ELECTROSPRAY MASS SPECTROMETRY ANALYSIS OF HAEM PIGMENTS

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SUMMARY

In order to assess the use of haem pigments for the identification of meat species by Electrospray mass-spectrometry (ESMS), the heat stability of these pigments in model systems and in fresh and cooked sheep meat was studied. Later the effect of added ingredients (salt, curing salt, monosodium glutamate, rusk, white pepper and glucose) on the ESMS analysis was investigated.

Electrospray ionization dissociates haem proteins to give the haem and globin components as different entities. When myoglobin was heated in model systems, the globin moiety seems to be heat-stable and little degradation was detected, even after heating at 121°C for 60 minutes. In fresh and cooked sheep meat, myoglobin globin chain was the major peak present in the ESMS trace (mass range 4 to 20 KDa), and as time of cooking was increased , the peak became broad with higher mass tail indicating the presence of unidentified components in the sample, probably derived from protein denaturation. The same trend was observed in minced meat products, as the spectra became complex with more peaks being detected as cooking time and temperature were increased. This could be due to interactions with added ingredients, formation of adducts or protein degradation. However in all the samples the globin chain could be detected.

Introduction

Meat is an extremely complex system. Its composition, quality and functional properties differ between species, breed, age, sex and diet as well as the anatomical location for a specific muscle. On the other hand improvements in technology and processing have led to the use of deboned and frozen meats that are easily transported and handled, and as a result, the use of undeclared meats has been facilitated. Consequently a rapid identification of meat species has become an important task in the meat industry, not only due to the possibility that minced meat products may contain animal or vegetable proteins not indicated on the label but also because of cultural or religious reasons.

Standard analytical procedures, such as gel electrophoresis and immunoassay tests have been investigated for the analysis of raw or mildly heated meat products; all of them are based on differences in physical, chemical and serological properties of proteins. These properties are altered during cooking and processing and consequently the sensitivity and accuracy of these techniques may be diminished. Preliminary experiments on native and heated myoglobins and haemoglobins from different animal species (Taylor et al., 1993), have suggested that electrospray-mass spectrometry (ESMS), can be used as a rapid technique for the identification of meat species by measuring the precise molecular weight of the globins. The differences in the molecular weights are due to small variations in the amino acid composition characteristic to each species.

Materials and Methods

Model systems

Solutions of haemoglobin and myoglobin (Sigma Chemical Co., Poole, UK) were prepared in screw top glass vials. The vials were heated in a steam autoclave (Benchtop 50 LTE Sci), at 100 or 121°C during 10min intervals from 0 to 60min. A water bath was used to heat vials at 80°C for the same time intervals. After cooling, the samples were then analyzed by ESMS.

Preparation and extraction of meat samples

Lean lamb was obtained from a local butcher and minced twice through a 5mm plate. Portions of minced meat (150g) were heated in sealed cans (75 x 60mm), either in a water bath (80°C), or in a canning retort (100°C and 121°C) for 10min intervals from 0 to 60min. After heating, the samples were cooled and subjected to extraction by mixing the meat samples (10g) with urea solution, (50ml; 8M). The resulting suspensions, after standing overnight were centrifuged at 4000g for 20min. The supernatants were filtered through Whatman No. 1 filter paper and finally dialysed against distilled water over 72h (12kDa cut-off).

Preparation of minced meat products

Burgers were used in this experiment as they are typical minced meat products and their formulation can easily be modified. The meat products were manufactured as follows: shoulder beef cuts were removed from any visible fat and connective tissue and then minced twice through a 5mm plate. The minced meat was mixed with the ingredients as a standard formulation (meat 90%, salt 2.75%, sodium glutamate 0.17% rusk 6.5% and white pepper 0.55%), using a Kenwood food processor. The mix was then pressed into approximately 90g burgers (85mm in diameter), using a hand operated burger press; then the burgers were frozen and stored at -20°C until they were cooked using a conventional electric grill, for either 0, 15, 20 or 25 minutes. During cooking, the burgers were turned every 5 min to allow uniform heating. Later they were subjected to urea extraction as in meat samples (Ponce et al., 1994). In a different trial, beef was mixed with either curing salt (2.25%) or glucose (3%), using a Kenwood food processor. The mixtures were cooked at the same conditions as before in meat samples.

Electrospray mass spectrometry analysis.

Before injection, all dialysed extracts were filtered through a 13mm 0.5µm filter unit. These filtered extracts (500µl) were mixed with acetonitrile (500µl), and formic acid (25µl). Solutions 10-40 µl were then assayed on a Quattro mass spectrometer fitted with the electrospray interface (VG Biotech, Altrincham, U.K.). The solvent stream (50% aqueous acetonitrile) was pumped via a Rheodyne valve into the source at 5 µl/min. After ionization the solvent was removed by a stream of nitrogen under reduced pressure. Ions were sampled through a sampling orifice to the high vacuum sector of the mass spectrometer, where the series of protein ions containing different number of protons were detected. Spectra were obtained by taking 20-25 scans. The molecular weight was determined by an averaging calculation, and the raw data was then plotted on a true molecular scale containing a single peak with the mass of each protein present in the sample. The mass scale was calibrated every three hours with horse heart myoglobin (Sigma Chem.).

