

Amino Acid Availability and Availability-Corrected Amino Acid Score of Red Kidney Beans (*Phaseolus vulgaris* L.)

Wu Wu,^{*,†} Woodie P. Williams,[†] M. Elizabeth Kunkel,[†] James C. Acton,[†] Yun Huang,[‡]
Foster B. Wardlaw,[†] and Lawrence W. Grimes[§]

Departments of Food Science, Poultry Science, and Experimental Statistics, Clemson University,
Clemson, South Carolina 29634

Thermal effect on availability of individual amino acids (AIAA) of red kidney beans was evaluated. Sulfur amino acids (SAA), methionine and cystine (Met + Cys), are the limiting amino acids (AA) and have the lowest availability among the AAs in nine treatments. The availability of SAA (ASAA) ranged from -18.6% in raw beans to 39.8–68.0% in thermally processed beans. Autoclaving at 121 °C for 10–90 min gradually reduced ASAA values. The mean availability for each AA (MAEAA) is the average of the AIAA values for the same AA. MAEAA values ranged from 82.1% (arginine) to 50.4% (Met + Cys). The mean availability in each treatment (MAET) is the average of the AIAA values in the same treatment. The difference between MAET and true digestibility of protein (TDP) was less than 7%. However, the differences between ASAA and TDP (16–37%) and between ASAA and MAET (14–30%) were large. The ASAA-corrected amino acid score (AAS_{ASAA}) for raw beans was negative (-29.4%) and ranged from 61.8 to 42.1% for thermally processed beans. From a comparison among the protein quality indexes, AAS_{ASAA} is the preferred method to evaluate protein quality of beans.

Keywords: *Amino acid availability; availability-corrected amino acid score; red kidney beans*

INTRODUCTION

The nutritional quality of a protein is primarily related to its amino acid (AA) composition. However, true digestibility of protein (TDP) and true digestibility (TD) of AAs are also key determinants, especially with regard to vegetable protein quality (Kies, 1981; Finley and Hopkins, 1985; Sarwar, 1987; Tanksley and Knabe, 1985; Vachan *et al.*, 1987; FAO/WHO, 1990).

Methods for evaluating vegetable protein quality have been discussed by the Codex Committee on Vegetable Proteins (CCVP, 1985, 1989). The use of amino acid score (AAS) corrected for TDP and/or TD (or availability) of AAs was the preferred method for assessing the quality of vegetable proteins (CCVP, 1985, 1989). Kuiken and Lyman (1948) proposed the fecal method for estimating the bioavailability of AAs. This procedure remains the most widely used animal assay for measuring both TDP and TD (or availability) of AAs at the same time (CCVP, 1982). TD (or availability) of AAs is difficult to evaluate; therefore, limited data are available with regard to TD (or availability) of individual AAs of beans, and even fewer data exist on TD (or availability) of AAs as affected by different processing conditions (Taverner *et al.*, 1981; Oste, 1990). TDP is often used with the assumption that the TD (or availability) of each AA of a food protein is more or less the same (Sauer *et al.*, 1977). However, wide differences between TDP and TD (or availability) of the limiting AA(s) in legumes have been reported (Eggum *et al.*, 1989; Sarwar 1984; Sarwar and Peace 1986; Sarwar *et al.*, 1989). Several

papers suggest that TDP in legume protein is a poor predictor of TD (or availability) of the first limiting AA(s) because processing reduces the availability of individual AAs more than it reduces overall nitrogen digestibility (Hurrell and Finot, 1985; Sarwar, 1987). Therefore, AASs of legume proteins should be corrected for TD (or availability) of the limiting AA instead of TDP (CCVP, 1985, 1989; Sarwar, 1984, 1987; Sarwar and Peace, 1986). The TD (or availability) of the limiting AA-corrected AAS method meets the criteria of being accurate and applicable to a wide variety of foods (Sarwar, 1984).

Most people use true digestibility, availability, and bioavailability synonymously referring to AAs, when evaluated by animal growth and balance assays. However, some scientists contend that in a strict sense they do not mean the same thing (Tanksley and Knabe, 1985). While many workers use the term true digestibility, some use availability, or bioavailability, or true digestibility (or availability) combined when referring to AAs. Since AAs will not further digest before absorption, to prevent confusion, the term availability is used in this paper.

The thermal effect on *in vitro* protein digestibility (IVPD), IVPD-corrected AAS (AAS_{IVPD}), TDP, and TDP-corrected AAS (AAS_{TDP}) of red kidney beans were reported previously (Wu *et al.*, 1994, 1995a). The thermal effect on corrected relative net protein ratio (CRNPR) of the beans was also studied (unpublished results). Objectives of this study were to investigate the effect of thermal processing on the availability of individual amino acids (AIAA) and on the availability of sulfur amino acids (ASAA)-corrected AAS (AAS_{ASAA}) of red kidney beans and to compare values among protein quality indices (AAS, AAS_{IVPD}, AAS_{TDP}, AAS_{ASAA}, and CRNPR) of the beans to evaluate the methods for protein quality measurement.

