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Chemical/biochemical detection of spoilage

R.H. Dainty*

MATFORSK, Norwegian Food Research Institute, Osloveien 1, N-1430, Aas, Norway

Abstract

Although sensory and/or microbiological analyses are widely relied on when assigning shelf-life of foods or trouble shooting problems with spoilage under storage, they do have drawbacks. Delay in obtaining results is one of them. The expense of the expert panels required to obtain meaningful sensory evaluations is another, while spoilage is not always of microbial origin. Even when it is, there are an increasing number of situations, including that of meats and fish packaged in modified atmospheres, where the relationships between microbial growth and spoilage onset is poorly defined.

Chemical analysis has long been recognized as a means of circumventing at least some of the drawbacks and its potential is reviewed below. From the data presented it can be concluded that chemical characterization of spoilage processes is presently of most value in trouble shooting i.e., establishing the causes of spoilage. Its value in assigning total or remaining shelf-life requires more knowledge of the chemical processes leading to reduced acceptability/spoilage and of their correlations with sensory and microbiological changes.

Keywords: Spoilage; Shelf-life; Chemistry

1. Introduction

Off-odours and off-flavours are a common cause of spoilage in all branches of the food industry and the economic consequences can be serious. Rapid and effective means of identifying the causes, trouble shooting and thereby being able to

* Corresponding author. Tel: +47 649 70100; fax: +47 649 70333.

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instigate remedial action with a minimum of delay are therefore essential. It is equally important to have reliable methods of assigning/predicting shelf-life during development of new or modified products. Being able to predict shelf-life of individual batches or consignments under routine production and to monitor remaining shelf-life under storage or display would also be a great boon.

Sensory and/or microbiological analyses are most widely used to serve these purposes in today's industry. Whilst sensory analysis is appropriate and indeed essential for product development, its reliance on highly trained panels to minimize subjectivity, makes it costly and therefore unattractive for the other more routine requirements. Nor, without supplementary studies of a detailed nature, do sensory studies establish the cause of problems. Microbiological methods, at least in their traditional form, give retrospective information which is satisfactory for product development but again less so for the other requirements. More rapid alternatives continue to be developed, e.g. epifluorescent microscopy, flow cytometry, electrical impedance, etc. Like the traditional methods they also presuppose that the specific spoilage organisms are known and detectable by the chosen technique. That this is not necessarily so is well illustrated by the demonstration of *Photobacterium phosphoreum* as the major spoiler of vacuum packed cod rather than *Shewanella putrefaciens* as had long been thought (Dalgaard et al., 1993). Clostridial spoilage of chilled vacuum packed raw beef provides another example (Dainty et al., 1989). And even when the specific spoilage organisms are well established, there are instances where their enumeration is of limited value. On vacuum and low O_2 gas packed meat, the supposed spoilers, lactic acid bacteria, can reach a maximum and remain at that level for a poorly defined period without outward signs of sensory spoilage. Furthermore, on high pH (> 6.0) meat, spoilage occurs at lower cell densities than on normal pH (< 5.8) meat.

An alternative or ancillary method to microbiological analyses involves the measurement of chemical changes associated with microbial growth processes in foods. Although the idea of their use is far from new, its application has not been so intensively researched as the sensory and microbiological methods in routine use today. The present status, with particular emphasis on more recent developments and potential for practical use is reviewed below. Although the intention has been to cover all foods the author's own practical experience is undoubtedly reflected in the choice of examples.

2. Diagnosis of spoilage problems-troubleshooting

2.1. Miscellaneous chemicals

The diagnostic power of chemical change for trouble shooting microbial spoilage is well illustrated by examples for a range of foods investigated by Whitfield and Tindale (1984).They found *bis*(methylthio) methane and trimethylarsine associated with garlic off-flavours in prawns and sand lobster; dimethyltrisulphide and indole with rotten onion-like flavours in prawns; 2-methyl isoborneol and geosmin with

earthy, muddy off-flavours in canned mushrooms; *p*-cresol, skatole and indole with pigsty-like flavours in french fries; and various chlorinated anisoles with muddy off-flavours in packaged cocoa beans. Although specific microbial sources were not unequivocally established for all of the compounds, compelling evidence that they were all of microbial origin, and suggested pathways of formation, were inferred from other studies as follows.

