SULFHYDRYL GROUPS IN MEAT PROTEINS

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In the mechanical and heat processing of comminuted meat products, physico-chemical characteristics of muscle proteins are essential for the water and fat binding properties of the meat emulsion.

The water-holding capacity of muscle under various conditions has been studied extensively by Wierbicki et al (1) and Hamm et al. (2,3). These studies suggested a correlation between the condition of the muscle proteins and the product quality.

Fukazawa et al. (4) established that myosin and actomyosin were indispensable among the myofibrillar proteins in developing binding properties of the product. F-actin alone did not affect the binding properties.

Recent work by Samejima et al. (5) pointed to a possible correlation between the heat gelling capacity and sulfydryl (SH) content of specific meat proteins. If SH groups were involved in the gelation, the formation of network system - as was observed by electron microscopy - could be ascribed to the oxidation of SH groups to intermolecular disulphide (S-S-) bridges in the native protein molecules.

In heating beef muscle myofibrils and actomyosin gels, Hamm and Hofmann (6) establisched that denaturation and heat gelling occured already at 45°C, although oxidation of SH to disulphide S-S- was not detectable until 70°C was reached. The number of SH groups reacting with $AgNO_3$ in an amperometric titration remained fairly constant and was found to be 90 Moles SH/g of protein. Under the experimental conditions used, all the SH groups in the native myofibrillar proteins were able to react with the Ag^+ ions.

In these native proteins only 35 Moles SH/g of protein reacted with the SH reagent N-ethyl-maleimide (NEM). Heat denaturation up to 70°C resulted in a considerable increase of SH groups, up to 70 Moles SH/g of protein being capable of reacting with NEM.

Apparently, the heat denaturation caused an unfolding of the peptide chains and "masked" SH groups, hidden within the folded structure of the native protein, became reactive towards the reagent.

Denaturation of myofibrillar protein with urea or ethanol also gave an increase in the number of SH groups reacting with NEM. However, in both chemical and heat denaturation the SH values obtained with NEM never reached the $AgNO_3$ value.

Under the experimental conditions used by Hamm and Hofmann, the presence of oxygen (air) had no influence on the quantity of SH groups determined with $AgNO_3$ or NEM.

When myofibrils were heated up to current sterilization temperatures of 115°C to 120°C, the number of SH groups reacting with Ag^+ decreased considerably. Heating for 30 min in the presence of air resulted in a 40% reduction of the SH value in native myofibrils.

If the heating process took place under nitrogen, the disappearance of SH groups diminished considerably.

Treating the myofibrils with $NaBH_4$ after heating restored the SH level and attained the value of unheated $NaBH_4$ reduced myofibrils. Apparently, SH groups disappeared by oxidation to S-S bridges and the reaction could be reversed by treatment with $NaBH_4$. Prolonged heating to 120°C (5hrs) of myofibrils resulted in substantial H_2S formation and subsequent reduction with $NaBH_4$ could not restore the SH level.

Recently an investigation was started in our Institute into the behaviour of sulfhydryl groups of meat proteins during the processing of meat products. The aim is to find out whether or not developing physico-chemical or rheological characteristics in various stages of meat products can be correlated with the SH/S-S household of the meat proteins. Our investigations started with the determination of SH groups in the myofibrillar model system described by Hamm and Hofmann (6).

Experiments.

Two methods have been developed for SH measurement in the samples used: a modification of Hamm and Hofmann's amperometric titration procedure and a spectrophotometric method with Ellmann's reagent 5,5'- dithiobis - (2-nitrobenzoic acid) DTNB (7).

In our amperometric titration procedure it was possible to carry out a direct titration of SH with 0.005 M $AgNO_3$, thus avoiding the use of reduced glutathione and indirect titration of excess $AgNO_3$. This was achieved by administering a short dose of $AgNO_3$ to the sample, just insufficient to reach the titration end-point, allowing it to react for about 45 min and subsequently continuously adding more $AgNO_3$ by means of a motor driven burette (Radiometer type ABU 16) with a reduced dosing speed of 0.07% ml/min. In this way very reproducible titration end-points were obtained.

The electrode system consisted of a stationary platinum electrode at potential of -0.3 V versus a saturated calomel electrode (SCE) as a reference. As buffer 0.25 M NH_4NO_3/NH_4OH - 0.001 M EDTA was used (pH=8.1).

In contrast to the findings of Hamm and Hofmann (6,8) our titrations had to be performed under oxygen-free conditions, i.e. by working under N_2. Without these precautions inconsistent results were obtained.

