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Promena belančevina za vreme salamurenja svinjskog mesa

Protein Changes in Producing Cured Pork

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PROMENA BELANČEVINA ZA VREME SALAMURENJA SVINJSKOG MESA*

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KRATAK SADRŽAJ

Praćene su promene ekstraktivnih belančevina u 1) svežem, 2) nesalamurenom (2 dana zrenja) i 3) mesu tretiranom ingredijencijama salamure pošto je ono odzrelo dva dana. Ispitivanja promena salamurenog mesa vršena su posle dvodnevnog delovanja ingredijencija. Frakcionisanje je vršeno usitnjavanjem uzoraka s peskom i ekstrakcijom smeše pesak-meso uz postepeno dodavanje sredstva za ekstrakciju (KCl). Iz izdvojenih frakcija dobijeni su podaci o ekstrakciji i frakcionisanju proteina, specifičnom dejstvu Ca^{++} i Mg^{++} ATF-aze na aktivaciju, ultracentrifugalnoj sedimentaciji i elektroforetskoj pokretljivosti. Rezultati pokazuju da je tehnika rada bila dobra. Gubitak nekih proteina koji se ekstrahuju pri maloj jonskoj jačini javlja se kao rezultat zrenja i salamurenja; pokazalo se da se formiraju dva nova proteina. Znatne promene su isto tako otkrivene kod proteina koji se ekstrahuju pri visokoj jonskoj jačini.

Upoznavanje faktora koji određuju kvalitet i karakteristike mesa značajne za preradu je osnovni problem pri ispitivanju mesa. Očigledno je da se najvažnija grupa faktora odnosi na sadržaj, sastav i svojstva proteina. Brojna ranija ispitivanja su pokazala da faktori koji izazivaju promene svojstava proteina, kao pH i prisustvo specifičnih jona, znatno utiču na meso. S druge strane, nije poznat stepen i uticaj varijacija u sastavu proteina različitih vrsta mesa. Učinjen je izvestan napredak u separaciji, identifikaciji i karakterisanju ovih proteina, koji se, u izvesnim slučajevima, znatno razlikuju od proteina svežeg tkiva pre mrtvačke ukočenosti. Ova ispitivanja će zahtevati velike napore većeg broja stručnjaka za duži period vremena. Takvo ispitivanje je, međutim, logičan preduslov za razvijanje naučne osnove na višem nivou u tehnologiji mesa. Laboratorijska istraživanja na ovom polju trajala su nekoliko godina. Najpre su ispitivani proteini mesa pre mrtvačke ukočenosti a u poslednje vreme proteini mesa uopšte.

Tema rada je problematika ekstrakcije i frakcionisanja proteina iz svežeg, odzrelog i salamurenog svinjskog mesa i određivanje promena u toku zrenja i salamurenja. Prilikom ispitivanja međusobnog dejstva faktora koji utiču na kvalitet mesa poželjno je prikupljanje brojnih podataka koji omogućuju adekvatne statističke analize. Zbog toga je potrebno analizirati veliki broj uzoraka. Analize proteina u uzorcima mesa bile bi znatno olakšane ako bi se razradio metod koji bi se sastojao od ekstrakcije i efikasnog frakcionisanja uzoraka uz minimalni utrošak vremena i rada. Imajući ovo u vidu, ispitivana je vrednost usitnjavanja smeše pesak-tkivo i sukcesivnost izdvajanja. Pored toga, ispitan je značaj određivanja aktivnosti adenozintrifosfataze (ATF-aze), naročito u pogledu uticaja aktivacije Ca^{++} i Mg^{++}, kao načina karakterisanja proteina u ekstraktima. Drugi metodi analiza obuhvatili su ispitivanja pomoću disk-elektroforeze i sedimentacije korišćenjem ultracentrifuge. Iako se može očekivati da će se u toku daljih ispitivanja poboljšati metode i način interpretacije rezultata, ipak će postignuti rezultati doprineti boljem poznavanju proteina mesa i metoda za ispitivanje njegovog sastava.