Erwinia caratovora and *Clostridium* spp. are known to be causative agents of soft rot in potatoes and to be able to produce one or more of the compounds responsible for the pigsty-like odours of the french fries. Fungal methylation of the chlorophenol-containing antimicrobial in the seam adhesives of the paper sacks containing the cocoa beans, was the suggested source of the beans' mouldy odour. Microbial methylation of chlorophenols in poultry house litter had previously been shown to be the cause of musty taint in chicken (Curtis et al., 1974). Each of the sulphur-containing compounds isolated from the prawns with garlic and rotten onion odours is a well established end-product of typical bacterial contaminants of a range of foods including sea foods. A likely source of the geosmin and isoborneol in the canned mushrooms was suggested to be actinomycetes in the water supply. In a later investigation of earthy off-flavours, in a bulk shipment of wheat flour and bread made from it, geosmin and 2-methyl isoborneol were again isolated (Whitfield et al., 1991). Their concentrations were above their respective detection thresholds in water and a strain of *Streptomyces griseus* isolated from the wheat was shown to produce the chemical and sensory spoilage characteristics.

There are many other reports of fungal production of the same two compounds in cereals. It is clear that their production, together with that of other sesqui- and mono-terpenes, is highly strain dependent and therefore of use for the detection of specific strains (Borjesson et al., 1992). Other volatile metabolites are produced by a wider range of fungi and therefore of greater potential value as general indicators of fungal growth. They include 3-methyl furan, which is of particular interest, 2-methyl propanol, 2- and 3-methyl butanols and 1-octen-3-ol (Borjesson et al., 1992). In Sweden, the odour of cereals is used as an official indicator of quality and a more objective means of detecting fungal growth would be of great value because determination of fungal colony forming units (cfu) is poorly correlated with biomass. Use of volatiles could serve this purpose and at the same time eliminate some sampling problems and avoid localized points of contamination being overlooked.

2.2. Volatile fatty acids

Differentiation between sporeformers and non-sporeformers as the cause of swelling of low acid canned foods has been suggested (Schafer et al., 1985) on the basis of the production of *n*-butyric acid and D(−) 2,3-butanediol by the sporeformers but not by the non-sporeformers (Schafer et al., 1985). Such information helps to distinguish between under processing and post-process recontamination as the cause of the problem and the method is the only way of examining samples where auto-sterilization has occurred. The method has been adopted by the

Association of Official Analytical Chemists (Anon, 1989). In a similar way, *n*-butyric acid has been successfully used to establish microbes as the cause of spoilage of a range of commercially sterile vegetable, fruit, meat, fish and dairy products (Eyles and Adams, 1986). The presence of *n*-butyric acid was in each case associated with the presence of clostridia. Furthermore, two of the meat samples had substantially different levels of *n*-valeric acid, a finding consistent with the isolation of two different clostridia from the respective products.

Detection of *n*-butyrate also played a key role in establishing *Cl. estertheticum* (Dainty et al., 1989; Collins et al., 1992) and *Cl. laramie* (Kalchayanand et al., 1993) as the respective causes of two separate incidents of spoilage of vacuum packed raw beef, and *Cl. algidicarnis* (Lawson et al., 1994) of an incidence of spoilage of salted, cooked, vacuum packed pork. There was no evidence of temperature abuse in any of the incidents, each of the three previously unrecognized causative organisms being cold tolerant. *Clostridium estertheticum* is psychrophilic, the other two are psychrotrophic.

Yet other isolations of clostridia from mild temperature abused sous-vide fish products (Hansen et al., 1995) and from chilled and mild temperature abused meat samples (Broda et al., 1996) suggest analysis of *n*-butyrate could be increasingly useful in this expanding area of convenience foods.

Accumulation of propionate and acetate in particular proportions, has been shown to have promise for the detection of *Pectinatus* spp. as agents of an off-flavour condition in beers which is accompanied by turbidity/haze (Membre et al., 1994). Both acids were produced at levels above their flavour thresholds by *Pect. frisingensis* from levels of glucose typical of beer.

Rapid analysis of volatile fatty acids, with *n*-butyric, 3-methylbutyric, 2-methylpropionic and valeric acids being of particular interest, also offers the possibility of differentiating between the causes of off-odours in paperboards (Ziegleder et al., 1995). The acids are formed by microbial action in the circuit water of paper mills and can lead to problems in packaged foods.

