides precision in estimating shelf life of foods. In addition, the combination of data on the environmental history of the product and mathematical models may lead to "intelligent" product management systems for the optimization of food quality and safety at the time of consumption (Giannakourou et al., 2001; Koutsoumanis et al., 2002; Koutsoumanis et al., in press, chap. 52, 2006).

Recently, the ability of using mathematical models that describe spoilage has been developed indirectly by developing and validating models that quantitatively estimate growth of these ephemeral organisms and as consequence are able to predict the shelf life of meat or other muscle foods (Koutsoumanis et al., 2002; Koutsoumanis et al., in press, chap. 52, 2006) (Table 8). Despite this progress however, predictive spoilage models remain a research tool rather than an effective application for meat industry (McDonald & Sun, 1999). The reasons for this include:

The lack of information required for the application of models for predicting shelf life of foods (e.g., ephemeral spoilage organisms, spoilage domain, spoilage level).

Most models are developed based on observations in a well-controlled laboratory environment using microbiological media. However, the complexity of the meat matrix, the quantification or even the categorization some of its features and their potential effects on microbial population dynamics or the ability to recover a target organism from a meat sample have been proved a difficult task and certainly influence the accuracy of the prediction. An example is the effect of food structure, of which findings confirm, that growth may be limited due to food matrix (Koutsoumanis et al., 2004; Koutsoumanis, Dourou, Nychas, unpublished; Wilson et al., 2002).

The majority of models have focused on the effect of the environmental factors on maximum specific growth rate without taking into account the lag phase. It has been shown, however, that the lag phase duration of the ESO can be a significant part of the total shelf life of foods (Koutsoumanis & Nychas, 2000; Koutsoumanis, 2001). Ignoring lag phase may lead to underestimated shelf life predictions with significant economic losses for the food industry.

Most models are developed and validated under static temperature conditions. In practice, however, temperature fluctuations may be frequent during storage and distribution of foods. Thus, validation at changing temperatures is of great importance for evaluating the performance of the model in predicting shelf life under real chill chain conditions. In fact, there are limited [Combase (Combase www.combase.cc), Koutsoumanis and Nychas Microbial Spoilage Predictor (MicroSPred), Dalgaard, Seafood Spoilage Predictor (SSP)], successfully validated models for the growth of ESOs that have been included in application software and this has facilitated prediction of food shelf life under constant and dynamic temperature storage conditions.

Table 8
Software available for modelling microbial spoilage

Kinetics characteristics of certain spoilage bacteria are available from the Growth Predictor (UK) – www.ifr.ac.uk/Safety/GrowthPredictor/ (Based on data previously used in the FoodMicromodel software)
The French approach 'Sym'Previus – www.symprevius.org' under development; kinetic data are also available in this database
Seafood Spoilage (Shelf life of seafoods and growth of specific spoilage organisms)
Safety monitoring and assurance system (Greek predictive microbiology application software under development); software is based on kinetic data of spoilage bacteria derived from meat (minced pork) *in situ*
Pathogen Modelling Program (USA) – www.arserrc.gov/mfs/pathogen.htm 37 models of growth, survival and inactivation; frequently updated (version 7.0); Available free of charge during the last 15 years; ~5000 downloads per year

Modified from Nychas et al. (2007), and www.arserrc.gov/mfs/pathogen.htm; www.ifr.ac.uk/Safety/GrowthPredictor/ and http://www.dfu.min.dk/micro/sssp/Home/Home.aspx.

3.2. Chemometrics

It is well recognized that there is lack of general agreement on the early signs of incipient spoilage for meat. This issue makes all more difficult the task to evaluate it objectively mainly due to changes in the technology of meat preservation (e.g., vacuum, modified atmosphere, etc.). The use of microbial metabolites as consequence of microbial growth in meat has been continuously recognized as a potential means for assessing meat quality (Dainty, 1996; Ellis, Broadhurst, Kell, Rowland, & Goodacre, 2002; Jay, 1986; Nychas et al., 1998; Sutherland, 2003).

The attempts (Table 9) that have been made over the last two decades to associate given metabolites with the microbial spoilage of meat have not been very much appreciated, due to low understanding of the phenomena (Nychas et al., 2007). The basic concept for these methods, that has been reviewed recently (Ellis & Goodacre, 2001; Nychas et al., 2007) is that as the bacteria grow on meat, they utilize nutrients and produce by-products. The quantification of these metabolites could provide us information about the rate of spoilage. The identification of the ideal metabolite(s) that can be used for spoilage assessment has been proven a difficult task. This is due to the fact that the specificity of most metabolites to certain organisms is not always the correct approach. Moreover, when these organisms are not present or inhibited by the natural or imposed from man, food ecology, this provides incorrect spoilage information. Metabolites contributing to spoilage, which is the result of the consumption of a specific substrate but their absence or their presence in low quantities, do not preclude spoilage. Additionally, the accurate detection and their measurements require sophisticated procedures, high educated personnel, time and equipment and even though many of them give retrospective information which is not satisfactory for the industry.