TEHNIKA RADA

Uzorci mesa.— Odmah posle klanja uzimane su šunke svinja žive vage od oko 100 kg. M. semimembranosus je analiziran posle pola do jednog časa (sveži uzorci). Delovi mišića su skladišteni u toku 2 dana pri 3°C, neki delovi su analizirani posle 2 dana zrenja, dok su drugi uzorci salamureni 2 dana pre analize. Salamurenje je vršeno u salamuri pri 20°C sastava: 732: g NaCl, 6,72 g $NaNO_2$, 448 g $NaNO_3$ i 112 g šećera rastvorenog u 3530 g vode.

Usitnjavanje peskom i peščana kolona.— 25 g šunke je dobro usitnjeno sa 50 g dobro opranog i osušenog morskog peska (za analize) u tarioniku. Smesi mesa i peska dodato je novih 50 g peska a zatim je usitnjavanje nastavljeno. Posle toga je dodano još 100 g peska i promešano — špatulom. 225 g smeše je tada ravnomerno i oprezno sabijeno u kolonu (6,5 cm široku, 15 cm visoku), čije je dno bilo načinjeno od grubog sintezovanog stakla; dodan je rastvor KCl, puferovan na pH 7,0 (počinjući s vodom i održavajući maksimalnu koncentraciju od 1,2 M KCl — 0,05 M imidazol, pH 7; ukupne zapremine varirale su od 1 do 2 l, a obično 1200 ml). Pošto je sveže tkivo prvo hlađeno, to temperatura tkiva i ekstrakta nije bila viša od 5°C. U nekim eksperimentima, kao onom opisanom u tabl. 1, mešavina pesak-meso je ekstrahovana sa 300 ml 0,05 M KCl, 0,002 M imidazolom pre pripremanja kolone. Kasnije, ovo se pokazalo kao nepotrebno i sve ekstrakcije su vršene na koloni za izdvajanje, kao što je ranije opisano. U cilju spajanja eluata radi dobivanja 15 frakcija za analizu, eluat je apsorbovan pri 280 mμ automatskim registracionim apsorpcionim aparatom.

Količina proteina je određivana biuretskom reakcijom koja je standardizovana prema mikro-Kjeldahl metodu. Aktivnost ATP-aze je određivana koristeći ranije opisane uslove (1), zajedno sa metodom određivanja neorganskog fosfora uz povećanu osetljivost (2). Disk-elektroforeza je vršena na ekstraktima dobijenim ekstrakcijom tkiva sa 0,05 M KCl, 0,002 M imidazolom i ekstrahujući ostatak sa 0,6 M KCl, 0,025 M imidazolom, koristeći 7% i 3% akrilamid gel. Korišćeni uslovi su bili nešto modifikovani u odnosu na ranije opisane (3). Kod ultracentrifugiranja je korišćena Beckman-Spinco Model E analitička centrifuga**).

REZULTATI I DISKUSIJA

Usitnjavanje tkiva s peskom i postepeno izdvajanje.— Usitnjavanje tkiva s peskom se mnogo primenjivalo u ranijim ispitivanjima tkiva pošto ono omogućuje njegovu potpunu dezintegraciju uz minimalnu denaturaciju belančevina. Stoga, osnova eksperimentalnog izvođenja ovog metoda je direktna ekstrakcija tkiva pomešanog sa peskom, koristeći uzlazno kretanje KCl rastvora u cilju frakcionisanja. Metod ekstrakcije pesak-tkivo, ako bi se usavršio, smanjio bi operacije potrebne u konvencionalnijim metodima ekstrakcije i frakcionisanja, kao što su one koje su primenjivane u

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poslednje vreme (4). Efektivnost stupnjevitog izdvajanja proteina u uzlaznoj hromatografiji je potpuno dokazana; primer je frakcionisanje miozina B pomoću hromatografije na DEAE-celulozi (5).

