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Amino Acid Analysis and Acrylamide Gel Electrophoresis Patterns of Bovine Hemopoietic Marrow

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Amino acid analysis and SDS polyacrylamide gels were used to characterize the proteins present in hemopoietic marrow. Amino acid composition of hemopoietic marrow remained relatively constant with changes in age of animal and proximate composition of the marrow. Major protein bands in hemopoietic marrow included albumin, a protein band at 20 000 daltons, and hemoglobin. Actin percentages in muscle and hemopoietic marrow mixtures increased in a linear manner as muscle content of the mixture increased. Since mechanically deboned meat (MDM) is a mixture of muscle and hemopoietic marrow, it is possible to determine the actin percentage in MDM and, from the actin percentage, estimate the amount of muscle or marrow present.

Marrow is the largest organ in the body, comprising 3.0 to 5.9% of the body weight (Reich, 1946; Winthrobe, 1974; Woodward and Holodny, 1960). At birth all bones contain red marrow (Custer, 1933) but in mature animals red marrow is found only in the proximal epiphysis of the long bones such as the femur and humerus and in flat bones such as the sternum, ribs, vertebrae, and pelvis. The metamorphosis of red to yellow marrow in long bones is a function of increasing age.

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Although the consumption of marrow by humans is well documented (Scrimshaw and Young, 1976; Souron, 1975; American National Cow Belles, Inc., 1973), the literature is devoid of data on marrow proteins. Characterization of marrow protein has become more important with the advent of mechanically deboned meat (MDM). The process of mechanical deboning removes much of the hemopoietic marrow from the interspaces of spongy bones at the same time the meat clinging to the outside of the bones is removed. Chang and Field (1977) believe that much of the variation in the protein quality of MDM is a result of variable amounts of marrow present.

This research was undertaken to characterize the protein

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in bovine hemopoietic marrow. It was anticipated that the data would be useful in elucidating the nutritional value of marrow and in identifying specific proteins which could be used to determine the marrow–muscle ratio in MDM.

EXPERIMENTAL SECTION

Bovine cervical vertebrae were removed from carcasses chilled at 2–3 °C for approximately 24 h postmortem. The bone cortex was removed with a band saw and the spongy bone was cut into pieces approximately 1 × 3 × 5 cm. Pieces of spongy bone containing marrow were placed on perforated platforms inside centrifuge tubes and spun at 17 000*g* for 1 h to separate hemopoietic marrow from bone. The bone remained on top of the perforated platform inside the centrifuge tube. Marrow, which made up about 40% of the weight of the spongy bone, was homogenized and then frozen in air-tight containers. Marrow from each of ten Good grade bullock carcasses, four Utility grade cow carcasses, and four Choice grade veal carcasses was frozen separately.

Protein, moisture, fat, and ash content of the marrow were determined (AOAC, 1970). A 0.5-g portion of each marrow sample was hydrolyzed with 20 mL of 8 N HCl in a sealed tube at 120 °C for 10 h; the hydrolysate thus obtained was filtered and then evaporated to dryness under reduced pressure. The dried residue was dissolved in distilled water and diluted to 25 mL, and an aliquot of 0.1 mL was used for the determination of amino acids using a Technicon auto-amino acid analyzer.

Cylinder and slab sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was used to characterize the protein banding patterns of muscle, hemopoietic marrow, and mixtures of muscle and marrow. Known amounts of fresh muscle and marrow were mixed and electrophoresed so the banding patterns obtained could be used to estimate the muscle:marrow ratio in mechanically deboned meat. Tissues and purified standard proteins (ovalbumin, bovine serum albumin, myoglobin, gamma globulin, and myosin) were solubilized by stirring overnight in an 8 M urea buffer solution, filtered, and run at the same time on cylindrical 5% acrylamide gels following the procedures of Weber and Osborn (1969). Relative mobilities of the standard proteins were plotted against the log of their molecular weights and found to be linear. Therefore, apparent molecular weights of proteins in the major bands of bone marrow were estimated.

After the protein banding pattern of hemopoietic marrow had been determined and compared to that of muscle, slab SDS polyacrylamide gel electrophoresis was used to determine the percentage of actin in mixtures of marrow and muscle containing 100, 75, 50, 37, 25, 12, or 0% marrow.

Gradient slab gels containing 4% acrylamide at the top and increasing in density to 10% acrylamide at the bottom were prepared. A 3% acrylamide stacking gel was layered on top of the separating gel and a comb with 1.4 cm wide slots was inserted to form wells for the solubilized protein. Approximately 150 μg of protein which had been solubilized by stirring overnight in 8 M urea was applied to each gel slot. A total of seven gels containing each of the muscle–marrow mixtures and samples of 100% muscle and 100% marrow were run using fresh muscle and marrow from the 14-month-old bullocks.