Table 9
Compounds potentially useful for the assessment of shelf life of raw meat and fish under different packaging conditions

Compound	Test	Packaging conditions[a]	Red meat and poultry
Glucose	Enzymatic kit	Air, VP, MAP	Y
Acetate	Enzymatic kit, HPLC	VP, MAP	Y
Gluconate	Enzymatic kit	Air, VP, MAP	Y
Total lactate	HPLC	VP, MAP	Y
D-Lactate	Enzymatic kit	VP, MAP	Y
Ethanol	Enzymatic kit, GLC	VP, MAP	Y
Free amino acids	Chromatometric	Air	Y
Ammonia	Enzymatic, colorimetric	Air	Y
Acetone, methyl ethyl ketone, dimethyl sulfide, dimethyldisulfide, hydrogen sulfide	GLC, GC/MS, Sulfur Selective detector	VP, MAP	Y
Diacetyl, acetoin	Colorimetric	VP, MAP	Y
Biogenic amines	HPLC, sensors, enzymic test, GLC, Enzyme electrodes, test strips	Air, VP, MAP	Y
Diamines	Amperometric electrodes (enzymatic systems)	Air	Y
Microbial activity	Enzymic/Resazurin	Air	Y
Volatiles (odors)	Electronic noses, PTR-MS (chemical sensors)	Air, VP, MAP	Y
Proteolysis (amides, amines, etc.)	FT-IR, NIR, MIR	Air, VP, MAP	Y

Modified from Nychas et al. (2007).
[a] VP = vacuum packaged; MAP = modified atmosphere packaged.

Recently, some interesting analytical approaches are being forwarded for the rapid and quantitative monitoring of meat spoilage. These are the biosensors (enzymatic reactor systems), electonic noses (array of sensors), Fourier transform infrared (FT-IR). Integration of the FT-MIR attenuated total reflectance biosensors or other biosensors and information platform and development of an "expert system" to automatically classify the sensorial input into a "diagnosis" based on extracted pre-processing features. However, the enormous amount of information provided by the last mentioned technology makes the data produced unmanageable. The application of advanced statistical methods (discriminant function analysis, clustering algorithms, chemometrics) and intelligent methodologies (neural networks, fuzzy logic, evolutionary algorithms and genetic programming) can be used as qualitative indices rather quantitative since their primary target is to distinguish objects or groups or populations (Goodacre, Vaidy-

nathan, Dunn, Harrigan, & Kell, 2004). This is an *unsupervised* learning method (Ellis & Goodacre, 2001). Nowadays, the modern machine learning procedures use *supervised* learning algorithms (Beavis et al., 2000; Goodacre, 2000; Shaw et al., 1999). The last mentioned approach together with the development of artificial neural networks (ANN) could be shortly used to implement the evaluation of meat spoilage.

4. Conclusions

Regardless of the methodology used for the quantitative evaluation of spoilage for control purposes, factors such as (i) food structure and physicochemical parameters (e.g., type, concentration and nutrient availability, diffusivity, etc.) (ii) microbial competition and physiological stage of the bacterial cells as well as effects of dynamic storage (fluctuation of temperature, packaging in vp/map, film permeability, etc.) conditions, and (iii) understanding of microbial ecology and determination of the mechanism (bacterial communication, decipher of function of genes-genomics) of growth/survival of established and emerging pathogens and spoilage bacteria in stressful food environments should be taken into account (Nychas & Skandamis, 2005). Thus by understanding *where* specific metabolites (metabolomics) originate from (i.e., responsible organism, substrate) *how* these are regulated at the cell level (genomics–proteomics), *what* is the effect of meat characteristics as well as the microbial association on the rate and the type of the metabolites formation, we will be able to know *when* and *how* to exploit them for the benefit of the industry, authorities and consumer. Indeed the meat industries need rapid analytical methods or tools for quantification of these indicators to determine what kind of processing is suitable for their raw material and to predict remaining shelf life of their products. *Inspection authorities* need reliable methods for control purposes. *Retail and wholesale* need these valid methods to ensure the freshness and safety of their products and in case of disputes between buyers and sellers. Reliable indication of the safety and quality status of meat in retail and until consumed is desirable. It is therefore crucial to have *valid methods to monitor* freshness and safety to be able to ensure *what* the quality is, regardless of *whose* perspective you take, i.e., that of the consumer, the industry, the inspection authority, or the scientist.

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