Kao što je ukazano razlikama u promenama aktivnosti ATF-aze vidljivih frakcija u podacima iz tabl. 1. i 2, frakcionisanje je izvršeno stupnjevitim izdvajanjem. Brzina protoka kroz smešu pesak-tkivo je bila odlična. Korišćena je brzina protoka od 3 m/min., međutim, kao što će kasnije biti pomenuto, nije utvrđena optimalna brzina protoka. Pri kontinualnom registrovanju apsorpcije u funkciji od zapremine eluata obično nisu postojali maksimumi, kao što bi se očekivalo, u pogledu male varijacije u količinama proteina ekstrahovanih u sukcesivnim frakcijama (tabl. 1). Izuzetak je bila stalna pojava maksimuma kod ekstrahovanih salamurenih uzoraka kad je rastvor za ekstrakciju bio 0,25—0,35 M KCl. Količina ukupno ekstrahovanog proteina, poredeći ukupnu količinu proteina ekstrahovanog iz mešavine pesak-tkivo s ekstrahovanom količinom iz uzorka usitnjenog s peskom i mućkanog cele noći sa 8 zapremina 0,6 M KCl, pH 7 — iznosio je 75 do preko 90 odsto. Potpuniji podatak bi se verovatno dobio kad bi se zapremina rastvora povećala pri stupnjevitom izdvajanju.

Nađeno je da sadržaj proteina ekstrahovanih datom količinom eluata u velikoj meri zavisi od brzine protoka. Tako, pri korišćenim uslovima, nije došlo do ravnoteže, tj. rastvori za ekstrakciju se nisu zasitili proteinom. Da bi se potpunije ocenila mogućnost ekstrakcije s peskom, u daljem se radu ispituju rezultati dobijeni korišćenjem različitih brzina protoka.

Frakcionisanje na osnovu aktivnosti adenozintrifosfataze.— Ispitivanje prečišćenih proteina mišića pre mrtvačke ukočenosti je pokazalo da miozin pokazuje dejstvo ATF-aze u pogledu aktivacije Ca^{++}, a aktomiozin ili miozin B u pogledu Mg^{++} (6). Analize koje su se zasnivale na ovim činjenicama efektno su poslužile u ispitivanjima brzine formiranja aktomiozina, što se ogledalo u simultanom opadanju dejstva aktiviranih Ca^{++} i povećanju dejstva aktiviranih Mg^{++} jona (1). Pri ispitivanju su korišćene slične analize. Rezultati ukazuju da su takve analize pogodne kod ispitivanja mesa za određivanje svojstava komponenata proteina, mada se posmatrana aktivnost adenozintrifosfataze u zrelom i salamurenom mesu može zapaziti i kod izmenjenih proteina koji se razlikuju od proteina svežeg tkiva pre mrtvačke ukočenosti.

Relativno povećano dejstvo ATF-aze proteina ekstrahovanih pri maloj jonskoj jačini (tabl. 1, frakcija 1) na aktivaciju Mg^{++} odraz je prisustva enzima koji aktiviraju Mg^{++}, a koji su ekstrahovani iz pojedinačnih materijala; jedina razlika u pogledu prisustva proteina zapažena je između mešavina pesak-meso koje su prethodno ispirane sa 0,05 M KCl (tabl. 2) i onih kod kojih je ova procedura izostavljena (tabl. 1). Smanjenjem frakcija 3 do 7, dejstvo aktiviranog Mg^{++} se značajno povećalo u eluiranim frakcijama. Dejstvo aktiviranog Ca^{++} pojedinih frakcija odvijalo se na uobičajen, sličan način, ali je bilo znatno izraženije u frakciji 7 u odnosu na dejstvo aktiviranog Mg^{++}. Podaci pokazuju da se odvijalo određeno frakcionisanje, dajući frakciju ekstrahovanu pri maloj jonskoj jačini i frakcije povećanog dejstva kao rezultat napredovanja stupnjevitog izdvajanja. Podaci u tabl. 1. i 2. pokazuju da proteini s najvećom enzimatskom aktivnošću nisu bili ekstrahovani sve dok je jonska jačina bila relativno visoka u poređenju sa onom koja se obično koristi za ekstrakciju mišićnih proteina. Ispitivanja izdvajanja pri različitim brzinama protoka mogu dati objašnjenje ove tendencije.

