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ABSTRACT

Veränderungen an Proteinen aus gepökeltem Schweinefleisch

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Es wurden Veränderungen an den extrahierbaren Proteinen aus folgenden Proben untersucht: 1.) Frisch, 2.) ungepökelt, zwei Tage gelagert, 3.) zwei Tage gelagert, danach zwei Tage gepökelt. Fraktionierung wurde dadurch erreicht, dass die Proben mit Sand gemahlen und die Sand-Fleischgemengsel durch eine Extrahiermittelgradiente (KCl) extrahiert wurden. Von den so eluierten Fraktionen wurden Messwerte für Proteinextrahierung und -fraktionierung, spezifische von Ca^{++} und Mg^{++} activierte ATPasen-Wirksamkeit, elektrophoretische Mobilität und Ultrazentifugensedimentierung erhalten. Die Resultate zeigen, dass diese Verfahrungsweisen von Wert sind. Verlust einiger bei niedriger Ionenstärke extrahierbaren Proteine fand statt; zwei Neue wurden anscheinend erzeugt. Auch an Proteinen, die bei hoher Ionenstärke extrahierbar sind, wurden deutliche Veränderungen wahrgenommen.

Protein Changes in Producing Cured Pork[1/]

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During recent years our knowledge of the hydration and dehydration of meat, the manufacture of sausage emulsions, and color has greatly increased. These and properties such as tenderness depend on the proteins present. However, only limited progress has been made in the basic problem of separating, identifying, and characterizing these proteins, which, to an undetermined extent, are different from those present in fresh tissue. Development of new methods for improving meat quality and meat processing may well be accelerated if an adequate knowledge of meat proteins is obtained.

The complexity of problems in investigating proteins soluble in water or at low ionic strength appears much less than that met in investigating the myofibrillar group. While the water soluble proteins have yet to be thoroughly investigated, methods capable of fractionation and characterization are available. Mainly, systematic application of these methods to meats of different types, ages, and that subjected to various types of processing is required. Electrophoresis has been used to detect as many as 22 components (1).

[1/] This paper was prepared for discussion at the 11th European Meeting of Meat Research Workers. It should be regarded more in the nature of a progress report and as material for discussion than as a completed publication.

Ion-exchange on cellulose has been used to separate as many as 14 fractions from post rigor meat (2, 3). Other workers have made excellent contributions using one or the other of these methods (4, 5, 6, 7, 8, 9). The work needed to ascertain the role and importance of these numerous proteins in meat is considerable owing to the need for thorough physical chemical and enzymatic analyses.

In contrast, much less success has resulted from attempts to characterize the myofibrillar proteins. Moreover, the value of much of that has been decreased because unjustified assumptions have frequently been advanced which would be valid only if meat proteins bear a closer likeness to those of fresh tissue than is probable. It is more likely that a considerable portion of the proteins of meat can only be rigorously characterized through the application of a similar variety of physical chemical methods and tools as has been used in investigating the proteins of fresh muscle tissue.

In the investigation discussed in the present paper attention has mainly been directed towards the myofibrillar proteins. Of initial concern was the need to extract proteins as completely as possible from the different samples to avoid the possibility that important fractions would be overlooked. Also, facility and avoidance of artifacts was desired. Since grinding tissue with sand is an approved method of disintegrating biological material, it was thought that the method could be applied to meat and that preliminary fractionation could be obtained by extracting sand-tissue mixtures with a suitable gradient of electrolyte solution (KCl). This report presents some of the results obtained with

this technique as applied to the detection of changes produced in storing and curing pork. The short period of storage (2 days) and brief cure (2 days) used is typical of many commercial operations. Methods used in the analysis of protein fractions includes measurements of specific adenosine triphosphatase activity, electrophoretic mobility and of ultracentrifugal sedimentation.

EXPERIMENTAL AND METHODS

Meat Samples. Hams from 200 pound pigs were obtained immediately after slaughter. Analysis of the semimembranosus muscles was begun within 1-1/2 hours (fresh samples). Portions of the muscles were stored at 3°C for 2 days; some portions were analyzed as 2-day old samples, and others were cured for 2 days before analysis. Curing was done in 68° pickle, containing 732 g. NaCl, 6.72 g. $NaNO_2$, 448 g. $NaNO_3$ and 112 g. sugar dissolved in 3530 g. water.

