DETERMINATION OF THE THERMAL TREATMENT OF THREE DIFFERENT MEAT SPECIES BY SDS-PAGE ELECTROPHORESIS

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Introduction

The determination of the final cooking temperature of meat aims to establish that meat has been cooked to at least 65°C to ensure destruction of harmful microorganisms and viruses that causes diseases in humans. Another crucial point linked with cooking is that internal cooking end point temperature of meat is associated with palatability and acceptability of meat. It is therefore important to establish the final cooking temperature of meat in a wide range of temperatures in order to assess the eating quality. Enzymatic essays have been proposed to measure the end point temperature (TOWNSED and BLANKENSHIP 1989). However a test with a specific enzyme will not allow the determination on a wide temperature range and the enzymatic activity is sensitive towards the environement (pH, salt). Among the electrophoretical methods, the use of sarcoplasmic proteins showed a potential to be used as temperature dependent markers. Results obtained with sarcoplasmic proteins and gradient SDS-PAGE (STEELE and LAMBE 1982) and SDS-PAGE (LEE et al. 1974, CALDIRONI and BAZAN 1980, CRESPO and OCKERMANN 1977) showed the possibility to use these techniques as a rapid method to determine the maximum internal temperature. The aim of this work was to define an analytical tool based on the SDS-PAGE technique to determine the end point cooking temperature of different meat species.

Materials and Methods

Three different meat sources with two different muscle types for each species were considered : beef M.semitendinosus and L.dorsi, pig M.semitendinosus and L.dorsi, chicken thigh and breast. The muscles were trimmed from visible fat and connective tissue and cut in pieces of approx. 5 g and minced through a 5 mm plate. The minced meat samples were introduced with a 50 ml syringe into stainless steel tubes of 150 mm length and 10 mm diameter. The tubes were hermetically closed and introduced in the tubular heating device. The apparatus was equipped with water and steam in and outlet and allowed a heat treatment with very short heating and cooling times. The system was cooled down by the mean of a cold water circuit.
Samples were heated during 30 min at given temperature between 50°C and 120°C with 10°C intervals. After the thermal treatment the samples were collected and the water soluble proteins were extracted by homogenizing 1 g sample with 10 ml dist.water in a Sorvall Omni Mixer at top speed for 90 sec. The sample was then centrifuged on a Beckman J2- 21 M at 23700 x g for 15 min. The control sample was obtained with sarcoplasmic proteins extracted from raw meat. The protein content of the supernatant was determined following the Bradford method (BRADFORD M.1976) and samples for SDS-PAGE were prepared by precipitating proteins with 3 M trichloroacetic acid. After centrifugation the pellet was dissolved in 62.5 mM Tris/HCl pH 6.8, 0.4% dithiothreitol, 10% glycerol, 2% SDS and 0.01% bromphenol blue, and heated for 5 min at 100°C. SDS-PAGE was performed with a Protean II (Bio-Rad) slab cell using a discontinous buffer system (LAEMMLI 1970) with a 12% separating gel. The gels were stained with Coomassie Brillant Blue G 250.

Results and Discussion

Beef

The protein pattern for beef L.dorsi and M.semitendinosus is shown in Figures 1 and 2. Heating at 50°C and 60°C was characterized, for both muscles, by the absence of the band at 42 KDa (creatine kinase) and 46 KDa (enolase) respectively. The M.semitendinosus additionally showed the disappearance at 50°C of the band at 26

KDa and at 60°C the disappearance of the band at 27 KDa. The bands located between 75 and 57 KDa were absent in the pattern of both muscles after heating at 70°C. At this temperature the M.semitendinosus showed the presence of a double band at 150 KDa. The band corresponding to myoglobin (18 KDa) was present in both pattern and was particularly strong for L.dorsi. The myoglobin band disappeared at 80°C in M.semitendinosus whereas for L.dorsi the band was less strong but still present. LEE et al. (1974) investigated the influence of cooking temperature between 65 - 90°C for 10, 20 and 30 min on several beef muscles, including M. semitendinosus, by SDS-acrylamide gel electrophoresis. The authors indicated the presence of six bands at 65°C and five bands at 70°C, including the myoglobin band, which was still present at 90°C. CALDIRONI and BAZAN (1980) analysed four beef muscles located in the upper part of the leg by SDS-PAGE at temperatures between 60 - 90°C for 10 min. From 68°C towards higher temperatures the authors observed bands at 72 and 55 KDa, the band at 41 KDa was present until 74°C and the myoglobin band was not observed above 80°C. ROBERTS and LAWRIE (1974) heated beef L.dorsi between 45 - 90°C during 10 to 70 min and ran electrophoresis on acrylamide gels. After heating at 65°C for 20 min or more, most of the sarcoplasmic proteins fail to appear, the only visible band was myoglobin. No bands were visible after heating to 85°C during 10 min. BAUER and HOFMANN (1990) showed that isoelectric focusing and silver staining allow to differentiate beef samples between 65 and 100°C.

