Esters Present in Pre-ferments. The only esters detected in the Fleischmann pre-ferment were those of acetic acid. The ethyl ester was presumably produced because of the high concentration of ethyl alcohol in the fermentation mixture. Wiseblatt (26) found only ethyl esters in bread crumb.

The change in ethyl acetate concentration in the pre-ferment with time is shown in Figure 2. The ester reached its maximum concentration after 6 to 8 hours of fermentation and decreased to zero after 48 hours. The concentration of ethyl acetate in American Dry Milk Institute pre-ferments was not determined because it was necessary to adjust the pH of that pre-ferment to 10.0 to precipitate a part of the nonfat dry milk. The ester was hydrolyzed at this pH.

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Literature Cited

- (1) Baker, J. C., Food Engineering 25, 60, 183 (1953)
- (2) Baker, J. C., Mize, M. D., Cereal Chem. 16, 295-7 (1939).
- (3) Baker, J. C., Mize, M. D., *Ibid.*, **18**, 19-34 (1941).
- (4) Baker, J. C., Parker, H. K.,

Fortmann, K. L., Ibid., 30, 22-30 (1953).

- (5) Barnard, T. H., Baker's Dig. 28, 61-4, 79 (1954).
- (6) Barnard, T. H., Trans. Am. Assoc. Cereal Chemists 13, 43-53 (1955).
 (7) Butterworth, S. W., Bakers' Natl. Assoc. Rev. 211 No. 27, 1-7 (1935).
- (8) Carroll, L. P., Miller, B. S., Johnson, J. A., Cereal Chem. 33, 303-10 (1956).
- (9) Choi, R. P., Koncus, A. F., and Staff, ADMI Stable Ferment Process (Chemical Study-A Progress Report), American Dry Milk Institute, Chicago, April 3, 1954.
- (10) Corcoran, G. B., Anal. Chem. 28, 168-71 (1956).
- (11) Denison, F. W., Phares, E. F., Ibid., 24, 1628-9 (1952).
- (12) Food Processing 16 (10), 23-5, 32 (1955).
- (13) Johnson, A. H., Cereal Chem. 2, 345-64 (1925).
- (14) Johnson, J. A., Miller, B. S., Refai, F. Y., Miller, D., J. Agr. Food Chem. 4, 82-4 (1956).
- (15) Magasanik, B., Umbarger, H. E., J. Am. Chem. Soc. 72, 2308-9 (1950).
- (16) Miller, B. S., Johnson, J. A., Kansas Agr. Expt. Sta., Bull. No. 76 (1954).
- (17) Pirrie, P., Bakers' Weekly 164 (2), 26-9 (1954).
- (18) Pirrie, P., Glabau, C. A., *Ibid.*, 163 (5, 6, 7), 25-8; 163 (9), 25-8; 163 (10), 29-31 (1954).

- (19) McLaren, L. H., Baker's Dig. 28, 41-2, 48 (1954).
- (20) Manewal, R., Baking Ind. 104 (1310), 43-5 (1955).
- (21) Pence, E. A., Master's thesis, Kansas State College, Man-hattan, Kan., 1952.
- (22) Randall, H. H., Fowler, R. G., Fuson, N., Dangl, J. R., "Infrared Determination of Organic Structures," Van Nostrand, New York, 1949.
- (23) Shriner, R. L., Fuson, R. C., "Identification of Organic Compounds," 3rd ed., Wiley, New York, 1948.
- (24) Stark, J. B., Goodban, A. E., Owens, H. S., Anal. Chem. 23, 413-5 (1951).
- (25) Underkoffler, L. A., Hickey, R. J., Industrial Fermentations, Vol. 1, Chemical Publishing Co., New York, 1954.
- (26) Wiseblatt, L., "Identities of Substances Which Contribute to the Flavor of Bread," American Association of Cereal Chemists, 41st Annual Meeting, New York, N. Y., 1956.

