The Availability for the Rat of Methionine and Cystine Contained in Dry Bean Seed (*Phaseolus vulgaris*)

Robert John Evans,* Doris H. Bauer, Kathleen A. Sisak, and Patricia Ann Ryan[1]

Growing rats were fed diets containing 10% protein in which all of the protein was supplied as dry bean seeds or soybean meal or these diets supplemented with methionine. Maximum growth and protein efficiency ratios (PER) were obtained when the bean diet contained 0.33% of methionine plus cystine and when the soybean diet contained 0.26% methionine plus cystine, which indicated that the methionine and cystine contained in the bean seeds were more poorly utilized by growing rats than those contained in soybeans. Methionine and cystine balance studies were also conducted with growing rats. Approximately 49% of the methionine and 25% of the cystine contained in beans were excreted with the undigested protein in the feces. About 26% of the methionine and 11% of the cystine of soybean meal were excreted in the feces by the same rats. Only small amounts of methionine (1%) or cystine (2–9%) were excreted in the urine. The amount of methionine and cystine in the undigested portion of the beans accounted for most of the poor utilization of methionine and cystine of the beans. The data obtained indicated that the dry beans also contained something which interfered with the utilization by the rat of methionine and/or cystine added to the diet.

Dry beans have been used for many years as a source of protein in the human diet and in a number of areas constitute a significant proportion of the total daily protein intake. Beans must be properly cooked to inactivate heat-labile growth inhibitors which include the phytohemagglutinins and trypsin inhibitors (Kakade and Evans, 1965). Kakade and Evans (1965) observed that rats fed a diet containing autoclaved beans as the sole source of protein supported some growth but that when the diet was supplemented with methionine, growth and feed efficiency were as good as for rats fed a diet containing casein as the sole source of protein. Evans and Bandemer (1967) analyzed several bean and soybean samples for the naturally occurring amino acids and determined the relative nutritive values of some of the bean and soybean proteins. A Sanilac bean sample used contained 1.3% of methionine and 2.0% of methionine plus cystine in the protein and a sample of Harosoy soybeans contained 1.0% methionine and 2.2% methionine plus cystine in the protein. The properly heated beans promoted only about 38% of the growth of rats that the properly heated soybeans did. According to the FAO pattern of amino acid requirements (Food and Agriculture Organization, 1957), the limiting amino acids of both beans and soybeans are methionine plus cystine.

The purpose of the present investigation was to determine the reason or reasons for the poorer utilization of the methionine and cystine from the proteins of optimally heated dry beans than from those of soybeans.

EXPERIMENTAL SECTION

Rats which were 21 days of age were divided into eight groups of six rats per group. They were housed in individual cages and distributed into groups so that the rats of each group were comparable in weight to those of the other groups. The diet fed was composed of 30% sucrose, 6% corn oil, 4% Hegsted salt mixture, 2% vitamin mixture, finely ground beans or soybean meal to furnish 10% protein, and cornstarch to make the total weight of the diet to 100%. The soybean meal used was a commercial meal of good quality. The dry bean (*Phaseolus vulgaris*) seeds used were Michigan-grown Sanilac beans. They were finely ground, spread on an enamel pan in a layer about 1–2 cm thick, and heated in an autoclave with moist heat at 121° for 10 min, unless otherwise noted. The rats were weighed at the start of each experiment and twice each week for the duration of the 4-week experiment. The amount of diet consumed by each rat each week was also determined. The protein efficiency ratio (PER) was calculated as the g of gain in weight/g of protein consumed.

Three experiments were carried out in which the bean seed or soybean meal containing diets were supplemented with different levels of commercial DL-methionine. The levels of added methionine used were 0.05, 0.10, and 0.15% in the soybean meal diet and 0.05, 0.10, 0.15, 0.20, and 0.25% in the bean diet.

The fourth and fifth experiments were methionine and cystine balance studies. Sixteen rats were used in these experiments. The rats were 35 days of age when started on the fourth experiment. The same rats were used for the fifth experiment as for the fourth, and they were 70 days of age when started on the fifth experiment. Half of the rats were fed the basal diet containing finely ground beans which had been autoclaved for 10 min at 121°, and half were fed the basal diet containing soybean meal in the fourth experiment. All of the rats were fed the respective basal diet for 3 days before the start of the balance study. Feed was removed from the rats for about 4 hr before the start of the balance experiment, which lasted for 4 days, and again for 4 hr at the conclusion of the experiment. Rats were weighed at the start and at the end of the experiment. Feed consumption was determined for each rat. All feces and urine were collected from each rat for the 4 days. The feces were dried and weighed. The volume of the urine was determined and feed, feces, and urine samples were analyzed for methionine and cystine by microbiological assay.

