

Microbial and chemical markers of meat spoilage

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Food is frequently classified on the basis of its susceptibility to microbial spoilage as non-perishable, semi-perishable and perishable. Non-perishable foods include, for example, sugar and canned foods, whereas low moisture foods such as flour, dry fruits, dry vegetables and baked goods are semi-perishable. Fresh foods (for example meat, fish, eggs, moist fruits and vegetables) are classified as perishable.

Microbial spoilage of foods is influenced by two factors: the nature of the food (intrinsic factors; pH, type of acid, water activity, oxidation-reduction potential and nutrient content) and the environment in which it is stored (extrinsic factors; temperature, relative humidity, and gaseous atmosphere).

Microbial spoilage is recognisable in several different ways; visible growth may be apparent or a loss of texture by degradation of the structural components of a food, but most commonly spoilage will be evident by gas production, off-odours and/or flavours, and the presence of pigments, or polysaccharides (slime), from microbial metabolism.

Generally, microbial spoilage exhibits a relatively sudden onset, reflecting the exponential nature of microbial growth, and therefore of microbial metabolism. If a microbial metabolite, for example an off-odour, has a certain detection threshold, although its concentration may be well below the threshold for most of the product's shelf life, once that threshold has been reached it will be rapidly exceeded and a consumer would then consider the product to be profoundly spoiled.

A good marker of food quality/spoilage should:

- Correlate well with organoleptic assessments.
- Detect incipient spoilage when organoleptic criteria may yield doubtful results.
- Be continuous from fresh to the spoiled state.
- Allow for a rapid and easy-to-conduct measurement of the indicator.

Changes in concentrations of various chemicals produced during the storage of meat and the relationship with product

acceptability have been studied in some detail, as described below.

Microbial spoilage in meat

Spoilage of meat and poultry is indicated most commonly by development of off-odours and off-flavours, but can include colour and texture changes, slime or any other undesirable characteristic.

Chilled storage allows psychrotrophic bacteria to grow, for example pseudomonads, but if the surface of fresh meat becomes dry, bacterial growth can be restricted and fungal spoilage may occur, producing a whiskey, hairy, or cottony white, green or grey to black growth on the surface. Under aerobic conditions, the spoilage microflora of meat with high moisture content is predominantly Gram negative, oxidase positive rods.

$$k_0 = \frac{\text{inosine} + \text{hypoxanthine} + \text{xanthine}}{\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{adenosine} + \text{inosine} + \text{hypoxanthine} + \text{xanthine}}$$

Table 1. The k_0 value has been proposed as suitable for meat.

Initially, off-odours become apparent when the aerobic plate count (APC) reaches about 10^7 cfu/g or /cm². Generally spoilage starts with a buttery or cheesy odour (enterobacteriaceae, lactic acid bacteria), then a sweet or fruity odour develops (pseudomonas, moraxella). As spoilage progresses meat develops a putrid odour (pseudomonas, acinetobacter, moraxella).

When the APC reaches 10^8 cfu/g or /cm², visible slime is apparent. In vacuum or modified atmosphere packed or ground meat, lactic acid bacteria dominate the microflora, resulting in the development of sour acid and 'cheesy' odours.

Chemical markers of meat

In meat, the total volatile basic nitrogen (TVB-N) consists almost entirely of ammonia, with only traces of trimethylamine, probably due to the psychrophilic bacteria producing ammonia by deamination of amino acids under aerobic conditions.

In the case of broilers, onset of spoilage at about 10^7 - 10^{10} cfu/g is generally accompanied by a rapid increase in ammonia.

Measurement of ATP breakdown products has also been used as a marker of meat quality. However, the k_0 value (Table 1) has been proposed as more suitable for meat than the K value used for fish. The k_0 value also takes into account adenosine and xanthine concentrations.

Inosinic acid, which degrades to inosine and hypoxanthine, is abundant in chicken muscle and its concentration directly related to the flavour of the meat. Inosinic acid has been suggested as a more suitable indicator of freshness than hypoxanthine, since hypoxanthine formation is dependent on the degradation of the intermediate compound, inosine.