Sand Grinding and Sand Column. 25 g. of ham was thoroughly ground with 50 g. of thoroughly-washed sea sand in a mortar. Approximately 50 g. of sand was added and the grinding continued. Then 100 g. of sand was added and mixed, using a spatula. The entire 225 g. of mixture was then pressed uniformly and lightly into a column (6.5 cm. width, 15 cm. heighth), the bottom being made of coarse sintered glass; and a solvent gradient of KCl, buffered at pH 7.0, was introduced (beginning with water and attaining a maximum concentration of 1.2 M KCl-.05 M imidazole, pH 7, total volumes varying from 1 L. to 2 L., but usually 1200 ml.). After the fresh tissue was initially cooled, the temperature of tissue

and extracts was not higher than 5°C. In some experiments, such as that described in Table 1, the sand-meat mixture was extracted with 300 ml. 0.05 M KCl, .002 M imidazole prior to preparing the column. Subsequently, this was found unnecessary and all extractions were accomplished by elution of the column, as described above. As a guide in combining portions of eluate to provide 1 to 15 fractions to be analyzed, the absorbance of eluate was monitored at 280 mμ by an automatic absorbance recorder.

Concentrations of protein were determined by the biuret reaction checked by the micro-kjeldahl method. ATPase activities were determined using previously described conditions (10), combined with a method for determining inorganic phosphorus with increased sensitivity (11). Disc electrophoresis was undertaken with extracts of tissue obtained with 0.05 M KCl, .002 M imidazole using 7% acrylamide gel and on extracts obtained with 0.6 M KCl, .025 M imidazole using 3% gel. The conditions used were a slight modification of those previously described (12). In ultracentrifugation, the Beckman-Spinco Model E analytical centrifuge was employed.

RESULTS

As shown by differences and changes in the ATPase activities of fractions described in Table 1, the gradient elution accomplished appreciable fractionation. An excellent rate of flow of eluant through the sand-meat mixture was possible; a rate of approximately 3 ml. per minute was used. However, the fractionation obtained was less at high rates of flow.

Plots of absorbance vs. volume of eluant served mainly as a guide in combining fractions of eluate because they tended towards a general similarity. An exception was the consistent appearance of a peak in extracted cured samples when extracted at 0.25-0.30 M KCl. This was not observed in extracting uncured samples. The yields of protein extracted, comparing the total extracted with that which could be extracted from samples ground with sand and allowed to stand overnight in 0.6 M KCl, pH 7, ranged from 75% to over 90%, depending on length of the extraction period rather than on the concentration of solvent.

In extracts from fresh tissue, preferential Ca^{++}-activated specific ATPase activity has been associated with myosin and preferential specific Mg^{++}-activated activity with actomyosin or with myosin B (9). Mg^{++}-activated activity is also to be expected of particulate extracts. However, these generalizations need not apply to proteins from aged or cured tissue and are not assumptions in the present discussion. The Mg^{++}-activated ATPase activity was elevated in the extracts at low ionic strength (Fractions 1 and 2), reflecting activity of particulate material. After decreasing in Fractions 3 to 7, activity increased markedly in the fractions which followed. Ca^{++}-activated activity followed a generally similar pattern, but was noticeably higher in Fraction 7 in proportion to Mg^{++}-activated activity. The data show a definite fractionation yielding at least three groups of components: (1) the fraction extracted at low ionic strength, (2) fractions

in which the Ca^{++}-activated protein is relatively elevated, and (3) fractions in which the Mg^{++}-activated material is present in relatively increased proportions. As shown by data presented in Table 2, ATPase decreased generally and markedly during 2 days of storage of the tissue, but 2 days of curing produced only slight changes. Also, ATPase activity of proteins extracted at lower ionic strength decreased during aging and curing more sharply than that of proteins extracted at high ionic strength.

The results obtained with disc electrophoresis on acrylamide gels are shown in Table 3. It should be noted that this work was done with extracts obtained with 0.05 M KCl, 0.002 M imidazole, and with 0.6 M KCl, 0.05 M imidazole, independent of fractions prepared by gradient elution. The data indicate that seven fractions were separated from extracts obtained with 0.05 M KCl and two with 0.6 M KCl. From the proteins extracted with 0.05 M KCl, two fractions, R_m values of 0.11 and 1.50, were obtained only from the fresh tissue. One fraction, R_m 0.87, increased during the two days of storage and even more on curing. Another, R_m 1.20, increased appreciably during two days of storage and subsequent curing. Two fractions, R_m 0.91 and 1.10, were substantially unaltered by either storage or curing. The results indicate appreciable change occurred, including destruction and creation of proteins.