Samples of 0.5 g of feed or feces were weighed into 50-ml Erlenmeyer flasks and aliquots of urine were measured into 25-ml Erlenmeyer flasks and the water was removed by drying. Ten milliliters of 20% hydrochloric acid was added to each flask containing feed or feces samples and 3 ml of 20% hydrochloric acid was added to the dried urine residue; the flask and contents were heated in the autoclave for 30 min at 121°. The hydrolyzed samples were rapidly cooled by packing in ice as soon as they were

Department of Biochemistry, Michigan State University, East Lansing, Michigan 48823.

[1] Participant in Michigan State University High School Honors Science Program, summer of 1972.

Table I. Weight Gains and Protein Efficiency Ratios (PER) of Rats Fed Dry Bean and Soybean Meal Containing Diets Supplemented with Methionine (Experiment 2)

Protein source	Supplemental methionine, %	Weight gain,[a] g	Feed intake,[a] g	PER[a]
Soybean meal	0	91b[b]	407bc[b]	2.24b[b]
Soybean meal	0.05	145d	509d	2.85c
Soybean meal	0.10	149d	454cd	3.28d
Beans[c]	0.05	53a	301a	1.76a
Beans	0.10	100b	381b	2.62c
Beans	0.15	123c	409bc	3.01cd
Beans	0.20	132cd	453cd	2.91cd
Beans	0.25	141d	429bc	3.28d

[a] Average of six rats fed the diet. [b] Means followed by the same letter in a column are not statistically significant ($p > 0.05$). [c] Dry beans heated 10 min.

removed from the autoclave. While in the ice, sodium hydroxide solution was added to adjust the pH to 6.2–6.5 using a pH meter. The neutralized digests were made to volume and filtered, and aliquots were used for the amino acid assay. Methionine was determined microbiologically by assay with *Leuconostoc mesenteroides* P-60 using prepared media from Difco Laboratories (Steele *et al.*, 1949). Cystine was determined by microbiological assay with *Leuconostoc mesenteroides* P-60 using the oxidized peptone medium of Lyman *et al.* (1946). Growth was determined by turbidity measurements in the Beckman Model B spectrophotometer at 700 nm.

Methionine and cystine intake, methionine and cystine in the undigested protein, methionine and cystine excreted unchanged in the urine, and methionine and cystine absorbed and not excreted were calculated.

The second balance experiment was carried out in a slightly different manner. The diets were supplemented with $ZnCl_2$ to give a level of 0.0006% zinc. All rats were fed the bean diet for 1 week, including a 4-day balance study followed by soybean meal diet feeding for 1 week, including the 4-day balance study.

The rat growth, feed intake, PER, and digestibility data were analyzed statistically by analysis of variance (Snedecor, 1959), with significant difference between means determined by the multiple range test of Duncan (1955).

RESULTS AND DISCUSSION

Supplementation of Diets Containing Dry Beans or Soybean Meal with Methionine. The dry beans used in the first experiments contained 0.93% methionine and 0.42% cystine on crude protein basis (N × 6.25). The diets containing the dry beans thus contained 0.093% methionine and 0.042% cystine or 0.13% methionine plus cystine. Similarly the soybean meal diet contained 0.113% methionine, 0.092% cystine, and 0.205% methionine plus cystine. The bean diet and the soybean meal diet were supplemented with 0.00, 0.05, 0.10, or 0.15% of methionine in the first experiment. Maximum growth on the soybean diet appeared to have been obtained with 0.10% added methionine or 0.305% total methionine plus cystine and maximum PER with 0.05% added methionine or 0.255% total methionine and cystine. Growth or feed efficiency of the bean diet supplemented with 0.10 or 0.15% of methionine was not equal to that of the optimally supplemented soybean meal diet, and the bean diet with 0.15% of supplemental methionine supplied 0.28% of methionine plus cystine.

Higher levels of supplemental methionine were used with the bean diet in the second experiment (Table I). The 0.25% level of added methionine produced growth and PER equal to that of the optimally supplemented soybean meal diet but these values were not statistically significantly different from those given by the 0.20% level of added methionine. The bean diet with 0.25% of supplemental methionine contained 0.385% of total methionine plus cystine and that with 0.20% supplemental methionine contained 0.335%, which is higher than the total methionine and cystine content of the soybean meal diet which gave optimal growth.