2.3. Indole and hydrogen sulphide

Increases in indole attributed to growth of Enterobacteriaceae have been detected during aerobic and vacuum packed storage of bovine tripe (Giaccone et al., 1994) and suggested as a reliable indicator of the product's sensory and hygienic quality.

In another example, vacuum packed ham samples supporting a microbial flora dominated by lactic acid bacteria, but having a very unpleasant odour, were received for investigation in the author's laboratory (Dainty et al., unpublished). Gas chromatographic detection of indole together with H_2S suggested Enterobacteriaceae could be the cause, but their presumed numbers as indicated by counts on violet red-bile-glucose agar appeared too low ($<$ log 1.5). On the other hand, numbers on cephaloridine-fusidin-chloramphenicol agar (CFC), included to enumerate pseudomonads, were unusually high (log 4.8). The majority of isolates on CFC were however oxidase negative and identified as *Morganella morganii*, which in accord with its known ability to produce the two identified metabolites, was shown to be the culprit in inoculation studies.

Production of H_2S in aerobically stored meats can be used as an indication of high numbers of Enterobacteriaceae, and therefore hygiene problems because it is not produced by pseudomonads. Likewise, high levels in vacuum packed normal pH meat point to the growth of particular stains of lactic acid bacteria (Egan et al., 1989; Borch and Agerhem, 1992).

2.4. Metabolites of sorbate

Off-flavours variously described as hydrocarbon-, kerosene-, paint- and solvent-like, occur from time to time in a number of products including cheese, wine and non-carbonated soft drinks (Daley et al., 1986). The cause is accumulation of *trans*-1,3-pentadiene formed by microbial decarboxylation of the preservative sorbic acid (*trans,trans*,-2,4-hexadienoic acid). The reaction is catalysed by mould and *Penicillium* spp. in particular, but growth of *Paeciliomyces varii* and *Debaromyces hansei* in margarine, and of an unidentified yeast(s) in Cresceni cheese has led to the same problem (Sensidoni et al., 1994). Both types of organism were able to grow and carry out the transformation in the presence of 3000 ppm sorbic acid.

A different transformation of sorbic acid, leading to 2-ethoxyhexa-3,5-diene and the development of a 'geranium' defect in wine, has been attributed to lactic acid bacteria (see Daley et al., 1986). Detection of both metabolites of sorbate therefore immediately suggests their likely source and the implementation of appropriate action.

2.5. 4-Vinylguaiacol

The presence of 4-vinylguaiacol (ferulic acid) in most lagers, beers and stouts can lead to phenolic tastes and consumer complaints. The cause is contamination with a wild yeast strain or bacteria with the ability to decarboxylate ferulic acid, a component of barley grains, to the offending guaiacol (Madigan and McMurrough, 1994). Its detection therefore allows rapid implementation of appropriate hygiene measures.

2.6. D-alanine

D-Amino acids are important components of bacterial peptidoglycans. Results on a variety of fruit juices showed that the presence of > 1 ppm D-alanine indicates bacterial as opposed to yeast contamination, and that it could be used as a quality indicator (Gandolfi et al., 1994).

The above examples illustrate the powerful diagnostic value of analysis of chemical changes arising from microbial growth in foods. In all cases the analyses were done using simple gas chromatographic analysis of aqueous or solvent extracts of foods. Thus a relatively simple, rapid technique, with great resolving power is available to be put to routine trouble shooting of spoilage problems.

3. Shelf-life assignment

3.1. Use of microbial growth substrates

3.1.1. Glucose

The results of a series of studies by Gill and co-workers showed glucose to be the initial substrate supporting growth of all the major types of bacteria making up the storage flora of red meats of normal or high pH, stored chilled in air, vacuum packs or modified gas atmospheres (see Gill, 1983). Other studies have confirmed and extended these findings (Nychas and Arkoudelos, 1990; Borch and Agerhem, 1992; Drosinos and Board, 1994, 1995a). Depending on its initial concentration, glucose may become depleted and only at this point do other substrates begin to be metabolised. These include lactate, amino acids and creatine under aerobic storage and lactate and arginine during vacuum and gas pack storage.

