K/2

Some observations on the water-holding capacity of meat

Norihide ANDO*, Tomio OHASHI** and Tatsumi ITO
Laboratory of Chemistry and Technology of Animal Products,
Faculty of Agriculture, Kyushu University, Fukuoka, JAPAN.

Summary

A quantitative investigation on the relationship between the acidic and basic polar groups of meat proteins and the water-holding capacity of meat was performed with beef samples treated with 0,2,5 and 10 % of sodium chloride, respectively. The amounts of acidic and basic groups of meat proteins were determined by the dye-binding method using safranine O for the estimation of acidic groups and orange G for basic groups. In addition, the amount of main acidic groups, i.e., carboxyl groups of meat proteins was determined by estimating the amount of methanol used for esterifying the carboxyl groups to form methoxycarbonyl groups.

According to the results of the present work, the water-holding capacity of meat was improved in the order treated with 0,10,2 and 5 % of sodium chloride, and the better the water-holding capacity of meat became, the more the amount of meat proteins existing in sol state in particular that of acto-myosin in sol state and the amounts of acidic, basic and carboxyl groups of those meat proteins in sol state obviously increased.

From the above observations, it may be suggested that when the amount of polar groups of meat proteins in sol state significantly increased by the addition of a favourable amount of sodium chloride, the amount of water molecules binding to those increased polar groups by hydrogen bonds may have conceivably increased, and thus the improvement in the water-holding capacity of meat may have taken place.

* Present address : Takamiya 2-14-20, Minami-ku,
815 Fukuoka-shi.
** Faculty of Agriculture, University of Miyazaki, Miyazaki.

EINIGE BEMERKUNGEN ÜBER DAS WASSERBINDUNGSVERMÖGEN DES FLEISCHES

Norihide ANDO, Tomio OHASHI
und Tatsumi ITO,

JAPAN

Zusammenfassung :

Eine quantitative Bestimmung der Beziehung zwischen den saueren und basischen Eiweisspolargruppen und dem Wasserbindungsvermögen des Fleisches wurde mit verschiedenen Proben von Rindfleisch durgeführt, die vorher mit bzw.
0, 2, 5 und 10% Natriumchlorid zugesetzt wurden.

Die saueren und basischen Gruppen wurden durch die Färbungsmethode mit dem Safranin O für die Sauergruppen und mit dem Orange G für die basischen gruppen bestimmt.

Ausserdem wurde die Menge der wichtigsten Sauergruppen (d. h. Carboxylgruppen) durch die Menge von Methanol bestimmt, die für die Veresterung der Carboxylgruppen in Methoxylkarbonylgruppen verwendet wird.

Die Ergebnisse der Untersuchung zeigten, dass das Wasserbindungsvermögen mit einem Zusatz von bzw. 10, 2 und 5 % Natriumchlorid verbessert wird ; gleicherweise wurde eine bedeutende Vermehrung der Muskelproteine (und besonders Aktomyosin) als Sol und der saueren, basischen und Carboxylgruppen der Fleischproteine als Sol ermittelt.

Darüber hinaus kann man behaupten, dass eine gesicherte Vermehrung der Polargruppen der Fleischproteine als Sol durch einen bestimmten Zusatz von Natriumchlorid zu einer Vergrösserung der Zahl der Wassermoleküle führt, die mit diesen Polargruppen durch Wasserstoffbindungen verbunden sind ; das Wasserbindungsvermögen des Fleisches wird also dadurch verbessert.

QUELQUES OBSERVATIONS SUR LE POUVOIR DE RETENTION D'EAU DE LA VIANDE

NORIHIDE ANDO, TONIO OHASKI et TATSUMI ITO
JAPON

Résumé :

Une recherche quantitative de la relation entre les groupes polaires acides et basiques des protéines et le pouvoir de rétention d'eau de la viande a été effectuée sur des échantillons de viande de boeuf additionnée respectivement de 0, 2, 5 et10 % de chlorure de sodium.

