Studies on the Digestion of Meat

V. Influence of heat upon the digestibility of muscle proteins

FUMIO OHTAKA and FUJIYA HONGO

Since 1907 when Grindley presented the report on the digestibility of raw, roasted, fried, and boiled beef, a number of papers on the effect of heat upon the digestibility of meat have been reported. With respect to proteins of animal sources, the method of heating appears to determine whether a loss of nutritive value occurs. With certain plant proteins, moderate heat has been shown to enhance the food value, whereas more severe treatment lowers the nutritive value. Again, there is evidence that actual destruction of essential amino acids from protein occurs during heating, whereas other experimental data indicate that reducing sugars combine with amino groups of protein to yield products largely resistant to the action of proteolytic enzymes[1]. Schroeder *et al.*[2] reported that the *in vitro* digestibility of the air-dried meat proteins decreased with increasing carbohydrate concentration upon dry-autoclaving, but when the samples were reconstituted with water such decreases were largely eliminated and the proteins showed increased susceptibility to proteolysis. Previous work in this series[3] has shown the influence of heat upon the digestibility of beef. In the present paper we wish to report further the influence of heat and glucose on the digestibility of muscle proteins (actin, myosin, and actomyosin fractions).

Materials and Methods

Materials. Crystalline trypsin and pepsin were obtained from Nutritional Biochemicals Corporation. Actin was prepared from fresh pork muscle by the method given by Szent-Györgyi[4]. Myosin was extracted with Straub's solution from fresh pork muscle and purified by the method of Mommaerts *et al.*[5], and dissolved in 0.5 M KCl solution. Actomyosin was prepared also from fresh pork muscle by a slight modification[6] of the method of Szent-Györgyi, and dissolved in 0.6 M KCl solution. The concentration of the proteins used in this study was determined by micro-Kjeldahl analysis, assuming a nitrogen content of 16 per cent for all the proteins.

Methods. Enzyme hydrolysis of actin, myosin, and actomyosin was carried out in the similar reaction systems. For peptic hydrolysis, the substrate solution was buffered with 0.2 M KCl-0.2 M HCl solution at pH 2.0; for tryptic hydrolysis, 0.2 M phosphate buffer of pH 8.0 was used.

Samples of 2 m*l* of protein solution were pippeted into test tubes; the test tubes were closed with a rubber stopper holding a glass capillary which acts as a vent, and heated in a water bath or autoclaved for 30 minutes. The weight of the test tube containing 2 m*l* of

sample solution was measured before boiling and the loss of evaporation was corrected with distilled water after boiling. After cooling to a temperature of 37°C, 2 m*l* of buffer solution and 1 m*l* of enzyme solution containing 0.04 mg pepsin or 0.01 mg trypsin were added to the sample solution. Both enzyme and buffer solution were prewarmed in a constant water bath to the experimental temperature before mixing together. The digestion mixture was incubated at 37°C for 30 minutes with constant shaking. The reaction was stopped by adding 1 m*l* of 1 M trichloroacetic acid (TCA) after digestion. The trichloroacetic acid precipitate was removed by filtration, and 1 m*l* of the filtrate was made alkaline with 5 m*l* of 0.4 M Na_2CO_3 solution prior to the addition of 1 m*l* of dilute Folin-Ciocalteau reagent[7]. The intensity of the resulting blue color was measured at 660 mμ in a Hitachi Model EPU-2 spectrophotometer. A blank was used in which trichloroacetic acid was added before the enzyme was introduced.

Results and Discussion

Effect of heat. The digestibilities of the muscle proteins heated at different temperatures are shown in Tables 1 and 2. Trypsin hydrolyzed the heat-denatured actin or actomyosin to about the same extent of unheated one, though the value of the actomyosin heated at 120°C was somewhat higher than that of unheated one. The heated myosin, especially the one heated at 60°C, was hydrolyzed to a lesser extent than was unheated one by trypsin. But the

Table 1. The effect of heat on tryptic digestion of muscle proteins (optical density of TCA filtrate).