* Address correspondence to this author at the Department of Food Science, University of Arkansas, Fayetteville, AR 72704 [e-mail mowu@comp.uark.edu; fax (501) 575-6936].

† Department of Food Science.

‡ Department of Poultry Science.

§ Department of Experimental Statistics.

Table 1. Amino Acid Composition (Milligrams per 16 g of N) of Thermally Processed Red Kidney Beans^a

treatment ^b	raw	home	can	121-10	121-20	121-40	121-60	121-90	128-20	MSE	casein
Essential Amino Acids ^c											
Lys	6.77 ^a	6.85 ^a	6.66 ^a	6.89 ^a	6.69 ^a	6.61 ^a	6.52 ^a	6.54 ^a	6.47 ^a	0.104	7.49
Met + Cys	2.32 ^{ab}	2.29 ^{ab}	2.24 ^{ab}	2.25 ^{ab}	2.38 ^a	2.29 ^{ab}	2.22 ^{ab}	2.24 ^{ab}	2.22 ^b	0.012	3.46
Thr	4.03 ^{abc}	3.98 ^{abc}	4.15 ^{abc}	3.98 ^{cd}	3.90 ^{cd}	3.92 ^{cd}	3.97 ^{cd}	3.83 ^d	4.23 ^{ab}	0.038	3.84
Ile	4.32 ^b	4.38 ^b	4.64 ^a	4.51 ^{ab}	4.34 ^b	4.34 ^b	4.35 ^b	4.44 ^{ab}	4.46 ^{ab}	0.028	4.54
Leu	8.01 ^c	8.20 ^{bcd}	8.21 ^{bcd}	8.41 ^{abc}	8.44 ^{ab}	8.46 ^{ab}	8.35 ^{abc}	8.55 ^a	8.27 ^{a-d}	0.042	9.01
Val	5.06 ^a	5.19 ^a	5.19 ^a	5.22 ^a	5.14 ^a	5.21 ^a	5.17 ^a	5.31 ^a	5.24 ^a	0.038	5.64
Phe + Tyr	8.30 ^f	8.34 ^{ef}	8.85 ^{ab}	8.47 ^{c-f}	8.50 ^{b-f}	8.27 ^f	8.40 ^{def}	8.68 ^{a-e}	8.81 ^{abc}	0.058	9.27
Trp	1.25 ^a	1.27 ^a	0.99 ^a	1.17 ^a	1.20 ^a	1.18 ^a	1.32 ^a	1.29 ^a	0.96 ^a	0.082	1.15
Nonessential Amino Acids ^c											
Asp	12.39 ^a	12.06 ^{ab}	11.86 ^{abc}	11.86 ^{ab}	12.00 ^{ab}	11.68 ^{ab}	12.05 ^{ab}	11.13 ^b	12.01 ^{ab}	0.528	6.55
Glu	17.77 ^{ab}	17.76 ^{ab}	16.97 ^{abc}	17.55 ^{ab}	17.52 ^{ab}	17.77 ^{ab}	17.42 ^{ab}	17.96 ^a	17.47 ^{ab}	0.795	22.50
His	2.64 ^{cd}	2.71 ^{a-d}	2.81 ^{abc}	2.68 ^{bcd}	2.62 ^{cd}	2.69 ^{bcd}	2.95 ^a	2.68 ^{bcd}	2.74 ^{a-d}	0.018	2.52
Arg	6.06 ^{ab}	5.93 ^{abc}	5.47 ^{abc}	6.30 ^a	5.97 ^{ab}	6.17 ^{ab}	5.82 ^{abc}	5.94 ^{abc}	5.77 ^{bc}	0.116	3.24
Ser	5.79 ^a	5.92 ^a	5.94 ^a	5.80 ^a	5.83 ^a	5.90 ^a	5.85 ^a	5.92 ^a	5.83 ^a	0.092	5.25
Pro	4.04 ^a	3.89 ^a	4.24 ^a	4.18 ^a	4.31 ^a	4.26 ^a	4.20 ^a	4.09 ^a	4.06 ^a	0.013	9.74
Ala	4.16 ^a	4.31 ^{abc}	4.26 ^{abc}	4.41 ^{ab}	4.33 ^{abc}	4.38 ^{ab}	4.45 ^a	4.46 ^a	4.31 ^{abc}	0.018	2.27
Gly	4.06 ^{abc}	4.02 ^{bc}	4.00 ^{abc}	4.11 ^{ab}	4.11 ^{ab}	4.12 ^{ab}	4.08 ^{abc}	4.18 ^a	4.08 ^{abc}	0.008	1.62

^a Data from Wu *et al.* (1994). ^b raw, uncooked dry beans; home, home-made beans (boiled in water, 100 °C for 120 min); can, commercially canned beans, Progresso; 121(128)-(10-90), autoclaved beans, at 121 (128) °C for 10-90 min, respectively; MSE, mean square for error; casein, ANRC casein. ^c Amino acid contents were determined in quadruplicate; lsd test only for the bean treatments and not for casein; means in a row having different letters are significantly ($P < 0.05$) different.

