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RESEARCH ARTICLE

VITAMIN A, C, AND RIBOFLAVIN CONTENT OF BOVINE MARROW FROM CERVICAL AND THORACIC VERTEBRAE OF GOOD AND CHOICE GRADE STEERS

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ABSTRACT

This paper is based on a study to determine Vitamin A, C and riboflavin content of red marrow from Good and Choice grade steers so that the nutritional contribution of marrow to Mechanically Separated Meat (MSM) could be more fully defined. Vitamin A analysis was also performed on ten liver samples from the same steers to more fully define the nutritional status of the animals. Total vitamin A of twelve marrow samples ranged from 9,057-24,727 IU/l00g with a mean of 13,927. Vitamin C values of sixteen marrow samples ranged from 3.05-4.63 mg/l00g with a mean of 3.67. Riboflavin of nine marrow samples ranged from 0.136-0.325 ug/g with a mean of 0.215. Since MSM is a muscle/marrow mixture, marrow's contribution of vitamin A, C and riboflavin to MSM is insignificant. Therefore, mechanically separated meat would be expected to have about the same vitamin A, C and riboflavin content as lean meat.

INTRODUCTION

Red marrow is incorporated into Mechanically Separated Meat (MSM) during deboning because marrow, like lean meat, is soft and is forced through the screened surface of the deboner (Field, 1981). No work has been done to investigate the vitamin content of bovine marrow or MSM. Limited data indicate that rat marrow is high in vitamin C (Lutwak-Mann, 1952; Cox *et al.,* 1960), but bovine muscle is devoid of ascorbic acid. Organ meats like liver contain high amounts of Vitamins A and C which are devoid in muscle and since marrow is an organ associated with the liver in normal production of red blood cells; there is reason to expect that marrow may have vitamin content closer to organ meats than to muscle. Knowing the vitamin content of marrow in addition to other nutrients that are already known in marrow, would be useful in more satisfactorily defining the nutritional value of MSM.
The vitamin values could also be used for comparative purposes between hand-deboned meat and MSM. The nutritional value of MSM is a major concern to the meat industry which is expected to produce wholesome and nutritious meat products, to the USDA that make and monitor regulations, and to consumers who expect to get meat and meat products which are high in nutritional quality. The specific purpose of this study was to investigate vitamin A, C and riboflavin content of bovine red marrow from cervical and thoracic vertebrae of Good and Choice grade steers. Proximate analysis and analyses of vitamin A and carotene content in the liver were also carried out to more completely define the nutritional status of the steers that furnished the marrow in this study.

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Bone Marrow

Bone marrow is a soft, fatty substance located in the interior cavities of bones (Stein, 1973). Bone marrow constitutes nearly *5%* of the body weight (Windle, 1976*)* and is classified as red marrow or yellow marrow (Sanchez, 1979) depending on its location and activity. Bone marrow, in general, is involved in the production of blood corpuscles and platelets, bone formation and reconstruction (particularly in young individuals), erythrocyte destruction, lipid storage and immunological responses (Windle, ibid). The chemical composition of bone marrow varies according to age, species, location of the bone and the functional demand so that values which have been reported vary greatly (Huggins *et al., 1940;* Dietz, 1946). Variations occur within the same individual depending on the location of the marrow and between individuals because of functional demand and age. The marrow cavity of all bones consists essentially of red hemopoietic tissue at birth, but during postnatal growth, warmer cavities retain more red marrow (Windle, *ibid*). Huggins and Blocksom (1936) documented distribution of red and yellow marrow by age in the rat, rabbit and dog and concluded that the presence of yellow marrow was correlated with a reduction in blood formation. Gong and Arnold (1963) reported that vertebrae are the greatest contributors to total skeletal marrow in the dog. Their data shows that flat bones generally have red marrow while long bones generally have yellow marrow.

Mechanically Separated Meat (MSM)

The USDA (1982) defined Mechanically Separated Meat as the finely comminuted product resulting from mechanical separation and removal of most of the bone from attached skeletal muscle of livestock

carcasses and parts of carcasses. The muscle removed by a deboner is that left on irregularly shaped bones from the vertebral column during hand-deboning operations (Field, 1981). The composition of MSM varies because its sources are not homogenous. Carcasses used come from different grades and from different anatomical parts that have been cut and trimmed by a variety of different methods (Field, *ibid*).

