EFFECT OF COOKING ON PROTEIN QUALITY OF GROUND BEEF AND A BEEF-SOY BLEND

by

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INTRODUCTION

Class A school lunches are served to many children in the United States. A requirement of the lunch program is that a 2 oz. serving of meat or a meat alternate be served. Ground beef is often used to fill this requirement because of its versatility in meat dishes and its widespread acceptance by children.

In 1971 the Food and Nutrition Service (1) allowed up to 30% of the meat or meat alternate in the Class A school lunch to be replaced by rehydrated textured vegetable products. Soy protein, high in nutritive value, is the main textured vegetable protein (TVP) presently used. The greatest difference between the amino acid levels of beef and soy protein is lysine content. Beef is higher in lysine than TVP, but TVP has adequate lysine for growth and maintenance (2).

Ground beef can be prepared by frying, broiling, char-broiling and baking to make hamburgers, casseroles, chili, taco filling, egg roll filling, spaghetti sauce, meat balls and pizza topping. Some browning of meat products is usually desirable for good flavor and it does not affect protein value of ground beef to any extent. However, Wilding (3) theorized that extended cooking may decrease protein value of soy mixtures by increasing browning reactions.

The two mechanisms causing non-enzymatic browning are the Maillard reaction (amino-sugar reaction) and caramelization (heating sugars). The omega group on an amino acid is the reactive group in the Maillard reaction. The epsilon amino group on lysine is in the omega position and it is available for reaction with reducing sugars. Consequently lysine is the most reactive amino acid in Maillard reactions. According

to Pearson et al. (4), both mechanisms of browning may be responsible for the brown color in cooked meat.

The purpose of this study was to investigate the effect of two cooking times on the digestibility and utilization of protein of ground beef and a beef-soy blend when fed to rats. In addition, the effect of cooking time on available lysine was determined.

REVIEW OF LITERATURE

Soy Protein

Historical aspects. The soybean has been an important food in eastern Asia for many centuries, but it only became important in the western world during the present century. Soybeans were grown in the U.S. as early as 1804, but the shortage of oil around the time of World War I spurred the growth of the soybean industry in the U.S. (5). After the oil was removed, the defatted meal was used for animal feed since it had a high protein content of good nutritional value (6). Presently, the soybean is a major protein source for animal feed and is the most important source of vegetable oil in industrialized countries of North America and Europe (7).

In 1970, 69% of U.S. food grade proteins were from animal sources and 31% were from plants (8). The volume of hydrated textured vegetable protein used in school lunches increased from 28 million lb. in 1971-72 to 60 million lb. in 1973-74 (8).

According to the present U.S. Secretary of Agriculture, Earl Butz, (9), trade estimates of U.S. soybeans used for human consumption in both domestic and foreign markets are placed at approximately 1 million metric tons per year. Of the crushed and exported U.S. soybeans, 85% goes into

animal feeds, 12% goes into industrial uses and only about 3% is used for human food. Butz also said that vegetable proteins could furnish the equivalent of 8% of the U.S. total red meat protein production by 1980 and up to 20% of the 1980 meat supply may be made of vegetable protein meat analogues.

Soybeans are a good food source since soy products are free from problems such as contamination from mold toxins (e.g. aflatoxin). Also, the high yield of soybeans per acre is advantageous for helping meet the protein demands of the growing world population (6).

Production of soybeans. World demand for soybeans increases by about 7% each year while yield / acre / year averages only 1% increase. For this reason, more acreage must be planted to soybeans to increase production and meet the demand (7). The major soybean producers in the world are the U.S., mainland China, Brazil, the U.S.S.R. and Indonesia (5).

Total soybean production in the U.S. increased from about 5 million bushels in 1924 to about 1.1 billion bushels in 1971 (approximately 2/3 of the world production). Yield per acre increased from 11.0 bushels in 1924 to 27.6 in 1971 (5). Approximately 1/6 of the total U.S. acreage is planted with soybeans currently (7).

Processing soybeans. Whole soybeans are screened to remove weed seed. The moisture is reduced to 10% and the beans can be stored until needed. Then the large trash is removed, the beans are cracked, dehulled, steam softened and flaked. The full-fat flakes can be ground into full-fat flour, or they can be solvent extracted (usually with hexane) to remove the lecithin and oil. The defatted flakes can be used for animal feed, toasted flakes, grits (which can be made into granular concentrates) or flour. The defatted flour can have a) oil added to

make refatted soy flour, b) lecithin added to make lecithinated soy flour, c) the fiber and sugars extracted and be dried to make protein isolate which can be spun into fibers, d) the sugars extracted and be dried to make a protein concentrate, or e) water and other additives added and heat and pressure applied to expand it and make textured vegetable protein. The flour also can be hydrolyzed to make hydrolyzed vegetable protein (10).

Textured soy protein can be processed by fiber spinning or thermoplastic extrusion (5). In the spinning process, fibers are spun from isolated soy protein and held together with binders; colors, flavors, seasonings and supplementary nutrients and ingredients are added; and fabricated products simulating familiar animal type products such as beef, bacon, ham, fish and chicken are made. The thermoplastic extrusion process uses soy flour which is processed in a cooker-extruder, and the thermoplastic protein material is forced through a die that regulates the shape and size of the product.

Nutritional value. The soybean has a high protein content (39-44%) of good nutritional quality (11). Soy protein has a high content of essential amino acids, especially lysine, leucine and isoleucine; however soy is somewhat low in sulphur containing amino acids, with methionine being the first limiting amino acid (6).

Soy flours and grits have a minimum protein content of 40 to 50% that varies with the fat content (12). Defatted soy flour has 51.5% protein, 8.2% nitrogen, 7% moisture, 4.5% fat, 3% fiber, 5.8% ash and 30.0% carbohydrates (13). Approximately one-half of the flour carbohydrates are the oligosaccharides - sucrose, stachyose and raffinose; the other half are polysaccharides (12).