Uticaj zrenja i salamurenja na specifičnu aktivnost ATF-aze. Kao što se vidi iz podataka u tabl. 2, aktivnost ATF-aze je opadala. U frakcijama koje su sadržavale glavni deo proteina (frakcije 6-10), ATF-aza je znatno opadala u toku 2 dana skladištenja i nešto više u toku 2 dana salamurenja. Pošto se odnos specifičnog dejstva aktiviranog Mg^{++} prema specifičnom dejstvu aktiviranog Ca^{++} povećao u toku zrenja i salamurenja, podaci ukazuju da su komponente proteina koje se pojavljuju u toku zrenja i salamurenja bile tipa aktomiozina, bar toliko koliko se moglo zaključiti iz aktivnosti ATF-aze.

Analize ultracentrifugom.— Nekoliko frakcija dobijenih izdvajanjem iz mešavine pesak-meso analizirano je ultracentrifugom (frakcije 6, 9 i 10, tabl. 2). Pri sutni proteini su pokazali da su mešavine koje sadrže manje količine proteina koji se talože pri malim brzinama (24.600 o/min.), a veće količine proteina koji se talože pri većim brzinama (59.700 o/min.) kao široki

Tipične analize frakcija izdvojenih iz salamurenog svinjskog mesa

Tabl. 1.

Frakcija	Sakupljena zapremina ml	Ekstrahovani protein mg	Specifično dejstvo ATF-aze na [2])	
			aktivirani Ca^{++}	aktivirani Mg^{++}
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) Ovaj uzorak je ekstrahovan dva puta sa 0,05 M KCl, pH 7,0
2) Mikromola P_i/mg protena/min. $\times 10^{-3}$
3) Tkivo je dalo 11,17 mg/ml kad je ekstrahovano preko noći sa 200 ml. 0,6 M KCl i 4,24 mg/ml kad je ekstrahovano sa 300 ml 0,05 M KCl, pokazujući 11,17 mg $\times$ 200 — 4,24 mg $\times$ 300 = 962 mg proteina ekstrahovanih slanim rastvorom visoke jonske jačine, koji ne mogu biti ekstrahovani pri maloj jonskoj jačini. Na osnovu ovoga, 74 odsto je otkriveno u gornjem ekstraktu.

Promena specifičnih ATF-aza aktivnosti [1]) u toku skladištenja i salamurenja

Tabl. 2.

Frakcija	Aktivirani Ca^{++}			Aktivirani Mg^{++}		
	sveže	zrelo	salamu.	sveže	zrelo	salamureno
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	45,0	10,7	8,7	59,9[2])	18,1	25,5
7	24,6	7,2	10,6	42,6	20,3	27,4
8	73,8	24,0	7,4	93,0[2])	43,3	14,9
9	73,2	32,0	23,8	97,0	62,8	58,3
10	73,2	73,5	52,3	97,3[2])	83,8	80,0

1) Mikromola P_i/mg proteina/min. $\times 10^{-3}$
2) Frakcije analizirane ultracentrifugovanjem.

maksimumi koji su težili da se dalje šire centrifugiranjem i da se talože bez frakcionisanja. Nedostatak tipičnih maksimuma normalnog miozina ili aktomiozina (miozini A i B) ne može da se objasni.

Uticaji zrenja i salamurenja dobiveni na osnovu frakcionisanja elektroforezom.— Dobijeni rezultati sa disk-elektroforezom na akrilamid gelima prikazani su u tabl. 3. Treba naglasiti da su frakcionisani ekstrakti dobijeni direktnom ekstrakcijom tkiva a ne postepenim izdvajanjem iz mešavine pesak-meso. Otkrivanje manjih komponenata se pokazalo da je ograničeno količinom proteina frakcionisanih ovom metodom (uzorci od 200/μg), pošto tragovi ne mogu da se oktriju pri bojenju. Skrob-gel-elektroforezom iz svinjskog mesa je dobijeno samo 7 glavnih frakcija, dok je prethodno uočeno 35 traka na frakcijama goveđeg mesa koje su služile za poređenje (7). Podaci pokazuju da je elektroforezom ekstrakata dobijenih ekstrakcijom uzoraka svinjskog mesa sa 0,05 M KCl utvrđeno 7 frakcija. Dve frakcije, sa R_m (odnos pokretljivosti) vrednostima od 0,11 i 1,50, dobijene su samo iz svežeg tkiva. Jedna frakcija, R_m 0,87, povećala se u toku 2 dana skladištenja, a čak i još više u toku salamurenja. Drugi R_m 1,20 vidljivo je porastao u toku dva dana skladištenja i zatim salamurenja. Dve frakcije, R_m 0,91 i 1,10 nisu se bitno menjale ni skladištenjem ni salamurenjem. Rezultati ukazuju da je došlo do vidne promene uključujući destrukciju i formiranje komponenata različitih pokretljivosti.