Vitamin A

In 1918, both McColium and Davis, and Osborne and Mendel noted that a factor occurring in certain fats was necessary for growth of rats. Rats fed purified proteins, carbohydrates, lard and salts alone did not grow well and developed soreness of the eyes. However, when an ether extract of butter or eggs was added to the diet, the rats grew well and the soreness of the eyes disappeared (Wagner and Folkers, 1964; Johnson *et al.,* 1974; Robinson, 1978). McColium named the complex present in the ether extract 'fat-soluble A' (Robinson, 1978). With time, the fat-soluble A was named vitamin A. At the beginning of the 20th century, it was learned that xeropthalmia and nyctalopia were associated with vitamin A deficiency. During the same period, it was demonstrated by Steenbock (1919) that carotenes had vitamin A activity. The carotenes are vitamin A precursors and are referred to as pro-vitamins-A (Robinson, 1978). ß-ionone occurs both in vitamin A and carotene. One molecule of B-carotene can give two molecules of vitamin A upon halving the molecule. Other isomers of carotene, like α and ϐ forms, can only yield one molecule of vitamin A. The presence of ß-ionone and the unsaturated polyene side chain, is essential for vitamin A activity (Waisman and Elvehjem, 1941; Lee, 1975). There are four forms of vitamin A: vitamin A alcohol which is retinol; vitamin A aldehyde which is retinal; vitamin A acid which is reti-noic acid and vitamin A esters, also called retinyl esters (Robinson, 1978).

Vitamin A is measured in retinol equivalents (RE) or international units (IU). The equivalents are:

1RE = 1 ug retinol (3.33 IU).
1RE = 6 ug beta carotene (10 IU).
1RE = 12 ug other carotenoids (10 IU).

Vitamin A has four main functions. It is necessary for normal visual cycle and for health and maintenance of the eye (Waisman and Elvehjem, 1941; Moore, 1957; Wald, 1960; Wagner and Folkers, 1964; Johnson *et al.,* 1974; Lee, 1975 and Ojner, 1977). Vitamin A is necessary for healthy epithelial and skeletal tissues (Waisman and Elvehjem, 1941; Wagner and Folkers, 1964; Ofner, 1977; Robinson, 1978). Studies on experimental animals have shown that vitamin A is essential for spermatogenesis in the male and normal oestrus cycle in the female (Robinson, 1978). It is also necessary for development and maintenance of the placenta (Wagner and Folkers, 1964; Robinson, 1978). Vitamin A and carotene are stable to mild heat treatment during processing and cooking (Siedler *et al.,* 1963; Johnson *et al.,* 1974; Osborne and Voogt, 1978). They are, however, destroyed at high temperatures or in sunlight and in presence of oxygen. Patty peroxides oxidize vitamin A and carotenes and, therefore, they can be destroyed under conditions that favour the oxidation of fats, such as the presence of iron and copper (DeRitter, 1976; Osborne and Voogt, 1978; Robinson, 1978). Food-approved antioxidants, such as butylated hydroxy-anisole and butylated hydroxytoluene, are added to liquid products that contain vitamin A and carotene to protect them from oxidation. A combination of antioxidants and protective coatings are used to protect dry products (DeRitter, 1976). DeRitter (1976) reported retention of vitamin A in different foodstuffs and in butter stored at 28°C for 12 months. Retention was 64 to 68% in food stuffs while in butter stored at 5°C for 12 months retention was 66 to 98%. More carotene was retained than vitamin A in these products, Kramer (1974) reported an average retention value for carotene of over 80% after canned carrot and tomato juices had been stored for 12 to 24 months. As a fat-soluble vitamin, vitamin A is susceptible to destruction by irradiation. Also, the extent of destruction is greatly influenced by media in which they are treated. The acetate and palmitate esters of vitamin A, as well as the fish-oil concentrates are more stable to irradiation than are mixtures of esters combined with unsaturated fatty acids. Carotene bound to protein is less affected by irradiation than is a "free" carotene (Thomas and Josephson, 1970). In humans, the efficiency of the utilization of B-carotene is estimated to be about 1/6 of the total amount consumed. Each ug of B-carotene is therefore estimated to be equivalent to 0.167 ug of all transretinol (Moore, 1957). Although there are no reports on vitamin A content in bone marrow, Krause (1913) suggested that some unsaponifiable portion of bone marrow lipids was vitamin A.