Fraction of protein	Temperature of heating (°C)				
	Unheated	60	80	100	120
Actin (1.8 mg/m*l*)	0.151 0.155 0.161	0.161 0.166 0.168	0.156 0.144 0.151	0.166 0.150 0.151	0.162 0.168 ——
Myosin (7.6 mg/m*l*)	0.183 0.161 ——	0.116 0.105 0.115	0.138 0.137 0.142	0.139 0.143 ——	0.152 0.151 0.160
Actomyosin (7.9 mg/m*l*)	0.148 0.161 0.157	0.162 0.169 0.166	0.161 0.151 0.164	0.160 0.150 ——	0.190 0.197 0.208

Table 2. The effect of heat on peptic digestion of muscle proteins (optical density of TCA filtrate).

Fraction of protein	Temperature of heating (°C)				
	Unheated	60	80	100	120
Actin (3.2 mg/m*l*)	0.329 0.380 0.334	0.220 0.197 0.193	0.179 0.224 0.228	0.193 0.163 0.176	0.182 0.191 0.191
Myosin (7.6 mg/m*l*)	0.234 0.250 ——	0.151 0.120 0.124	0.114 0.105 ——	0.164 0.184 0.177	0.166 0.166 0.168
Actomyosin (7.9 mg/m*l*)	0.292 0.294 0.287	0.127 0.149 0.122	0.138 0.127 ——	0.132 0.139 0.155	0.160 0.160 0.151

difference in the extent of hydrolysis between heated and unheated myosin was not so large. In the case of peptic digestion (Table 2), heat had great influence on the extent of solubilization of the muscle proteins, i. e., the digestibilities of unheated proteins were considerably higher than those of the muscle proteins heated at different temperatures (60, 80, 100, and 120°C). Especially the unheated actin or actomyosin was hydrolyzed by pepsin to about twice the extent of the heated one. This clearly demonstrates the difference in the methods of attack on muscle proteins by these two proteolytic enzymes.

The rate of hydrolysis of the muscle proteins autoclaved at 120°C was also determined.

Figure 1. The rate of tryptic hydrolysis of heat-denatured muscle proteins.

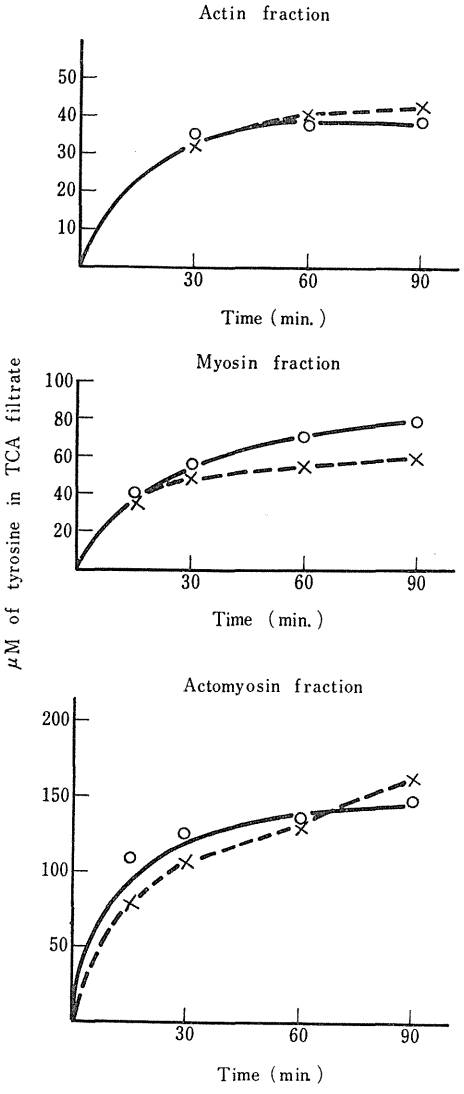
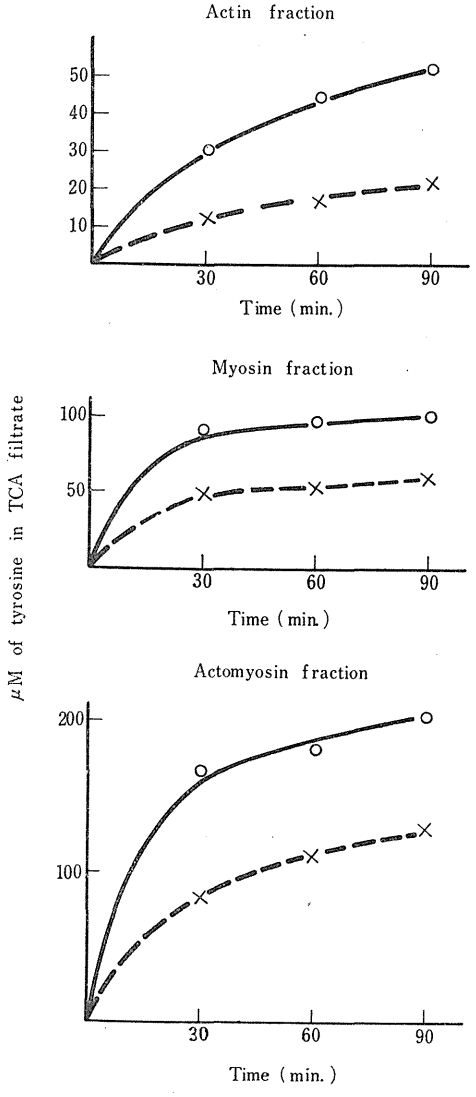