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WATER CONTENT OF MEATS

Determination of Water-Holding Capacity of Fresh Meats

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The water-holding capacity of nondisintegrated muscles and ground and comminuted fresh lean meats was determined on fresh and heated meat. By pressing a 400- to 600mg. fresh muscle sample on No. 1 Whatman filter paper of constant humidity in a specially made press operating under 500 p.s.i., the area of the paper wetted in 1 minute by the expressed juice is directly proportional to the weight of water in the press juice. The method gives the reproducible results within 2 to 5%. The amount of free water in beef, pork, veal, and lamb varies from 30 to 50% of the total moisture content, depending on the kind of meat and period of aging.

 ${f R}$ ecent studies on consumer qualities of meats, such as tenderness, texture, drip on freezing and thawing, and shrinkage on cooking indicate that these qualities depend on the degree of hydration of muscle proteins (1, 17, 36-40). The highly polar water molecules are attracted to the muscle proteins by ionizable basic and acidic groups as in arginine, histidine, lysine, glutamic acid, and aspartic acid or by polar nonionic groups such as in cystine, cysteine, serine, methionine, threonine, tyrosine, and tryptophan. The mechanism of the

protein hydration is not well understood. Some pioneering work on hydration of various proteins, other than muscle proteins, and polypeptides has been done by Bull (2), Pauling (33), Mellon and Hoover (29), and others (4, 5, 26).

Lean meat contains about 3.5 grams

of water per gram of protein, about 10 times as much as the water of hydration of commonly known proteins (26). Consequently, muscle like other biological material contains water of hydration, or electrostatically bound water, and physically absorbed water, held on the proteins by the secondary forces, such as water dipole-dipole induction, hydrogen bonds, and capillary and surface attractions.

In this paper, free water is considered to be that portion of the total moisture which has been released by pressing or heating the meat under specified laboratory conditions which are presented here. The remaining portion of the total mixture of meat is then the bound water, consisting of the water of hydration and that portion of the physically absorbed water which has not been so released. The exact amount of bound or free water cannot be determined in meat, for it contains different protein components and the water of hydration of each is not known. Furthermore, the amount of the physically absorbed water is changed by various laboratory and processing techniques. However, by considering the various muscle proteins as a single protein component and by using the same method under the same experimental conditions, relative changes in the waterholding properties of meat can be measured.

A simple centrifugal method for measuring the water-holding capacity during heating, freezing, and thawing of meat has been recently published by Wierbicki, Kunkle, and Deatherage (40). The method is very useful for measuring relative shrinkage of meat under different experimental conditions (36). However, when this is applied to the meat samples heated below 100° F. or not heated at all (fresh meats), the amount of juice, if any, collected in the centrifuge tubes was within the range of the experimental error (0.1 to 0.3 ml.).

In 1953 Grau and Hamm, at the Bundesforschungsanstalt für Fleischwirtschaft in Kulmbach, reported a simple filter paper method for the determination of water-holding capacity of fresh meats (12). Since that time, while using this method, Grau and Hamm and their associates have published several papers dealing with the theory of protein of hydration (17, 18, 22) and the effects of pH (14, 17, 18, 20), adenosine triphosphate (ATP) (16, 20), meat aging (10, 20), sodium chloride (7-9, 12, 17-19), calcium, magnesium, zinc, and potassium ions (15, 18, 19, 21, 22), various phosphates (6, 13, 15, 17, 18, 23), and various organic and inorganic anions (22) on the hydration of meat proteins. The usefulness of this method for meat research has been reported also from Finland (30-32, 34), Poland (24, 25), and Hungary (27, 28), but it has not been used in the study of cooked meats.

This paper presents a modification of the Grau and Hamm's original method for the determination of the waterholding capacity of fresh meats. The pressing by hand has been replaced by a pressing device which controls the pressure and assures a greater accuracy of the determination. Inasmuch as the centrifugal method (40) is useful in studying heated, frozen, and defrosted meats, and the German method (12)is particularly adapted to fresh meats, the two methods have been applied to the same meat. The limitations of each method appear to be complemented by the usefulness of the other method.

