Table X. Calculated and Observed Loss in Nutritive Value in Evaporated Milk Diets

Expt.	Protein Efficiency: Wheat + sweetened condensed milk	Protein Efficiency: Wheat + evaporated milk	Decrease in Nutritive Value with Evaporated Milk, %: Observed	Decrease in Nutritive Value with Evaporated Milk, %: Calculated 1.54 × x-value
1	3.25	3.06	6	15.4
2	2.81	2.42	14	12.3
3	2.77	2.40	13.5	11.3
4	3.30	2.91	12	15.7
5	3.70	3.12	15.5	15.4

anticipated from the previous investigation (*10*) on the relationship between in vitro lysine availability and in vivo protein evaluation in milk powder, when the regression equation of loss in nutritive value y on lysine deterioration x was:

$$y = -31.71 + 1.54x \quad (1)$$

Lysine deterioration is defined here as reduction of in vitro lysine availability compared to the reference standard and loss in nutritive value as the decrease in protein efficiency in comparison to the same standard. This equation was established with milk + methionine as sole protein source. Such a diet has an initial excess of lysine of 20.6% as may be seen by solving equation 1 for $y = 0$, when x becomes 20.6%. The value of −31.71 for the intercept of the regression line on the y-axis is the expression for this lysine excess in milk. Because of this excess, lysine deterioration in evaporated milk does not affect the nutritive value of the latter. This is, however, not the case when milk is used as a supplement to wheat.

When lysine is present in adequate amount or is limiting in the reference diet, the regression goes through the origin and equation 1 becomes

$$y = 1.54x \quad (2)$$

In the wheat-milk mixtures used here, lysine is always limiting, so that equation 2 can be used throughout to estimate the decrease in nutritive value from lysine deterioration. However, the units for x must remain those established for equation 1—i.e., lysine deterioration x is expressed as per cent of available lysine in reference milk (Table II).

The diet containing wheat + sweetened condensed milk is the reference for calculation of lysine deterioration and loss in nutritive value in the corresponding diet with evaporated milk. The wheat part of the diet remains unchanged in all comparisons, so that its content in available lysine is per se irrelevant. For computation of lysine availability in the wheat–milk mixtures used, available lysine content of white wheat flour was taken as equal to lysine content, namely 1.9% of the protein. Lysine availability in sweetened condensed and evaporated milk is taken from Table II. The computation of lysine deterioration in the evaporated milk diets used is shown in Table IX.

The last column of Table IX corresponds to the x-value in equation 2. The loss in nutritive value y can now be calculated and compared to the observed decrease in protein efficiency (Table X).

With the exception of experiment 1, the agreement between observed and calculated values is good. This shows that the diminution of nutritive value observed with evaporated milk can be predicted from the results of the enzymatic in vitro digestion confirming the value of this method for evaluating the quality of milk proteins.

Acknowledgment

The authors are indebted to Eliane Bujard for the chromatographic determination of lysine content.

Literature Cited

(1) Allison, J. B., *J. Am. Med. Assoc.* **164,** 283 (1957).
(2) Block, R. J., *Borden's Rev. Nutr. Res.* **17,** 75 (1956).
(3) Dustin, J. P., Czajkowska, C., Moore, S., Bigwood, E. J., *Anal. Chim. Acta* **9,** 256 (1953).
(4) Food and Agricultural Organization of the United Nations, Rome, *Production Yearbook* **13,** pp. 33, 194, 199, 219, 231 (1959); *Yearbook of Fishery Production Statistics* **IX,** a-3 (1958).
(5) Gale, E. F., *Biochem. J.* **39,** 46 (1945).
(6) Hawk, P. B., Oser, B. L., *Science* **74,** 369 (1931).
(7) Howard, H. W., Bauer, C. D., Block, R. J., J. Agr. Food Chem. **8,** 486 (1960).
(8) Howard, H. W., Monson, W. J., Bauer, C. D., Block, R. J., *J. Nutr.* **64,** 151 (1958).
(9) Mauron, J., *Médecine et Hygiéne (Geneva)* **17,** 59 (1959).
(10) Mauron, J., Mottu, F., *Arch. Biochem. Biophys.* **77,** 312 (1958).
(11) Mauron, J., Mottu, F., Bujard, E., Egli, R. H., *Ibid.*, **59,** 433 (1955).
(12) Moore, S., Stein, W. H., *J. Biol. Chem.* **192,** 663 (1951).
(13) Osborne, T. B., Mendel, L. B., Ferry, E. L., *Ibid.*, **37,** 223 (1919).