Soy contains a trypsin inhibitor (TI) and a hemaglutinin which need to be inactivated (usually done by heating in the presence of moisture to denature the proteins) for good growth (14). Mustakas et al. (15) found that 89% TI inactivation produced a PER of 2.15. Heating during extruder-processing, which inactivated deleterious components, did not decrease available lysine in extruded flours much from the original soybean meal which contained 6.5% lysine.

Beef and Soy Blends

Cooking qualities. Bowers and Engler (16) found that addition of 15% and 30% textured soy protein to ground beef decreased cooking losses. The beef-soy blends had more moisture, less ether extract, less meat flavor and aroma, and more cereal-like flavor and aroma than all-beef patties. Nielsen and Carlin (17) found that raw and cooked all-beef loaves and beef-soy loaves were similar in moisture content. They also found that raw beef loaves were higher in fat but beef-soy loaves lost less fat during cooking so the final cooked loaves were similar in fat content. Quality characteristics of ground beef and turkey meat loaves were not adversely affected by 30% soy substitution (18).

Nutritional value. Debry et al. (19) found meat was slightly superior to textured soy protein when they compared digestibility, net protein utilization and biological value of the two products. Human nitrogen balance studies using a basal diet with 0.8 g N /day and TVP and beef conducted by Kies and Fox (20, 2) resulted in positive nitrogen balances when adults were fed either TVP or beef at 8.0 g N / day and negative balances on 4.0 g N / day. As the ratio of beef to TVP nitrogen decreased (4/0, 3/1, 2/2, 1/3 and 0/4), the mean nitrogen balances of

subjects fed 4.8 g N / day also decreased linearly (-0.44, -0.56, -0.75, -0.90 and -1.11 g N per day, respectively). Soybean protein (TVP) fed at a level of 4.0 g N / day had a crude protein digestibility of 79.4 compared with 81.4 for beef fed at the same level. At 8.0 g N / day, crude protein digestibilities were 81.6 for soybean protein and 82.7 for beef.

Methionine is the first limiting amino acid for soy proteins (20, 21, 6). According to Kies and Fox (2), methionine is probably the first limiting amino acid in beef also. They observed that lysine content is the largest difference between beef and textured vegetable protein (TVP), although TVP has sufficient levels of lysine for growth and maintenance. Beef contained 2.2 g lysine / 4 g N and TVP had 1.6 g lysine / 4 g N. Methionine levels were 0.7 g / 4 g N in beef and 0.3 g / 4 g N in TVP.

With casein equal to PER 2.5, lean beef had a PER of 2.8 and soy concentrate had a PER of 2.2 (22). Happich (22) also reported an 80/20 lean beef-soy concentrate blend and a 70/30 beef-soy blend had PER slightly above 2.5, while a 60/40 blend had a PER slightly below 2.5. Further increases in soy concentrate resulted in decreased PER. Wilding (3) determined PER for chicken patties, meat loaf, meat balls, cooked beef patties, uncooked beef patties and chili with 0, 12, 21 and 30% levels of hydrated textured soy. The PER for the meat mixtures were generally higher than for the casein controls. All the products had negative regression slopes for PER as the soy content increased, but the chili had the greatest negative slope, perhaps caused by increasing browning reactions. Wilding also presented a table (Table 1) comparing essential amino acids in beef, textured soy and a 70/30 beef-soy blend. The percentage of the adult requirements of amino acids

was lower for the beef-soy than for the beef except for tryptophan and phenylalanine. However, only methionine was limiting in the beef-soy blend.

Table 1

Percentage of adult requirements of amino acids found in 45 g protein[a] from beef, textured soy, and beef soy blends (3)

Essential amino acids	Beef	Textured soy	70% Beef 30% Soy
Lysine	490	330	440
Threonine	400	330	380
Valine	310	280	300
Methionine	100	50	90
Total sulfur AA	150	120	140
Isoleucine	340	330	330
Leucine	370	350	340
Phenylalanine	170	190	170
Tryptophan	210	220	220

[a]New U.S. recommended daily allowance (adult) is 45 g protein (protein efficiency ratio 2.5).

Nonenzymatic Browning during Cooking

Nonenzymatic browning may result from caramelization (heating of sugars) or from the Maillard reaction between a reducing sugar or aldehyde with an amine. Lysine is particularly reactive in the browning reaction because of its free epsilon-amino group (23). Adrian (24) explained the influence that heat, moisture content, pH and presence of reducing sugars have on the Maillard reaction. Adrian showed that soy flour had good heat stability and that meat products and dried yeast are the most resistent to Maillard reactions.

Willits et al. (25) and Lento et al. (26) found that the presence of basic pH and lysine caused increased browning of glucose solutions.

Spark (27) used a sugar-glycine mixture and found that loss of N in the form of free amino groups during browning depends on the type of sugar (pentose, aldohexose or ketose). He suggested that monoketose amines are a major intermediate in browning reactions involving loss of amino nitrogen. In lysine and sugar mixtures, around 80% of the original lysine was recoverable as lysine after 8 hours heating at 103°C, but lysine heated on paper without added sugar showed about 4 to 5% loss after 8 hours (28).

Reducing sugar and ether extract were important in affecting browning in pork -- browning increased as reducing sugar and ether extract increased (29). Pearson et al. (4) found that the level of sugar in the tissues of pork was closely related to color development. A high relationship between free sugar content and degree of brownness suggested that sugar is partly responsible for the brown color developed upon heating. Color production may be attributed to the Maillard reaction, to caramelization, or to both mechanisms.

Warmed-over flavor (WOF) in cooked meats was inhibited by reductic acid and reductones (30). The browning reaction between amino acids and sugars produced these products in retorted meat.

As the heat intensity or duration of heating is increased, the possibility of loss of protein quality is also increased. Presence of reducing sugars or a source of reducing sugars also has been found to contribute to protein denaturation or damage (31). Lysine involved in the Maillard reaction is complexed and unavailable for absorption, thus creating a decreased biological value.