Experimental

Apparatus. This method of Grau and Hamm involves pressing a freshly cut 400- to 600-mg. sample of muscle or well-minced lean meat onto filter paper under constant pressure and for a fixed time and measuring the area occupied by the water, which diffused from the meat sample into the filter paper. The amount of the free or "loose" (12) water expressed is then calculated from the area by using an appropriate conversion factor. In the original version of the method (12), two Plexiglas plates screwed together as firmly as possible by hand were used. The pressure thus developed is about 60 to 70 kg. per sq. cm. (853 to 996 p.s.i.) and the variation in pressure, 30 to 70 kg. per sq. cm. (427 to 996 p.s.i.), has no effect on the wetted area. However, Figure 2 indicates that this is not strictly so. Pohja and Niinivaara (34) in their modification of the method (pressing by the balance weights) showed also that the area varies with the pressure, particularly at the acid side of the meat, pH 5.0.

An apparatus has been built for pressing the meat samples under constant pressure. The apparatus, shown by Figure 1, consists of an 8-ton capacity hydraulic jack (Model CG-9, Blackhawk Manufacturing Co., Milwaukee, Wis.), which is built into the 1/4-inch steel frame with the side walls 15 inches high and 8 inches wide, welded on the bottom and the top with 8×8 inch steel plates; a movable 8×8 inch steel plate is located inside the frame, just over the hydraulic jack, which can be pressed to the top plate of the frame during the operation. A pressure gage reading from 0 to 1000 p.s.i. with increments of 10 p.s.i. is tapped to the base of the jack. Two 8 \times 8 \times $^{1/4}$ inch Plexiglas



Figure 1. Hydraulic meat press with pressure gage



 Figure 2.
 Effect of increasing pressure on free water area

 X Semimembranosus pork muscle
 • Semimembranosus beef muscle

plates are placed between the movable steel plate and the top plate of the frame: these are separated from the steel plates by rubber cushions to protect the Plexiglas plates and assure an equal distribution of the pressure through the plates.

A 400- to 600-mg. meat sample is weighed on a 9-cm. No. 1 Whatman filter paper of constant moisture content, $10.18 \pm 0.10\%$, obtained by holding the filter paper in a desiccator over saturated potassium chloride solution as suggested by Grau and Hamm (11, 12). The filter paper and meat are then placed between the Plexiglas plates and pressed immediately at a constant pressure for a fixed period of time. A pressure of 500 p.s.i. and a pressing time of 1 minute were most suitable. By pressing, the muscle material is squeezed to an almost circular film (meat film), while the expelled water is absorbed by the filter paper forming a circular brown- or redcolor area (free moisture area).

Immediately after the pressing has been accomplished, the Plexiglas plates with the meat sample are removed and the meat film area is marked with a colored pencil on the other side of the filter paper before removing the Plexiglas plate from the meat side of the paper, as the meat film usually adheres to the plate. The filter paper is then removed from the upper plate and the meat film taken off. The filter paper can now be stored for the surface measurement, if necessary, for a long period of time.

For the calculation, the surface of the free moisture area (juice ring) around the pressed muscle is determined by subtraction of the surface of the meat film from the entire surface. For the surface measurement, an Ott compensating planimeter with vernier range of 0.01 square inch (Type 16, Charles Bruning Co.) was satisfactory.

The centrifugal method was used as previously reported (40).

Pressure. Samples, 500 mg., of lean beef semimembranosus and pork longissimus dorsi, 2 days post-mortem, were accurately weighed on an analytical balance and then pressed for 1 minute at varying pressures. Free moisture area was calculated and plotted as a function of the pressure from 100 to 1000 p.s.i. The results (Figure 2) indicate that the free moisture area varies with the pressure, usually the amount of the pressed-out juice increased with the increased pressure. However, the increase of the free moisture area is not directly related to the magnitude of the pressure, but rather a stepwise increase of the pressed-out juice occurred within the pressure range investigated. Presumably, the water present in meat is bound or fixed by the muscle proteins with different forces forming several water layers around the protein molecules, which are held by different

Table I. Effect of Pressing Time on Free Moisture Area^a

	Area, Square Inches			
Time, Min.	Total	Meat film	Free moisture	
0.5	4.38	1.18	3.20	
1	4.50	1.26	3 24	
2	4.86	1.28	3.58	
3	5.24	1,31	3.93	
4	5.38	1.30	4.08	
5	5,40	1.28	4.12	
6	5.38	1,27	4.11	
a 500-m	ig, sample	s of pork	(semimem-	

branosus) at 500 p.s.i. pressure.

water-binding energies, each water layer requiring a different pressure for being released by the protein molecules.

