3733

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EFFECT OF COOKING ON CERTAIN NITROGEN FRACTIONS OF BEEF AND THE CHANGES OCCURRING SUBSEQUENTLY IN THESE FRACTIONS DURING ASEPTIC STORAGE AT 37°C.

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It was shown in earlier work that in the course of aseptic autolysis during storage of raw beef at 37°C, there was continuous production of non-protein-nitrogen, which seemed to be derived mainly from the soluble sarcoplasmic proteins (1). The almost complete absence of collagen fractions soluble in 0.1M KCl indicated that no major autolytic change took place in the connective tissue fraction.

Inthe present work, the changes occurring in the same nitrogen fractions of beef on cooking and during subsequent sterile storage were studied.

Experimental.

The procedures given in detail in the report of the earlier work (1) for the preparation, storage and analysis of samples of bovine longissimus dorsi, muscle were followed closely, the only major modification being the inclusion of the cooking step. Briefly, the sample was homogenised and extracted in 0.1M KCl. The trichoroacetic acid fraction, the KCl soluble fraction and the insoluble residue were analysed for all or some of the following components - total nitrogen (TN), non-protein-nitrogen (NPN), tyrosine and hydroxyproline (OHP). No free OHP was found in any of the samples. The quoted values therefore represent OHP found in the fractions after acid hydrolysis.

The two degrees of cooking were (a) "mild" cook, in which the samples were held at 70° for 15 or 25 minutes and (b) "full" cook in which the samples were held at 100° for 45 or 60 minutes. These conditions were sufficient according to recent work to cause complete inactivation of the catheptic enzymes present (2).

The samples were stored aseptically in nitrogen at 37°.

Results.

The more important analytical values are given in Table 1.

Changes occurring on cooking fresh meat.

After cooking, only 1 - 2% of the total protein N remained soluble in 0.1M KCl. On mild cooking, (samples N2, T1, R5, S4) there were increases of the order of 7% in the non-protein N soluble in trichloroacetic acid (TCA) and also changes in the collagen fraction which resulted in 2-5% of the total OHP being present in the fraction soluble in 0.1M KCl.

On fully cooking, (samples T3, R7, S7), similar increases in TCA-soluble N were observed but the OHP soluble in 0.1M KCl had increased to 10 - 21% of the total OHP.

The degradation of the proteins caused by pressure cooking for 3 hours at 126° (sample T4) was reflected in increases of 85% and 60% respectively in the values for non-protein N and tyrosine soluble in TCA, and also in an increase from 1.0 to 16.6% in the protein N soluble in 0.1M KCl. In addition, the collagen had become entirely soluble, all the OHP being present in the fraction soluble in 0.1M CKl; 62% of the OHP was also present in the TCA soluble fraction. The absence of any detectable OHP in the insoluble residue of this sample indicated that the proportion of elastin present was relatively very small.

Changes occurring on cooking meat after a period of asptic storage in the raw state (Samples R2, R4, Q2, S2, S3).

The main finding in these tests was that the storage history of the samples in the raw state did not make any appreciable difference to the overall effect of cooking. Thus, on mild cooking samples which had been stored 8, 30 and 172 days at 37°, the non-protein N which was initially high due to autolysis did not change, the protein N soluble in 0.1M KCl was reduced from 10% to about 3% of the total after 30 days and from 4% to about 0.3% after 172 days at 37°.

The OHP soluble in 0.1M KCl remained at the low level of 2 to 5% found in cooked fresh samples, but there was a suggestion that a small fraction of collagen amounting to 0.5 to 2% of the total OHP had become soluble in TCA. In addition, the tyrosine soluble in TCA showed increases from 6 to 21%, the increases being in inverse proportion to the percentage of soluble tyrosine present before cooking.

On fully cooking sample S3 after 30 days at 37° in the raw state, the only difference from the corresponding mild cooked sample was the usual increase to about 9.5% in the OHP soluble in 0.1M KCl, part of which was also soluble in TCA. In addition, there was a further increase of approximately 12% in the tyrosine soluble in TCA.