MATERIALS AND METHODS

Diets. Red kidney beans were thermally processed as described previously (Wu *et al.*, 1994, 1995a). Briefly, nine treatments, raw, home, can, 121-10, 121-20, 121-40, 121-60, 121-90, and 128-20, were utilized. Diets were formulated according to the procedure of McDonough *et al.* (1990) and contained about 10% protein, 10% fat, 2% vitamin mix, 3.5% mineral mix, 0.005% BHA, 5% cellulose, and cornstarch to equal 100%. A protein-free diet was used to estimate metabolic amino acid output, and a diet with 10% protein from ANRC casein was used as control.

Animals. Forty-four male weanling Sprague-Dawley descended rats weighing 60 ± 5 g were housed in individual stainless steel cages with filter paper placed below the cages to minimize contamination of feces with urine and to catch spilled food during the balance period. The cages were in a room with temperature 22 ± 1 °C, relative humidity $65 \pm 5\%$, and a 12-h light, 12-h dark cycle. The rats were fed a standard pelleted diet for an acclimation period for 4 days and then completely randomly distributed into 11 blocks, each on the basis of equal mean body weights. The rats were provided distilled water *ad libitum*, but dietary intake was limited to 15 g/day. The rats were fed the diets for a 4-day preliminary period and a 5-day balance period with daily feed intake recorded. On each day of the balance period, feces and spilled food for each rat were collected and deposited in open containers. Feces were dried in a vacuum oven at 40 °C for 24 h, weighed, ground to pass a 20-mesh screen, and stored in a freezer (-20 °C).

Chemical Analysis. Crude nitrogen, petroleum ether extractable material (total fat), ash, acid detergent fiber, and moisture of diet and feces were determined according to procedures described by the AOAC (1990). True N conversion factors (F_i) for diets and feces were obtained by using AA composition and Kjeldahl nitrogen content (Wu *et al.*, 1995b). The protein content of diets and feces for each treatment was calculated by multiplying the Kjeldahl nitrogen content and factor F_i .

Amino Acid Analysis. Diet and fecal material were acid hydrolyzed using 6 N HCl according to the methods of Moore and Stein (1963) for analysis of most AAs except tryptophan (Trp), methionine (Met), and cystine (Cys). Trp was determined using 4.2 N NaOH hydrolysis of the protein (Hugli and Moore, 1972). Met and Cys were determined by pretreating the protein with performic acid followed by hydrolysis with 6 N HCl (Moore, 1963). The hydrolysates were analyzed by ion-exchange chromatography (DioneX AA Analyzer, Model D-300).

Calculation and Statistical Analysis. Availability of individual amino acid (AIAA) was calculated as follows:

$$AIAA = \{[AA_i - (AA_f - AA_{mf})]/AA_i\} \times 100 \quad (1)$$

AA_i is the AA intake of animals fed the test diet, AA_f is the fecal AA output of animals fed the test diets, and AA_{mf} is the fecal AA output of animals fed the protein-free diet. AA composition in diet proteins (Table 1) and in fecal proteins (Table 2) and the protein intake and fecal protein output (Table 3) were given. Therefore, the values of AA_i (AA_f and AA_{mf}) can be calculated by multiplying AA content in diet protein (fecal protein) and the protein intake (output), respectively. Sulfur AAs (Met + Cys), the limiting AAs, had the lowest availability among the treatments (Table 4). Therefore, the availability of sulfur AAs (ASAA) other than TDP should be used to correct AAS to evaluate protein quality of the beans. ASAA-corrected AAS (AAS_{ASAA}) was calculated by multiplying the values of AAS and the availability factor as

$$AAS_{ASAA} = AAS \times ASAA \quad (2)$$

where AAS is the lowest amino acid ratio (AAR) of the beans. AAR = (mg of AA in 1 g of test protein)/(mg of AA in 1 g of reference protein) \times 100. The FAO/WHO/UNU (1985) suggested pattern of AA requirement for preschool children (2-5 years) was used as reference protein.