The data given in Figure 2 do not represent the characteristic pattern for the water binding of all pork and/or beef muscles. The absolute amounts of juice were found to vary from muscle to muscle, however, all the samples investigated showed more or less definite stepwise binding of the water by the muscle substance. At 500 p.s.i. pressure a plateau always resulted and, therefore, this pressure was selected as the constant pressure for the determination of the water-binding capacities of fresh meats.

Pressing Time. Table I shows the effect of the pressing time on the meat film and the free moisture area from pork semimembranosus muscle. The free moisture area increases with the pressing time, up until about 4 minutes. However, as the spreading of the meat film reached its maximum after 1 minute of pressing, pressing time was standardized at 1 minute.

Pressing Delay. During the preliminary experimentation, the first sam-

Table III.

Table II. Effect of Pressing Delay on Free Moisture Area

Min. between Weighing	Area, Square Inches					
and	Total	Meat	Free			
Pressing		film	moisture			
BEEF, LONGISSIMUS DORSI ^a						
10	2.69	1.88	0.81			
7.5	4.15	2.66	1.49			
5	4.19	2.67	1.52			
2.5	4.35	2.68	1.67			
BEEF, SEMIMEMBRANOSUS ^a						
10	3.32	1.40	1.92			
7.5	3.59	1.65	1.94			
5	3.81	1.70	2.11			
2.5	3.95	1.85	2.10			
¢ 500-mg. s	samples a	at 500 p.s	.i. pressure			

ple out of the four samples weighed for the simultaneous pressing gave a somewhat smaller free moisture area. Evaporation appeared to be responsible for this error. Consequently, the effect of the time of the exposure of the samples to the air was studied with the results presented in Table II.

Air exposure for longer than 5 minutes causes significant evaporation of moisture from the sample and probably from the filter paper. A change in temperature of the samples is another factor affecting the protein-water relationship, as the same beef samples taken from the refrigerator (7° to 10° C.) gave somewhat smaller free moisture areas than after bringing them to room temperature (25° C.) before pressing. Therefore, in the course of further experimentation, meat samples taken from a cooler were placed first in a $7^{\circ} \pm 2^{\circ}$ C. refrigerator for a few hours, and then promptly

Free H₂O Area Area, Square Inches Relative per 500 Mg. Pair Sample Meat Free Error, ±% Wt., Mg. Sample No. Total film moisture Av. BEEFa 2.28 2.13 2.065 3.1 4 50 2 32 1 535 495 2.00 4.51 2.53 1.98 534 2.40 2.45 4.49 2 09 1 96 2.0755.5 2 2.33 2.19 4.78533 4.73 4.79 2.12 2.05 3 514 2.55 2.18 2.085 1.7 493 2.772.02 Porkb 3.74 2.48 2.725 1.3 1 467 1.26 2.68 2.77464 4.03 1.49 2.54 3.16 3.07 3.010 2.02 513 4.30 1.14 2,95 472 4.10 1.32 2.78 3.41 3.28 3 501 4.63 1.21 3.42 3.345 1.6 5.05 1.23 3.82 584

Reproducibility of Duplicate Determinations

^a Same beef sample used for all three pairs. Pressure, 500 p.s.i. per min.

weighed (within the time interval, not longer than 5 minutes), and pressed.

Filter papers were weighed in advance and covered with dry beakers; the samples weighed first were also covered before weighing the remaining samples. All samples were taken from freshly cut meat. With filter papers weighed in advance and the apparatus made ready for pressing, four samples can be easily weighed and pressed by an experienced operator within 5 minutes. By doing so, the reproducibility of the method was maintained within the desired limits.

The area of the Plexiglas plates is great enough to press four 9-cm. No. 1 Whatman filter papers at a time. However, the apparatus was made to hold five Plexiglas plates, or 16 samples of meat, if all 16 samples can be weighed within the time interval of 5 minutes, say by three or four operators simultaneously.