Figure 2. The rate of peptic hydrolysis of heat-denatured muscle proteins.



The results are shown in Figures 1 (tryptic digestion) and 2 (peptic digestion). On the basis of these data, it seemed reasonable to assume that the digestibility of muscle proteins was diminished by heating, and the difference in digestibility between the heated and the unheated samples was more distinct in peptic hydrolysis than in tryptic hydrolysis.

It has been pointed out before[8] that many of the globular proteins in their native state are resistant to proteolytic enzymes, but that they become susceptible to the enzymes after denaturation. The experimental work of Haurowitz and his coworkers[9] had demonstrated that ovalbumin and pseudoglobulin heated at 100°C for 30 minutes were hydrolyzed more rapidly than the native ones by trypsin, while the rate of hydrolysis of myosin and fibrinogen remained almost unchanged after heat denaturation. Native collagen is hardly attacked by trypsin, while heated collagen is readily hydrolyzed. The resistance of native globular proteins to the action of proteolytic enzymes is probably due to the absence of points of attack for the enzyme molecules; when the peptide chains of the protein molecule are unfolded by denaturation, the structures required for enzyme action become accessible and proteolysis occurs. The groups required for the action of trypsin are the basic side chains of arginine or lysine[8]. These are, apparently, beneath the surface of the globular molecules of the native protein inaccessible to the enzymes, although they are titratable, i. e., accessible to the small hydrogen ions. On the other hand, fibrous proteins have an extended or folded polypeptide chains, but presumably the structures are not so folded as globular proteins. Therefore, it is assumed that the rate of hydrolysis of fibrous protein remains almost unchanged after heat denaturation.

But in the peptic hydrolysis (Fig. 2), the rate of hydrolysis of heated actin, myosin, and actomyosin was significantly lower than that of unheated ones. For this reason, it may be considered that the surface area of protein particles contacting with enzymes diminished due to the heat coagulation of the proteins; the action of enzymes was disturbed because of the formation of some compound or compounds of proteins with the impurities in the sample.

Myosin and actomyosin were dissolved in KCl solution. Therefore, in order to see if KCl has any influence on the digestion of muscle proteins heated with KCl, myosin solution containing 0.25 to 0.5M KCl was put to test. The results are summarized in Tables 3 and 4.

As shown in Tables 3 and 4, the extent of hydrolysis of myosin decreased apparently as the concentration of KCl increased. But the difference in digestibility between heated and unheated myosin was conspicuous regardless of the addition of KCl.

Table 3. The effect of KCl on tryptic digestion of myosin.

Treatment	Concentration of KCl solution (M)		
	0[a]	0.25[b]	0.5[c]
Heated*	0.203 0.217	0.156 0.164	0.153 0.142
Unheated	0.276 0.278	0.243 0.234	0.210 0.209

a Myosin was dispersed in distilled water.
b Myosin was dispersed in 0.25M KCl soln.
c Myosin was dissolved in 0.5M KCl soln.
* Autoclaved at 120°C for 30 minutes.

Table 4. The effect of KCl on peptic digestion of myosin.