Pork

Figures 3 and 4 show the protein pattern of pig L.dorsi and M.semitendinosus. At 50°C a low molecular weight protein of 25 KDa disappeared in both muscles. The heat temperature of 60°C was characterized by the disappearance of the bands at 46 KDa (enolase) and 26-27 KDa for both muscles. The band at 41 KDa (creatine kinase) disappeared between 50°C and 60°C, at 60°C this band was definitely not visible in both muscles whereas at 50°C a very thin band for M.semitendinosus subsisted. At 70°C, the bands between 64 KDa and 57 KDa disappeared. At this temperature the M.semitendinosus showed the presence of a double band at 150 KDa as well as the presence of a band in the area of 41 KDa which disappeared at 80°C. Myoglobin (18 KDa) was particularly heat resistant, the band disappeared at 70-80°C in M.semitendinosus, whereas in L.dorsi the band was still present at 80°C but disappeared at 90 °C. Myoglobin is known to be one of the most heat stable sarcoplasmic proteins and is denatured at relative high temperature, between 80 - 85°C, therefore it can be used to determine whether or not meat has been heated to 90°C. Mc CORMICK et al. (1987) separated water soluble proteins by reverse phase HPLC and identified the proteins by separating RP-HPLC eluent fractions corresponding to absorbance peaks in SDS-PAGE. They reported that insolubilisation of creatine kinase for pig *L. dorsi* occurred at 50 - 55°C and enolase at 60°C. STEELE and LAMBE (1982) heated pig muscle at 65 - 90°C during 20 min. Proteins were analysed by SDS gradient electrophoresis. Between 65 and 80°C bands located at 13, 26, 52, 74, 87 and 101 KDa gradually disappeared. No significant differences were observed in protein bands between 80 and 90°C. DE WREEDE et al. (1982) could not obtain a clear identification by SDS-PAGE for scalded sausages samples heated at 80 - 130°C for 10, 20, 40 min because of diffused bands. We observed the presence of diffused bands at 120°C. Between 80 and 110°C no changes in bands were present and the determination of the heat treatment in these range was not possible.

Chicken

As shown in Figures 5 and 6 the behaviour of chicken breast and chicken thigh muscle to heat treatment was very different. At 50°C only chicken breast showed a change in protein pattern compared to the control with the almost complete disappearance of the band at 41 KDa. Coagulation of sarcoplasmic proteins in chicken muscled started at 60°C where bands started to disappear. However no common marker could be found for thigh and breast. The breast muscle showed absence of bands at 64 KDa, 59 KDa, 36 KDa and 26-27 KDa whereas the thigh muscle was characterized by the disappearance of the band at 44 KDa. At 70°C the bands at 46 KDa and 30 KDa were absent for breast muscle, only a band at 34 KDa was present, this band disappeared at 80°C. The thigh muscle showed at 70°C absence of bands at 70 KDa and 47 KDa. A particular behaviour was observed for leg muscles at high temperatures, between 90 and 100°C two thick bands at 36-38 KDa were present. CRESPO and OCKERMAN (1977) heated extracted sarcoplasmic proteins from thigh and breast between 40 and 80°C for 15 min. The result of the peaks obtained by densitometry on acrylamide gels showed a different behaviour of the proteins of the two muscles as observed in our experiments indicating that the heat sensibility of dark and light muscles is different. Major changes in peak intensities also occurred at 60°C. In chicken thigh three peaks could be still detected at 80°C but only one small peak was present in the chicken breast samples at low MW. STEELE and LAMBE (1982) observed between 65 and 90°C, by gradient

gel electrophoresis, six bands at 188, 155, 56, 48, 35 and 24 KDa with different intensities . Bands at 48 and 56 KDa disappeared at 75°C.

Conclusion

Protein markers have been used to assess the end point temperature of cooked meat : the method is based on the presence or absence of the markers which allow a rapid and clear detection. The protein markers can be used in a temperature range between 50 and 80°C for pork, beef and chicken breast and between 50 and 100°C for chicken thigh. The same protein markers can be used for muscles located in different parts of the animal for beef and pork. Chicken thigh and breast proteins showed a different behaviour towards temperature. In this case specific markers for each type of muscle have to be used.

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Figure 1. Pattern of proteins extracted from heated beef L.dorsi.
C is control, water soluble proteins from raw meat. The molecular weights (KDa) are indicated on the left side.

Figure 2. Pattern of proteins extracted from heated beef M.semitendinosus.
C is control, water soluble proteins from raw meat. The molecular weights (KDa) are indicated on the left side.

Figure 3. Pattern of proteins extracted from heated pig L.dorsi.
C is control, water soluble proteins from raw meat. The molecular weights (KDa) are indicated on the left side.

Figure 4. Pattern of proteins extracted from heated pig M.semitendinosus.
C is control, water soluble proteins from raw meat. The molecular weights (KDa) are indicated on the left side.

Figure 5. Pattern of proteins extracted from heated chicken breast.
C is control, water soluble proteins from raw meat. The molecular weights (KDa) are indicated on the left and right side.

Figure 6. Pattern of proteins extracted from heated chicken thigh.
C is control, water soluble proteins from raw meat. The molecular weights (KDa) are indicated on the left side.