Methods of Protein Quality Evaluation

Weight gain and protein efficiency ratio. Osborne et al. (32) and Chapman et al. (33) found that differences between body weight gain per gram of protein eaten were much less than those between absolute body weight gains. However, Hegsted and Worcester (34) found a very high correlation between weight gain and protein efficiency and claimed that calculating PER (protein efficiency ratio) added little information.

In order to get reproducible results and to be able to compare results with those of other workers, Chapman et al. (33) proposed a procedure to standardize conditions for PER. A reference diet containing 10% protein from casein would be fed to a control group of rats and test diets containing 10% protein would be fed to the test groups. Rats should be 20 to 23 days old, should be housed individually, should have weekly weight and feed consumption records kept, and should be fed ad libitum for 4 weeks. The PER of test diets would be adjusted to 2.5 for casein.

PER is a simple and convenient method of measuring protein quality. It has, however, been criticized (33, 35, 36) because it fails to allow for body maintenance; it assumes that the increase in body weight is proportional to the protein retained, which may not always be true depending on the type of diet; results vary with the level of protein in the diet and with food intake; and proteins cannot be evaluated which do not produce growth.

Biological value. Mitchell (37) studied nitrogen metabolism, measuring the dietary nitrogen wasted in digestion and metabolism and assessing the body's contribution from tissue catabolism to the urinary

and fecal nitrogen excretions. He defined biological value of protein as the percentage of absorbed nitrogen (N intake - fecal N of dietary origin) that is not eliminated in the urine.

Net protein utilization. Net protein utilization (NPU) is the percentage of food nitrogen which is retained in the body or the biological value times digestibility (the amount of N absorbed from the food N) (36). Miller and Bender (38) gave a shortened method for determining NPU which involves body nitrogen (whole carcass) of a test group, body nitrogen of a non-protein group and nitrogen consumed by both groups.

NPU is independent of protein and food intake (39, 35); however, Rippon (40) was dissatisfied with the tedium of carcass analysis and the fact that animals had to be destroyed. Bender (35) found that PER and NPU were highly correlated and that NPU can be correlated with negative PER.

Net protein ratio. The net protein ratio (NPR) is obtained by adding together the loss in weight of a group of rats receiving a no-protein diet and the gain in weight of the test group and dividing by the protein consumed by the test group (33, 36, 41). Advantages of NPR are brevity, simplicity, independence from food intake and high correlation with NPU (41).

Chapman et al. (33) found that PER, NPR and NPU placed the nutritive value of foods in the same order, but NPR and NPU tended to have larger standard errors. Protein quality is related to the first limiting amino acid (36). A disadvantage of PER, NPU and NPR is that they give no indication of the amounts of non-limiting essential amino acids in proteins.

Chemical determination of available lysine. Chemical methods for determining biologically available lysine are based on the hypothesis that the epsilon-amino group must be intact for lysine to be available in vivo (42). Carpenter (43) developed a procedure to measure possible nutritional damage to foods that contain lysine. The free epsilon-amino groups on lysine reacted with 1-fluoro-2, 4-dinitrobenzene (FDNB), the dinitrophenyl-proteins were hydrolyzed with acid and the hydrolysates were ether extracted. The DNP-lysine was measured colorimetrically along with a blank of methoxycarbonyl chloride. Heat-treated samples which had shown a decreased nutritional value in feeding tests showed lower available lysine values using the chemical procedure.

Carpenter's FDNB method was modified by Booth (44); he filtered the refluxed mixture while it was hot rather than cooling it first. This modification prevented adsorption of DNP-L by residues. Also, a smaller correction factor (1.05) for loss of DNP-L during hydrolysis was determined for animal proteins.

Various materials were assayed for available lysine by Booth's revision and by total lysine minus inaccessible lysine (TLMI). Values were closer for animal than for vegetable materials and for undamaged than for heat-damaged materials. Animal protein resulted in a smaller loss of DNP-lysine during acid digestion than did vegetable protein (44).

If a sample's inferiority was caused by heat-damage during processing or storage, total amino acid values might have failed to reveal the damage and would not have reflected the extent of damage (45). This was true for both lysine and methionine. Normally in undamaged protein, a close correlation existed between the available and total values for both lysine and methionine, with the mean value for available lysine about

10% below that of total lysine.

Hurrell and Carpenter (46) compared analytical tests for determining early Maillard, advanced Maillard and protein-protein damage on lysine content of various materials. They thought the direct FDNB and MIU (methyl isourea) procedures were the methods of choice for the full range of possible damage. The direct FDNB procedure was thought to measure the full extent of changes in biologically available lysine in early Maillard reactions, and it was thought to be a sensitive method for advanced Maillard and protein-protein damage but may not measure the full nutritional change.

Concerned with the behavior of deoxyketosyl derivatives (Amadori products) in different chemical procedures for analysis of available lysine, Finot and Mauron (42) found that the guanidination method of using O-methyl-isourea was the best method and that Carpenter's FDNB method was second-best, being rapid and suitable for routine use.

Lysine Availability

Amino acid requirements for rats. Rama Rao et al. (47) established the essential amino acid requirements of the growing rat. Different levels of one amino acid were fed, keeping others constant, to determine the minimum requirements for maximum growth. With 10% protein diets, the requirements for lysine, histidine, tryptophan, isoleucine, valine, threonine, methionine and cystine, and phenylalanine and tyrosine are: 0.19, 0.21, 0.11, 0.55, 0.69, 0.56, 0.51, 0.49 and 0.72% of the diet, respectively.

Reevaluating the preceding levels, Stockland et al. (48) found that the lysine requirement to give maximum weight gain / feed consumed was

0.60% of the diet. Jansen (23) summarized different researchers' findings for lysine requirements for man and rat for both growth and maintenance. For growth, the values for rats ranged from 5.2 - 8.1 g lysine / 16 g N. According to McLaughlan (49), rat growth requires 19% lysine and 10% methionine-cystine of the total essential amino acid content. Maintenance requires 4% lysine and 24% methionine-cystine.