However, hypoxanthine is associated with a bitter off-flavour and therefore may be a useful spoilage indicator.

When lipid deterioration products are measured, free fatty acid levels in extracted fat of less than 2% have been suggested as denoting meat of acceptable quality.

Acceptable values for peroxides (a measure of lipid oxidation) in fresh beef have been suggested as less than 1mEq peroxide per kilogram of fat, with a cut-off value for acceptability as 5mEq peroxide per kilogram of fat.

In a study of minced beef stored aerobically at 4°C and 25°C over a maximum 15 day period the TVB-N value and TBA (marker of lipid oxidation) correlated well with psychrotrophic bacteria counts and off-odour. TVB-N and TBA were regarded as good spoilage and rancidity indicators respectively.

However, other studies have shown that TVB-N and free fatty acids were unsuitable indicators of acceptability of beef for consumption.

In this instance however, the storage was much longer and studied meat vacuum-

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packed and shipped for export, kept deep chilled for up to 90 days.

Biogenic amines

The concentrations of some biogenic amines, for example tyramine, putrescine and cadaverine, normally increase during processing and storage of meat and meat products, whereas others, such as spermidine and spermine, decrease or remain constant.

The usefulness of biogenic amines as freshness indicators depends on the nature of the product. A combination of putrescine and cadaverine has been suggested as a quality index as their concentration increases prior to spoilage and correlates well with microbiological load. The biogenic amine index (BAI) consists of the sum of putrescine, cadaverine, histamine and tyramine, with upper limits of 500mg/kg recommended for minced beef and pork.

BAI limits for fresh meat have been suggested as follows: <5mg/kg indicates good quality meat; between 5 and 20mg/kg, acceptable meat; between 20 and 50mg/kg, low meat quality; >50mg/kg, spoiled meat.

Slightly different biogenic amine profiles were observed between aerobic and vacuum-packed (VP) beef stored at 4°C for 12 and 35 days. While putrescine increased with storage time, cadaverine was not detected until day eight and thereafter levels were very variable although tended to increase.

Histamine was not detected until day 15 in the VP samples and was not detected in the unpacked meat. According to the BAI, the aerobic samples reached levels of unacceptability by day eight although were not rejected on sensory criteria until day 12.

VP samples were acceptable until day 35 although the biogenic amine levels were close to 5g/g by day 12 and were >50g/g, the level suggested for spoiled meat, by day 26.

Tyramine and cadaverine concentrations could be used as a spoilage index of fresh beef packed in an aerobic atmosphere. In a study in which beef samples were stored at 4°C for eight days, tyramine and cadaverine increased significantly with storage time and to a lesser extent putrescine and spermidine levels also increased.

Tyramine concentration correlated well with pseudomonas and brochothrix thermosphacta counts and cadaverine with Enterobacteriaceae counts.

For MAP chicken, tyramine, putrescine and cadaverine could serve as quality indicators, especially if stored at chill abuse temperatures. Storage temperature significantly affected the rate of formation of tyramine; above 6°C, levels of these amines increased after five days up to nine days.

Tyramine was also formed below 6°C, but putrescine and cadaverine were absent. The increase in mesophilic bacterial count was

highly correlated with the increase in the amount of tyramine.

When the growth of enterobacteriaceae, proteolytic bacteria, hydrogen sulphide producing bacteria and clostridia was restricted, there was no putrescine and cadaverine formation, indicating a correlation between production of these amines and microbial growth. Similar results were obtained from a study on chicken stored aerobically and under modified atmosphere at 4°C up to 23 days, putrescine being the main amine formed; lactic acid bacteria were the dominant microflora and enterobacteriaceae were not detected.

A study on smoked turkey breast fillets stored at 4°C for 30 days under aerobic, vacuum, modified atmosphere and skin packaging, identified tryptamine, histamine and tyramine as spoilage indicators. In contrast to the other studies discussed, levels of putrescine, cadaverine, spermidine and spermine were low throughout the whole storage period.