Results and Discussion

Just before ESMS analysis, extracts were mixed with concentrated formic acid to avoid formation of formic acid esters (Schindler, 1993) that can affect the determination of molecular weight. Formic acid enhances the solubility of hydrophobic proteins and acts a proton source for ionization.

Aqueous solutions of standard sheep myoglobin were made in distilled water and heated at 80, 100 and 121°C. These represent the cooking, boiling and canning temperatures used in food processing. Electrospray ionization dissociates haem proteins to give the haem and globin components. Figure 1 shows the ESMS analysis of myoglobin heated at 121°C for 0, 30 and 60min. In all the samples, sheep myoglobin globin chain (16923.2 Da) remained intact even after being heated at 121°C over 60min, but a new peak 170 units smaller than the globin was detected and its intensity increased with the severity of the heat process.

Portions of lamb meat were heated at 80, 100 or 121°C for times ranging from 0 to 60 min at 10 min intervals and, after urea extraction and dialysis, were subjected to ESMS analysis. Although that myoglobin can be extracted from fresh meat using water, in cooked meat it becomes insoluble and stronger agents are required for its extraction. Figure 2a shows the ESMS trace of fresh lamb meat extracted with urea and dialysed. The major peak corresponded to the globin chain of myoglobin (16923.2 Da). Figures 2b and 2c shows the spectra of lamb meat heated at 100°C for 30 and 60min respectively. The results were similar in all the tested temperatures and, it was seen that, as time of heating increases, the trace becomes more complex with more components being present. This is probably due to breakdown of muscle proteins into smaller fragments that

can be extracted with urea solution or may be due to interactions between myoglobin and other heat-denatured meat components leading to formation of agglomerates (Ledward, 1979).

The effect of added ingredients in the ESMS analysis was studied using burgers as typical minced meat products. Beef burgers were cooked using a conventional grill, for either 0, 15, 20 and 25min. These times represent the raw meat and the typical grades of cooking (rare, medium and well done). Figure 3a shows the transformed spectra where one peak related to the beef myoglobin globin (16946.25 Da) was detected. In cooked samples (Figures 3b & 3c), as time of cooking was, increased the myoglobin peak became broad with higher mass tail indicating the presence of unidentified components. However in all the analyzed samples, myoglobin was still detected after 25 min of cooking time. The presence of other components, (besides myoglobin) could be due to interactions with the added ingredients as the presence of salts increase the solubility of myofibrillar proteins, but tend to decrease protein heat stability or adduct formation. Addition of curing salt results in a broadening of the myoglobin peak and generation of new peaks as the heat, time and temperature were increased. The same behaviour was observed when glucose was added, but in samples heated at 100°C the spectra showed several peaks making the myoglobin globin chain difficult to resolve.

Conclusions

In aqueous solutions of pure sheep myoglobin, the globin chain seems to be stable after heating at 121°C for 60 minutes with little degradation being observed. In both beef and lamb fresh meat, myoglobin was the only protein detected in the mass range 4-20kDa. However in cooked meat and meat products, this protein seemed to undergo several reactions and physical transitions during heating (including protein denaturation, aggregation, degradation and Maillard reactions), that alter the molecular weight and so the ESMS analysis. The level of these reactions varies according with the thermal treatment.

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References

Hamm, R. (1977) Changes of muscle proteins during the heating of meat. In Physical and Biological Changes in Food caused by Thermal Processing. Edited by Tore Hϕyem and Oskar Kvåle. Applied Sci. Publishers Ltd London 101-127.

Ledward, D.A. (1979) Meat. in "Effects of heating of Foodstuffs". edited by R.J. Priestley. Applied Science Publishers Ltd London 157-193

Ponce, E.; Linforth, R.; Hall, M.; Guerrero, I. & Taylor, A.J. (1994) Stability of haem pigments in model systems and cooked meat. Meat Science in press.

Schindler, P.A.; Van Dorsselaer, A. & Falick, A.M. (1993) Analysis of Hydrophobic Proteins and Peptides by Electrospray Ionization Mass Spectrometry. Anal. Biochem. 213: 256-263.

Taylor, A.J.; Linforth, R.; Weir, O.; Hutton, T. & Green, B. (1993) Potential of electrospray mass spectrometry for meat pigment identification. Meat Sci 33:75-83.

Figure 1. ESMS of standard sheep myoglobin heated at 121°C for (1a) 0 min, (1b) 30 min and (1c) 60 min.

Figure 2. ESMS of lamb meat heated at 100°C for (2a) 0 min, (2b) 30 min and (2c) 60 min.

Figure 3. ESMS traces of beef burgers cooked for (3a) 0 min, (3b) 15 min and (3c) 20 min.