The collagen in the single sample, R4, cooked after 19 days at 5°, had the same order of solubility in 0.1M KCl as the collagen in the samples stored at 37°.

Changes occurring during aseptic storage of cooked meat at 37°.

During storage of mild cooked meat, (Samples R5, R6, S4, S5, S6) the non-protein N increased slowly from 11.2 to 12.9% of the total N over

35

a period of 97 days. The proportion of protein soluble in 0.1M KCl did not change, but remained at 2 to 3% of the total N. The tyrosine soluble in TCA increased by approximately 35%.

In the corresponding fully cooked samples, (R7, R8, S7, S8, S9) the increase in non-protein N was similar, the protein soluble in 0.1M KCl remained unchanged at 1.1 to 1.4% of the total N and the tyrosine soluble in TCA increased by only 20%.

The most interesting observation in these tests was the change which took place in the solubility of the collagen fraction. The values plotted in Figure 1 show a steady production of fractions soluble in 0.1M KCl derived from collagen. The values for samples R6 and R8 (Table 1) suggest that there may be an initial lag period of 8 days or more before the collagen becomes increasingly soluble.

A certain proportion of the KCl soluble collagen was also soluble in TCA, amounting to 7.7 and 13.9% of the total OHP in the mild and fully cooked samples respectively after storage for 96 days.

DISCUSSION.

The intermediary stages of the degradation of insoluble collagen in muscle to the soluble derived gelatin are not well defined. The present results show that on mild cooking only 1.9 to 4.7% of the collagen becomes soluble in 0.1M KCl: on fully cooking for 45 to 60 minutes at 100°, 10.9 to 21.6% of the collagen becomes soluble. Cooking the meat for 3 hours at 126° however, causes a high degree of degradation of the collagen which becomes 100% soluble in 0.1M KCl and 62% soluble in 10% TCA.

The results from the cooking of the samples stored in the raw state are interesting since after storage for 30 and 172 days the presence of traces of collagen soluble in TCA in the mild and fully cooked samples indicates that the collagen in the stored samples suffers greater degradation than the collagen in the unstored samples. It may be that during storage, the structure becomes more susceptible to change on cooking. The overall effect of storage for 172 days at 37° on the collagen fraction is however very small.

The other interesting effect of storage in the raw state is the increased formation on cooking of tyrosine soluble in TCA. This is much greater than in the unstored meat and indicates that changes take place during storage which produce a greater susceptibility of the protein or peptide fractions to release fractions which contain tyrosine and are soluble in TCA.

It is realised that values for tyrosine obtained by absorption at λ 293 in 0.2N NaOH may not be true values but should be accepted for the present rather as "tyrosine index" values until more reliable values are available.

When meat is stored aseptically in the cooked state, the results are quite different. The tyrosine soluble in TCA shows a significant increase indicating autolysis of protein during storage, particularly in the mild cooked samples.

The collagen fraction becomes altered during storage and produces fractions not only soluble in 0.1M KCl but soluble also in TCA. The rates of production of OHP soluble in 0.1M KCl in mild and fully cooked samples over 30 days at 37° were 2.2 and 5.0 µg/g/day respectively. After storage for 97 days, 22.6 and 55% respectively of the total OHP presentin these samples had become soluble in 0.1M KCl. This change is not likely to be due to enzymic action but is most probably due to purely physical changes in the collagen. The effect has been observed by Gustavsen with hide powder stored at 37° in water and in solutions of various salts (3). In 4M solutions of sodium or potassium chloride for example, approximately 25% of the collagen became soluble after storage for 120 days; Gustavsen suggests that this solubilisation is due to the disruption of secondary valencies since there is no evidence that primary valency bonds are broken.

