IXth CONFERENCE OF EUROPEAN MEAT RESEARCH WORKERS
Budapest, September 4-11, 1963

393

28

THE EFFECT OF MEAT FLUIDS AND PROTEASES UPON THE STABILITY OF BEEF COLLAGEN

by

J. W. Giffee, Helen L. Madison, and W. A. Landmann

American Meat Institute Foundation

The University of Chicago

The fact that less tender meats require moist heat cooking methods has been known for a long time. The content of collagen in such meats is relatively high and its conversion to gelatin is much more rapid with the moist heat procedure than with dry heat. Before a desirable rate of collagen conversion will occur the temperature of the fibers must be sufficient to cause shrinkage. This shrinkage has been shown to be a melting point phenomenon (1, 2, 3), and as such is influenced by environmental factors. Winegarden (4) et al. found that connective tissues from beef softened when heated at 65°C but not much change occurred at 60°C. Since heating was performed with the tissues bathed in water rather than at the pH and salt concentration characteristic of meat it may be predicted that the temperatures reported were too high.

The use of proteolytic enzymes in less tender meats is expected to shorten the time and to reduce the final temperature required to obtain tenderness. Since most enzymes are incapable of altering undenatured collagen (5) little or no effect upon collagen should be anticipated before heating to the region of the shrinkage temperature.

We have observed that the juice expressed from beef does depress the shrinkage temperature of collagen and have confirmed these findings with proteolytic enzymes.

Materials

Collagen. -- Center corium slices were obtained from steerhide which was extracted with 10% NaCl 4 times, defatted with ether, and again extracted with 1 molal NaCl. It was then washed with water until chloride free.

Epimysial membrane regions containing a layer of adjacent muscle fibers were dissected from beef bottom round and extracted 5 times with ether at 2°C. The ether was removed at the same temperature with acetone and the latter removed under vacuum.

Fresh epimysial and perimysial collagen fibers were teased from the membranes of cuts of bottom round obtained from a local market.

Meat juice. -- Meat juice was expressed from fresh bottom round at pressures ranging from 7 to 56 kg/cm^2 in a stainless steel piston and cylinder device, which was slotted to permit escape of the juice. The juice was heated to 65°C and centrifuged to obtain a clear solution. Heating time was long enough that when the solution was reheated to 65°C gross coagulation of protein did not occur.

Methods

Shrinkage temperature. -- Shrinkage temperature, T_s, measurements were performed with a Fisher-Johns melting point apparatus which contains a thermostatically controlled temperature block. The well of the block was flooded with the test solution and the collagen fibers were placed on glass cover slips which were then inverted on top of the solution. The cover slips helped maintain a uniform fluid temperature by decreasing the evaporation surface.

Temperature correction. -- A calibration factor of -1.3°C was determined with purified diphenyl amine (m.p. 52.8-53.2°C).

Effect of pH on shrinkage temperature. -- The effect of pH on shrinkage of hide collagen was observed in the range, pH 4.0-6.5 using the Na_2HPO_4 -- citric acid buffers of Mc Ilvaine (6).

Effect of salts on shrinkage temperature. -- The effects of NaCl, $MgCl_2$, and $CaCl_2$ in varying concentrations were determined with hide collagen.

Action of papain relative to shrinkage temperature of solution. -- A purified preparation of papain was dissolved in heat-clarified meat juice to produce a range of concentrations, 0.6 to 5.0 mg/ml. The effects of the solutions upon hide collagen were observed under the same conditions used to determine T_s. The effects of the solutions upon fresh perimysial fibers were also observed.

Results

The reduction in T_s due to pH followed a relatively smooth curve and amounted to almost 20 degrees at pH 4.0 (Table I). While it appears that all the electrolytes have some depressing effects upon T_s the nature of the ions is obviously important (Table II).

When the observation of shrinkage was attempted without prior clarification of the meat juice the fibers became obscured by the coagulation of proteins. However, the pH of the juice was not altered by the procedure employed and it is assumed that shrinkage temperatures characteristic of the media were not affected. The actual shrinkage occurs over a range of 2 to 3 degrees but the temperatures reported here represent the values at which the

most active shrinkage was apparent when the heating rate was approximately 1 degree per minute (Table III).