- les groupes acides et basiques ont été déterminés par la méthode de coloration avec la sofranine O pour les groupes acides et l'orange G pour les groupes basiques.

En plus,la quantité des principaux groupes acides (c à d les groupes carboxyl) a été déterminée par la quantité de méthanol utilisé pour estérifier les groupes carboxyl en groupe methoxy-carbonyl.

Les résultats de l'étude ont montré que le pouvoir de rétention d'eau était amélioré, de façon croissante avec une addition de 0, 10, 2 et 5% de chlorure de sodium; parallélement à cette amélioration la quantité de proteines musculaires (et en particulier d'actomyosine) à l'état de sol et les groupes acides basiques et carboxyl des proteines de la viande, à l'état de sol, augmentaient nettement.

On peut suggérer, à partir de ces observations, que quand la quantité de groupes polaires des protéines de la viande à l'état de sol augmente significativement par addition d'une quantité convenable de chlorure de sodium, la quantité des molécules d'eau retenues à ces groupes polaires par les liaisons hydrogéne peut augmenter,améliorant ainsi le pouvoir de retention d'eau de la viande.

It is well known that sodium chloride remarkably improves the water-holding capacity (WHC) of meat. In the previous works, it has been observed that the addition of 4.6 - 5.8 % of sodium chloride (ionic strength 0.8 - 1.0) exhibited the most favourable effect on the WHC of meat[1-7], and also it has previously been pointed out that changes in the charged groups and structure of meat proteins may chiefly affect the WHC of meat[8]. As to the effect of sodium chloride on the charged polar groups and structure of meat proteins, the following several suggestions have been offered, i.e., the addition of a favourable amount of sodium chloride may have resulted in the binding of Cl^- ions to positively charged polar groups and the increase in the electrostatic repulsion between negatively charged polar groups of meat proteins[5], the binding of both Na^+ and Cl^- ions to the counter charged polar groups and the cleavage of salt linkages between positively and negatively charged polar groups of meat proteins[9,10], the increase in the amounts of free bivalent metal ions (Ca^{2+}, Mg^{2+} and Zn^{2+} ions) presumably liberated from meat proteins by the cleavage of cross linkages in meat proteins, neither of Na^+ and Cl^- ions binding to the counter charged polar groups of meat proteins[11], and in all of the above cases, it has been considered that the loosening of the structure of meat proteins may have taken place, and accordingly the WHC of meat may have been improved.

Nevertheless, it seems that hardly any detailed quantitative investigation on the relationship between the acidic and basic polar groups of meat proteins and the WHC of meat has so far been performed. In the present work, therefore, some observations have been made on this point with beef samples treated with sodium chloride.

Experimental

In the present experiments, beef samples were comminuted first, then treated with 0(control),2,5 and 10 % of sodium chloride at 4°C for 72 hours respectively, and the pH and ionic

strength of each solution used in preparing beef homogenate, extracting meat proteins, and dialyzing beef homogenate, i.e. the pH and ionic strength of the outer liquid used in dialysis, were adjusted to those of each beef sample employed for each experiment with M/7.5 phosphate buffer and sodium chloride in the same manner as described in the previous paper[12], which will be termed A-solution in the present paper.

Since the ionic strength of meat has been reported to be about 0.22 - 0.26 in general[13], and it is also well known that it is very difficult to estimate accurately the ionic strength of meat, in the present work the ionic strength of A-solution used in the experiment on each control sample was adjusted to 0.22 with sodium chloride and that of each A-solution used in each of the experiments on each of the beef samples other than control was adjusted with each amount of sodium chloride with which each sample was treated plus that of sodium chloride used for the adjustment of the ionic strength of A-solution in the case of control beef sample.

In each of the present experiments, therefore, the meat proteins extracted with each A-solution prepared by the procedure described above may be considered as those existing in sol state in each beef sample, and the extract obtained itself may be considered as a sol, and thus the meat proteins in the extract will be termed sol meat proteins, sol actomyosin and so on, and the extract obtained itself will be termed sol in the present paper.