The use of the specific SH reagent DNTB was based on the question whether or not the increased reactivity of "masked" SH groups resulting from the unfolding of the peptide chains during heat treatment at 70°C was a general phenomenon for molecules of specific SH reagents larger than the Ag^+ ion. Furthermore, the nitromercaptobenzoic acid ion as a reaction product could be measured spectrophotometrically at a wavelength at which dissolved proteins gave a negligible contribution to the absorption value. Treatment with trichloroacetic acid, indispensable in the NEM method (6,9) could, therefore, be omitted.

Maximum color formation was obtained in a 0,2 M Tris - 0.02 M EDTA buffer at pH = 8.2. Protein concentration was chosen in such a way that the SH concentration was in the range 1 to 5×10^{-6} Mol. A linear relationship exists in that range between absorbancy and SH concentration.

We used beef muscle myofibrils from M. longissimus dorsi, 3 to 5 days post mortem, insolated according to the method of Hamm and Hofmann (8,9). In the final washing procedure ice-water was replaced by 0.1 M KCL.

Resluts and Discussion.

When determining SH groups in beef muscle myofibrils by our direct amperometric titration method with $AgNO_3$, we found a SH value in native myofibrils of 85 to 90 Moles SH/g of protein, which was in agreement with Hamm and Hofmann's experimental data (8). Upon heating myofibrils up to 70°C we found a less pronounced increase of SH groups reactive with DNTB when compared with the findings obtained by these authors with the NEM reagent (6), as is shown in table 1.

Table 1. SH levals in myofibrils in Moles/g of protein after 30 min heating or denaturation in 8 M urea

reagent	30°C	50°C	70°C	8 M urea
$AgNO_3$	85-90	84-90	85-90	82
DTNB	60-65	65	85	85-90
NEM	35	50	70	45

In native myofibrils the quantity of DTNB reactive SH groups was 60 to 65 Moles SH/g of protein, being about 70% of the value detected by $AgNO_3$. In myofibrils heated under nitrogen to 70°C all SH groups seem to have become reactive towards DTNB, since the SH value attained 85 Moles SH/g of protein (table 1). If the samples were heated in air, lower values, which were badly reproducible, were obtained. Apparently, oxidation of SH takes place during this heating process.

In 8 M urea denatured myofibrils all SH groups, reacting with $AgNO_3$, are able to react with the DTNB reagent. The conclusion can be drawn that DTNB can react with some SH groups, inaccessible for the NEM reagent. Therefore, a difference in reactivity of myofibrilar protein SH groups towards Ag^+ and NEM cannot be interpreted exclusively in "steric" terms.

In an experiment in which the influence of the time of comminuting on the SH level in beef emulsion was studied, no significant difference in SH could be detected with the DTNB reagent and $AgNO_3$ between beef-salt-water mixtures comminuted in a chopper for 2 min and 11 min. The level of SH in micromoles/g of protein was strongly reduced in both cases. Further experiments on this behalf will be carried out in the near future.

REFERENCES

1. Wierbicki, E., L.E. Kunkle & F.E. Deatherage, Changes in waterholding capacity and cationic shifts during the heating and freezing of meat as revealed by a simple centrifugal method for measuring shrinkage. Food Technol. 11 (1957)69-73.

2. Hamm, R., Biochemistry of meat hydration. Proc. 11th Res. Conf. A.M.I.F. 50 (1959) 17.

3. Hamm, R. & F.E. Deatherage, Changes in hydration and charges of muscle proteins during freeze-dehydration and heating of meat. Food Res. 25 (1960) 573-610; 623-629

4. Fukazawa, T., Y. Hashimoto & T. Yasui, Effect of storage conditions on some physicochemicals properties in experimental sausage prepared from fibrils. J. Food Sci. 26 (1961) 331-336; 541-549

5. Samejima, K., Y. Hashimoto, T. Yasui & T. Fukazawa, Heat gelling properties of myosin, actin, actomyosin and myosin-subunits in a saline model system. J. Food Sci. 34 (1969) 242-245.

6. Hamm, R. & K. Hofmann, Changes in the sulfhydryl groups in beef muscle proteins during heating. Nature 207 (1965) 1269-1271.

7. Sedlak, J. & R.H. Lindsay, Estimation of total, protein-bound and non-protein sulfydryl groups in tissue with Ellman's reagent. Anal. Biochem. 25 (1968) 192-205.

8. Hamm, R. & K. Hofmann, Bestimmung von Sulfhydryl- und Disulfid-Gruppen in Myofibrillen und Muskelgewebe mit Hilfe der amperometrischen Titration. Z. Lebensm. Untersuch. Forsch. 130 (1966) 133- 144.

9. Hamm, R. & K. Hofmann, Bestimmung von Sulfhydrylgruppen in Muskelgewebe und Myofibrillen mit N-Äthylmaleinimid. Z. Lebensm. Untersuch. Forsch. 130 (1966) 85-92.