Under aerobic storage, spoilage is most frequently associated with the post-glucose utilization of amino acids by pseudomonads. Together with knowledge of bacterial growth rates, glucose concentration would therefore appear to offer a way of determining the time to onset of the spoilage determining reactions, and therefore a measure of expected shelf-life. In support of this is the onset of spoilage at lower cell numbers in high pH meat, which has a naturally lower glucose content than normal pH meat (Gill, 1983). An alternative (Kress-Rogers et al., 1988) is measurement of the glucose gradient which develops in meat as bacteria grow at the surface (Gill, 1983). The slope of the gradient was shown to correlate with bacterial numbers and could be related to incipient spoilage, thus providing a real time evaluation of remaining shelf-life. Natural variation in the glucose content of meat precludes determination of the actual concentration from being used in this way. To sense the gradient a linear array of electrodes coated with glucose oxidase and ferricenium ions was mounted behind the cutting edge of a spear-shaped probe. When inserted to a predetermined depth into a meat sample, the glucose gradient could be determined via the ferricenium ion mediated transfer of electrons from the enzyme to the electrode elements. At the last reported stage of development, sensitivity restricted use of the technique to the immediate pre-spoilage stage of storage (ca. log 6.0 bacteria/g).

The relevance of glucose determination to shelf life of vacuum packed normal pH meat is a little more complicated. Although depletion of surface glucose coincides with attainment of maximum cell numbers,which typically equates with lactic acid bacteria, there is an ill-defined lag before the onset of spoilage. Determination of surface depletion or initial concentration is therefore presently of limited value. Both may be more relevant for high pH meat whose spoilage typically results from the growth of Gram negative bacteria like *Shewanella putrefaciens* and Enterobacteriaceae.

An initial glucolytic phase probably characterizes bacterial growth on poultry (Kakouri and Nychas, 1994) and fish (Beatty and Collins, 1939). However, the relatively low levels in both foods probably limits their potential use.

The same applies in the case of fortified sweet wines (portwines) where the metabolism of glucose and fructose by ethanol tolerant lactobacilli e.g. *Lact.hilgardii* results in so-called 'mannite' spoilage (de Revel et al., 1994). A fall in a known initial concentration would imply that spoilage was already under way and the method therefore of confirmatory rather than predictive value. The authors have however demonstrated the potential predictive value of measuring L-malate disappearance in a malolactic fermentation by the same organism, though the end product, L-lactate is probably better (see below).

3.2. End-products of microbial growth

The range of end-products of microbial growth which have potential for use in the determination/prediction of shelf-life is far wider than that for substrates. Because of its key role as a substrate for microbial growth, the end-products of glucose have received a lot of attention.

3.2.1. Gluconic and 2-oxogluconic acids

In accord with classical studies, oxidation of glucose by pseudomonads during the aerobic storage of meat has been shown to lead to the extracellular accumulation of gluconic and 2-oxogluconic acids in sliced beef (Farber and Idziak, 1982) and of gluconic acid (the oxo-acid was not determined) in minced beef (Nychas et al., 1988). The variable glucose content of meat coupled to the transient nature of the phenomenon, with the acids being themselves oxidized upon glucose depletion, could negate any possible use for the acids in determining spoilage. However, the results of an unpublished study with aerobically stored beef sirloin steaks suggest otherwise (Dainty et al., unpublished). Steaks of normal pH were stored in air at 1°C and five analysed using HPLC on each of twelve consecutive days. Of 27 samples having a total viable count (TVC) $<$ log 6.0, just two showed detectable levels of gluconate, whilst the 33 samples having TVC $>$ log 6.5 each contained the acid. This included many samples with TVC $>$ log 8.0 and exhibiting obvious signs of spoilage. Though there was no closer correlation between TVC and gluconate concentration, the presence of gluconate was an indicator of approaching spoilage. A very similar division between samples was apparent from their 2-oxogluconate content, whose concentrations, based on HPLC peak heights, were ca. 5-fold lower than those of gluconate. The latter was also detected in 12 samples of minced lamb stored in a high O_2/CO_2 atmosphere and having TVC values between log 5.3 and log 8.9 (Drosinos and Board, 1995b).

3.2.2. L- and D-lactic acids, acetic acid and ethanol

In the case of red meats stored in vacuum packs or low O_2 modified atmospheres, there is, as mentioned previously, a poorly defined lag between attainment of maximum cell numbers and the onset of sensory spoilage. In view of this, chemical indicators of spoilage would be of enormous value. To this end the major end-products of fermentation of glucose by lactic acid bacteria, i.e. L- and D-lactic acids, acetic acid and ethanol have received a lot of attention. For ground beef

samples stored at 7°C in relatively impermeable material, increases in total lactic acid (presumed by the present author to be predominantly the L-isomer) were observed (Nassos et al., 1983). A statistically significant correlation was found between lactate content and odour acceptability with 50% of the panelists rejecting all beef samples containing > 704 mg lactate/100 g meat. Microbial counts were all > log 7.0.