1. Beef samples

Beef samples (M.adductores) at three days after slaughter were used in the determinations of acidic and basic polar groupes, and beef samples (M.vastus fibularis) at three days after slaughter were used in the investigations on the distribution of carboxyl groups, sol meat proteins and bivalent metal ions (Ca^{2+}, Mg^{2+} and Zn^{2+} ions) respectively.

2. Figures for pH and the WHC of beef samples were determined by the methods described in the previous papers[14,15].

3. Determination of acidic and basic polar groups of

meat proteins

From 5g of each beef sample 100ml of each beef homogenate was prepared by homogenizing with its respective A-solution in a Waring blendor for 3 minutes, cooling with ice water, and each beef homogenate prepared in this way was employed for the determination of the total acidic and basic polar groups of meat proteins in beef.

Each sol sample was prepared by centrifuging 50ml of each beef homogenate for 10 minutes at 10,000 rpm then filtering the supernatant through glass wool, and the resulting each sol sample was employed for the determination of acidic and basic polar groups of meat proteins in the sol in beef.

On reference to the method of Fraenkel-Conrat and Cooper[16], the amounts of acidic and basic polar groups of meat proteins in beef and those in the sol in beef were determined by the dye-binding method using safranine O for the estimation of acidic polar groups and orange G for basic polar groups following the procedures described in detail in the previous paper[12].

4. Determination of total sol protein nitrogen, sol myosin nitrogen and sol actomyosin nitrogen

The total protein, myosin and actomyosin in the sol prepared by the procedure described above was precipitated following the methods of Barnstein (total protein) and Khan[17] (myosin and actomyosin) respectively, and then the amount of nitrogen of each precipitate was determined by the micro Kjeldahl method.

5. Determination of carboxyl groups of meat proteins

Each 75g of comminuted beef was treated with 0,2,5 and 10 % of sodium chloride respectively, and then each of them was homogenized with 75ml of its respective A-solution in a Waring blendor for 5 minutes, cooling with ice water. The resulting each homogenate sample was dialyzed against 3 liters of its respective A-solution for 72 hours at 4°C using a cellophane membrane, renewing the outer each A-solution at 24-hour intervals.

Each 2g of the dialyzed homogenate was lyophilized and then employed for the determination of the total carboxyl groups of meat proteins in beef.

Another each 10g of the dialyzed homogenate was homogenized again for 3 minutes in the same manner to prepare 100ml of a secondary dialyzed homogenate, which was separated into two fractions, i.e. the insoluble residue and the sol by centrifuging for 10 minutes at 10,000 rpm and then filtering through glass wool.

Each insoluble residue was homogenized over again as before to prepare 100ml of a homogenate of insoluble residue, and 20g of which was lyophilized, then employed for the determination of carboxyl groups of meat proteins in insoluble residue.

Each 20ml of the sol prepared by the procedure described just above was also lyophilized, and then employed for the determination of carboxyl groups of meat proteins in the sol in each beef sample.

According to the method of Fraenkel-Conrat and Olcott[18], each lyophilized sample was first treated with methanol and hydrochloric acid at 4°C for 48 hours to esterify the carboxyl groups to form methoxycarbonyl groups, and then according to the method of Mathers and Pro[19], the methoxycarbonyl groups formed from the carboxyl groups were hydrolyzed with sulphuric acid to liberate again the methanol used for the esterification, then the amount of methanol liberated again from the methoxycarbonyl groups was determined colorimetrically following the procedures described in detail in the previous paper[20], and from which the amount of carboxyl groups of meat proteins was calculated.

6. Determinations of Ca^{2+}, Mg^{2+} and Zn^{2+} ions in beef

The amount of each of the above bivalent metal ions in each beef sample and that in each of the dialyzed homogenate samples prepared by the procedure described above were determined by the procedures described in detail in the previous paper[21], and the former was expressed as the total amount of each of the bivalent metal ions and the latter was expressed as the amount of each of the bound bivalent metal

ions in beef. The amount of each of the free bivalent metal ions in beef was calculated by subtracting the latter from the former.