Vitamin C

Vitamin C (ascorbic acid) is a water-soluble vitamin. It is easily and reversibly oxidized to dehydroascorbic acid which, like vitamin C, is active but less stable. Dehydroascorbic acid can be reduced to ascorbic acid again, but if it is oxidized further, it becomes diketogulonic acid which is an inactive form of the vitamin (Waisman and Elvehjem, 1941; Johnson *et al.,* 1974; Lee, 1975; Osborne and Voogt, 1978). Vitamin C was isolated for the first time in the early 1930s by Dr. Charles King and co-workers at the University of Pittsburgh and by Dr. Szent Gyorgi of Hungary. Two Norwegian scientists, Hoist and Trolich, had however, produced scurvy in guinea pigs in 1907 (Robinson, 1978). Scurvy, as a disease, had been known since the middle ages when it caused a heavy death loss among sailors (Sharman, 1974). Scurvy is the term which refers to the disease resulting from a deficiency of vitamin C in the diet (Lee, 1975). It is characterized by multiple haemorrhages (Marks, 1968). Hodges *et al.* (1971) found that human male subjects developed clinical scurvy in 64 to 97 days, manifested by signs and symptoms of fatigue, haemorrhagic phenomena, swollen bleeding gums, follicular hyperkeratosis, muscular aches and pains and emotional changes. The Minimum amount of ascorbic acid necessary to prevent or cure scurvy is slightly less than 10 mg daily (Hodges *et al.,* ibid). Cox *et al.* (1960) observed significant reduction in the bone marrow concentration of ascorbic acid in all patients with megaloblastic anaemia.

Ascorbic acid is involved in numerous metabolic processes (Johnson *et al.,* 1974). It is essential for the formation of collagen which is essential for healthy bones and strong teeth (Johnson *et al.,* 1974; Robinson, 1978). It builds and maintains tissues and blood. It helps to maintain, the body's resistance to infection (Johnson *et al.,* ibid) and acts as an antioxidant. It increases the absorption of iron, helps release transferritin so that it can be incorporated into tissue ferritin, and deactivates adipose tissue lipase (Robinson, ibid). Chen *et al.* (1975) studied the effects of ascorbic acid deficiency on growth and calcification of bone in whole 18-day foetal rat radii and ulnae cultured in a chemically defined medium. They found that 200 ug/ml of ascorbic acid caused maximum formation of labelled hydroxyl-proline. Bones cultured in media without added ascorbic acid showed a decrease in labelled hydroxy-proline formation after 2 days. After 4 days, the rate was less than 15% of the ascorbic acid-treated controls. There was no significant increase in shaft weight and cartilage weight. Calcification, however, was not affected by ascorbic acid absence. Vitamin C is widely used as an antioxidant in food. One frequent method of applying ascorbic acid and its sodium salt is by spraying or dipping meat in a solution of either of these compounds. Hood (1975) reported that intravenous pre-slaughter injection of sodium ascorbate

into beef animals 5 to 10 min before slaughter is successful in distributing the ascorbate throughout the musculature where it has an effect of inhibiting oxidation of myoglobin to metuyoglobin. The shelf life was 6 days when this procedure was followed instead of 3-4 days.
Vitamin C is highly labile in aqueous solution and is easily oxidized in an alkaline medium or on exposure to heat, light and traces of metals like copper and iron. Acid solution, particularly in the presence of metal chelating agents, does not destroy vitamin C (Johnson *et al.,* 1974; DeRitter, 1976; Osborne and Voogt, 1978; Robinson, 1978). Vitamin C that is in food can be reduced during storage and processing and is sensitive to enzymatic oxidation on heating. Since vitamin C is soluble in water, processing may lead to losses through the leaching action of excess water (Osborne and Voogt, ibid).

Kramer (1974) reported that at a storage temperature of -20.5°C asparagus, green and lima beans and peas maintain at least 90% of their original vitamin C for 12 months. He also pointed out that at -20.5°C for 12 months or more, broccoli, cauliflower, spinach and peaches may lose 20 to 50% of their vitamin C. On the other hand, some juices, such as orange juice, lose less than 10% of the vitamin C at temperatures as high as 4.5 C. Feister *et al.* (1950) showed that high temperatures accelerated the loss of vitamin C in grapefruit juice. Juice stored at 46°0 lost more of its original vitamin C than the juice stored at 22°C. Rate of leaching can also be decreased in canned food by storing at low temperatures (Kramer, 1974). Frozen foods tend to retain more vitamin C than canned foods. However, if the food is not handled correctly, during and after freezing it will lose more of vitamin C. Frozen, cooked and drained vegetables lost only 26% of the original vitamin C (Fennema, 1977). Davis (1956) showed that low temperatures for freezing ensured high retention of ascorbic acid. Only 5% ascorbic acid was lost after 6 months at -29°C, while 75% was lost at -12°C. Loss of vitamin C also depends on the type of package used. Daoud and Luh (1967) compared two types of pouches used in packaging freeze-dried pepper in relation to quality of the product. They found that pepper packaged in aluminium-film combination pouches retained more vitamin C than that packaged in mylar-saran-polyethylene plastic laminate since the latter type of package was permeable to water vapour and oxygen. In meat the conditions under which it is cooked has an effect on vitamin C retention. Liver and brains retain 63 to 100% of their vitamin C after cooking and little or no vitamin C was contained in the drip from samples used. Presimmering caused larger amounts of the vitamin to appear in the drip. Lachanoe *et al.* (1973) reported that time and temperature play a part in determining how much vitamin C is lost during heat conditioning of convenience foods.