In the third experiment two heating times of the beans were used, 10 or 20 min in the autoclave at 121°. The bean diets were supplemented with 0.10, 0.15, or 0.20% methionine. The time of heating of the beans did not influence significantly the growth and feed efficiency of the rats fed them (Table II). Growth and feed efficiency of rats fed beans supplemented with 0.15 or 0.20% methionine were similar to those of rats fed soybeans supplemented with 0.05% methionine.

The results of the three experiments indicate that the best growth (140 g) and PER (3.3) were obtained with the rats fed the soybean meal diet supplemented with 0.05% of methionine and with the bean diet supplemented with 0.20% methionine, although the 0.25% level of supplemental methionine gave better results than the 0.15% level but the 0.20% level did not. It is therefore possible that the requirement for methionine and cystine on the bean diet is closer to 0.25% added methionine than to 0.20%. The supplemented soybean meal diet contained 0.255% of methionine plus cystine and the supplemented bean diet contained 0.335% of methionine plus cystine. One might assume, therefore, that at least 0.080% of the methionine plus cystine of the bean diet was not available for the growing rat. Since the bean diet contained only 0.135% methionine plus cystine when unsupplemented, over half of these amino acids present in the beans thus appeared to be unavailable to the rat.

Methionine and Cystine Balance Studies with Rats Fed Diets Containing Beans or Soybean Meal. The first balance study was carried out to determine what happens to the methionine and cystine of dry beans when they are fed to rats if they are not utilized by the rats. The bean diet contained 1.13 mg of methionine and 0.42 mg of cystine/g of diet, and the soybean diet, which was fed for

Table II. Weight Gains and Protein Efficiency Ratios (PER) of Rats Fed Dry Bean and Soybean Meal Containing Diets Supplemented with Methionine. Dry Beans Heated for 10 or 20 min (Experiment 3)

Protein source	Heating time, min	Supplemental methionine, %	Weight gain,[a]	Feed intake,[a]	PER[a]
Soybean meal		0	98bc	345a	2.84bcd
Soybean meal		0.05	136d	402b	3.38e
Beans	10	0.10	89ab	338a	2.62ab
Beans	10	0.15	116cd	413b	2.81bc
Beans	10	0.20	127d	405b	3.14de
Beans	20	0.10	72a	308a	2.34a
Beans	20	0.15	120d	389b	3.08cde
Beans	20	0.20	135d	423b	3.19e

[a] Means followed by the same letter in a column are not statistically significant ($p > 0.05$).

Table III. Methionine and Cystine Digested and Utilized or Metabolized by Rats Fed Dry Beans or Soybean Meal as the Sole Source of Protein in the Diet

First balance study[a]	Beans	Soybean meal
Percent of methionine not digested	37[c]	25
Percent of cystine not digested	21[c]	10
Second balance study		
Percent of methionine not digested	49[c]	26
Percent of cystine not digested	25[c]	11

[a] Average of eight rats fed the diet. [b] Average of 16 rats fed the diet. [c] Values for beans and soybean highly significantly different ($p < 0.001$).

comparative purposes, contained 1.13 mg of methionine and 0.92 mg of cystine/g of diet. On the average 37% of the methionine and 21% of the cystine of the beans were excreted in the feces with the undigested protein, compared to 25% of the methionine and 10% of the cystine of the soybean meal (Table III). There was considerable variability among the rats in their abilities to digest the methionine- and cystine-containing proteins, especially among the rats which were fed the bean diet, where values ranged between 23 and 43% of the total methionine and 11 and 28% of the total cystine of the beans excreted as methionine and cystine in the feces.

Small amounts of methionine and cystine were excreted unchanged in the urine by rats fed either soybean meal (1% of the methionine and 2% of the cystine) or dry beans (1% of the methionine and 4% of the cystine). Rats fed the dry bean containing diet thus absorbed and did not excrete 62% of the dietary methionine and 75% of the dietary cystine, while rats fed the soybean meal containing diet absorbed and did not excrete 74% of the dietary methionine and 88% of the dietary cystine.

All of the rats were used for the balance studies with both beans and soybeans in the second balance study so that each animal could serve as a control for itself and any animal differences would be eliminated. The bean diet used in this experiment contained 0.96 mg of methionine and 0.50 mg of cystine/g of diet, and the soybean meal diet contained 1.10 mg of methionine and 0.77 mg of cystine/g of diet. The beans used in this experiment were of the same variety but from a different source than those used in the first balance experiment, and more of the methionine and cystine were excreted unchanged in the feces (Table III). The rats excreted 49% of the methionine and 25% of the cystine contained in the beans as part of the protein which was not digested by the rat. This compares with 26% of the methionine and 11% of the cystine of soybean meal which was not digested by these same rats.