Results and Discussion

It has been observed in the present work that the WHC of beef was improved in the order treated with 0,1 0,2 and 5 % of sodium chloride, as was to be expected.

The results given in Table 1 indicated that the difference in the amount of sodium chloride added did not seem to bring about any appreciable changes in the total amounts of acidic and basic polar groups of meat proteins in beef, but the amount of total sol protein nitrogen and also the amounts of acidic and basic polar groups of the sol meat proteins in beef evidently increased with the increase in the WHC of beef.

Each amount of the sol meat proteins determined in the present work, as shown in Table 2, increased with the increase in the WHC of beef, and of all of them the amount of sol actomyosin did seem to have the most close relation to the variation in the WHC of beef resulted from the addition of varied amounts of sodium chloride, viz., the WHC of beef obviously increased with the increasing amount of sol actomyosin.

The behaviour of carboxyl groups of meat proteins shown in Table 3 seemed to be substantially similar to that of acidic and basic polar groups of meat proteins shown in Table 1, namely, the total amount of carboxyl groups of meat proteins in beef remained almost unchanged by the addition of varied amounts of sodium chloride, while the amount of carboxyl groups of sol meat proteins in beef increased manifestly as the WHC of beef increased.

According to the results given in Tables 4,5 and 6, the amounts of free Ca^{2+} and Mg^{2+} ions in beef increased by the addition of sodium chloride up to 5 %, accompanying the decreases in the amounts of bound Ca^{2+} and Mg^{2+} ions. A similar tendency was also observed in the case of Zn^{2+} ions, though

Table 1. Effect of sodium chloride on the WHC of beef, the amount of total sol protein nitrogen in beef and the binding-amount of dye to beef and the sol in beef

Sample [a]	NaCl added (%)	WHC (%)	Total sol protein N (%)	Total binding-amount of dye to beef		Binding-amount of dye to the sol in beef	
				Safranine O [b]	Orange G [c]	Safranine O [b]	Orange G [c]
A	None	47.43	0.535	0.108	0.208	0.030	0.051
	2	57.03	0.832	0.107	0.205	0.050	0.081
	5	59.65	0.960	0.104	0.202	0.069	0.093
	10	51.64	0.630	0.102	0.200	0.033	0.057
B	None	49.84	0.617	0.118	0.228	0.040	0.058
	2	60.32	0.926	0.113	0.225	0.061	0.089
	5	61.88	1.210	0.112	0.219	0.079	0.103
	10	53.31	0.784	0.108	0.215	0.045	0.071

a) Treated at 4°C for 72 hours.
b) Expressed as the figure for absorbance at 520mμ per 0.2mg of each beef sample.
c) Expressed as the figure for absorbance at 480mμ per 1.0mg of each beef sample.

Table 2. Effect of sodium chloride on the WHC of beef and the distribution of sol protein nitrogen in beef

Sample [a]	NaCl added (%)	WHC (%)	Total sol protein N (%)	Sol myosin N (%)	Sol actomyosin N (%)	Other sol protein N [b] (%)
A	None	46.75	0.541	0.023	0.046	0.472
	2	58.43	0.956	0.049	0.358	0.549
	5	60.77	1.074	0.060	0.448	0.566
	10	51.81	0.846	0.043	0.318	0.485
B	None	46.68	0.512	0.031	0.045	0.436
	2	55.73	0.942	0.038	0.397	0.507
	5	58.82	1.022	0.042	0.439	0.541
	10	51.46	0.824	0.035	0.311	0.478

a) Treated at 4°C for 72 hours.

b) Sol protein N other than sol myosin N and sol actomyosin N.