This highly promising result and others (Nassos et al., 1988) have not generally been confirmed in subsequent work in which the L-isomer has been specifically analysed. These include studies of naturally contaminated minced beef of normal and high pH stored in 100% CO_2 or N_2 (Nychas and Arkoudelos, 1990); beef slices inoculated with a *Lactobacillus* sp. or a *Leuconostoc* sp. and stored in 95% CO_2/5% N_2 (Borch and Agerhem, 1992); and chicken breast and thigh meat stored in vacuum, 100% CO_2, 100% N_2, or 80% O_2/20% CO_2 (Kakouri and Nychas, 1994). In the latter study, overall decreases in concentration at the end of storage were preceeded by clear increases, but in all the other cases decreased or unchanged concentrations were reported. The majority of samples of minced lamb stored in a high O_2/CO_2 atmosphere also showed decreases in concentration (Drosinos and Board, 1995b). However, in the few samples in which *Brochothrix thermosphacta* dominated rather than lactic acid bacteria as in all the other samples, increases in L-lactic acid were recorded. Clearly more results are needed to clarify these apparent discrepancies.

More consistent and promising results have been obtained for D-lactate. Unlike the L-isomer it is not found in fresh foods, making its production far easier to detect. It is also a far better diagnostic feature of the specific presence of bacteria. Its potential value can be inferred from its demonstrated accumulation in a range of meat products, including vacuum packed Bruhwurst (Sinell and Luke, 1979), vacuum packed pork (de Pablo et al., 1989), pork stored in CO_2 and O_2 enriched atmospheres (Ordonez et al., 1991), beef stored in 95% N_2/5% CO_2 (Borch and Agerhem, 1992), and minced lamb stored in a high O_2/CO_2 atmosphere (Drosinos and Board, 1995b). In a study of a total of 72 beef joints of which 8 were sampled each week over a period of 9 weeks, the vast majority of samples having a TVC > log 6.4 had a D-lactate content in excess of 100 mg/g (Table 1). The acid was not detected in the majority of samples with lower microbial count, thus indicating its potential as an indicator of microbial quality.

In several of the studies referred to above (Ordonez et al., 1991; Borch and Agerhem, 1992; Drosinos and Board, 1995b; Dainty et al., unpublished) acetate levels were also determined. The general consensus was that acetate concentrations increased during storage and showed promise as an indicator of shelflife/microbial quality of vacuum and gas packed meat. From the author's unpublished study it was again possible to define a concentration, in this case 8 mg/100 g meat, separating samples with microbial loads above and below log 6.4 (Table 1). It is therefore difficult to explain reported decreases in acetate levels in naturally contaminated ground beef stored in 100% CO_2 (Nychas and Arkoudelos, 1990).

Ethanol, the other major end-product of heterofermentative metabolism in lactic acid bacteria, was found in higher concentrations in unacceptable than in accept-

able samples of beef inoculated with a *Leuconostoc* sp. and stored in 95% N_2/5% CO_2 (Borch and Agerhem, 1992). Readily detectable levels were also detected in the author's unpublished study of vacuum packed samples of beef referred to above. As for D-lactate and acetate it was possible to differentiate samples with bacterial numbers above and below log 6.4, this time at a concentration of 1.5 mg/100 g meat (Table 1).

The concentrations of the three end-products were of similar magnitude in each of the studies referred to above, thereby increasing the general potential value of their determination. Only in the pure culture inoculation study of Borch and Agerhem (1992) was any attempt made to equate concentrations with sensory data. More data of this kind, but for naturally contaminated samples, is needed to be fully able to evaluate the potential of the chemicals as indicators of shelf-life.