Ascorbic acid oxidase, which has a maximum activity at 40°C and is almost inactivated at 65°C, can destroy vitamin C through oxidation. Bender (1974) pointed out that rapid heating of food protects the vitamin because the enzyme is inactivated quickly. Dehydration of food is rarely done in the United States, but it is widely used in other areas as a means of preserving food. It is difficult to protect ascorbic acid during drying because the temperature of the product and nonenzymatic browning increase the loss of the vitamin. Morgan *et al.* (1945) reported that dehydrated vegetables containing 4 to 5% moisture lost 50 to 80% of their ascorbic acid after 6 months storage at 35°C, to 40.5°C. Those stored at 4.5°C to 10°C lost less than 20%. Morgan's study indicates that storage temperature plays an important role in the overall retention of vitamin C. Kramer (1974) also indicated that low storage temperatures assure a higher retention of vitamin C in dried foods. Vitamin C in food is less radiosensitive than the pure compound and can be protected somewhat by freezing if sterilizing doses are utilized (Thomas and Josephson, 1970). Cain (1967) reported that vitamin C retention during irradiation depends on the amount of ionizing radiation and the concentration of vitamin C. If the product has' high vitamin C concentration it will lose less, but if it has a lower concentration it will lose more. With doses below 80 Krad, no measurable amounts of vitamin C are lost. Although data on vitamin C content of mechanically separated meat is limited, Kruggel and Field (1977) and Chant *et al.* (1977) have analyzed vitamin C content in MSM. Kruggel and Field (ibid) found vitamin C values ranging from 2.08 to 2.67ug/l00g from MSM of beef and 2.38 to 2.61ug/l00g from MSM of pork. Chant *et al.* (ibid) reported 1.37ug/100g in MSM of beef. Previously, Lutwak-Mann (1952) reported 2 4 mg/100g in fresh bone marrow from the rat and Cox *et al.* (1960) reported 13-15 mg ascorbic acid per 100 human bone marrow cells. Figures obtained from MSM samples are reportedly low because ascorbic acid is easily oxidized in presence of air, which is incorporated into the meat and marrow during mechanical deboning (Kruggel and Field, ibid). No data on the vitamin C content of bovine marrow is available.

Riboflavin

Riboflavin is a water-soluble vitamin. It is a bitter tasting orange-yellow odourless compound in its pure state, with needle-shaped crystals. It is slightly soluble in water and exhibits a greenish-yellow fluorescence (Robinson, 1977). The fluorescent pigments of milk, which include riboflavin, were investigated as early as 1879 by Blyth though it was not until they were related to biological oxidation in 1932 that intensive biochemical research was done (Anonymous, 1942; Robinson, ibid). Riboflavin was differentiated from thiamin (B^), which is heat labile, in the 1920s. The yellow-green pigments were named flavins. They were named according to what they were extracted from, such as, ovoflavins from eggs and lactoflavins from milk. Later, when it was known that the factor contained a ribose attached to an isoalloxazine nucleus, the term riboflavin was used to refer to all the flavins (Eddy, 1949; Wagner *et al.,* 1964; Robinson, ibid).

Riboflavin, which exists in two forms, either as flavin mononucleotide (FMN) or as Flavin adenine dinucleotide (FAD), is necessary for growth and general health of people of all ages and animals. Before it can be absorbed, it must be phosphorylated in the gastrointestinal tract (Johnson *et al.,* 1974; Robinson, ibid). FMN and FAD form the prosthetic groups of different enzyme systems. These enzymes group with their prosthetic groups (flavoproteins) are all concerned with hydrogen transport. First, FAD acts as accessory redox system by acting as a link between the intermediary metabolites and the cytochromes. It also acts as a link between the intermediary metabolites and the cytochrome systems. Secondly, FAD is an hydrogen carrier (Marks, 1968; Lee, 1975; Robinson, 1977). Ariboflavinosis is believed to be one of the common deficiency diseases (Robinson, 1977). It is characterized by cracking of the skin at the corners of the mouth and an oily dermatitis around the folds of the nostrils. Another symptom of riboflavin deficiency is an abnormally smooth condition of the tongue accompanied by a change in its colour to purplish red (Anonymous, 1942; Eddy, 1949; Marks, 1968; Johnson *et al.,* 1974; Robinson, 1977). In animals, deficiency symptoms of riboflavin are fatty liver, kidney congestion, general muscular and nervous debility and corneal vascularization (Waisman and Elvehjem, 1941; Marks, 1968). Patients who have undergone total or subtotal gastrectomy and patients being treated with chloramphenicol, and other