Table 3. Effect of sodium chloride on the distribution of carboxyl groups in beef

Sample *	NaCl added (%)	Cardoxyl groups (m moles/100g beef)		
		Total	Insoluble residue	Sol
A	None	38.26	34.02	3.34
	2	38.10	31.16	6.11
	5	37.99	29.81	7.38
	10	38.08	32.61	4.78
B	None	39.15	34.77	3.48
	2	39.43	32.86	5.77
	5	39.68	30.90	8.08
	10	39.39	34.17	4.62

* Treated at 4°C for 72 hours.

Table 4. Effect of sodium chloride on the distribution of calcium ion in beef

Sample *	NaCl added (%)	Total Ca^{2+} (mg/100g beef)	Bound Ca^{2+} (mg/100g beef)	Free Ca^{2+} (mg/100g beef)
A	None	5.27	3.62	1.65
	2		2.85	2.42
	5		2.47	2.80
	10		2.43	2.84
B	None	5.76	3.83	1.93
	2		2.97	2.79
	5		2.66	3.10
	10		2.58	3.18

* Treated at 4°C for 72 hours.

Table 5. Effect of sodium chloride on the distribution of magnesium ion in beef

Sample *	NaCl added (%)	Total Mg^{2+} (mg/100g beef)	Bound Mg^{2+} (mg/100g beef)	Free Mg^{2+} (mg/100g beef)
A	None	25.53	18.81	6.72
	2		15.63	9.90
	5		12.45	13.08
	10		11.83	13.70
B	None	26.87	18.51	8.36
	2		16.47	10.40
	5		13.23	13.64
	10		13.07	13.80

* Treated at 4°C for 72 hours.

Table 6. Effect of sodium chloride on the distribution of zinc ion in beef

Sample *	NaCl added (%)	Total Zn^{2+} (mg/100g beef)	Bound Zn^{2+} (mg/100g beef)	Free Zn^{2+} (mg/100g beef)
A	None	4.73	3.85	0.88
	2		3.70	1.03
	5		3.62	1.11
	10		3.60	1.13
B	None	5.15	4.54	0.61
	2		4.48	0.67
	5		4.40	0.75
	10		4.37	0.78

* Treated at 4°C for 72 hours.

the amounts of which found in both free and bound forms were fairly smaller. These observations are in good agreement with those observed by Berman and Swift[11].

In each case of the above bivalent metal ions, however, any appreciable increases in the amounts of both of their free and bound ions could hardly be observed when the amount of sodium chloride added increased from 5 to 10 %.

Since the WHC of beef markedly decreased by increasing the amount of sodium chloride added from 5 to 10 % accompanied by the considerable decreases in the amounts of sol meat proteins, especially sol actomyosin, and the acidic and basic polar groups of sol meat proteins in beef, in the present case the behaviours of the above bivalent metal ions did not seem to have played any substantial role in the change in the WHC of beef, whereas the salting-out effect of the undesirably increased amount of sodium chloride added may have principally been responsible for the significant decrease in the WHC of beef.

Since the pH values of the beef samples used in the present experiments were higher than the isoelectric point of meat proteins and the addition of sodium chloride shifts the isoelectric point of meat proteins to a lower pH range in general, the amount of charged acidic polar groups of meat proteins may have increased by the addition of sodium chloride. Accordingly, it may be considered possible that not only Cl^- ions but also Na^+ ions bind to the respectively counter charged polar groups of meat proteins, as reported by Sherman[9,10], and this may conceivably lead to the cleavage of the electrostatic linkages between positively and negatively charged polar groups of meat proteins, viz., the salt linkages in meat proteins.

Further, it has been observed in the previous work of Berman and Swift[11] and also in the present work that the addition of a favourable amount of sodium chloride seems to have been appreciably effective in increasing the amount of free bivalent metal ions in meat presumably liberated from meat proteins by the cleavage of cross linkages in meat proteins

possibly due to an ion exchange reaction with Na^+ ions from sodium chloride added, and this may have conceivably promoted the loosening of the structure of meat proteins as pointed out by Hamm[22-24].