3.2.3. *Biologically active amines*

Unlike lactate, acetate and ethanol, tyramine, another compound produced by some lactic acid bacteria, has no known sensory properties of relevance to spoilage. However, concern has been expressed from time to time about its production in view of its vasoactive properties. Its accumulation at bacterial numbers above log 6.0 has been reported in many studies of vacuum packed beef (Dainty et al., 1987; Smith et al., 1993; Krizek et al., 1995; Yano et al., 1995), of pork stored in CO_2 and O_2 enriched atmospheres (Ordonez et al., 1991) and of tuna steaks stored in various atmospheres enriched with O_2 and CO_2 (Lopez-Galvez et al., 1995). In the unpublished study of Dainty et al. referred to above, all 52 samples of vacuum packed beef with a TVC above log 6.4 had levels of the compound ranging from 0.1–1 mg/100 g meat. It was detected in only one of the 20 samples with a lower TVC. Yano et al. (1995) used a tyramine sensor based on tyramine oxidase activity in addition to a HPLC method in their study. Samples were passed through a column of glass beads coated with the enzyme and O_2 consumption measured

Table 1
Relationship between bacterial numbers and the acetate, D-lactate and ethanol content of vacuum packed joints of beef stored at 1°C for up to 8 weeks[a]

Compound	Concentration mg/100 g	Number of samples with total viable count (log_{10} number/g)	
		<6.4	>6.4
Acetate	<8	18	4
	>8	1	48
D-lactate	<100	18	1
	>100	1	52
Ethanol	<2	19	7
	>2	0	46

[a] Data from unpublished study of 72 samples (Dainty et al.).

electrochemically. Although less sensitive then the HPLC method, tyramine was detectable before off-odour development in the samples. With real time answers thus available the method has clear potential for routine use as an indirect indicator of shelf-life.

3.2.4. Volatile compounds

All of the analyses described so far require extracts of foods to be made. Analysis of volatile compounds above foods, which will include the actual chemicals responsible for spoilage, does not. At least some of the problems inherent in sampling are thereby avoided and the food itself is not disturbed. These obvious attractions have been clearly illustrated in the development of a rapid and objective method for predicting the shelf-life of milk (Vallejo-Cordoba and Nakai, 1994a). Volatile compounds produced during an 18 h. incubation of refrigerated milk samples of varying ages were analysed by dynamic headspace capillary gas chromatography. Using principal component regression (PCR) a high correlation was established between the complex mixtures of volatile compounds formed and the shelf-life of the refrigerated milk as determined by sensory analysis. The standard error of estimate of shelf-life, using the regession equation developed, was less than 2 days. This compared very favourably with that determined in standard storage trials and was better than that based on standard microbiological tests on the same samples. For a product with a normal expected shelf-life of 14 days the method is therefore rapid enough to be of practical use. It has the further advantage of taking into account the microbiological status of the initial product, information which, even if it could be obtained on the same time scale, would not necessarily give a satisfactory estimation of shelf-life. Use of artificial neural networks to analyse similar data gave an even better correlation between sensory and volatile compound data (Vallejo-Cordoba et al., 1995).

Applying a different mathematical treatment to the results allowed the milk samples to be grouped, with only a small degree of overlap, into categories of good, marginal and poor quality or into normal, fruity, malty and rancid flavour groups (Vallejo-Cordoba and Nakai, 1994b). Such possibilities are of value in deciding what the milk will be used for and in helping to pinpoint likely causes of poor quality and appropriate remedial action. At least nine of the 27 volatiles used in the analyses could be associated with known spoilage bacteria. These were 2- and 3-methylbutanals, 2-propanol, ethyl hexanoate, ethyl butanoate, 1-propanol, 2-methylpropanol and 1-butanol. Other compounds were identified as oxidation production of lipids. This points to another big advantage of the analysis of volatiles over other methods, namely its ability to give information about different problems e.g. chemical, enzymatic and microbiological, simultaneously.

Progress in the use of volatiles for other foods is less advanced, although experiments with naturally contaminated and inoculated samples have provided good background information for aerobic, vacuum packed and gas atmosphere packed meat (Dainty et al., 1985; Edwards and Dainty, 1987; Stutz et al., 1991; Jackson et al., 1992), and for aerobically stored chicken (Viehweg et al., 1989a,b) and fish (e.g. Miller et al., 1973). Complex mixtures of compounds, with varying

numbers of components in common, were found to be associated with the aerobic storage of each of these foods. Stutz et al. (1991) suggested acetone, methyl ethyl ketone, dimethyl sulphide and dimethyl disuphide as possible indices of spoilage of ground beef. Dainty et al. (1985) reported a defined time sequence of production of volatiles in naturally contaminated non-comminuted beef samples. Amongst those appearing first, and therefore with the greatest potential for early detection of spoilage, were acetoin and diacetyl, though their detection was rapidly followed by that of esters and the sulphur-containing compounds found by Stutz et al. (1991). It appears that the presence of H_2S is a clear indication of the growth of an atypical flora including Enterobacteriaceae because as mentioned earlier, pseudomonads do not produce this compound.