Dve glavne komponente su razdvojene disk-elektroforezom proteina ekstrahovanih iz svežih, odzrelih i salamurenih uzoraka sa 0,6 M KCl. Odnos količine pokretljivijeg (A) u odnosu na manje pokretan (B) protein dokazan je bojenjem gela sa Amido Schwartz bojom i registrujući apsorpciju uređajem za merenje gustine. Odnosi proteina menjaju se u toku zrenja i salamurenja, kao što je pokazano u tabl. 3, opadanjem odnosa — 0,58, 0,39 i 0,27 — više pokretljivih prema manje pokretljivim frakcijama. Mada frakcionisanje miofibrilarnih proteina elektroforezom nije bio naročito pogodan način u ispitivanju proteina, rezultati ukazuju da disk-elektroforeza može da dâ korisne informacije u ispitivanjima koja treba još da se obave.

Disk-elektroforeza ekstrakata šunke [1])

Tabl. 3.

Relat pokretljivost (R_m)	Sveže	Zrelo	Salamureno
Ekstrahovano sa 0,05 M KCl, pH 7[2])			
0,11	1[3])	0	0
0,87	0	1	3
0,97	8	8	7
1,00	2	4	1
1,10	7	8	6
1,20	2	5	7
1,50	4	0	0
Ekstrahovano sa 0,6 M KCl, pH 7[4])			
Odnos komponenata A i B[5])	0,58	0,39	0,27

1) 25 g tkiva usitnjenog sa 50 g peska i ekstrahovanog sa 300 ml 0,05 M KCl, pH 7, ili 200 ml 0,6 M KCl, pH 7
2) Frakcionisano na 7% akrilamid gelu
3) Relativni udeo frakcije
4) Frakcionisano na 3% akrilamid gelu
5) Odnos količina komponenata A i B dobijenih integracijom površina ispod maksimuma koje je registrovao merač gustine

REZIME

Ispitivani su ekstrakcija i frakcionisanje komponenata proteina uzoraka svežeg, odzrelog i salamurenog m. semimembranosus svinja koristeći tehniku koja se sastoji od usitnjavanja s peskom i ekstrakcije mešavine pesak-tkivo izdvajanjem s KCl pomoću uzlazne hromatografije. Rezultati ekstrakcije i frakcionisanja dobijeni su određivanjem sadržaja proteinskog azota, specifičnih dejstava Ca^{++} i MG^{++} aktiviranih ATF-azom i sedimentacijom ultracentrifugom. Efekti zrenja i salamurenja na komponente proteina su isto tako određivani analizirajući ekstrakte, koristeći disk-elektroforezu. Na osnovu rezultata mogu se izvući sledeći zaključci:

1. Moguće je usavršavanje tehnike rada koja se zasniva na usitnjavanju mesa peskom i stupnjevitom izdvajanju komponenata iz mešavine pesak-tkivo.

2. Određivanje prvenstva kod aktivacije ATF-azom je neobično značajan način ispitivanja sastava mesa.

3. Aktivnost ATF-aze svinjskog mesa opala je znatno u toku 2 dana skladištenja. Na nju su, međutim, samo u osrednjoj meri uticala 2 dana salamurenja. Aktivnost ATF-aze proteina pomerena je od aktivnosti »miozin-tipa« ka aktivnosti »aktomiozin-tipa«.

4. Rezultati disk-elektroforeze su pokazali značajne promene koje su utvrđene u obe grupe analiziranih proteina, ekstrahovanih rastvaračima pri nižoj ili višoj jonskoj jačini. One su rezultat zrenja i salamurenja.