Reproducibility of the Method. After the operating conditions were standardized at 500 p.s.i. pressure with a pressing time of 1 minute, and weighing time not longer than 5 minutes, the reproducibility of the method was checked on different kinds of meat and different muscles of the same kind of meat. The reproducibility of the method was within $\pm 5\%$. For the series of analyses, the number of samples taken from the same muscle was reduced to two. Table III represents the accuracy of the duplicate determinations. The sample size of 400 to 600 mg. was required to get this accuracy. Smaller samples (200 to 300 mg.) gave usually relatively greater free moisture area than the larger ones (700 to 800 mg.). The accuracy of the determination requires also that the



juice front does not reach the edge of the filter paper, as found originally by Grau and Hamm (11, 12).

Loss of Free Moisture under Meat Film. For the calculation of the free moisture only the free moisture area is considered. Yet, the filter paper under the meat film may absorb some of the free moisture. Grau and Hamm (11-13) state that the amount under the meat film is negligible, because of a greater pressure exercised on the meat sample than on the filter paper outside the meat film. Meat samples, 500 mg., were pressed on the filter paper before and after waxing both sides of the filter paper area occupied by the resulting meat film. Paraffin wax was used. The results given in Table IV indicate that the total moisture area increased by 1.4 to 5.4% as the result of the waxing of the meat film area of the filter paper.

Figure 3. Relationship between wetted area (free water area) and weight of water (free water) being pressed onto No. 1 Whatman filter paper at 500 p.s.i. per minute

Α = distilled water: X = $43.64 \times Y$ B = water in various meat juices: ● juice from fresh beef; x juice from the beef after freezing and thawing; \blacktriangle Juice from fresh lean pork; and X = 61.10 \times Y

This increase is within the experimental error of the method (Table III) and confirms the statement of Grau and Hamm.

Standard Curve. Free Moisture Area vs. Free Moisture. For the calculation of the free and bound water in a meat sample, the conversion factor of the free moisture area in the amount of free moisture must be known. For this purpose, a sample of chilled beef semimembranosus muscle 2 days post-mortem was ground. Several 20-gram samples of the meat were placed in the centrifuge tubes used for shrinkage determination (40), warmed at 27° C. for 30 minutes, and then centrifuged. The juice which collected at the bottom of the centrifuge tubes was removed, filtered through a coarse filter to remove suspended solid particles, and the clear juice obtained in this way was stored for a few hours in a

Table V. Standard Curve^a: Free Moisture Area vs. Free Moisture

Juice, Fresi	h Beef	Juice, Be Freezing and	ef after I Thawing	Juice, Fres	h Pork	Distilled	Water
H ₂ O, Mg. ^b	Area	H ₂ O, Mg. ^c	Area	H ₂ O, Mg. ^d	Area	H_2O , Mg.	Area
90	1,60	42	0.70	30	0.53	27	0.94
92	1.68	65	1.16	31	0.54	28	0.98
125	2.15	111	1,84	62	1.08	55	1,63
153	2.59	158	2.49	91	1.53	65	1.96
187	3.08	186	2.99	109	1.80	95	2.71
203	3.30	211	3.31	110	1.86	104	2.80
211	3.40	283	4.82	112	1.92	144	4.02
221	3.70	317	5.31	118	1.95	155	4.34
258	4.55	363	6,10	151	2.65	201	5.00
265	4.60	399	6.49	187	3.08	224	5.89
298	5.02			192	3.11	262	6.44
357	5.61			249	4.06	292	6.52
362	5.78			261	4.56	334	7.64
385	6.38			270	4.42	372	8.81
389	6.45			326	5.38	381	9.37
				331	5,42	413	10.14
				385	6.36		
				392	6.45		
be	62.35		59.94		61.18		43.64

^b Mg. juice \times 0.883 (moisture of the juice = 88.3%). ^c Mg. juice \times 0.890.

^d Mg. juice \times 0.892.

Regression coefficient, b: X = bY where $X = \text{area, sq. in. and } Y = \text{mg. free H}_2O$. b – value for all 43 meat samples = 61.103 ± 0.311.