antibiotics are likely to show symptoms of riboflavin deficiency. Ariboflavinosis may be prevented with a daily dose of 3 mg riboflavin (Marks, 1968). Riboflavin is very unstable in solution and its decomposition is greatly influenced by light (Ultra violet and visible light), temperature and pH of the solution. Slight destruction of riboflavin occurs by heating (Cain, 1967; Johnson *et al.,* ibid). Many studies have been done concerning the effect freezing, thawing and cooking has on riboflavin retention. Several studies on the influence of dehydration and irradiation on riboflavin retention are also available. Reported affects of freezer storage on the riboflavin content of meats vary by storage time and temperature, preparation method, method of analyses and species (Stocking, 1977). In this respect, Kotschevar (1955) reported a 34% loss of riboflavin in calf liver frozen for 2 months. Fennema (1977) also reported a 35% loss of riboflavin in sliced liver frozen at -20°C for 2 months. Westerman *et al.* (1955) observed an 18% riboflavin loss in pork loins after 8 weeks of storage at -1°C and 20% riboflavin loss after 32 weeks of storage. Westernan *et al.* (1952), Meyer *et al.* (1963) and Fennema (1977) however, have reported a significant increase of riboflavin upon freezing the meat over some time. Fennema (ibid) attributed the increases to either analytical errors or to release of bound nutrients during the freezing process. The rate of freezing has been reported to have little effect on the retention of riboflavin in pork (Lee *et al.* 1954). Westernan *et al.* (ibid) reported that storage up to 48 weeks at temperatures varying from -2°C to -29°C had little effect on the riboflavin content of pork.

Riboflavin usually is stable when meat is thawed, but considerable amounts of the vitamin are transferred from muscle to the thawing drip (Angler and Bowers, 1976). Pearson *et al.* (1959) determined that 4.15% of the B-Complex vitamins were lost in the drip obtained upon thawing frozen pork. Larson (1956) reported appreciable amounts of riboflavin in the drip of defrosted poultry. Slicing tends to cause more dripping because of the increased surface area. Larson (1956) observed that there was more drip in cut chicken, but none in whole eviscerated Turkeys. Effects of cooking on riboflavin contents of meat are influenced by factors which include cooking method, moisture content, time and temperature of cooking method, moisture content, time and temperature of cooking and the cut of meat being prepared.

Schweigert and Payn (1956) stated that vitamin content with cooking methods give typical vitamin retention figures for cooked meat plus drippings of 100% for riboflavin. Tucker *et al.* (1946) found the plus drippings in individual experiments ranging from 91-129% in all methods of braising, pot-roasted slices of chuck showed retention ranging from 88-111% of riboflavin. Riboflavin losses in beef, veal, lamb, pork or chicken are negligible, regardless of cooking method (Angler and Bowers, 1976). Mclntire *et al.* (1943a) reported 79-86% riboflavin retention on roast veal and lamb and 79% riboflavin retention on broiled lamb. Mayfield and Hedrick (1949) obtained 83-102% riboflavin retention at 149°C and 85-98% at 260°C. Asp *et al.* (1953) stated that total riboflavin retention was within experimental error of 100% although roasts consistently retained greater percentages of riboflavin than did the braised cuts. Retention of riboflavin in dry cooking methods is generally higher than in wet cooking methods, like braising and stewing. However, Mclntire *et al.* (1943b) found 85% retention of riboflavin after roasting and braising. Dong *et al.* (1980) analyzed the vitamin content of selected foods, as served, and found that braised liver contained the highest riboflavin content, ranging from 0.689 to 1.060 mg/100g.

Final internal temperatures affect vitamin retention in meat. Cover *et al.* (1944) found more riboflavin is rare than in well-done roasts. They also found that beef roasts cooked for a shorter time contained more riboflavin than those cooked for longer time. Stewing did not cause any destruction of riboflavin (Cover and Dilsaver, 1947b). Bowers and Fryer (1972) studied the effects of cooking and reheating (after 1 day of refrigerated and 5 weeks of freezer storage) on riboflavin content of turkey meat. They reported more riboflavin in muscle cooked with heat than in that heated by microwave. They also reported that muscles which had been refrigerated for 1 day had a higher riboflavin retention than those which had been frozen for 5 days. Baldwin *et al.* (1976) compared microwave and conventional ovens. They found that there was no significant difference in vitamin retention, although meat heated in conventional ovens retained more riboflavin than meat heated in microwaves.