Values for tryptamine, histamine and tyramine correlated well with microbiological data and sensory analysis. These three amines were present in all packaging conditions, the highest level being attained in aerobic samples and lowest levels in the skin-packed samples after 30 days of storage. Overall, biogenic amines may be useful spoilage indicators for meat and meat products, but their formation depends on the dominant microflora which will vary depending on intrinsic and extrinsic factors.

Validation of the selected markers would be necessary, carefully replicating actual handling and storage practices.

Volatile sulphur compounds

Growth of spoilage organisms in a meat product will result in the accumulation of microbial metabolites primarily end products of glucose metabolism (the initial substrate for most organisms) and in some cases sulphur compounds. Some volatile sulphur compounds have a remarkable effect on sensory qualities of meat products due to their pungent odour and low odour threshold. The headspace of MAP raw chicken legs contained the following volatile compounds: butane, ethanol, acetone, pentane, dimethylsulphide, carbon disulphide and dimethyl disulphide. Ethanol and dimethyl disulphide were major and butane a minor component of the volatiles.

Odour, microbiological quality, ethanol and sulphide volatiles were strongly affected by storage time and temperature (1°C, 4°C or 7°C). Sulphide volatiles were more dependent on temperature than storage time, whereas microbial counts and ethanol were more affected by storage time than temperature. Ethanol and dimethyl disulphide were suggested as good spoilage markers for raw chicken legs.

A study on marinated MAP chicken breast stored at 5°C showed similar results. The

volatiles detected were ethanol, acetone, 2-propanol, dimethylsulphide, 3-(methylthio)-1-propene and aliphatic straight and branched chain and cyclic hydrocarbons.

During storage, amounts of ethanol and acetone increased and acetaldehyde, an oxidation product of ethanol, was produced. The amount of dimethylsulphide had increased in all samples. Dimethylsulphide has an unpleasant, musty smell and probably caused the spoilage odour.

After 19 days of storage, the concentration of ethanol in the head space of all samples was at least two-fold greater than after day 11 and the amount of dimethylsulphide and pentane had almost doubled. Ethanol was probably produced by lactic acid bacteria or coliforms.

VP beef joints stored at 1°C for eight weeks showed a decrease in L-lactic acid content and an increase in D-lactic acid, acetic acid and ethanol content. These changes were evident after about three weeks when the background flora, dominated by lactic acid bacteria, exceeded six logs. The amount of D-lactic acid exceeded 100mg/100g but did not lead to a net increase in the total amount of lactic acid.

Acetic acid exceeded 8mg/100g and ethanol 2mg/100g of meat in spoiled samples, but the amounts produced depended on the background flora present in the meat sample. With measurable amounts of these compounds in fresh meat, these compounds are not so attractive as freshness indicators. D-lactic acid was the most promising indicator of freshness especially since D-lactic acid cannot be detected in fresh beef.

In general, the accumulation of end products of glucose fermentation by lactic acid bacteria, especially ethanol and lactic acid, may potentially be useful spoilage indicators of meat. The volatile sulphur compounds seem to be the most promising freshness/spoilage indicators. Utility of the indicators depends on the intrinsic and extrinsic factors of the meat.

Products of proteolysis

Evidence indicates that proteolysis (protein degradation) is associated with spoilage of meat, but methods based on monitoring products of proteolysis as freshness indicators, have received little attention. Alomirah et al (1997) using electrophoresis of meat proteins, showed a decrease of myosin heavy chain (201kDa) and an increase in a proteolytic product of myosin (188kDa), between days 12-16 in whole beef and between days 8-12 in ground beef.

Analysis of sarcoplasmic proteins demonstrated the disappearance of a protein fraction (40kDa) after day eight in both whole and ground meat.

Ground beef is characterised by higher microbial activity than intact meat, suggesting that loss of this protein is a result of autolytic rather than microbial activity and may be a potential indicator of freshness. ■