The proportions of proteins extracted with 0.6 M KCl and representative of the myofibrillar proteins changed during aging and curing, as shown by the ratios 0.58, 0.39 and 0.27. Interpreted

in the classical manner, this trend could indicate a tendency for myosin to decrease and actomyosin to increase. However, the identity and characteristics of these proteins is conjecture and ascertaining the facts will require continued investigation

Some fractions obtained by elution of sand-meat mixtures were subjected to ultracentrifugal analysis (see fractions marked with asterisks, Table 2). The fractions proved to be mixtures containing only small amounts of material sedimenting at low speeds (24,600 r.p.m.) and material sedimenting at higher speeds (59,700 r.p.m.) as broad peaks which tended to broaden with centrifugation and to sediment without fractionation. Measurements of two of these peaks, Fractions 6 and 10, were calculated to have sedimentation constants corresponding to S_s 3.5 and S_s 4.3, respectively.

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Table 1. A Typical Analysis of Fractions Eluted from Cured Pork[1/]

Fraction	Volume collected	Protein extracted, mg.	Specific ATPase activity[2/]	
			Ca^{++}-activated	Mg^{++}-activated
1	100	85	2.3	13.7
2	100	106	1.3	6.9
3	100	89	1.9	5.0
4	125	87	4.6	5.6
5	100	51	5.4	7.8
6	100	45	3.2	9.5
7	100	51	7.8	3.5
8	100	43	4.6	10.5
9	100	51	10.4	13.9
10	100	41	23.1	44.2
11	100	31	19.6	37.8
12	75	30	19.1	34.7
		710[1/3/]		

[1/] This sample was extracted twice with 0.05 M KCl, pH 7.0, prior to above extraction.

[2/] μMoles P_i per mg. per min. x 10^{-3}.

[3/] Tissue yielded 11.17 mg/ml. when extracted overnight with 200 ml. of 0.6 M KCl and 4.24 mg/ml. when extracted with 300 ml. 0.05 M KCl, indicating 11.17 mg. x 200 – 4.24 mg. x 300 = 962 mg. protein salt extractable. On this basis, 74% was recovered in the above extraction (which was not conducted exhaustively).

Table 2. Change of Specific ATPase Activities[1/] During Storage and Curing

Fraction	Ca^{++}-activated			Mg^{++}-activated		
	Fresh	Aged	Cured	Fresh	Aged	Cured
1	3.9	2.0	2.2	17.5	13.5	9.8
2	17.1	4.6	9.2	29.9	20.2	-
3	19.7	8.5	9.8	29.4	28.3	31.9
4	19.8	7.5	9.2	30.3	17.7	27.9
5	38.2	7.1	8.8	51.1	17.5	23.5
6	38.2	10.7	8.7	59.9*[2/]	18.1	25.5
7	45.0	7.2	10.6	42.6	20.3	27.4
8	24.6	24.0	7.4	93.0*	43.3	14.9
9	73.8	32.0	23.8	97.0	62.8	58.3
10	73.2	73.5	52.3	97.3*	83.8	80.0

[1/] μMoles P_i per mg. per min. x 10^{-3}.

[2/] Fractions analyzed by ultracentrifugation.

Table 3. Disc Electrophoresis of Ham Extracts[1/]

Relative mobility (R_m)	Fresh	Aged	Cured
Extracted with 0.05 M KCl, pH 7[2/]			
0.11	1[3/]	0	0
0.87	0	1	3
0.91	8	8	7
1.00	2	4	1
1.10	7	8	6
1.20	2	5	7
1.50	4	0	0
Extracted with 0.6 M KCl, pH 7[4/]			
Ratio R_m A/B	0.58	0.39	0.27

1/ 25 g. tissue ground with 50 g. sand and extracted with total of either 300 ml. 0.05 M KCl, pH 7, or 200 ml. 0.6 M KCl, pH 7.

2/ Fractionated on 7% acrylamide gel.

3/ Relative proportion of fraction.

4/ Fractionated on 3% acrylamide gel.