It would be expected that this type of solubilisation would take place more readily in the thermal denatured collagen present in the cooked meat samples. It was observed in these samples that the cohesion of the connective tissue between the fibre bundles, (perimysium) became weaker with duration of storage.

References.

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TABLE 1. CHANGES IN VARIOUS FRACTIONS OF BOVINE MUSCLE (LONGISSIMUS DORSI) ON COOKING AND DURING SUBSEQUENT STERILE STORAGE. N VALUES AS % TN.

Animal	Storage Days(°C)	Cooking Conditions °C(Mins)	N sol. in TCA (NPN)	Total OHP µg/g	% of total OHP sol. in 0.1M KCl.	% of total tyrosine sol. in TCA
N(Control)						
1	2 (0°)	Raw	10.9	550	Nil	10.9
2	Ditto	70°(25)	11.7	550	4.7	11.6
T 1	2 (0°)	70°(25)	12.5	535	4.7	-
2	Ditto	70°(65)	13.3	514	5.3	-
3	Ditto	100°(60)	13.3	500	21.6	-
4	Ditto	126°(180)	20.2	494	100	17.5
R 1	8 (37°)	Raw	17.5	1110	Nil	15.7
2	Ditto	70°(25)	16.3	780	3.9	19.0
3	19 (5°)	Raw	-	-	-	-
4	Ditto	70°(25)	13.3	701	3.3	13.9
Q 1	172(37°)	Raw	31.4	735	<1	34.9
2	Ditto	70°(25)	32.3	675	5.9	37.0
S 1	30 (37°)	Raw	24.2	790	Nil	21.0
2	Ditto	70°(15)	24.6	644	2.9	23.5
3	Ditto	100°(45)	24.6	707	9.5	26.8
R 5	0	70°(25)	11.6	678	3.4	10.4
6	8 (37°)	70°(25)	12.3	705	4.2	13.7
7	0	100°(60)	11.8	595	10.9	12.0
8	8 (37°)	100°(60)	12.4	709	10.5	13.4
S 4	0	70°(15)	11.2	765	1.9	10.5
5	30 (37°)	70°(15)	12.2	646	12.6	14.2
6	97 (37°)	70°(15)	12.9	690	22.6	14.2
7	0	100°(45)	11.6	627	11.9	10.0
8	30 (37°)	100°(45)	12.0	704	31.9	11.1
9	97 (37°)	100°(45)	12.6	670	55.0	12.0