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PROTEIN CHANGES IN PRODUCING CURED PORK*

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ABSTRACT

Changes were investigated in the proteins extractable from samples that were (1) fresh; (2) uncured, aged 2 days; and (3) aged 2 days, then cured 2 days. Fractionation was obtained by grinding samples with sand and extracting sand-meat mixtures with a gradient of extractant (KCl). Data on protein extraction and fractionation, specific Ca^{++} and Mg^{++} activated ATPase activities, electrophoretic mobilities and ultracentrifugal sedimentation were obtained on the fractions eluted. The results indicate the techniques to be of value. Loss of some proteins extractable at low ionic strength occurred as a result of aging and curing; two new proteins appeared to be formed. Marked changes also were detected in proteins extractable at high ionic strength.

Understanding the factors that determine the quality and processing characteristics of meats is a basic

1) Presented at the 11th European Meeting of Meat Research Workers, Belgrade, Yugoslavia, August 16-21, 1965.

problem in meat research. It is evident that the most important group of factors concerned are the content, composition, and properties of the proteins. Considerable past research has shown that factors which modify the properties of proteins, such as pH and the presence of specific ions, markedly affect meat. On the other hand, the extent and effect of variations of protein composition in meat of different kinds is not known. Only limited progress has been made in separating, identifying, and characterizing these proteins, which, in some instances, are significantly different from those present in fresh (prerigor) tissue. The task will demand the efforts of numerous investigators for a considerable period of time; however, such research is a logical prerequisite to the development of a more scientific basis for meat technology. Research in this area has been conducted for several years at the authors' laboratory, first, emphasizing investigations of the proteins of prerigor tissue, but, more recently, those obtained from meat.

The present report is concerned with the problem of extracting and fractionating proteins from fresh, aged, and cured pork and determining some of the changes which occur on aging and curing. In investigating the relationships that affect meat quality, it is generally desirable that one develop enough quantitative data to permit adequate statistical analysis. In such investigations analysis of numerous samples is required. The analysis of proteins in samples of meat would be greatly facilitated if a method were developed that produced thorough extraction and efficient fractionation of samples with a minimum of manipulation. With this purpose in mind the value of sand-tissue grinding and gradient elution was explored. In addition, the usefulness of determinations of adenosine triphosphatase (ATPase) activity, taking advantage of the effects of preferential Ca^{++} and Mg^{++} activation, has been investigated as a means of characterizing proteins in extracts. Other procedures consisted of analyses by disc electrophoresis and ultracentrifugal sedimentation. While continued investigation can be expected to improve procedures and interpretation of data, result have been obtained which should contribute to a better understanding of the proteins of meat, and to methods available for investigating composition.

EXPERIMENTAL AND METHODS

Meat Samples. Hams from 200-pound pigs were obtained immediately after slaughter. Analysis of the semimenbranosus 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 and dried sea sand (reagent grade) 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. height), the bottom being made of coarse sintered glass; and a linear gradient of KCl solution, 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 up to 15 fractions to be analyzed, the absorbance of eluate was monitored at 280mμ by an automatic absorbance recorder.

Concentrations of protein were determined by the biuret reaction which had been standardized by the micro-Kjeldahl method. ATPase activities were determined using previously described conditions (1), combined with a method for determining inorganic phosphorus with increased sensitivity (2). Disc electrophoresis was undertaken on extracts obtained by extracting tissue with 0.05 M KCl, .002 M imidazole and by extracting the remaining residue with 0.6 M KCl, .025 M imidazole, using 7% and 3% acrylamide gels, respectively. The conditions used were a slight modification of those previously described (3). In ultracentrifugation, the Beckman-Spinco Model E analytical centrifuge[2]) was employed.

RESULTS AND DISCUSSION

Sand-Tissue Grinding and Gradient Elution. Grinding tissue with sand has been widely used in previous tissue studies because it provides a thorough disintegration of tissue with minimum denaturation of proteins. Therefore, the principal experimental aspect of the method presently used was direct extraction of the tissue while still mixed with sand, using an ascending gradient of KCl solution to obtain fractionation. The sand-tissue extraction method, if perfected, would reduce the operations necessary in more conventional methods of extraction and fractionation, such as that which has been applied in a relatively recent approach to the problem (4). The effectiveness of ascending gradient elution in the chromatography of proteins is thoroughly proven, an example being the fractionation of myosin B by chromatography on DEAE-cellulose (5).