Table IV. Effect of Waxing Meat Film Area on Total Area

	Total Area, S	Effect of	
No.	Before waxing	After waxing ^a	Waxing + Rel., %
	R	ound ^b	
1	4.43	4.67	5.4
2	4.37	4.44	1.6
3	4.41	4.61	4.5
4	4.38	4.56	4.1
5	4.40	4.55	2.5
	F	LANK ^b	
1	3.62	3.77	4.1
2	3.45	3.59	4.1
3	3.64	3.69	1.4
4	3.77	3.83	1.6
Av			3.26%

^a Standard Parowax household wax.

^b 500-mg, samples at 500 p.s.i. per min. pressure.

 7° C. refrigerator. The juice was then transferred, drop by drop, in increasing amounts on Whatman paper, weighed on an analytical balance, and pressed under the constant conditions of 500 p.s.i. per minute. The area of the juice on the filter paper was then measured and tabulated along with the corresponding weight of water in the juice, obtained by multiplying the juice weight by the moisture fraction in the juice.

The corresponding results are given in Table V, along with the data for distilled water (control) and two other juices: juice obtained after freezing $(-1^{\circ} \text{ C}.)$ and thawing $(25^{\circ} \text{ C}.)$ of the beef and the juice of fresh pork (semimembranosus). The amounts of solids in the corresponding juices were 11.7, 11.0, and 10.8%, respectively. By plotting the areas, X, against the corresponding moisture weights, Y, the standard curves for the juices and distilled water were obtained (Figure 3). The area wetted by the juices is directly related to the weight of water in the press juices and this proportionality is independent of the kind of muscle used.

The proportionality constant b (regression coefficient) for the three kinds of meat juices given in Table V was statistically equal to 62.35, 59.94, and 61.18, respectively. For all 43 juice samples tabulated, the b value was 61.10, with the standard deviation from the regression equal to ± 0.31 . Thus, the free moisture area is related to the free moisture in meat juices by the equation X = bY. One inch of the area is equal to 61.10 ± 0.31 mg, of free moisture.

The reference is made to all meats, as by pressing veal and lamb meat juices the same proportionality between the area and free moisture has been reconfirmed. This agrees with the results by Grau and Hamm found for various mammalian muscles (11). However, the proportionality constant, b, for distilled water is by about one third smaller (Table V, Figure 3). This means that the presence of solids in the meat juice has a pronounced effect on the spreading out of water onto the filter paper. Connell (3), while working with cod muscle press juices ranging in water content from 91.7 to 97% observed that the increase in water content of the juices over 94% resulted in a larger wetted area for equal weights of water.

Grau and Hamm in their recent papers (11, 13) investigated the effect of the dilution of meat juices on the free moisture area. They confirmed the observation of Connell that the dilution of the meat juices from 88 to 94% moisture did not affect the proportionality constant. However, on further dilution, a new proportionality constant should be established. To overcome this difficulty, Connell (3) in his modification of the method used a direct weighing of the water pressed out onto the filter paper.

This version of the method is not as fast and is less accurate than the free moisture estimation by the area measurement. It requires a standard drying of the filter paper before use and the immediate weighing of the moist paper after pressing, otherwise the moisture evaporated very rapidly from the filter paper on exposure to air. Also, removal of the filter paper from the muscle meat film from the total moisture area. The difference multipled by the regression coefficient of 61.10 mg. water per square inch gives the amount of free water in the meat sample being pressed. Another sample of the same meat should be run for the total moisture content. The results are best expressed as the per cent of the free water out of the total moisture content of the meat:

Per cent free H₂O = $\frac{\text{(total area - meat film area)} \times 61.10}{\text{total moisture (mg.) in muscle sample}} \times 100$

residue could not always be achieved. As various meat juices contain from 10 to 12% solids and as the method gives the same relationship between the wetted area and the free moisture for the juices containing as low as 6% solids, an admixture of 5 to 40% water to meat does not affect the accuracy of the determination. This was confirmed in this investigation and by Grau and Hamm (11, 13).