Treatment	Concentration of KCl solution (M)		
	0[a]	0.25[b]	0.5[c]
Heated*	0.301 0.293	0.153 0.149	0.099 0.106
Unheated	0.410 0.408	0.370 0.368	0.320 0.332

a Myosin was dispersed in distilled water.
b Myosin was dispersed in 0.25M KCl soln.
c Myosin was dissolved in 0.5M KCl soln.
* Autoclaved at 120°C for 30 minutes.

Effect of glucose. Table 5 shows the effect of glucose on the digestion of muscle proteins. From the data with pepsin and trypsin it would appear that the results of Table 5 on the digestibility of heated and unheated muscle proteins were similar to those of Table 1 and 2 and the addition of glucose had no effect. It is known that when milk proteins are dry-heated in the presence of reducing sugars, glyconyl peptides are formed which are hydrolyzed only with difficulty by the digestive proteases, with the result that growth is retarded, nitrogen balance becomes negative and the *in vitro* liberation of amino nitrogen is inhibited. The presence of water in minimal amounts appears to prevent the sugar-peptide formation during heating. Schroeder *et al.*[2] showed that the digestibility of air-dried meats decreased with increasing carbohydrate concentration upon dry-autoclaving with glucose, but when the samples of meats were reconstituted with water such decrease were largely eliminated and the proteins showed increased susceptibility to proteolysis.

Table 5. The effect of heating with glucose on the digestion of muscle proteins.

Enzyme	Fraction of protein	Glucose	Temperature of heating (°C)		
			Unheated	60	120
Pepsin	Actin	None	0.196 0.209	0.126 0.142	0.130 0.118
		Added*	0.203 0.185	0.114 0.135	0.136 0.148
	Myosin	None	0.277 0.280	0.195 0.199	0.143 0.122
		Added**	0.288 0.284	0.198 0.207	0.164 ——
	Actomyosin	None	0.392 0.392	0.177 0.196	0.218 0.209
		Added***	0.387 0.406	0.194 0.213	0.206 0.219
Trypsin	Actin	None	0.161 ——	0.133 0.133	0.148 0.152
		Added*	0.156 ——	0.125 0.136	0.199 0.185
	Myosin	None	0.190 0.187	0.169 ——	0.172 0.172
		Added**	0.209 0.209	0.176 0.176	0.180 0.173
	Actomyosin	None	0.130 0.130	0.129 0.135	0.157 0.135
		Added***	0.138 0.126	0.141 0.129	0.189 0.178

* To 2 m*l* of 0.23 % actin, 0.5 m*l* of 2 % glucose was added and heated at 60 or 120°C for 30 minutes.

** To 2 m*l* of 0.72 % myosin, 0.5 m*l* of 6 % glucose was added and heated at 60 or 120°C for 30 minutes.

*** To 2 m*l* of 0.81 % actomyosin, 0.5 m*l* of 6 % glucose was added and heated at 60 or 120°C for 30 minutes.

Table 6 shows the effect of glucose concentration on the tryptic and peptic hydrolyses of myosin. To 5 m*l* of 0.26 % myosin, 1 m*l* of 0.06 to 0.48 M glucose was added, and the mixture was autoclaved at 120°C for 30 minutes. After autoclaving, 1 m*l* of enzyme solution was added, and the digestion mixture was incubated at 37°C for 30 minutes. As shown in Table 6, the effect of amount of glucose added was not found on the digestion of myosin under these conditions.

Table 6. The effect of amount of glucose added on the digestion of myosin.

Enzyme	Concentration of glucose soln. (M)				
	0	0.06	0.12	0.24	0.48
Trypsin	0.179 0.165	0.177 0.179	0.176 0.174	0.178 0.173	0.177 0.176
Pepsin	0.124 0.134	0.150 0.157	0.143 0.132	0.159 0.141	0.142 0.153

Effect of pH. The digestibility of the muscle proteins autoclaved with 0.5 % glucose at different pH levels is shown in Tables 7 and 8. As the pH value of the myosin solution used in this experiment was 6.9, the specimens of pH 2.0, 5.4, or 8.0 were ajusted with HCl or NaOH to the desired pH levels before autoclaving. And the solution of myosin was autoclaved at 120°C for 30 minutes with or without glucose. After autoclaving, the myosin was hydrolyzed at 37°C for 30 minutes by trypsin or pepsin.