About 1% of the methionine of dry beans or the soybean meal was excreted in the urine in the second balance study, and 9% of the cystine of the dry beans was excreted, compared to 5% of the cystine of the soybeans. The values were higher than were those of the first balance study and might be related to the age of the rats because the rats were older in the last study or to the presence of added zinc chloride in the diets used in the second balance study. The zinc was added because it has been shown that supplemental zinc is necessary for proper utilization of plant-seed protein diets (Oberleas and Prasad, 1969) and bean seeds have been found to be low in zinc content (Leucke, 1973).

Rats fed dry beans in the second balance experiment absorbed and did not excrete 50% of the bean methionine and 66% of the bean cystine, compared to 73% of the methionine and 84% of the cystine of the soybean meal. Variability among rats fed the dry bean diet in the percent-

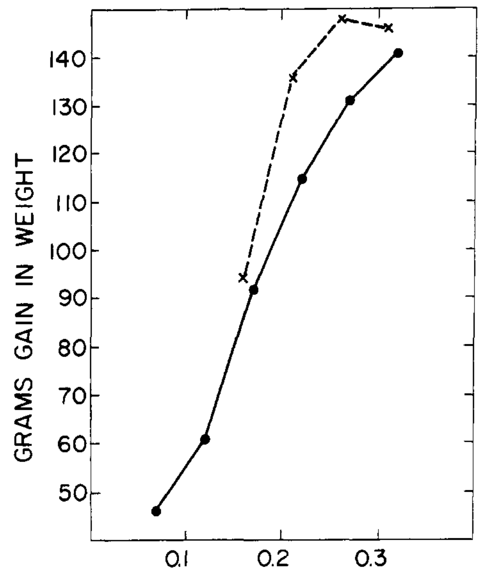

Figure 1. Gains in weight of rats fed different levels of dietary available methionine plus cystine. The gains in weight of the three rat-feeding experiments were adjusted so that all points are compatible. Bean diet (——). Soybean meal diet (- - - -).

ages of methionine and cystine absorbed was much greater than among the same rats fed the soybean meal diets. The poor digestibility of the methionine- and cystine-containing proteins was not caused by the bean trypsin inhibitor because it is destroyed by the heat treatment given the beans (Kakade and Evans, 1965).

Methionine and Cystine Requirements for Growing Rats. The soybean meal-containing diets used in the supplementation experiments contained 0.113% methionine and 0.092% cystine. Using the value of 73% availability of the methionine and 84% of the cystine of soybean meal, the soybean meal diet contained 0.082% available methionine and 0.077% available cystine or 0.159% of available methionine plus cystine. Then if we consider that the supplemental methionine is completely available, we obtain a value of 0.259% or roughly 0.26% as the level of available methionine in the diet required for optimal growth of rats.

Similarly, the dry bean diet contained 0.093% of methionine and 0.042% of cystine. Using the value of 50% availability of the bean methionine and 66% of the cystine, the bean diet contained 0.046% available methionine and 0.028% of available cystine or 0.074% of available methionine plus cystine. Assuming that the supplemental methionine is completely available, with optimal growth obtained with 0.20% supplemental methionine, a methionine plus cystine requirement of 0.274% or roughly 0.27% is obtained. However, the data indicate that 0.25% of supplemental methionine was better than 0.20%, even though the difference was not statistically significant and that the methionine plus cystine requirement might be more than 0.27%.

Most of the poor utilization of methionine and cystine of dry beans thus appeared to be caused by failure of the rat to digest and absorb some proteins or peptides which contain considerable amounts of the bean methionine and cystine.

Average adjusted gains in weight for the three growth experiments are plotted against the percent of available methionine plus cystine in Figure 1 and the average adjusted PER are plotted against these in Figure 2. Although the curves for bean-fed rats and soybean meal-fed rats do not cross, if the soybean curves are extrapolated as straight lines they intersect the bean curves at about 0.12% of available methionine plus cystine. The slopes of curves

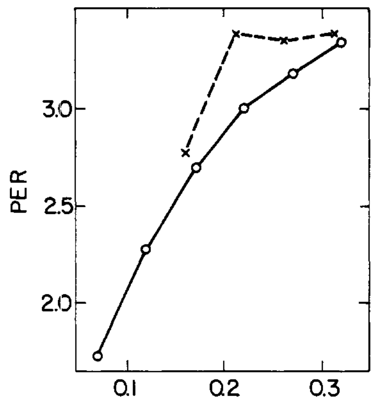

Figure 2. Protein efficiency ratios (PER) of rats fed different levels of dietary available methionine plus cystine. The PER of the three experiments were adjusted to be comparable. Bean diet (——). Soybean diet (- - -).

indicate that methionine added to the soybean meal diet was more fully utilized for growth than that added to the bean diet using either rat growth or rat PER as criteria. The curves indicate the presence of an as yet unknown substance which interfered with the utilization of dietary methionine.