Canning and corning, according to Mayfield and Hedrick (1949), ensure higher conservation of riboflavin. Meat canned in metal retained 213-280% riboflavin while that canned in glass retained 171%. Corned meat retained 90-114% riboflavin. Dehydration process causes only moderate losses of B-vitamins, approximately 5% in the case of riboflavin. Normally, riboflavin and other B-vitamins are retained quite well in steam-blanched dehydrated vegetables (Cain, 1967). In this respect, Kramer (1974) pointed out that ambient temperatures utilized for drying potatoes and carrots caused increased retention of riboflavin. Thomas and Josephson (1970) reported the effect of irradiation on vitamin content and suggested that low temperatures during irradiation would cause high retention of B-complex vitamins. The values of riboflavin in lean meat from either beef or pork are almost the same. They range from 1.3 ug/g to 2.5 ug/g. Sheep lean, however, can contain up to 78ug/g riboflavin (Michelson *et al.,* 1939; Waisman and Elvehjem, 1941; Cheldelin and Williams, 1942; Lawrie, 1980). A search of the literature reveals no information on the riboflavin content of MSM and bone marrow.

MATERIALS AND METHODS

Marrow samples for vitamins A, C and riboflavin analyses were obtained from cervical and thoracic vertebrae of 20 Good and Choice grade steers weighing 499-567 kg from the University of Wyoming beef herd. Each steer consumed approximately 9 kg/day of a concentrate mixture consisting of 1588 kg corn, 680 kg barley, 340 kg beet pulp and 254 kg soy protein. In addition, an alfafa-grass hay mixture containing 18% protein was fed free choice. The steers were slaughtered in the University of Wyoming abattoir and the carcasses were split in half and aged for 7 days at approximately 2O ºC before the cervical and thoracic vertebrae were removed. The cortex of the cervical and thoracic vertebrae was removed with a band-saw, and the spongy bones were cut into rectangular pieces of approximately 5 x 1 x 1 cm and frozen in plastic bags. Before centrifugation, the spongy bones were thawed overnight in a refrigerator and individual pieces were scraped clean of all muscle, fat, connective tissue and bone dust. Two to three pieces of the clean spongy bones were placed on a platform inside a centrifuge tube (Field *et al.,* 1978). The perforated stage allowed marrow to collect at the bottom of the 2.7 cm diameter centrifuge tubes during centrifugation. A refrigerated (4°C) centrifuge was used to collect marrow for vitamins A and C analyses while marrow used for riboflavin and proximate analyses was collected at room temperature. All marrow was removed from the spongy bones by centrifugation

at 35,000 x G for 30 min. After approximately 50g of marrow was collected from each steer, it was homogenized in a Virtis homogenizer. Marrow collected at refrigerated temperatures was homogenized for 3 to h min while marrow collected at room temperature was homogenized for 1 min. The homogenized marrow was weighed into airtight small brown bottles and frozen at -30°C for 1 week to 3 months before analysis. A composite sample of marrow from all steers was used for proximate analysis. Protein, moisture, fat and ash were determined in quadruplicate following proximate analysis procedures of the AOAC (1975). Vitamin A and carotene were determined on marrow from 12 different steers following the USDA (1979) procedure. To validate the procedure, liver samples were analyzed and the results were compared to data available in the literature. Portions from the same liver were analyzed and the results compared to check repeatability. After the procedure was tested, several marrow samples were analyzed and the procedure was slightly modified so that it could be used successfully to analyze vitamin A and carotene in marrow. Modification was necessary because the bone marrow has a high fat content and more blood than lean meat and liver. Moore (1957) indicated that blood needs to be saponified longer than liver to give complete saponification. During extraction, the ether layer was washed at least 5 times with water before the washings (water layer) was neutral to phenolphthalein. Eluted vitamin A from the column was placed in a hot water bath while a stream of nitrogen was blown onto the sample to drive off the acetone hexane mixture. The dry sample was then taken up with 10m chloroform. Before photometric measurement, two drops of acetic anhydride were added to the sample in a cuvette to absorb the moisture. Antimony trichloride reacts with moisture causing turbidity which distorts the absorbance readings. The amount of vitamin A in marrow was determined spectrophotometrically using a Bausch and Lomb Spectronic 2,000 at a wavelength of 620nm.