Changes in lysine levels or availability. Complexed amino acids may be unavailable for absorption, thereby decreasing the biological value, even though the true availability would not be reflected by chemical analysis or calculation of protein scores (31). Changes in availability are reflected by measurements such as biological value, gross protein value, net protein utilization and protein efficiency ratio only when changes have occurred in the limiting amino acid, and provide no information on changes in nonlimiting essential amino acids present (50).

Womack et al. (50) observed that autoclaved lactalbumin decreased the availability of 9 of the essential amino acids. Critical levels for the amino acids were determined in an attempt to develop a testing system to show which amino acids decreased in nutritional availability. Lysine (0.487% in the diet) tested for heat damage in the autoclaved lactalbumin was 81% of the critical level (0.60% of the diet) and had a PER that was 64% of the control.

Hackler et al. (51) found that PER of heat-processed soymilk was dependent on both time and temperature of treatment. Cooking soymilk 1-6 hours at 93^{o}C did not have adverse effects on protein efficiency, growth or available lysine. Cooking for 32 minutes at 121^{o}C caused a definite decline in PER and indicated that available lysine was declining.

At 121°C available lysine (g / 16 g N) went from 6.0 at 0 minutes to 5.7 at 40 minutes to 5.0 after 120 minutes.

Mauron and Mottu (52) concluded that small losses of lysine in heat-treated milk can be determined in feeding tests only when methionine (the first limiting amino acid) is added since the small lysine losses which occur would otherwise be masked by the methionine deficiency in milk. According to Said and Hegsted (53), data are accumulating which indicate a conservation of amino acids since the protein quality is not lowered proportionately as the essential amino acid falls below the amount in a reference protein.

Heat Effect on Protein

Mitchell and Block (54) reported that the nutritive value of proteins subjected to heat may be depressed without involving amino acid destruction. Reasons are that protein digestibility may be depressed, that a decreased digestibility may be caused by excretion of a protein fraction containing disproportionate amounts of certain amino acids and that heating a protein may cause certain combinations between terminal groupings that are resistant to proteolytic action, resulting in unnatural peptides that may be absorbed intact and excreted in the urine.

PROCEDURES

Beef and Beef-Soy Blend

Approximately 40 pounds of freshly ground beef (about 20% fat) were obtained from the KSU Animal Science and Industry Department. Twelve 400 g portions of beef were wrapped in heavy foil, frozen and stored

at -2°C. The rest was used in the beef-soy blend (70:30). One thousand g Ultra Soy[1] was rehydrated with 2000 g water. Seven thousand g beef and 3000 g hydrated soy were combined and mixed for 2 minutes on speed 1 (low) and for 1½ minutes on speed 2 in a floor model Hobart mixer. After mixing, the blend was packaged in foil in 400 g portions and frozen. The beef and beef-soy blend will be referred to as meat.

Treatments were: 1) beef cooked 5 minutes (B-5), 2) beef cooked 15 minutes (B-15), 3) beef-soy cooked 5 minutes (BS-5) and 4) beef-soy cooked 15 minutes (BS-15). The meat was thawed in a refrigerator for 3 days so that its temperature was 5.5 - 7.5°C before cooking. Twelve 400-g portions of each product were cooked for each of the four treatments. One 400 g portion was cooked at a time at 121°C in an uncovered, preheated, small electric skillet for the specified time with occasional stirring. The meat and drippings were transferred to a mixing bowl and the skillet was washed. This process was repeated until all twelve 400-g portions (representing one treatment) had been cooked.

The meat was stored in a refrigerator overnight and then mixed with a floor model Hobart mixer for 4 minutes (½ minute on speed 1, 1 minute on speed 2 and 2½ minutes on speed 3). From the blended, cooked meat, four 50 g samples were removed, packaged and frozen for protein, fat and moisture analyses (Appendix, Table 6). The remaining meat was frozen in 200-g packages, which were then freeze-dried to a suitable form for incorporation in rat diets. The freeze-dried meat was ground in a meat grinder, then mixed for 2 minutes on speed 2 of a Hobart table model mixer.

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[1]Far-Mar-Co, Hutchinson, Kansas.

Biological Evaluation

Preparation of Diets. Moisture, fat and protein analyses were done on the four freeze-dried treatments (Table 6). Moisture was determined by calculating weight loss after drying a sample of approximately 2.5 g for 2 hours in a drying oven. A slight modification of the AOAC method (55) for ether extracts was used for lipid analysis. Protein content (N x 6.25) was determined by macro-Kjeldahl analysis.

Diets were formulated, using the method recommended by the National Academy of Science - National Research Council (56), for each of the four meat treatments, for a casein control and for a low protein (4%) egg albumin diet (Table 2).

The freeze-dried meats were mixed for 2 minutes on speed 2 of a Hobart table model mixer to break up chunks which formed. Ingredients for each diet were then weighed, combined and blended for 20 minutes with a Hobart floor model mixer on speed 1. The six diets were then bagged and the bulk stored in a refrigerator during the feeding study.

Rat Feeding Study. Fifty-two Sprague-Dawley male weanling rats were ordered for the study. The rats were weighed and arranged in cages so that there were 8 rats per diet, two rats from each diet on each of the four levels of cages and total weights of the group were about equal. The remaining 4 rats were sacrificed and their carcasses frozen for later analysis.

During the 3-week feeding period, rats were given food and water ad libitum. Rats, feed containers and spilled food were weighed weekly to calculate cumulative weight gain and the amount of feed consumed. Feces were collected during the third week, dried in the air, weighed and ground for determination of digestibility.