As indicated by differences and changes in the ATPase activities of fractions apparent in the data given in Tables 1 and 2, gradient elution accomplished some fractionation. An excellent rate of flow through the sand-tissue mixtures was possible. A rate of flow of approximately 3 ml. per minute was used; however, as noted below, the optimal rate of flow has not been established. Continuous plots of absorbance vs. volume of eluant generally lacked peaks, as would be expected in view of the small variation of amounts of proteins extracted in successive fractions (Tabl 1). An exception was the consistent appearance of a peak in extracting cured samples when the extracting solution was 0.25-0.35 M KCl. The yield of total protein extracted - comparing the total protein extracted from sand-tissue mixtures with that extracted from samples ground with sand and stirred overnight with 8 volumes of 0.6 M KCl, pH 7 — ranged from 75 to over 90%. More complete recovery would probably have been obtained had the volume of solution been increased in gradient elution.

It was found that the amount of protein extracted by a given amount of eluant was markedly influenced by the rate of flow. Thus, under the conditions used, equilibration did not occur, i. e., the extracting solutions did not become saturated with protein. To more completely evaluate the capability of sand-tissue extraction, results obtained using various rates of flow are being investigated in further work.

Fractionation Obtained Based on ATPase Activities. Investigation of purified proteins prepared from prerigor muscle tissue has shown that myosin exhibits

2) It is not implied that the USDA recommends the above company or its product to the exclusion of others in the same business.

a Ca^{++} -activated ATPase activity and actomyosin or myosin B a Mg^{++} -activated ATPase activity (6). Analyses based on these facts have been effectively used in studies of the rate of formation of actomyosin in which formation was marked by a simultaneous decrease of Ca^{++} -activity and an increase of Mg^{++} -activated activity (1). The application of similar analyses in the present investigation was exploratory. The results suggest that such analyses are of value in meat research for determining the properties of protein components, even though the observed ATP-ase activity in aged or cured meat may be produced by modified proteins which are different from those of fresh, or prerigor, tissue.

The relatively elevated Mg^{++} -activated ATPase activity of proteins extracted at low ionic strength (Table 1, Fraction 1) reflected the presence of Mg^{++} -activated enzymes, which are extracted from particulate materials; the presence of proteins from this source was the only difference noted between prewashing sand-meat mixtures with 0.05 M KCl (Table 2) and omitting this procedure (Table 1). After decreasing in Fractions 3 to 7, the Mg^{++}-activated activity increased markedly in the fractions eluted thereafter. The Ca^{++} -activated activity of fractions followed a generally similar pattern but was noticeably higher in Fraction 7 in proportion to Mg^{++} -activated activity. The data show that a definite fractionation occured, yielding the fraction extracted at low ionic strength and fractions of increasing activity as the gradient elution proceeded. The data in Tables 1 and 2 show that proteins of highest enzymic activity were not extracted until the ionic strength was relatively high compared with that usually used for extracting muscle proteins. Investigations to be conducted of the effects of eluting at different rates of flow may provide an explanation of this tendency.

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

Fraction	Volume collected, ml.	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	19.6	34.7
		710[1],[3])		

1) This sample was extracted twice with 0.05 M KCl, pH 7.0, prior to gradient extraction.

2) μ Moles P_i per mg. protein per min. $\times 10^{-3}$.

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 x 300 = 962 mg. protein extractable by salt solution of high ionic stength, unextractable at low ionic strength. On this basis, 74% was recovered in the above extraction (which was not conducted exhaustively).

Table 2. Change of Specific ATP-aze 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	45.0	10.7	8.7	59.9[2])	18.1	25.5
7	24.6	7.2	10.6	42.6	20.3	27.4
8	73.8	24.0	7.4	93.0[2])	43.3	14.9
9	73.2	32.0	23.8	97.0	62.8	58.3
10	73.2	73.5	52.3	97.3[2])	83.8	80.0

1) μ Moles P_i per. mg. protein per min. $\times 10^{-3}$

2) Fractions analyzed by ultracentrifugation.