Table 7. The effect of pH of heating on tryptic digestion of myosin.

Glucose	pH			
	2.0	5.4	6.9	8.0
None	0.255 0.259 0.238	0.148 0.145 0.180	0.149 0.157 0.157	0.183 0.178 0.189
Added	0.238 —— ——	0.171 0.173 0.154	0.179 0.173 0.165	0.233 0.241 0.227

Table 8. The effect of pH of heating on peptic digestion of myosin.

Glucose	pH			
	2.0	5.4	6.9	8.0
None	0.228 0.208	—— ——	0.110 0.104	0.149 0.137
Added	0.222 0.218	0.087 0.090	0.090 0.095	0.153 0.134

Except for the result of pH 8.0 by trypsin, there was no effect of the addition of glucose on the digestion of autoclaved myosin. But, at pH 8.0 in Table 7, the digestibility of myosin autoclaved with glucose was higher than that of myosin autoclaved without glucose. Therefore it will be necessary to investigate further with respect to this question. The digestibilities of myosin autoclaved at pH 2.0 or 8.0 were apparently higher than those of myosin autoclaved at pH 6.9 or 5.4. This is probably due to the denaturation of myosin by acid or alkali followed by subsequent enzymatic attack. It is known that most proteins are denatured below pH 3 and above pH 10, though numerous exceptions exist. The isoelectric precipitation zone of myosin is pH 4.8 to 7.5. Therefore, myosin may coagulate readily on heating at pH 5.4 or 6.9, and consequently resists to enzymatic attack. In either case, it is assumed that glucose had no effect on the digestion of myosin if the myosin was heated with water.

Summary

The effects of heat, potassium chloride, glucose, and pH level of heating on the digestibility of muscle proteins (actin, myosin, and actomyosin fractions) were investigated. The results obtained are as follows.

1. The digestibilities of heated muscle proteins were lower than those of unheated ones. And the difference in digestibility between heated and unheated muscle proteins was more evident in peptic hydrolysis than in tryptic hydrolysis.

2. Potassium chloride had a negative effect on the digestibility of muscle proteins. The digestibility of myosin decreased apparently as the concentration of KCl increased. But the difference in digestibility between heated and unheated myosin was conspicuous regardless of the addition of KCl.

3. The addition of glucose had no effect on the digestibility of muscle proteins if the proteins were heated with water. When myosin was heated at different pH levels (2.0, 5.4, 6.9, and 8.0), the digestibilities of myosin autoclaved at pH 2.0 or 8.0 were apparently higher than those of myosin autoclaved at pH 6.9 or 5.4. This is probably due to denaturation of myosin by acid or alkali followed by subsequent enzymatic attack.

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摘　　要

肉の消化に関する研究

第5報　筋肉蛋白質の消化に対する加熱の影響

大高　文男・本郷冨士弥

筋肉蛋白質を加熱した時の消化性について検討するため，豚肉からアクチン，ミオシンおよびアクトミオシン各区分の筋肉蛋白質を調製してペプシンおよびトリプシンによる人工消化試験を行なった。

1．各区分の蛋白質とも，加熱による影響はトリプシン消化におけるよりもペプシン消化の時に著しかった。ペプシン消化の時は加熱による消化率の低下が明瞭であって，両酵素の作用の差が認められた。

2．ミオシンおよびアクトミオシン区分は塩化カリ溶液にとかして用いたので，消化に対する塩化カリの影響について実験した。その結果，塩化カリ濃度が高くなれば消化性の低下する傾向が認められた。しかし加熱による影響は塩化カリの有無にかかわらず前記の通りで，特にペプシン消化の時に著しかった。

3．グルコースが存在するときの筋肉蛋白質の消化に対する加熱の影響について実験したが，この実験におけるように水と共に加熱する方式の場合にはグルコース添加の影響は認められなかった。なお加熱時の pH をかえたところ，pH が酸性（2.0）またはアルカリ（8.0）側においては，酵素作用の前に酸またはアルカリによる加水分解が行なわれるためか，中性付近のものよりも消化されやすかった。