TABLE 2

Percent composition of the diets

Ingredients	Diets[1]					
	1	2	3	4	5	6
Beef cooked 5 min	22.2	-	-	-	-	-
Beef cooked 15 min	-	22.8	-	-	-	-
Beef-soy cooked 5 min	-	-	20.9	-	-	-
Beef-soy cooked 15 min	-	-	-	22.0	-	-
Casein	-	-	-	-	10.0	-
Egg albumin	-	-	-	-	-	4.0
Beef tallow	0.7	-	2.0	1.7	10.5	10.5
Water	1.0	-	1.3	1.1	3.0	3.0
Vitamins[2]	0.3	0.3	0.3	0.3	0.3	0.3
Salts[2]	4.0	4.0	4.0	4.0	4.0	4.0
Cellulose	5.0	5.0	5.0	5.0	5.0	5.0
Choline[2]	0.2	0.2	0.2	0.2	0.2	0.2
Cornstarch	66.5	67.7	66.3	65.7	67.0	73.0

[1]Diet 1) beef cooked 5-min., diet 2) beef cooked 15-min., diet 3) beef-soy cooked 5-min., diet 4) beef-soy cooked 15-min., diet 5) casein control, and diet 6) egg albumin, low protein diet.

[2]Provided in amounts recommended by NAS / NRC (56).

At the end of three weeks, food was removed from the rat cages and the rats were fasted for 16 hours. The rats were sacrificed with chloroform, reweighed and frozen for later analysis.

PER determinations. PER was calculated using the formula:

$$\text{PER} = \frac{\text{g body weight gain}}{\text{g protein ingested}} \times \frac{2.5}{\text{PER of casein diet}} \text{ (corrected to casein)}$$

Carcass nitrogen analysis for NPU. Using a modification of Hegsted's method (57), the frozen whole rat carcasses were digested by putting each in a glass mason quart jar, adding 300 ml of approximately 9 N hydrochloric acid, covering the jar and autoclaving for 3 hours at 120°C. The digestion mixture was poured into a 1000 ml volumetric flask and made to volume with distilled water after adding 5 ml toluene to help collect the fat. Weights of the empty and full flasks were recorded. The insoluble material floating at the top in the flask was suctioned off with little loss of soluble nitrogen and the flask was again weighed. The rest of the flask contents were mixed and filtered. Two pharmaceutical bottles were filled with the filtrate, which was analyzed for nitrogen by the macro-Kjeldahl method. The method used to determine the NPU for carcass nitrogen was that of Miller and Bender (38):

$$\text{NPU} = \frac{\text{body N of test group} - \text{body N of low protein group} + \text{N consumed by low-protein group}}{\text{N consumed by test group}} \times 100$$

Digestibility of diets. Fecal samples were analyzed for nitrogen by the macro-Kjeldahl method. Digestibility was calculated:

$$\text{Apparent digestibility} = \frac{\text{N intake on test diet} - \text{fecal N on test diet}}{\text{N intake on test diet}}$$

$$\text{True digestibility} = \frac{\text{N intake on test diet - fecal N of test diet -fecal N of low protein diet}}{\text{N intake on test diet}}$$

Chemical Evaluation

Amino acid analysis. The Biochemistry Department at Kansas State University used an amino acid auto-analyzer to determine the amino acid content of samples of the four freeze-dried meat treatments, freeze-dried raw beef and raw beef-soy. Values were calculated on a moisture-free, fat-free basis.

FDNB - available lysine. The FDNB - available lysine in samples of the 4 freeze-dried cooked meat samples and in samples of freeze-dried raw beef and raw beef-soy was determined by the Booth modification (44) of the Carpenter method. Freeze-dried samples were dried to a constant weight in a vacuum oven at room temperature to prevent browning of the meat. Samples were then ether extracted so that the moisture-free, fat-free samples could be pulverized, sent through a 0.5 mm sieve and then analyzed.

Results were calculated using the following formula:

$$C \text{ (corrected)} = \frac{w_s \times A_u \times v \times 100 \times 100}{w_u \times A_s \times a \times (cp)} \times 1.05$$

C = content as g lysine / 16 g N

w_s = weight of standard, expressed as mg lysine in 2 ml

w_u = weight of sample in mg

A_s = net absorbance of standard

A_u = net absorbance of unknown

v = volume of filtered hydrolysate

a = aliquot of filtrate

cp = crude protein, 6.25 x g N / 100 g material

RESULTS AND DISCUSSION

Chemical Evaluation

Essential amino acids (g / 16 g N) present in raw and cooked (5 and 15 minutes) beef and beef-soy blend (70:30) as determined in the auto-analyzer are shown in Fig. 1. Tryptophan was not analyzed. No statistical analyses were performed on the chemical data but some apparent differences that were observed will be discussed.

On the raw basis, beef-soy was lower in all of the essential amino acids measured than the beef. Cooking for 5 minutes resulted in decreases in essential amino acids (except lysine) in the beef but in increases in the beef-soy blend (except total sulfur AA). Cooking for 15 minutes resulted in essential amino acid values for the beef that generally were higher than those found with the beef cooked only 5 minutes but not as high as the values for the raw beef. Cooking of the beef-soy blend for 5 minutes increased the essential amino acid values somewhat; cooking for 15 minutes increased values even further so that they were close to the cooked beef values. Values of all amino acids determined are in the appendix (Table 7).