Two particular volatiles, acetoin and diacetyl, have been suggested as useful indicators of the microbial quality/spoilage of pork stored in O_2 and CO_2 enriched atmospheres (de Pablo et al., 1989; Ordonez et al., 1991). Both compounds were present as major early components of the headspace volatiles of beef, pork and lamb stored in 75% O_2/25% CO_2 (Dainty et al., unpublished). Although these latter studies cast some doubt on whether their initial production is microbial or not, there were clear increases in concentration throughout storage.

The potential for use of ethanol, which is readily detected by headspace analysis, has already been discussed as a potential indicator of spoilage of vacuum packed beef. Its use as a potential indicator of spoilage and leakage of modified atmosphere packs of marinated chicken breast and rainbow trout has also been suggested (Randell et al., 1995).

The advantages of use of analysis of volatile compounds in this way is increasingly being realized. Of particular interest in this context is the recent commercial availability of so-called electronic noses. With this technique volatiles are detected, but not identified, through their relatively non-specific adsorption to electronic sensors e.g., gas sensitive metal oxide semi conductor field effect transistors and conducting organic polymers. The responses are analysed within the instrument using pattern recognition techniques such as artificial neural networks and results printed out in real time. Initial results from a laboratory-built instrument have given promising results in the prediction of shelf-life of ground beef and pork stored in air (Winquist et al., 1993). The technology is such that portable instruments suitable for use during storage and display of products should be feasible.

Perhaps the most tested and discussed of all volatile compounds for use as an indicator of spoilage is trimethylamine, which is formed from the energy yielding metabolism of bacteria growing on fish. Opinions as to its usefulness differ despite its being thought to be largely responsible for the characteristic 'fishy' odours of spoiling seafoods (Gill, 1992). Reasons include the varying content of its precursor, trimethylamine *N*-oxide, in different fish species and seasonal variations within a species. Recent publications in which its production has been found to be of value as an indicator of spoilage include: Dalgaard et al. (1993) who studied cod stored in varying concentrations of CO_2; Reddy et al. (1994) who studied tilapia fillets packed in N_2/CO_2 mixtures; and Rehbein et al. (1994) who studied ice-stored redfish.

3.2.5. Microbial activity

Measurement of aminopeptidase activity in bacterial suspensions prepared from swabs of aerobically stored meat samples has been shown to correlate well with TVCs of aerobically stored meat, poultry and fish samples (Perez de Castro et al., 1988; Alvarado et al., 1992). After a 2 h incubation at 37°C the activity could be detected visually making it of value for determining incipient spoilage. For predictive purposes i.e., lower bacterial numbers, spectrophotometric measurement was necessary.

Another suggestion involves measuring the difference in rate of proton efflux from and influx into bacterial suspensions incubated with glucose or a mixture of glucose and peptone as a source of amino acids (Seymour et al., 1994). The measurements can be made within 2 h at room temperature using a standard pH meter. For samples of minced beef, rate differences were measurable as numbers exceeded log 6.0 and at log 8.0, which was equated with spoilage, the rate was typically found to be greater than a particular value (5 mV/h). The method would appear to have diagnostic and some predictive possibilities.

A commercial disposable oxygen electrode system, without covering membrane to allow the detection of reducible species in addition to oxygen, was used to detect the growth from low inocula ($< 1-60$ organisms/ml) of a range of Gram positive and negative organisms in commercial packs of UHT milk (Bell et al., 1995). In all cases growth was detectable within seconds of incubation at 30°C for 72 h. The samples showed no indication of pH change, another often used quality criterion in storage trials of this kind of milk. Given the extended shelf-life of the milk, the detection of reducible species shows great potential for routine determination of sterility and prediction of shelf-life problems

4. Conclusion

There are varying degrees of potential in many of the methods described above for assigning shelf-life. In the overwhelming majority of studies correlations have however only been sought between microbial numbers, the traditional indices of shelf-life/spoilage, and chemical change. What is now needed are correlations between both of these and sensory data to fully establish the value of the chemical changes.

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