Standard dilutions of pure B-carotene in concentrations of 5, 1.0, 2.0, 4.0 and 8.0ug/ml were made. Absorbance of each dilution was determined using a Bausch and Lomb spectronic 2,000 set at a wavelength of 436nm and used to plot a standard curve. The absorbance of the carotene fraction from each marrow sample was determined in the same manner as the standard dilutions. Ten samples of liver obtained from 10 of the same steers which furnished the marrow, were also analysed for vitamin A and Carotene. The USDA (1979) procedure was followed. All analyses of vitamin A and carotene were done in darkened room to protect vitamin A from destruction. Ascorbic acid was determined on marrow samples from nine steers by titration following the USDA (1979) procedure. Before the analyses were performed, several liver samples were used to check repeatability of the method. Riboflavin was determined on marrow samples from nine steers following the USDA (1979) procedure. Before riboflavin analysis, validation of the procedure was tested using liver samples from the same steers which furnished the marrow. Liver samples were used.

RESULTS AND DISCUSSION

Means and standard deviations for proximate analysis of a composite sample of red marrow from the steers are shown in Table 1. Protein, moisture, fat and ash percentages were et al. 14.50%, 58.32%, 22.52% and 2.73% respectively. However, some variation in composition of red marrow from beef animals would be expected due to anatomical location, grade and age. Total vitamin A content of bovine red marrow averaged 436 IU/100g (Table 2) and total vitamin A of bovine liver averaged 13,927 IU/100g (Table 3). As the data indicate, the vitamin A content of red marrow is much lower than it is in liver. It was expected that red marrow would have vitamin A content closer to that in other organs, such as the liver. The composite sample of red marrow which was sent to a commercial laboratory for analysis contained 620 TU/100g of total vitamin A (Table 5). This value is higher than the 436 IU/100g which is the reported average in Table 5 but both values are within the range reported for kidney. In red marrow, approximately 71% of the total vitamin A came from carotene (Table 2) but in liver only 8.6% came from carotene (Table 3). 42% of the vitamin A in values reported in Table 5 came from carotene. The difference between this study and the analysis by Hazleton Raltech with respect to carotene is fairly large and it is probably due to differences in methodology since the marrow came from the same animals.
Although the steers which furnished the marrow were on the same feed, considerable variation in vitamin A and carotene content of liver and marrow between steers was found. No correlation between vitamin A and carotene content among steers was apparent. Variation between animals and within each animal for vitamin A and carotene is probably, due to individual differences in metabolism, and the ability of individual steers to convert carotene to vitamin A.

Table 1: Means and standard deviations for proximate analysis of bovine marrow from cervical and thoracic vertebrae of Good and Choice grade steers

Content	Mean (%)	Standard Deviation
Protein	14.50	0.36
Moisture	58.32	1.71
Fat	22.52	1.97
Ash	2.73	0.45
Total	98.06	0.61

[a] Composite samples run in quadruplets.

Table 2: Vitamin content of bovine marrow from cervical and thoracic vertebrae of Good and Choice grade steers

Sample from Steer Number	Vitamin A IU/100g	Carotene Ug/100g From carotene	Vitamin A IU/100g	Total Vitamin A IU/100g
1	123	300	500	623
2	107	133	222	329
3	170	161	268	438
4	120	167	278	398
5	125	233	388	513
6	126	200	333	459
7	122	241	402	524
8	115	133	222	351
9	128	200	333	461
11	113	167	278	391
12	129	133	222	351
13	124	167	278	402
Mean	**125**	**186**	**310**	**436**
Standard Deviation	**16.2**	**53.3**	**85.2**	**86.8**

Marrow could be a fair source of vitamin A if 100% red marrow were consumed. However, marrow constitutes only 10 to 30% of MSM (R.A. Field, personal opinion) and only

Table 3: Vitamin A content of bovine liver of Good and Choice Grade Steers

Sample from Steer Number	Vitamin A IU/100g	Carotene ug/100g	Vitamin A From Carotene IU/100g	Total Vitamin A IU/100g
108	10800	668	1113	11913
138	8500	334	557	9057
209	11100	84	140	11240
2102	12700	585	975	13675
3084	12800	585	975	13775
5251	11700	835	1391	13091
7102	16200	1002	1670	17870
7105	8300	919	1531	9831
7114	22500	1336	2227	24727
8106	12700	835	1391	14091
Mean	**12730**	**718**	**1197**	**13927**
Standard Deviation	**4365**	**371**	**619**	**4805**

Table 4: Vitamin C and riboflavin content of bovine marrow from cervical and thoracic vertebrae of Good and Choice Grade Steers