Received for review September 25, 1961. Accepted January 29, 1962.

MINERAL CONTENT OF MEATS

Mineral Elements in Adipose Tissue of Lamb and Pork

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Although data are accumulating on the mineral element content of raw and cooked muscle meat (*4, 5*) and of organ meats (*3*), little is known about the mineral composition of the cellular matrix of the fatty tissues of meat.

In this study, separable lean and separable fat portions of cuts, essentially representing skeletal muscle and fatty tissue of lamb and pork, were analyzed for total content of nine mineral elements, using emission spectroscopy.

Experimental

Separable lean and the corresponding separable fat portions were obtained from 21 raw rib-loins and 18 raw legs of lamb and from 24 raw rib-loins and 20 raw whole hams (legs) of pork. Lambs were crossbreeds raised under two dietary regimens, feed lot and pasture, and were from 4 to 14 months of age at slaughter. Swine were of Duroc or Yorkshire breed, and were slaughtered when they reached 225 pounds live weight, regardless of age. For each cut, the muscle, adipose tissue, and bone with waste and gristle were separated, and all of the separable lean or separable fat thus obtained was weighed, ground, and thoroughly mixed. On one portion of each fat or lean compos-

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Amounts of nine elements in physically separable fat and separable lean portions from raw lamb and pork rib-loin and leg cuts were determined by emission spectroscopy. Calcium, copper, iron, and sodium levels per 100 grams of protein in separable fat tissue of lamb and pork cuts were significantly higher—generally about twice that of separable lean of the same cuts. However, on a fresh weight basis, because of the lower protein level of the adipose tissue, element content was about one-third that of separable lean. On an equivalent protein basis, separable fat of lamb contained more iron, boron, and phosphorus than did separable fat of pork.

ite, moisture, crude fat, and total nitrogen by micro-Kjeldahl were determined, using the procedures of the AOAC (*1*). Since the nitrogen of separable fat or lean tissue is assumed to be protein nitrogen, the factor 6.25 was used to convert nitrogen values to protein. Representative portions of each separable lean sample were dried under a battery of heat lamps, washed four times with petroleum ether, and ground in a Wiley mill, using procedures to minimize metal contamination. Residual moisture and fat determinations were made on each sample. Samples of lamb separable fat, after an hour under the heat lamps, were also fat-extracted with petroleum ether. Residues were dried overnight in a 70° C. convection oven and ground in an agate mortar. The pork separable fat samples were the combined dry, fat-free residues from triplicate Soxhlet crude fat determinations, also ground in an agate mortar. All prepared samples were stored in screw-cap bottles at 4° C. Test checks showed no measurable amounts of the nine elements present in the solvent-extracted fats.

Potassium was determined by flame photometry, using a Beckman DU spectrophotometer with flame attachment and photomultiplier. For the determination of boron, phosphorus, magnesium, iron, aluminum, calcium, copper, and sodium, the Bausch & Lomb medium quartz spectrograph and the analytical system of Hopkins and Eisen (*2*) were used with the following modifications. Weighed samples (usually 10 mg., range 5 to 15 mg.) of the separable lean or separable fat residues were transferred to preburned carbon electrodes. The samples in the electrodes were then ashed in a controlled furnace fitted with a quartz liner by gradually raising the temperature to a maximum of 540° C. for the last 10 minutes of a 2-hour period. Four arcings per sample were made, each on a separate day, and data from the four plates were averaged arithmetically.

Since calcium had not been detected in measurable quantities in the separable lean of lamb but was present in measurable amounts in the fatty tissue, calcium determinations were made on the lamb separable leans, using a Hilger Littrow spectrograph and a 5-level standard curve. This provided a three-fold increase in sensitivity of the element.

To eliminate such variables as moisture and extractable fat and to obtain a common basis for comparison, mineral element values were calculated as milligrams of element per 100 grams of protein.