Taking the above facts into consideration, it may be suggested from the results of the present experiments that the addition of a favourable amount of sodium chloride may have resulted in the increase in the amount of sol meat proteins and the increases in the amounts of acidic and basic charged polar groups of sol meat proteins presumably resulting from the loosening of the structure of sol meat proteins due to the increase in the electrostatic repulsion between positively and negatively charged polar groups of sol meat proteins and the cleavage of the salt linkages between positively and negatively charged polar groups of sol meat proteins and also the cleavage of cross linkages in sol meat proteins, and consequently the binding-amount of water molecules to those increased charged polar groups of sol meat proteins by hydrogen bonds may have possibly increased, which may have led to the increase in the hydrophilic affinity of those increased sol meat proteins.

The mechanism of the WHC of meat is certainly much complicated and the details of which still remain obscure, but the above facts may presumably be considered at least partly responsible for the improvement in the WHC of meat by the addition of a favourable amount of sodium chloride.

Furthermore, it has also been observed in our laboratory that the addition of sodium nitrite at the amount usually used in processing meat products, i.e. 0.02 % sodium nitrite, caused a slight but appreciable increase in the WHC of meat, and the effect of nitrite on the WHC of meat seems particularly of great interest in special connection with the interaction of nitrite and the ε-amino groups of lysyl residues of meat proteins. Preparing bovine myosin and actomyosin, an investigation in the effect of nitrite on certain characteristics of myosin and actomyosin is now in progress.

References

1) J.R.Bendal : J.Sci.Food Agr., 5,468 (1954)
2) R.Grau und A.Böhn : Fleischwirtschaft, 7,652 (1955)
3) C.E.Swift and R.Ellis : Food Technol., 10,546 (1956)
4) R.Grau und O.Fleischman : Fleischwirtschaft, 9,252 (1957)
5) R.Hamm : Z.Lebensm.Untersuch.u.-Forsch., 106,281 (1957)
6) R.Hamm und R.Grau : ibid., 108,28 (1958)
7) T.Ohashi and N.Ando : Bull.Faculty of Agriculture, University of Miyazaki, 14,390 (1967)
8) R.Hamm : Z.Lebensm.Untersuch.u.-Forsch., 109,337 (1959)
9) P.Sherman : Food Technol., 15,79 (1961)
10) P.Sherman : ibid., 16,4,91 (1962)
11) M.D.Berman and C.E.Swift : J.Food Sci., 29,182 (1964)
12) T.Ohashi, H.Sera and N.Ando : Bull.Faculty of Agriculture, University of Miyazaki, 15,194 (1968)
13) J.T.Edsall : J.Biol.Chem., 89,289 (1930)
14) N.Ando, Y.Kako and Y.Nagata : Bull.Meat and Meat Products, 1,1 (1961)
15) N.Ando, Y.Kako, T.Ohashi, Y.Hirakata, N.Suematsu and E.Katamoto : ibid., 2,1 (1963)
16) H.Fraenkel-Conrat and M.Cooper : J.Biol.Chem., 154,239 (1944)
17) A.W.Khan : J.Food Sci., 27,430 (1962)
18) H.Fraenkel-Conrat and H.S.Olcott : J.Biol.Chem., 161,259 (1945)
19) A.P.Mathers and M.J.Pro : Anal.Chem., 27,1662 (1955)
20) T.Ohashi, H.Sera and N.Ando : Bull.Faculty of Agriculture, University of Miyazaki, 16,202 (1970)
21) T.Ohashi, H.Sera and N.Ando : ibid., 19,261 (1972)
22) R.Hamm : Naturwissenschaften, 42,394 (1955)
23) R.Hamm : Z.Lebensm.Untersuch.u.-Forsch., 107,423 (1958)
24) R.Hamm : ibid., 110,95 (1959)
25) T.Ohashi and N.Ando : Bull.Faculty of Agriculture, University of Miyazaki, 14,381 (1967)