College	New students	Graduating students
108	4.58	0.170
138	3.05	0.248
209	3.05	0.325
3084	3.05	0.136
5251	4.58	0.293
7102	4.58	0.235
7105	3.05	0.209
7114	3.05	0.177
8106	4.58	0.145
2	3.11	---
3	4.63	---
6	4.63	---
8	3.17	---
11	3.21	---
12	3.21	---
13	3.21	---
Mean	**3.67**	**0.215**
Standard Deviation	**0.74**	**0.065**

Table 5[a]: Vitamin content in bone marrow from cervical and thoracic vertebrae of Good and Choice Grade steers

Assay	Analysis	Units
Beta Carotene	0.160	mg/100g
Vitamin A from carotene	260.0	IU/100g
Vitamin A	360.0	IU/100g
vitamin A, total	620.0	IU/100g
Vitamin C, total	<1.1	mg/100g
Thiamin	0.130	mg/100g
Riboflavin	2.30	mg/g
Vitamin B	0.0170	mg/g
Niacin	22.0	mg/g

[a]This table is the result of the essay analyses by Hazleton Raltech, Inc.

20% MSM is allowed as an ingredient for processed meat products (USDA, 1982). Therefore, the contribution of marrow to vitamin A in the diet is probably insignificant. The vitamin C content of marrow is shown in Table 4. Vitamin C values ranged from 3.05 to 4.63 mg/100g with a mean and standard deviation of 3.67 mg/100g and 0.74 respectively. The commercial laboratory obtained a value of less than 1.1 mg/100g from the composite marrow sample (Table 5). Methodology or destruction of vitamin C during shipping possibly accounted for the difference. The vitamin C values from this study are higher than vitamin C values of 2.08 to 2.67ug/100g from MSM reported by Kruggel and Field (1977) and the 1.37ug/100g of MSM reported by Chant *et al.* (1977). However, the difference between marrow and MSM was expected because marrow makes up part of MSM (Field, 1981) and during deboning, air is incorporated into MSM and vitamin C could be oxidized (Chant *et al.,* ibid). Fresh bone marrow from other species reportedly contains higher levels of vitamin C. An average of 24 mg/100g was found in fresh rat marrow (Lutwak-Mann, 1952) while Cox *et al.* (1960) found 13 to 16 mg/100g cells of human bone marrow. Thus, the low levels of vitamin C found in bovine marrow in this study could be due to species difference. All lower animals except the guinea pig synthesize ascorbic acid. Rats have the ability to synthesize ascorbic acid in the liver which has been reported to contain as much as 42.2 mg/l00g tissue (Salomon, 1961). Several investigations on vitamin C metabolism by species have been based on the concentrations of Ascorbic Acid 2-Sulphate (AAS) in various tissues. It is believed that high AAS concentrations may be associated with the high ascorbic acid level of particular tissues. Rat tissue reportedly has 12.3 mg AAS per 100g of tissue (Salomon, 1961; Hornig, 1974). In addition to species differences, vitamin C might have been oxidized in the present study because the beef carcasses were split down the middle after slaughter and marrow samples were not obtained until 7 days of aging. As evidenced in this study, marrow in MSM which is incorporated into meat products is not high enough to be beneficial as a vitamin C source.

Riboflavin values in marrow found in Table 4 ranged from 0.136 to 0.325 ug/g with a mean and standard deviation of 0.215 ug/g and 0,065 respectively. These values are much lower than riboflavin values in muscle and organ meats like liver, heart and kidney (Waisman and Elvehjem, 1941; Rice, 1971; Lawrie, 1980). The analysis by a commercial laboratory on the composite sample resulted in values similar to those in muscle (Table 5). The difference may be due to methodology. Because marrow is only 10 to 30% of MSM and because only 20% MSM can be added to processed meat products, riboflavin content of products containing MSM would not be changed significantly regardless of which analysis is correct. If the riboflavin values obtained from the commercial laboratory are correct, then the products containing MSM and those without MSM would have the same riboflavin content.

CONCLUSIONS

Vitamin A, C and riboflavin content of bovine red marrow from cervical and thoracic vertebrae of Good and Choice grade steers were investigated in order that the possible vitamin contribution of marrow from MSM could be estimated. Vitamin A in marrow was lower than that in liver but higher than that reported for muscle. Vitamin C in marrow was higher than that in MSM but less than that found in most organ meats like liver. Riboflavin values obtained from nine marrow samples analyzed were minimal. Since marrow makes up 10-30% of MSM and since only 20% MSM is allowed in red meat products, the vitamin content of products containing MSM should be similar to those containing only hand deboned lean. However, values for vitamin A and C may

be slightly higher in products containing MSM and values for riboflavin may be slightly lower than in those products which do not contain MSM.

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