At a concentration of 5 mg/ml of meat juice papain caused rapid dissolution of hide fibers at the shrinkage temperature previously determined for the solutions. When heated slowly the fibers dissolved without apparent shrinkage. At an enzyme concentration of 0.6 mg/ml shrinkage occurred with no apparent solution while at intermediate concentrations both shrinkage and solution were apparent.

When perimysial fibers were heated in the papain solutions their appearance was quite deceptive, particularly at the higher enzyme concentrations. Although shrinkage was not observed in the expected temperature region the form of the fiber remained visible. When the cover slip was disturbed, however, the form disintegrated showing that the collagen had been attacked. Furthermore, when the solutions were heated rapidly (5 to 10 degrees/min.) the fibers shrank dramatically in the expected range.

A preparation of beef muscle cathepsin fractionated through the DEAE cellulose step according to the procedure of Landmann (7) was also employed in a parallel study. However, the fibers were obscured due to protein coagulation. Further studies will be conducted with more highly purified muscle protease.

Conclusions

While muscle collagen characteristically shrinks in water in the range 65-67°C the fluid environment normally found in meat causes the phenomenon to occur nearer to 60 degrees. When special tenderizing agents are not employed this fact is most important when cooking is performed at low temperatures for long periods of time. Although it is known that enzymes may slowly attack collagen at temperatures below T_s, the rate of collagen breakdown is quite slow until the temperature of shrinkage is attained.

The contrast in behavior toward enzymes between hide collagen and perimysial fibers remains to be explained. Apparently the collagen from meat is loosely associated with enzyme-resistant material (perhaps mucopolysaccharide) which affords the fibers increased stability to heat.

Summary

1. Shrinkage of collagen fibers occurs near 60°C in meat rather than about 65 degrees which is the temperature of shrinkage in water.

2. The reduction in shrinkage is attributed to the pH and salt environment found in meat.

3. Enzymatic degradation of collagen occurs principally above the shrinkage temperature.

Bibliography

(1) Wohlisch, E. Biochem . 247 329 (1932)
(2) Wright, B. A., and Wiederhorn, N. M. J. Polymer Sci. 7 105 (1951)
(3) Garrett, R. R. and Flory, P. J. Nature 177 176 (1956)
(4) Winegarden, M. E., Lowe B., Kastelic, J., Kline, E. A., Plagge, A. R. and Shearer, P. S. Food Res. 17 172 (1952)
(5) Mandl, I. Advances in Enzymol. 23 963 (1961)
(6) McIlvaine, J. Biol. Chem. 49 183 (1921)
(7) Landmann, W. A. Proceedings of the Meat Tenderness Symposium sponsored by the Campbell Soup Company, Camden, New Jersey. 1963, p. 91.

Table I

The effect of pH upon the shrinkage temperature of hide collagen fibers

pH	Shrinkage temperature (°C) (corrected)
4.0	45
4.8	51
5.2	54
5.9	57
6.5	59
(H_2O)	64

Table II

The effects of certain salts upon the shrinkage temperature of hide collagen fibers

Salt concentration (Molarity)	Shrinkage temperature (°C) NaCl	$MgCl_2$	$CaCl_2$
0.02	--	59	57
0.05	--	57	53
0.08	59	--	--
0.10	--	54	52
0.15*	59	--	--
0.20	--	51	51
0.31	59	--	--
0.50	--	48	45
1.0	--	42	35
1.5	59	37	24
2.0	--	27	10

Table III

Shrinkage temperatures of collagen fibers in meat juice

pH	Shrinkage Temperature (°C) Hide fibers	Fresh perimysial fibers	Defatted perimysial fibers
5.45-5.62*	54(53.5-56)**	59(58-61)**	59(59-61)**
5.9	56(55-57)**	60(59-61)**	

* pH range from five different meat samples

** Observed range of shrinkage.