Preparation of linear gradient polyacrylamide gels, stacking gels, Tris–HCl buffer (pH 8.7), electrophoresis apparatus, fixing solution, Coomassie Brilliant Blue stain, and destaining solution was similar to that described by Owens and Haley (1976). After destaining, the gels were dried on typing paper using a Hoefer Scientific Instruments Slab Gel Dryer (Model SE 540). An Ortec Model 4130 densitometer was used for duplicate tracings on each muscle, marrow, or muscle–marrow sample for each of the seven gels which were similar to the gel shown in Figure 3.

A simple correlation coefficient, a linear regression equation, and a standard error of estimate relating the percentage of actin in each gel to the amount of fresh marrow in the tissue was determined as outlined by Steel and Torrie (1960). Seven different gradient gels each containing seven gel slots with fresh muscle:marrow mixtures of 0:100, 25:75, 50:50, 63:37, 75:25, 88:12, and 100:0 were run, and each gel slot was traced in duplicate; therefore, a total of 98 tracings were evaluated.

Table I. Means and Standard Deviations for Proximate Analysis of Cervical Vertebrae Marrow from 4-Month-Old Calves, 14-Month-Old Bulls and Mature Cows

Item	Calves (N = 4)		Young bulls (N = 10)		Mature cows (N = 4)	
	Mean	SD	Mean	SD	Mean	SD
Protein, %	16.1	0.9	12.6	1.4	15.9	0.6
Moisture, %	76.6	0.5	54.8	4.8	66.9	1.6
Fat, %	5.3	1.1	30.8	6.3	14.8	1.0
Ash, %	1.5	0.1	1.5	0.2	2.0	0.3

Table II. Means and Standard Deviations for Amino Acid Composition of Cervical Vertebrae Marrow from 4-Month-Old Calves, 14-Month-Old Bulls, and Mature Cows[a]

Amino acid	Calves (N = 4)		Young bulls (N = 10)		Mature cows (N = 4)	
	Mean	SD	Mean	SD	Mean	SD
Asp	7.3	1.7	7.8	1.2	8.6	1.0
Thr	4.8	0.1	4.5	0.6	4.9	0.6
Ser	4.8	0.3	4.6	0.2	5.3	0.3
Glu	10.4	0.9	10.0	0.6	9.2	0.1
Pro	5.1	1.7	4.4	0.4	4.0	0.4
Gly	5.2	0.4	5.8	0.4	5.4	0.2
Ala	7.6	0.2	6.6	0.1	7.3	0.1
Cys	2.6	0.2	2.7	0.3	2.8	0.3
Val	5.8	0.8	6.4	0.3	6.5	0.4
Met	2.0	0.6	2.2	0.1	1.7	0.1
Ile	2.2	0.7	2.9	0.1	2.5	0.2
Leu	11.1	1.0	10.0	0.9	13.2	0.3
Tyr	3.0	0.6	3.7	0.4	2.6	0.3
Phe	5.2	0.1	5.8	0.4	4.7	0.6
Lys	10.6	0.3	10.6	0.8	9.4	0.2
His	5.0	0.2	4.8	0.3	4.6	0.3
Arg	6.0	1.2	7.2	0.6	5.8	0.1
EAA[b]						
TAA	59.3	2.7	60.9	1.3	59.1	1.5

[a] Gram/100 g of protein. [b] EAA (essential amino acids) = threonine, total sulfur amino acids (cystine + methionine), valine, isoleucine, leucine, total aromatic amino acids (tyrosine + phenylalanine), lysine, histidine, and arginine; TAA = total amino acids.

RESULTS AND DISCUSSION

Means and standard deviations for protein, moisture, fat, and ash percentages in hemopoietic marrow from cervical vertebrae of the animals used in this study are shown in Table I. Fat percentage increased while moisture and protein percentages decreased in marrow from 14-month-old bulls when compared to marrow from 4-month-old calves. This finding supports the work of Dietz (1949) who found that the moisture and protein content of marrow decreased with increasing age. The lower fat and higher protein and moisture levels in hemopoietic marrow from mature cows when compared to that of the 14-month-old bulls is a reflection of differences in state

of animal nutrition. Marrow from animals on a high plane of nutrition has greater amounts of fat than marrow from animals on a low plane of nutrition (Verme and Holland, 1973). Therefore, it is not surprising that the marrow from thin Utility grade cow carcasses contained less fat than the marrow from Good grade carcasses of young bulls on a high plane of nutrition.