A comparison of the lysine content determined by amino acid auto-analysis and the FDNB method for available lysine of the raw and cooked beef and beef-soy blend is shown in Table 3. All lysine values by the FDNB method were larger than those of comparable products by the amino acid auto-analyzer.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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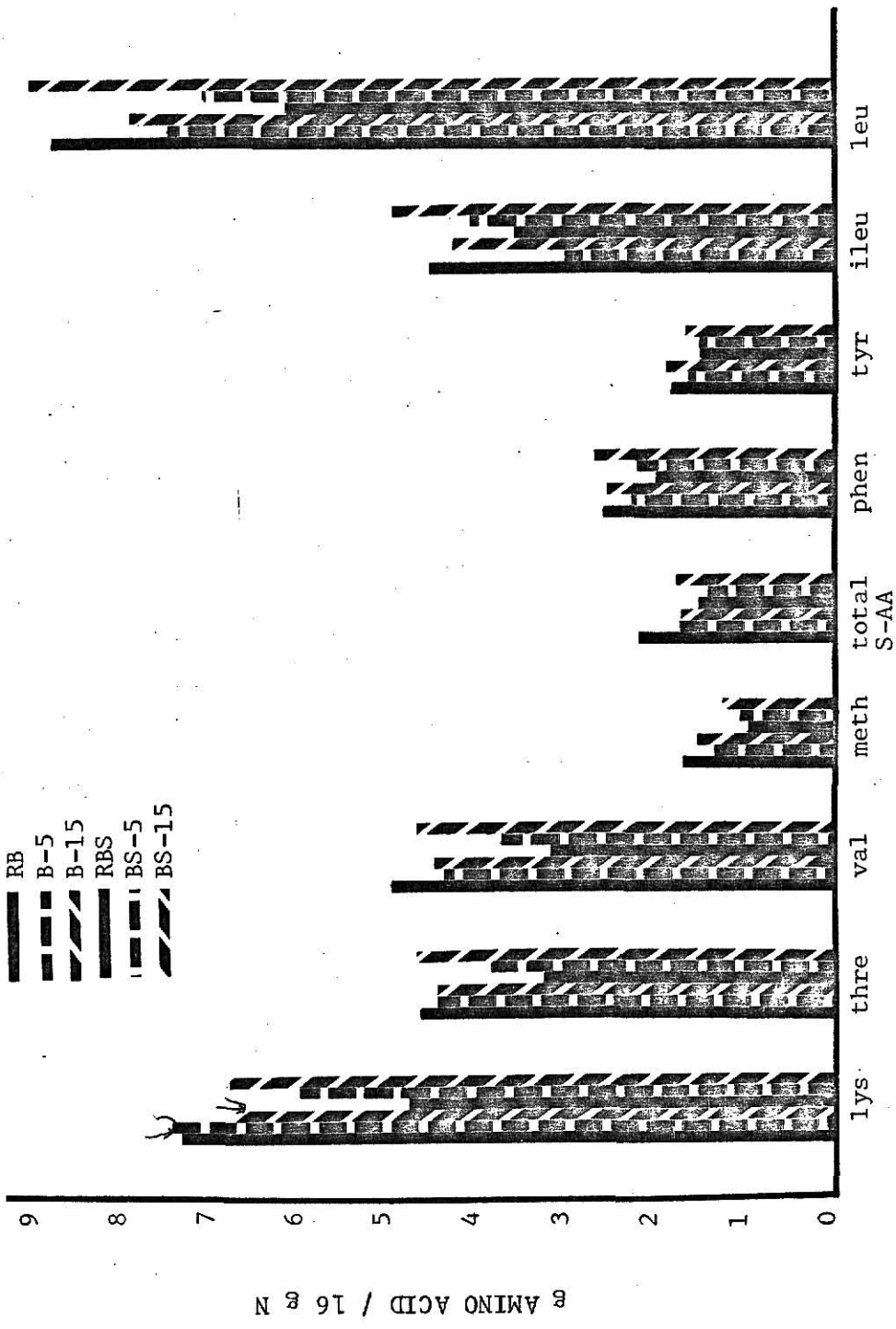

Fig. 1 Amount of essential amino acids (excluding tryptophan) in raw beef (RB), in beef cooked 5 (B-5) and 15 (B-15) minutes, in raw beef-soy (RBS) and in beef-soy (70:30) cooked 5 (BS-5) and 15 (BS-15) minutes as determined by an amino acid auto-analyzer.

TABLE 3

Lysine content of meats determined by amino acid autoanalyzer and the FDNB method.

Protein source	Lysine (g / 16 g N)	
	Amino acid analyzer	FDNB
Raw beef	7.27	8.19
Beef cooked 5 min	7.37	8.34
Beef cooked 15 min	6.65	8.00
Raw beef-soy	4.75	7.51
Beef-soy cooked 5 min	5.95	7.19
Beef-soy cooked 15 min	6.72	6.94

Lysine levels of the beef-soy by either method of analysis were lower than those of beef, except for analysis by the amino acid analyzer of products cooked 15 minutes. Results from both the auto-analyzer and the FDNB method showed an increase in lysine content of beef after 5 minutes of cooking and a decrease after 15 minutes of cooking when compared to raw beef. Analysis by the auto-analyzer showed an increase in lysine level in beef-soy as cooking time increased; FDNB analysis of beef-soy resulted in decreased lysine content with increased cooking time. The latter was anticipated because of damage caused by Maillard reactions with longer cooking.

Values from the auto-analyzer were expected to be equal to or higher than values for the FDNB method, since the amino acid analyzer estimates total lysine and the FDNB values estimate available lysine. Essential amino acid values obtained by the auto-analyzer in this study were generally lower than those presented by Kies and Fox (2). Using an

auto-analyzer, they found that beef had 2.2 g lysine / 4 g N (8.8 g / 16 g N) and TVP had 1.6 g lysine / 4 g N (6.4 g / 16 g N). Therefore, a beef-soy blend (70:30) should have about 8.1 g lysine / 16 g N. Assuming that raw samples had not undergone browning reactions, most of the total lysine should have been present as available lysine, and values by both the auto-analyzer and the FDNB method should have been fairly close.

Biological Evaluation

Mean weight gain, feed consumption, PER, NPU and digestibility for rats fed beef and beef-soy (70:30) cooked 5 and 15 minutes as their source of protein are in Table 4. Individual rat values are in the appendix (Table 8).

Homogeneity of variance within diets was tested using Bartlett's chi-square test; variances within diets were homogeneous except for PER when casein was equal to 2.50. One-way analysis of variance was computed for each measurement and two orthogonal comparisons were made (Table 5).

Weight Gain and Feed Consumption. There were no significant differences among weight gains that could be attributed to the type of product or to length of cooking time. However, diets containing beef or beef-soy produced significantly higher weight gains than that of casein. There were no significant treatment effects on feed consumption.