Table I. Element Content and Equivalent Protein Content of Raw Adipose Tissue and Separable Lean of Lamb and Pork Cuts

	Protein in Fresh Tissue, % (Nitrogen × 6.25)	Element Content, Mg. per 100 Grams of Protein ± Standard Error			
		Calcium	Copper	Iron	Sodium
Lamb leg					
Separable fat	3.5	111 ± 13	1.05 ± 0.12	13.4 ± 0.9	551 ± 31
Separable lean	20.0	25 ± 1	0.48 ± 0.03	7.2 ± 0.4	314 ± 14
Mean difference[a]	...	86 ± 13	0.57 ± 0.11	6.2 ± 0.9	237 ± 34
Lamb rib-loin					
Separable fat	3.2	261 ± 29	0.89 ± 0.07	12.3 ± 0.7	551 ± 15
Separable lean	19.3	55 ± 7	0.46 ± 0.03	6.6 ± 0.3	389 ± 12
Mean difference[a]	...	206 ± 28	0.43 ± 0.08	5.7 ± 0.7	162 ± 21
Pork leg					
Separable fat	3.2	69 ± 7	0.72 ± 0.09	9.1 ± 0.8	563 ± 23
Separable lean	21.1	29 ± 1	0.36 ± 0.01	4.4 ± 0.2	340 ± 11
Mean difference[a]	...	40 ± 8	0.36 ± 0.09	4.6 ± 0.7	223 ± 22
Pork rib-loin					
Separable fat	3.6	94 ± 13	0.71 ± 0.05	7.7 ± 0.4	439 ± 16
Separable lean	20.8	36 ± 2	0.32 ± 0.02	4.1 ± 0.2	365 ± 10
Mean difference[a]	...	58 ± 13	0.39 ± 0.05	3.6 ± 0.4	74 ± 12

[a] All differences highly significant, P = 0.01 or less.

Results and Discussion

Boron content per 100 grams of protein in the separable fat portion of cuts averaged 0.31 mg. in lamb and 0.16 mg. in pork. Aluminum values for the fatty tissues averaged 1.9 mg. in lamb and 1.3 mg. in pork on a comparable protein basis. Neither element was detected in the separable lean portion of cuts, nor was manganese detected in either fat or lean of the two species.

In both lamb and pork, phosphorus, magnesium, and potassium in fatty tissues were essentially the same as in separable lean portions of cuts on an equivalent protein basis. In lamb, values averaged 850, 84, and 1500 mg., respectively, per 100 grams of protein in separable fat compared to 820, 106, and 1500 mg. in separable lean. In pork, comparable values were 702, 72, and 1400 mg. in separable fat tissues compared to 772, 117, and 1300 mg. in separable lean.

Based on equivalent protein content (Table I), levels of four elements—calcium, copper, iron, and sodium—were considerably higher in fatty tissues than in lean of both lamb and pork. The coefficient of variation for the spectrographic procedure for these elements in lamb and pork samples was approximately 25%. In spite of this range in the replicated spectrographic data, practically no overlapping of individual data was observed between content in separable lean and in fatty tissue from the same cut. Using differences between content in fatty tissue and in separable lean of each individual cut, the significance of mean differences was tested by Student's method (*6*). For all comparisons for these four elements, differences between content of corresponding fatty tissue and lean were highly significant ($P = 0.01$), and in all comparisons but one, "t" values far exceeded requirements for significance at the 0.001 level. Protein content of separable fat of both lamb and pork, however, was about one-sixth that of separable lean. Therefore, on a fresh weight basis, the content of these elements was approximately one-third as great per gram in separable fat

tissues as in separable lean of various cuts.

Lamb adipose tissue from leg and rib-loin cuts contained more iron, boron, and phosphorus than did similar tissue from pork. Calcium was higher and aluminum lower in fatty tissue of rib-loin than of leg of lamb. In pork, sodium was higher in adipose tissue of leg than in that of rib-loin.

Literature Cited

(1) Assoc. Offic. Agr. Chemists, "Official and Tentative Methods of Analysis," 9th ed., pp. 207, 643, 1960.
(2) Hopkins, H. T., Eisen, J., J. AGR. FOOD CHEM. 7, 633 (1959).
(3) Hopkins, H. T., Murphy, E. W., Smith, D. P., *J. Am. Dietet. Assoc.* **38,** 344 (1961).
(4) Leverton, R. M., Odell, G. V., *Oklahoma Agr. Expt. Sta. Misc. Publ No.* **MP-49,** Okla. State Univ., 1958.
(5) Schweigert, B. S., Payne, B. J., *Am. Meat Inst. Foundation Bull. No.* **30,** Chicago, 1956.
(6) Snedecor, G. W., "Statistical Methods," 5th ed., p. 49, Iowa State College Press, Ames, Iowa, 1956.