Amino acid composition of hemopoietic marrow did not change appreciably with changes in age (Table II). Therefore, protein quality of marrow should be relatively constant regardless of age or percentage of fat or protein present in marrow. Variation in amino acid composition of marrow within animal age or between animals of different ages (Table II) is similar to variation in the amino acid composition of whole blood (Altman and Dittmer, 1971). Hemopoietic marrow does have higher concentrations of cystine and methionine when compared to whole blood (Altman and Dittmer, 1971) or when compared to the globin or plasma fractions of blood (Tybor et al., 1975). Since HCl was the only method of hydrolysis, some destruction of methionine and cystine may have occurred. Therefore, the values should be taken with some reservation until a more reliable assay of these amino acids is made. Marrow, like blood, is an excellent source of lysine and leucine and is characterized by a relatively high level of histidine which is required by human infants (Clark, 1965).

The amino acid composition of meat has been reported by Rice (1971) and Happich et al. (1975). When compared to the amino acid composition of meat, hemopoietic marrow is low in isoleucine. Chang and Field (1977) reported that MDM from bones having the most lean attached had higher contents of isoleucine than MDM from bones where very little lean was attached. Chant et al. (1977) evaluated the amino acid content of tissue mechanically separately from bones where all the visible lean had been removed. This tissue, which contained high amounts of hemopoietic marrow was similar in amino acid composition to MDM except for isoleucine which was deficient in mechanically separated tissue. Since protein efficiency ratios of red meat and commercially produced MDM which contains marrow are often similar (Field, 1976) it is apparent that marrow, when diluted with sufficient amounts of meat, ceases to be a problem with regard to isoleucine as a limiting amino acid.

Banding patterns typical of those obtained on 5% acrylamide gels for hemopoietic marrow, muscle, and hemopoietic marrow–muscle mixtures of 1:1 are shown in Figure 1. The myofibrillar proteins in muscle were lacking or found only in trace amounts in marrow. The heavy myosin and actin bands of muscle were replaced by faint bands in the gels containing marrow. Hemoglobin at the bottom of the gel was the major protein in hemopoietic marrow but muscle lacked hemoglobin in sufficient concentration to be evident. In muscle, myoglobin was the only heme pigment present. The band at the 85 000 dalton region of the gels containing hemopoietic marrow was probably albumin as reported by Orstein (1964) and Davis (1964) for blood. Some very faint bands in the 29 000 to 38 000 dalton region of hemopoietic marrow were also present along with a rather distinct band at 20 000 daltons. Fatty marrow, not shown in the figures, had banding patterns similar to hemopoietic marrow but the protein concentrations were much lower.

The gel with fresh muscle–fresh marrow mixtures of 1:1 in Figure 1 contained all the major bands found in marrow and muscle. The myosin and actin bands in muscle and the hemoglobin band in marrow were diminished by

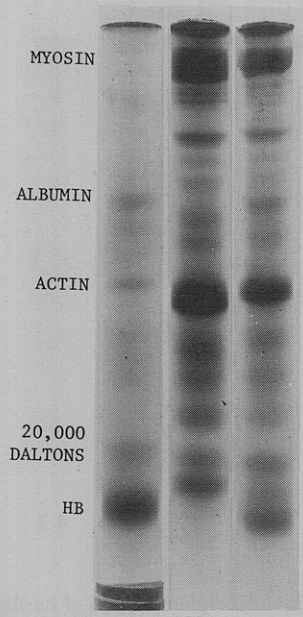

Figure 1. SDS 5% polyacrylamide gels of hemopoietic marrow (left), muscle (center), and a marrow–muscle mixture of 1:1 (right).

approximately half in 1:1 muscle–marrow mixtures. Since myosin, actin, and hemoglobin were present in the highest concentrations, these proteins were thought to be the most valuable for use in estimating the proportion of muscle to marrow in mixtures of the two. Further consideration ruled out hemoglobin because the amount of hemoglobin in gels containing marrow varied by age and physiological condition of the animal. Marrow from a 7-day-old calf had less than half the hemoglobin found in marrow from an 18-month-old steer. In addition, bands for hemoglobin and myoglobin, which were easy to separate and identify by use of authentic standards on 5% acrylamide gels, tended to merge on the 4–10% polyacrylamide gels. Size of the myosin band also varies according to experimental procedure (Rampton et al., 1976) and by conditions involved in postmortem aging (Yates et al., 1977). Arakawa et al. (1976) found a protease which cleaved myosin into heavy and light meromyosin. They concluded that the protease alters myosin banding patterns on acrylamide gels during postmortem aging. Therefore, actin produced the only major protein band in muscle–marrow mixtures which was considered suitable for use as an indicator of the amount of muscle or marrow present in mixtures of the two. In addition, the actin band varied in its concentration in different samples of MDM which were known to contain variable amounts of muscle.