Protein Efficiency Ratio. Beef cooked 15 minutes produced a significantly higher PER (corrected to 2.50 for casein) than any other treatment. The casein diet (PER 2.50) had a significantly lower PER than diets containing beef or beef-soy. Mean PER for the beef-soy diets cooked 5 or 15 minutes were not significantly different, nor were either

TABLE 4

Mean weight gain, food consumption, PER, NPU and true digestibility of different diets

Diet	Protein		No. of Rats	Weight gain	Feed Consumption	PER[2]	NPU	Digestibility True
	Source[1]	Percent						
				g	g			
1	B-5	10	8	105.1	294.0	2.98	74.4	96.0
2	B-15	10	8	106.8	285.2	3.12	77.1	95.8
3	BS-5	10	8	101.1	287.6	2.93	70.8	94.5
4	BS-15	10	8	99.2	276.8	2.99	72.7	94.3
5	Casein	10	7	87.0	290.3	2.50	62.2	96.0
	$LSD^{3}_{.05}$			11.37		.107	3.01	.521
	$LSD_{.05}$			10.99		.104	2.91	.503

[1] B-beef; BS-beef-soy; 5-5 min. cooking period; 15-15 min cooking period
[2] PER corrected to 2.5 for casein
[3] Larger LSD used when diet 5 (Casein) was compared

TABLE 5

Analysis of variance and individual comparisons

Source of Variation	D.F.	Weight Gain	Feed Consumption	PER[1]	NPU	Digestibility True
Treatments	4	442.7**	333.0	.4040**	233.9**	5.463**
Beef vs Beef-soy	1	264.5	442.5	.0621*	125.7**	17.25**
5-min cooking vs 15-min cooking	1	.1250	770.3	.0770*	41.23*	.4100
Error	34	117.2	601.4	.0126	8.216	.1365
Total	38					

[1]Error D.F.-28; total D.F.-32

of them significantly different from the beef cooked 5 minutes.

The beef treatments had significantly higher PER than the beef-soy treatments (Table 5). Beef and beef-soy cooked 15 minutes had significantly higher PER than their counterparts cooked only 5 minutes.

Net Protein Utilization. Casein produced a significantly lower NPU than those of diets containing beef or beef-soy. Beef cooked fifteen minutes gave the highest mean NPU, significantly higher than the beef-soy products. Beef cooked 5 minutes had a significantly higher NPU than beef-soy cooked 5 minutes, but mean NPU between 5 minute cooked beef and 15 minute cooked beef-soy were non-significant. Mean NPU for the two beef-soy treatments were not significantly different.

The 5 minute cooking time for beef and beef-soy produced significantly lower NPU than the 15 minute cooking time. Beef resulted in higher NPU than the beef-soy.

Digestibility. Five and 15 minute cooked beef and casein resulted in mean digestibilities which were about equal, and were significantly higher than those for the 5 and 15 minute cooked beef-soy. There was not a significant difference in true digestibility coefficients between the 5 minute and 15 minute cooked beef-soy.

Discussion

Since the digestibility and NPU of the beef-soy blend were significantly lower than the beef, data from this study agree with those of Debry et al. (19) who found that beef was slightly superior to textured soy protein in digestibility and net protein utilization. PER for both the 5 and 15 minute cooked beef-soy (70:30) were above the

approximately 2.5 PER found in another study (22).

Unlike the decreased PER which Wilding (3) observed in chili, thought to be due to increased browning reactions, in this study the more extensive cooking time (15 minutes) produced slightly, though not significantly, higher PER and NPU than the 5 minute cooking time for the beef-soy blend. Wilding (3) indicated that methionine was the limiting amino acid in a 70:30 beef-soy blend and was just adequate to meet adult requirements in beef, whereas lysine was present in both beef and beef-soy at a level over 4 times the adult requirement. Even with the loss of lysine caused by browning, adequate lysine was probably available, and methionine was the most limiting amino acid.

Amino acid analysis by the auto-analyzer (Fig 1) showed an increase in methionine in the beef-soy with increasing cooking time. Beef cooked 15 minutes appeared to have more methionine than beef cooked 5 minutes, though both had less than raw beef. With further cooking, available lysine in the beef-soy blend may be reduced enough to become limiting, regardless of whether or not methionine is still limiting. Another possible reason for the superior protein quality of beef and beef-soy cooked 15 minutes rather than 5 minutes is that the essential amino acid balance may become more desirable due to decreases and/or increases in some amino acids.

SUMMARY

Since soy is high in protein and also high in carbohydrate content, heating may produce browning by the Maillard reaction. If soy is combined with beef and cooked, protein quality may be affected. A rat study was conducted to compare the protein quality of ground beef and a beef-soy

blend (70:30) when cooked 5 minutes and 15 minutes. Amino acid content of the products was determined. Available lysine also was determined since lysine is often involved in the browning reaction.

Raw beef-soy was lower in essential amino acids than raw beef, as determined by the amino acid auto-analyzer. Amino acid content in beef-soy increased as cooking time increased; amino acid levels in beef decreased (except for lysine) after 5 minutes cooking but tended to increase after 15 minutes cooking, though the levels were still less than raw beef.

Lysine level in beef increased after 5 minutes of cooking, then decreased to less than the raw value when determined by both the auto-analyzer and the FDNB method. In the beef-soy, total lysine (auto-analyzer) increased and available lysine (FDNB method) decreased with increased cooking time.

Weight gain and feed consumption were not affected by type of product (beef or beef-soy) or length of cooking time. Beef cooked 15 minutes had a PER significantly higher than any other treatment and an NPU significantly higher than the beef-soy treatments. PER, NPU and digestibility were not significantly different between the 5 and 15 minute cooked beef-soy.

Beef treatments produced significantly higher PER, NPU and digestibility coefficients than beef-soy treatments. The 15 minute cooked products produced mean PER and NPU which were significantly higher than products cooked 5 minutes.