Increasing the solids in the meat fluids by adding various meat additives is without effect on the spreading of water on the filter paper (11). However, the additives which increase the viscosity of fluids, like Graham salt (metaphosphates) tend to decrease the wetted area for the same weight of water in the fluid (11, 13). On the other hand, the presence of the visible fat particles in the meat sample being pressed increases the moisture area around the meat film.

Calculation of Results. For the calculation, the free moisture area is determined by subtracting the surface of the

The per cent of bound water equals 100 minus per cent of free water. The amount of free or bound water can be also expressed as per cent of the meat weight, or as the amount of bound or free water per unit weight of protein of the muscle.

Application of Method. The method can be successfully used for studying the degree of water holding of fresh lean muscles. As very small muscle samples are required for the determination, the method is adequate for studying the relative water holding of different muscles of the same animal or of the same muscles of different animals.

It can also be used for studying the relative changes of water holding of muscles under different physiological and experimental conditions—i.e., setting in and resolution of rigor mortis, and effect of various meat additives or processing techniques.

Hamm (16) used this method for studying the biochemistry of muscular

Table VI. Free Moisture Content of Some Meats

Carcass No.	Muscle	Age Post-mortem (Days) at 33° F,	Total Moisture, %	Free H2O as % of Total H2O
		BEEF		
1	Vastus medialis Rectus femoris	2 2	75.52 75.80	38.5 35.1
2	Biceps femoris Adductor	2 2	74.40 74.16	44.3 42.5
3	Semimembranosus	0.5 1 2 5 7	76.32	29.5 34.2 40.6 36.2 34.2
4	Longissimus dorsi	2 8	74.86	38.0 30.9
		Pork		
5 6 7 8 9	Longissimus dorsi Rectus femoris Semimembranosus Cured hams	2 1 1	73.29 72.50 73.10 74.96 75.00	41.0 42.9 56.8 39.6 34.1
		VEAL		
10 11	Semimembranosus Biceps femoris	3 3	76.60 76.62	49.0 46.8
		Lamb		
12 13	Leg Leg	2 2	76.80 77.01	52.1 50.9

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Figure 4. Effect of chilling and aging on laboratory shrink, waterholding, and pH of beef biceps femoris muscle

• Free H₂O at 25° C. (filter paper method), as per cent of total moisture lost on pressing

X Shrink at 70° C. (centrifugal method), as per cent of total moisture lost on heating ▲ pH

contraction and relaxation by measuring the meat film area for the same weight of muscle. A very small area was found for the muscle in rigor, which then gradually increased with the resolution of the rigor caused either by the muscle aging or by the proper addition of adenosine triphosphate, analogous with the changes in the rigidity and elasticity of the glycerol isolated muscle fibers commonly used in studying biochemistry and physiology (35).

A few examples of the application of the method are given in Table VI. The water-holding properties of different muscles of meat animals vary from muscle to muscle, from animal to animal, and with the post-mortem aging of the muscles.

The complementary nature of the centrifugal method, as reported from this laboratory, and the filter paper method is shown in Figures 4 and 5. Figure 4 shows the changes in water-holding capacity of meat with post-mortem age. The upper curve is for the shrinkage at 70° C. and confirms earlier data obtained in Ohio by the centrifugal method (37), and the lower curve shows the changes in water-holding capacity of the same but unheated meat as determined by the filter paper method. Grau and Hamm (11-13) and the authors (39, 40)have previously reported on the effect of sodium chloride on the water-holding capacity of meat. Using the same meat, the two methods give the results shown in Figure 5. The parallelism of the data obtained is apparent. Each method can give useful and reproducible information on the water-holding capacity of meat. There remains much work to determine if information from one method carries directly to the other and to determine the quantitative relationship to consumer quality attributes, such as tenderness, shrinkage on cooking, and drip on freezing.