ACKNOWLEDGMENTS

The authors acknowledge the assistance of Mary Ashley in the statistical analyses of the data. The soybean meal was kindly furnished by Dr. Cal J. Flegal of the Poultry Science Department of Michigan State University and the beans by the Michigan Bean Shippers Association and by Dr. M. Wayne Adams of the Crop and Soil Science Department of Michigan State University.

LITERATURE CITED

Duncan, D. B., *Biometrics* 11, 1 (1955).
Evans, R. J., Bandemer, S. L., *J. Agr. Food Chem.* 15, 437 (1967).
Food and Agriculture Organization of the U. N., FAO Nutrition Studies, no. 16, 1957, p 28.
Kakade, M. L., Evans, R. J., *Brit. J. Nutr.* 19, 269 (1965).
Luecke, R. W., Michigan State University, East Lansing, Mich., personal communication, 1973.
Lyman, C. M., Mosely, O., Wood, S., Hale, F., *Arch. Biochem.* 10, 427 (1946).
Oberleas, D., Prasad, A. S., *Amer. J. Clin. Nutr.* 22, 1304 (1969).
Snedecor, G. W., "Statistical Methods," 5th ed., Iowa State College Press, 1959.
Steele, B. F., Sauberlich, H. E., Reynolds, M. S., Baumann, C. A., *J. Biol. Chem.* 177, 533 (1949).

Received for review April 6, 1973. Accepted October 29, 1973. Published as Michigan Agricultural Experiment Station Journal article no. 6388. This investigation was supported in part by a grant from the Michigan Bean Commission.

Lemon Juice Particulates: Comparison of Some Fresh Juices and a Commercial Concentrate

Wayne Venolia,* Shirley Peak, and Fred Payne

Techniques were developed for characterizing size distributions of lemon juice particles. Precision was sufficient to reveal patterns that may typify fresh, unpasteurized juice prepared in the laboratory. A commercial concentrate differed markedly from the fresh juices. Results of two sedimentation experiments are discussed.

The natural turbidity and some of the organoleptic properties of citrus juices are due to the presence of suspended particles. Stevens *et al.* (1950) stabilized the suspensions by heating. Baker and Bruemmer (1971) devised a treatment for orange juice that produced suspensions of enhanced stability and turbidity. Mizrahi and Berk (1970) reported some properties of orange juice particulates. The present work was directed toward the development of methods to facilitate characterization of a variety of lemon juice products, primarily from the standpoint of particle size.

EXPERIMENTAL SECTION

Fruit specimens were selected for similarity of size and color and for low incidence of visible defects such as scars, bruises, and infections. Each specimen was washed before juice was extracted; a glass reamer was used and care was taken to minimize contamination of the juice with peel oil. Seeds revealed by slicing were removed prior to juicing. Nearly all coarse solids were removed by straining through cheesecloth, and the rest were removed by filtration through fritted glass disks. C-Porosity frits were used for samples that were to be centrifuged. All other samples were filtered with EC-porosity frits. Frit filtration did not visibly alter the turbidity of lemon juice.

Fruit and Vegetable Chemistry Laboratory, Western Region, U. S. Department of Agriculture, Agricultural Research Service, Pasadena, California 91106.

Particle Sizing. Particle size was estimated with a Model A Coulter Counter using apertures having nominal diameters of 30, 50, and 200 μm. To facilitate accurate counting, juice specimens were diluted fourfold or more with a particle-counting electrolyte. This electrolyte was prepared from reagent grade chemicals and distilled deionized water; the stock solution was 0.125 *M* NaCl with 1 g/l. of Na_2SO_3 added as preservative. This solution was vacuum filtered through a sintered-glass disk of UF-porosity and boiled briefly. Electrolyte was discarded within 2 days of boiling. Dilutions were made gravimetrically.

In the counting procedure adopted, each range of particle sizes was scanned in one direction and then back with the electrode polarity control set for automatic reversal. The result was a pair of counts for each particle size with both polarities represented in each pair. Counts were replicated further because an overlap was needed when there was (a) a sample dilution or (b) an aperture change. These replicates consisted of an extra pair of counts at each of two adjacent particle sizes. There were two overlaps within the range of the 50- μm aperture and one for the 200-μm aperture.

To minimize error from aperture plugging, erratic or excessively long counts were discarded and apertures were