Three different MDM samples were evaluated using SDS polyacrylamide gel electrophoresis as shown is Figure 2. A Beehive mechanical deboner with 0.46 mm holes in the cylinder yielded 19% MDM from bones which had very little muscle attached; 36% MDM from bones where approximately one-third of the bone weight was muscle; and 43% MDM from bones where approximately one-half of the bone weight was muscle. The yields of MDM increased as more muscle was included in the MDM. The greater amounts of muscle in samples of MDM with higher yields is illustrated in Figure 2. Increased amounts of muscle on the bones increased the yield of MDM and the concentration of actin on the gels.

A SDS gradient 4–10% polyacrylamide gel of hemopoietic marrow, marrow–muscle mixtures, and muscle is shown in Figure 3. Known amounts of fresh muscle and hemopoietic marrow were mixed together and run on the gels so that the proportions of muscle to marrow in a given

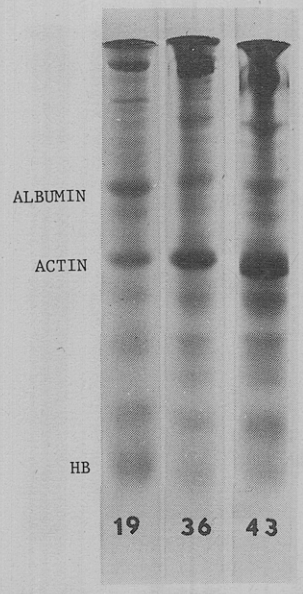

Figure 2. SDS 5% polyacrylamide gels of mechanically deboned meat with 19, 36, or 43% yields. The higher yields were produced by increasing the amounts of muscle on the bone prior to mechanical deboning. Greater amounts of muscle in mechanically deboned meat with higher yields is reflected by increases in size of the actin band.

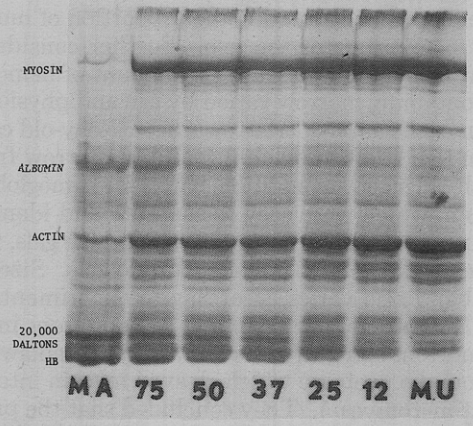

Figure 3. SDS gradient 4–10% polyacrylamide gels of hemopoietic marrow (MA), marrow–muscle mixtures containing 75, 50, 37, 25, or 12% marrow, and muscle (MU).

sample could be predicted from the concentration of the actin band on the gel. Duplicate densitometric tracings of seven samples of pure hemopoietic marrow showed that 2.5% of the total protein was in the region of 47 000 daltons (actin) while duplicate densitometric tracings of seven samples of pure muscle averaged 21.3% actin. The gels containing fresh muscle–fresh marrow ratios of 25:75, 50:50; 63:37, 75:25, or 88:12 decreased in concentration of the actin band as the marrow percentages in the mixtures increased (Figure 3).

The protein bands in each of seven gel slots on seven different gradient gels similar to the one shown in Figure 3 were traced in duplicate. Peaks for the actin band were expressed as a percentage of the total protein in the gel slot and compared to the marrow percentage in the fresh muscle–fresh marrow mixtures. The resulting linear regression equation based upon the 98 tracings was $Y = 113.6 - 5.33X$, where Y = percent marrow (wt/wt) in the fresh muscle–fresh marrow mixture, X = area of the actin band as a percentage of the total area of the protein bands in the gel. The simple correlation coefficient was −0.83 and the standard error of estimate was 12.5%. Therefore, the proportion of muscle to marrow in a mixture of the two, such as is found in MDM, can be estimated from the concentration of actin on acrylamide gels. The equation for estimating percent of fresh marrow in the muscle–marrow mixture from actin would be expected to change with changes in percent protein in marrow or muscle. However, composition of the marrow (12.6% crude protein, 54.8% moisture, 30.8% ether extract, and 1.5% ash) and muscle (21.7% crude protein, 71.7% moisture, 5.6% ether extract, and 1.1% ash) used in developing the equation is typical of the composition of muscle and marrow often found in MDM.

Determining the quantity of actin present might be of value to regulatory agencies who monitor the quantity of MDM added to meat products or to meat processors who by knowing the amount of hemopoietic marrow present in MDM could formulate meat products for uniform color, texture, flavor, and processing characteristics.

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