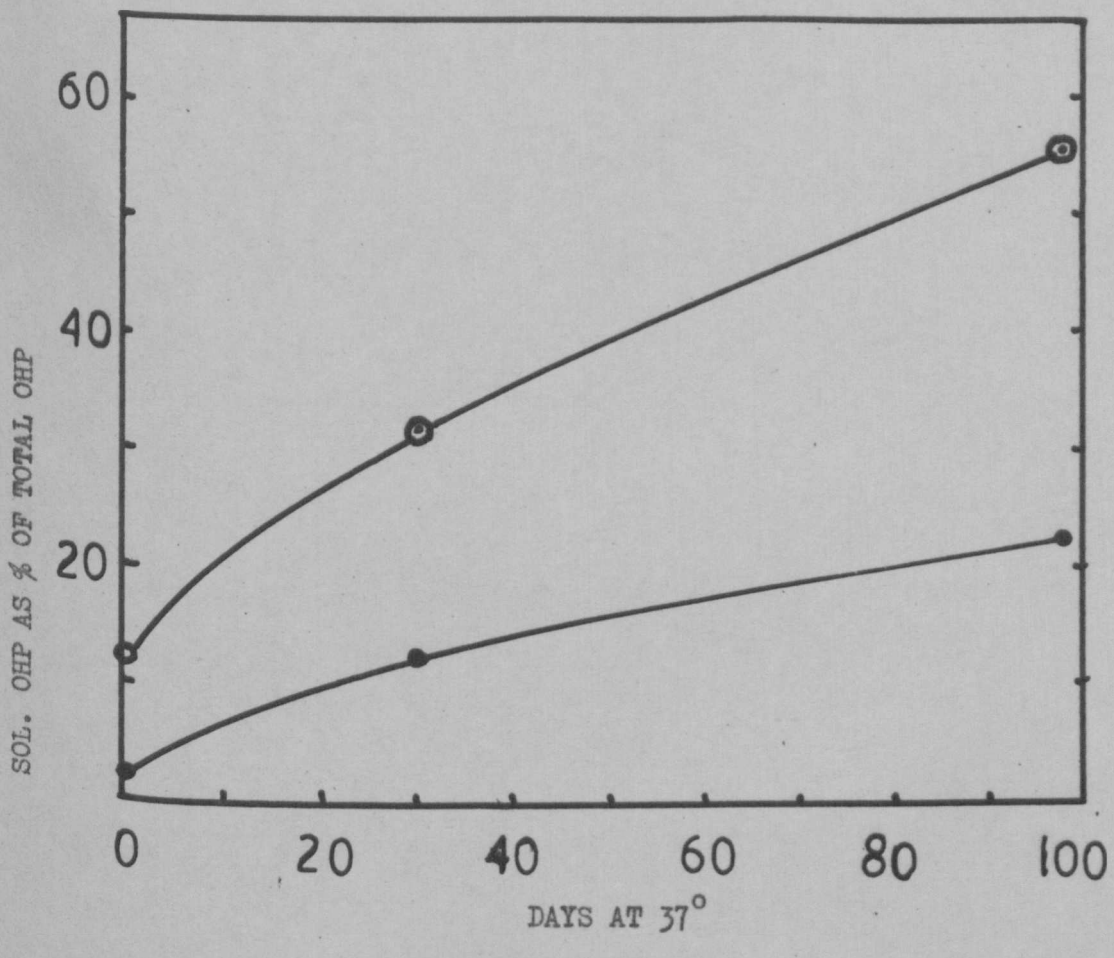

FIG. 1. CHANGES IN SOLUBILITY OF COLLAGEN FRACTION DURING STORAGE OF COOKED MEAT AT 37°.

●—● HEATED 15 MINS AT 70°
○—○ " 45 " " 100°

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SUMMARY.

On mild cooking samples of bovine, longissimus dorsi, only 2 - 5% of the collagen becomes soluble in 0.1M KCl: on fully cooking for 60 minutes at 100°, 11 to 23% of the collagen becomes soluble.

Sterile storage of meat in the raw state at 37°C for 6 months does not alter the solubility of the collagen in the cooked samples.

During sterile storage of cooked meat, however, there is continuous non-enzymic change in the collagen which produces fractions soluble in 0.1M KCl and in 10% trichloroacetic acid.

In mild and fully cooked samples, 23 and 55% respectively of the total collagen were soluble in 0.1M KCl after storage for 3 months at 37°C.

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Des effets de la cuisson sur certaines fractions azotées dans la viande de boeuf et les changements qui se sont produits dans ces mêmes fractions pendant préservation aseptique à 37°C.

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Résumé

Dans des échantillons de viande de boeuf (longissimus dorsi) moderément cuite le pourcentage de collagèn qui devient soluble dans 0,1M KCl est 2-5%. Après une heure de cuisson à 100°C, ce pourcentage s'élève à 11-23%.

La préservation aseptique de viande crue à 37°C pour 6 mois n'affecte pas la solubilité du collagèn dans les échantillons cuits. Cependant, dans la préservation aseptique de viande cuite, il se produit un changement continu et non-enzymatique, lequel produit des fractions de collagèn qui sont solubles dans 0,1M KCl et également dans l'acide trichloracétique (10%).

Dans les échantillons modérément et bien cuits les pourcentages respectifs de collagèn qui après un intervalle de trois mois à 37°C, étaient solubles dans 0,1M KCl s'élevaient à 23 et à 55.