Received for review September 8, 1961. Accepted December 18, 1961. Work performed at a laboratory of the Agricultural Research Service, U. S. Department of Agriculture.

FOOD STABILIZERS AND PROTEIN DIGESTION

The Effect of Carrageenin on the Peptic Hydrolysis of Various Proteins

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Pepsin inhibition by carrageenin was studied to determine whether the addition of the polysaccharide to foods interferes with normal protein digestion. Different levels of carrageenin were added to solutions containing various concentrations of egg albumin, hemoglobin, casein, or soybean protein dissolved in HCl–citrate buffer, pH 1.6. Carrageenin at 0.085% level or lower did not affect pepsin activity in any of the solutions containing 1.0% or more protein, but there was marked inhibition of pepsin activity with higher concentrations of carrageenin or lower concentrations of protein. The protein level at which inhibition by a given concentration of carrageenin occurred, and the effect of pH on the degree of inhibition, varied from one protein substrate to another. Experiments in vivo further indicated that the levels of carrageenin used in foods do not interfere with normal protein digestion.

THE PROTEOLYTIC ACTION of pepsin may be inhibited by certain sulfated polysaccharides. In 1954, Levey and Sheinfeld (*1*) reported that heparin and chondroitin sulfate inhibit the pepsin-catalyzed hydrolysis of proteins, and more recently Houck *et al.* (*3*) reported that carrageenin, a sulfated polysaccharide from seaweed, is almost equally effective as an inhibitor of pepsin action. Data were obtained from studies both in vitro and in vivo suggesting that carrageenin affects pepsin activity. These authors reported that carrageenin ingested in drinking water (5 mg. per ml.) will inhibit ulcerogenesis in rats with pylorus ligation or subcutaneous injections of cortisone and in dogs subjected to large doses of histamine.

Since carrageenin is used as a stabilizer in many food products, the conditions under which pepsin inhibition by carrageenin occurs have been studied to determine whether addition of the polysaccharide to foods interferes with normal protein digestion. The results of these studies are presented.

Reagents and Methods

Casein, Hammerstein quality; soybean protein, 88% protein; and pepsin (3× Crystalline) were obtained from Nutritional Biochemical Corp., and commercial grade carrageenin was obtained from Marine Colloids, Inc. Egg albumin was prepared from fresh hen eggs, and hemoglobin from fresh beef blood according to the procedure of Anson and Mirsky (*1*).

For tests in vitro, different levels of carrageenin were added to solutions containing various concentrations of egg albumin, hemoglobin, casein, or soybean protein dissolved in hydrochloric acid–citrate buffer, pH 1.6. The quantity of pepsin which was then added depended on the substrate and the assay used for following protein hydrolysis. Three different assays were employed, since no single procedure was suitable for measuring protein hydrolysis when different proteins are used as substrates. The Riggs and Stadie assay (*6*) was used with egg albumin, the Anson and Mirsky assay (*1*) with hemoglobin, and the Volhard and Lohlein assay (*2*) with casein or soybean protein as the substrate.

For studies in vivo, male rats weighing approximately 150 grams were fasted 18 hours, and then given, by stomach tube, 2 ml. of a solution containing milk proteins with or without carrageenin. The dosing solutions were preparations of cow's milk modified to resemble human milk (carrageenin is used in most prepared infant formulas as a stabilizer). Exactly 1 hour after receiving the dosing solutions, the rats were killed with chloroform, the abdomen opened immediately, and the gastrointestinal tract ligated in two places—the esophagus directly above the stomach and the intestine 20 inches below the pylorus. The ligated segment was then removed, and the contents of the stomach and intestine were washed into a beaker with water. The

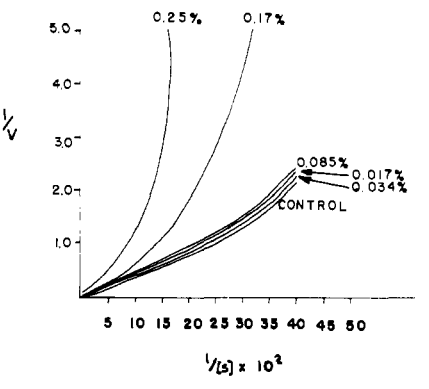

Figure 1. Effect of carrageenin on hydrolysis of egg albumin by pepsin

Egg albumin concentration [*s*], is expressed as mg. per ml. of incubation mixture, and velocity, *v*, is expressed in arbitrary units