Effects of Aging and Curing on Specific ATPase Activity. As shown by the data presented in Table 2, ATPase activity decreased generally. In the fractions containing the bulk of the proteins (Fractions 6-10), ATPase decreased markedly during 2 days of storage and slightly more during 2 days of curing. Since the ratio of Mg^{++} -activated specific activity to Ca^{++} -activated specific activity increased during aging and curing, the data indicate that the protein components appearing during aging and curing were of the actomyosin type, at least in so far as ATPase activity is concerned.

Ultracentrifugal Analysis. Some fractions obtained by elution of sand-meat mixtures were subjected to ultracentrifugal analysis (Fractions 6, 9, and 10, Table 2). The proteins present proved to be mixtures containing small amounts that sedimented at low speeds (24,600 r. p. m.), but mainly proteins sedimenting at higher speeds (59,700 r. p. m.) as broad peaks which tended to broaden further with centrifugation and to sediment without fractionation. A lack of peaks typical normal myosin or actomyosin (myosins A and B) was inexplicable.

Effects of Aging and Curing Based on Electrophoretic Fractionation. The results obtained with disc electrophoresis on acrylamide gels are shown in Table 3. It should be noted that the extracts fractionated were obtained by direct extraction of tissue, not by sand-meat gradient elution. Detection of minor components appeared to be limited by the amount of protein fractionated by the method (200 μ g samples), since traces escaped detection on staining. Owing to this, or the possibility that the method as used was capable of limited resolution, only seven major fractions were obtained from pork, whereas 35 bands have previously been noted on fractionating comparable material from beef by starch-gel electrophoresis (7). The data show that seven fractions were obtained by electrophoresis of extracts obtained by extracting pork samples with 0.05 M KCl. Two fractions, with R_m (mobility ratio) values of 0.11 and 1.50, were only obtained 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 that appreciable change occurred, including destruction and formation of components of different mobilities.

Two principal components were separated on disc electrophoresis of proteins extracted from fresh, aged and cured samples with 0.6 M KCl. The ratio of the

amount of the more mobile (A) to the less mobile (B) protein were determined by staining gels with Amido Schwartz stain and tracing the absorbance with a recording densitometer. The proportions of proteins changed during aging and curing, as shown in Table 3 by the decreasing ratios - 0.58, 0.39, and 0.27 - of the more to the less mobile fractions. Although fractionation of myofibrillar proteins by electrophoresis has not been an especially effective tool in protein research, the results suggest that disc electrophoresis may provide useful information in additional investigations to be undertaken.

SUMMARY

The extraction and fractionation of protein components of fresh, aged, and cured samples of pork semimembranosus muscle was investigated using a technique which involved grinding with sand and extraction of the sand-tissue mixtures by elution with an ascending gradient of KCl. The results of the extraction and fractionation were assessed by determining protein nitrogen content, specific Ca^{++}- and Mg^{++} -activated ATPase activities and ultracentrifugal sedimentation. The effects of aging and curing on protein components were also determined by analyzing extracts using disc electrophoresis. The conclusions reached were as follows:

1. The development of an analytical tool based on sand grinding and gradient elution of sand-tissue mixtures may be possible.

2. Determination of preferential activation of ATPase activity is a uniquely powerful tool for meat composition studies.

3. The ATPase activity of pork decreased markedly during 2 days storage, but was affected only to a moderate extent by 2 days of curing. The ATPase activity of the proteins shifted from a »myosintype« to an »actomyosin-type« of activity.

4. Results with disc electrophoresis showed marked changes occurred in both groups of proteins analyzed, those extracted with solvents at either low or high ionic strength, as a result of aging and curing.

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

Relative mobility (R_m)	Fresh	Aged	Cured
Extracted with 0.5 M KCl, pH 7[2])			
0.11	1[3])	0	0
0.87	0	1	8
0.97	8	8	7
1.00	2	4	1
1.10	7	8	6
1.20	2	5	7
Extracted with 0.5 M KCl, pH 7[4])			
Ratio, components A and B[5]	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.
5) Ratio of amounts of components A and B obtained by integration under peaks of densitometer tracings.

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