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APPENDIX

TABLE 6

Moisture, ether extract and protein content of meats

Treatment	Moisture (%)	Ether extract (%)	Protein (%)
Raw beef	61.29	19.74	20.18
Freeze-dried raw beef	4.24	50.62	48.60
Beef cooked 5 min	54.72	25.92	23.33
Freeze-dried beef cooked 5 min	8.88	43.98	45.14
Beef cooked 15 min	35.70	34.21	30.21
Freeze-dried beef cooked 15 min	13.49	46.03	43.79
Raw beef-soy	61.57	15.86	19.38
Freeze-dried raw beef-soy	4.29	40.78	49.22
Beef-soy cooked 5 min	54.69	18.19	21.41
Freeze-dried beef-soy cooked 5 min	8.00	40.72	47.80
Beef-soy cooked 15 min	49.44	22.72	26.34
Freeze-dried beef-soy cooked 15 min	8.48	40.03	45.50

TABLE 7

Amino acid content (g / 16 g N) of raw beef (RB), beef cooked 5 minutes (B-5), beef cooked 15 minutes (B-15), raw beef-soy (RBS), beef-soy cooked 5 minutes (BS-5) and beef-soy cooked 15 minutes (BS-15) as determined by an amino acid autoanalyzer

Amino Acid	RB	B-5	B-15	RBS	BS-5	BS-15
Lysine	7.27	7.37	6.65	4.75	5.95	6.72
Threonine	4.63	4.43	4.42	3.28	3.82	4.66
Valine	4.92	4.36	4.45	3.20	3.72	4.66
Methionine	1.71	1.35	1.55	0.98	1.09	1.26
Isoleucine	4.50	3.01	4.24	3.55	4.06	4.90
Leucine	8.68	7.39	7.80	6.06	6.98	8.90
Phenylalanine	2.59	2.29	2.51	2.04	2.21	2.68
Tyrosine	1.82	1.66	1.88	1.49	1.51	1.67
Histidine	1.77	1.69	1.60	1.22	1.41	1.77
NH_3	5.60	6.86	6.35	5.93	6.01	6.86
Arginine	3.81	3.76	3.84	2.86	3.36	4.47
Aspartic Acid	8.84	7.76	9.47	7.30	8.20	9.89
Serine	5.16	4.46	4.93	4.02	5.55	4.72
Glutamic Acid	17.15	15.78	17.15	13.07	15.32	21.77
Proline	3.97	3.88	4.29	3.17	3.80	3.38
Glycine	11.94	11.51	12.40	6.79	10.94	10.36
Alanine	9.02	7.68	8.71	5.63	7.52	9.74
Cysteine	0.49	0.41	0.18	0.56	0.32	0.54

TABLE 8

Cumulative weight gain (g), food consumption (g), protein efficiency ratio, net protein utilization and digestibility of beef and beef-soy diets

Diet	Rat no.	Body Wt. gain	Food intake	PER	NPU	True Digestibility
Beef cooked 5 minutes	1	109	307	2.96	74.89	96.10
	2	109	289	3.14	76.22	96.13
	3	123	341	3.01	74.65	96.03
	4	116	325	2.97	70.76	96.07
	5	96	280	2.86	72.39	95.75
	6	91	257	2.95	77.14	95.53
	7	99	273	3.02	75.87	96.26
	8	98	280	2.92	73.50	96.26
Beef cooked 15 minutes	1	104	287	3.02	75.32	96.00
	2	104	266	3.26	81.37	95.22
	3	120	318	3.14	74.90	96.14
	4	106	278	3.18	77.86	95.74
	5	96	269	2.97	77.46	95.72
	6	108	273	3.30	81.17	95.71
	7	119	317	3.13	75.64	95.72
	8	97	274	2.95	72.72	96.01
Beef-soy cooked 5 minutes	1	114	310	3.06	68.02	94.91
	2	97	299	2.70	68.47	95.39
	3	94	273	2.87	71.45	94.58
	4	96	273	2.93	70.86	94.74
	5	102	272	3.12	72.26	94.23
	6	101	286	2.94	73.57	94.35
	7	116	342	2.83	63.88	93.80
	8	89	246	3.01	78.05	94.33

TABLE 8 (cont'd)

Diet	Rat no.	Body Wt. gain	Food intake	PER	NPU	True Digestibility
Beef-soy cooked 15 minutes	1	108	302	2.98	70.82	95.08
	2	95	269	2.94	69.95	93.69
	3	110	297	3.09	73.24	94.45
	4	94	252	3.11	73.09	93.94
	5	92	268	2.86	72.79	94.35
	6	99	287	2.87	70.70	94.77
	7	95	264	3.00	76.27	94.11
	8	101	275	3.06	75.02	94.13
Casein	1	118	327	2.50	62.50	96.16
	2	99	308	2.50	64.64	96.37
	3	82	279	2.50	60.36	96.07
	4	-	-	-	-	-
	5	77	272	2.50	62.11	96.07
	6	73	266	2.50	63.57	95.48
	7	88	313	2.50	58.66	95.69
	8	72	267	2.50	63.55	96.23

EFFECT OF COOKING ON PROTEIN QUALITY OF GROUND BEEF AND A BEEF-SOY BLEND

by

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Since soy is high in protein and also high in carbohydrate content, heating may produce browning by the Maillard reaction. Protein quality may be affected in a cooked beef-soy blend. A rat study was conducted to compare the protein quality of ground beef and a beef-soy blend (70:30) when cooked 5 minutes and 15 minutes. Amino acid content of the products was determined. Available lysine also was determined since lysine is often involved in the browning reaction.

Raw beef-soy was lower in essential amino acids than raw beef, as determined by the amino acid auto-analyzer. Amino acid content in beef-soy increased as cooking time increased; essential amino acid levels in cooked beef were generally lower than in raw beef. In the beef-soy, total lysine (auto-analyzer) increased and available lysine (FDNB method) decreased with increasing cooking time.

Weight gain and feed consumption were not affected by the treatments. Beef treatments produced significantly higher PER, NPU and digestibility coefficients than beef-soy treatments. PER, NPU and digestibility were not significantly different between the beef-soy treatments. The 15 minute cooked products produced mean PER and NPU which were significantly higher than products cooked 5 minutes.