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Literature Cited

- (1) Arnold, Nancy, Wierbicki, E., Deatherage, F. E., Food Technol. 10, 245 (1956).
- (2) Bull, H. B., J. Am. Chem. Soc. 66, 1499 (1944),
- (3) Connell, J. J., Naturwissenschaften 42, 442 (1955).
 (4) Dole, M., McLaren, A. D., J. Am.
- Chem. Soc. **69,** 651 (1947).
- (5) Edsall, J. T., "The Proteins. Chemistry, Biological Activity, and Methods," by Hans Neurath and Kenneth Bailey, Vol. I, part B, 549-726, Academic Press, New York, 1953.
- (6) Grau, R., Fleischwirtschaft 7, 15 (1955).
- Ibid., 8, 20 (1956).
- (8) *Ibid.*, p. 180.
 (9) Grau, R., Fleischmann, O., *Ibid.*, 9, 252 (1957).
- (10) Grau, R., Hamm, R., Ibid., 6, 36 (1954).
 (11) *Ibid.*, **8**, 733 (1956).
 (12) Grau, R., Hamm, R., *Naturwissen*-
- schaften 40, 29 (1953).
- (13) Grau, R., Hamm, R., Z. Lebensm. Untersuch. u. Forsch. 105, 446 (1957).
- (14) Grau, R., Hamm, R., Baumann, A., Biochem. Z. 325, 1 (1953).
- (15) Grau, R., Hamm, R., Baumann, Naturwissenschaften 40, 535 A (1953).
- (16) Hamm, R., Biochem. Z. 328, 309 (1956).
- (17) Hamm, R., Deut. Lebensm. Rundschau 49, 153 (1953).
- (18) Hamm, R., Fleischwirtschaft 7, 196 (1955).
- (19) Ibid., 8, 266, 340 (1956).
- (20) Ibid., p. 539.
- (21) Hamm, R., Naturwissenschaften 42,



Figure 5. Effect of increasing salt additions on laboratory shrink and water-holding capacity of beef biceps femoris muscle, 2 days post-mortem, with 10% added water

> Shrink at 70° C. X Free H₂O at 25° C.

> > 394 (1955).

- (22) Hamm, R., Z. Lebensm. Untersuch. u. Forsch. 106, 281 (1957).
- (23) Hamm, R., Grau, R., Deut. Lebensm. Rundschau **51,** 106 (1955). (24) Janicki, M. A., Walczak, Z.,
- Przemysł Spożywczy 8, 197 (1954).
- (25) Ibid., p. 404.
- (26) Katchman, B., McLaren, A. D., J. Am. Chem. Soc. 73, 2124 (1951).
- (27) Koermendy, L., Élelmezési Ipar 9, 283; 345 (1955); 10, 7 (1956); Food Sci. Abstr. 29, No. 1, 20-1 (1957).
- (28) Koermendy, L., Gantner, G., Élelmezési Ipar 8, 172 (1954); Food Sci. Abstr. 29, No. 1, 20 (1957).
- (29) Mellon, E. F., Hoover, Sam R., *Ibid.*, 73, 3879 (1951).
- (30) Niinivaara, F. P., Fleischwirtschaft **6,** 357 (1954).
- (31) Niinivaara, F. P., Pohja, M., Ibid.,
- 6, 192 (1954).
 (32) Niinivaara, F. P., Ryynaenen, T., *Ibid.*, 5, 261 (1953).
- (33) Pauling, L., J. Am. Chem. Soc. 67, 555 (1945).
- (34) Pohja, M. S., Niinivaara, F. P.,
- (35) Szent-Gyorgyi, A., "Chemistry of Muscular Contraction," Aca-
- demic Press, New York, 1951. (36) Wierbicki, E., Cahill, V. R., Deatherage, F. E., Food Technol. 11, 74 (1957).
- (37) Wierbicki, E., Cahill, V. R., Kunkle, L. E., Klosterman, E. W., Deatherage, F. E., J. ÁGR. FOOD CHEM. 3, 244 (1955). Vierbicki, E., Kunkle, L. E.,
- (38) Wierbicki, E., Kunkle, L. E., Cahill, V. R., Deatherage, F. E., Food Technol. 8, 506 (1954).
- (39) Wierbicki, E., Kunkle, L. E., Cahill, V. R., Deatherage, F. E., Ibid., 10, 80 (1956).
- (40) Wierbicki, E., Kunkle, L. E., Deatherage, F. E., *Ibid.*, **11**, 69 (1957).

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