A total of 144 AIAA values were obtained from 16 amino acids in 9 treatments (Table 4). The average value of 16 AIAAs in each treatment is called the mean availability in each treatment (MAET). The average value of availability for each individual AA is called the mean availability for each AA (MAEAA). MAET values can be compared with the TDP and can be used to correct AAS. The calculation of MAET-corrected AAS (AAS_{MAET}) was the same as AAS_{ASAA}:

$$AAS_{MAET} = AAS \times MAET \quad (3)$$

The experiment was conducted in a completely randomized design. Data were analyzed using analysis of variance, and means were separated using the least significant difference (lsd) test procedures when significant ($P < 0.05$) F values were obtained. Pearson correlation coefficients were used to evaluate the relationship among values of AAS, AAS_{IVPD}, AAS_{TDP}, AAS_{MAET}, AAS_{ASAA}, and CRNPR to evaluate the protein quality of processed beans (Ott, 1988).

RESULTS AND DISCUSSION

Amino Acid Difference between Diets and Feces. Feces consist of indigestible bean protein, intestinal mucosal cell protein, and microbial protein (Beames and Eggum, 1981). AA contents of feces from rats fed

Table 2. Amino Acid Composition (Milligrams per 16 g of N) of Feces from Rats Fed Red Kidney Bean Diets

treatment ^a	raw	home	can	121-10	121-20	121-40	121-60	121-90	128-20	MSE	casein
Essential Amino Acids ^b											
Lys	5.55 ^c	6.09 ^{ab}	6.08 ^b	6.10 ^{ab}	6.22 ^{ab}	6.31 ^{ab}	6.07 ^b	6.49 ^a	6.18 ^{ab}	0.056	5.59
Met + Cys	3.16 ^c	4.39 ^{ab}	4.92 ^a	4.87 ^a	4.08 ^{abc}	3.85 ^{abc}	4.76 ^a	3.99 ^{abc}	3.62 ^{bc}	0.404	5.22
Thr	4.14 ^c	5.35 ^a	4.28 ^{bc}	4.92 ^{abc}	4.78 ^{abc}	5.28 ^v	4.58 ^{abc}	5.24 ^{ab}	5.04 ^{ab}	0.237	4.98
Ile	4.24 ^a	4.53 ^a	4.22 ^a	4.19 ^a	4.41 ^a	4.68 ^a	4.38 ^a	5.10 ^a	5.02 ^a	0.392	5.71
Leu	8.55 ^{ab}	7.11 ^c	7.77 ^{bc}	8.04 ^{abc}	8.34 ^{ab}	8.27 ^{ab}	8.54 ^{ab}	8.23 ^{ab}	8.88 ^a	0.419	5.93
Val	5.89 ^{de}	5.76 ^d	6.30 ^{bcd}	6.47 ^{abc}	6.67 ^{ab}	6.26 ^{bcd}	6.98 ^a	6.36 ^{a-d}	6.77 ^{ab}	0.139	5.65
Phe + Tyr	8.48 ^a	7.54 ^{bcd}	7.12 ^d	7.38 ^{cd}	8.06 ^{ab}	7.90 ^{abc}	7.91 ^{abc}	8.06 ^{ab}	7.91 ^{abc}	0.140	5.25
Trp	1.21 ^{ab}	1.12 ^{ab}	1.28 ^a	0.98 ^{abc}	1.13 ^{ab}	0.93 ^{bc}	0.78 ^c	0.92 ^{bc}	0.70 ^c	0.036	0.62
Nonessential Amino Acids ^b											
Asp	12.09 ^a	10.56 ^{cd}	11.20 ^b	10.26 ^d	10.53 ^{cd}	10.96 ^{bc}	11.06 ^{bc}	11.27 ^b	10.91 ^{bc}	0.124	9.69
Glu	15.34 ^a	13.24 ^b	13.54 ^b	13.40 ^b	14.00 ^a	13.85 ^b	13.64 ^b	13.61 ^b	13.69 ^b	0.643	0.66
His	1.99 ^b	2.15 ^{ab}	2.11 ^{ab}	2.10 ^{ab}	2.17 ^{ab}	2.13 ^{ab}	2.17 ^{ab}	2.20 ^a	2.14 ^{ab}	0.011	1.44
Arg	4.83 ^{ab}	4.12 ^b	4.47 ^{ab}	4.49 ^{ab}	4.35 ^{ab}	4.04 ^b	4.45 ^{ab}	4.14 ^b	4.08 ^b	0.083	2.84
Ser	5.86 ^a	4.81 ^d	5.61 ^{abc}	5.13 ^{a-d}	5.17 ^{a-d}	4.88 ^{cd}	5.64 ^{ab}	5.07 ^{bcd}	5.24 ^{a-d}	0.189	9.40
Pro	5.06 ^a	6.15 ^a	5.47 ^a	5.99 ^a	5.42 ^a	5.91 ^a	4.51 ^a	4.10 ^a	4.12 ^a	1.000	4.18
Ala	5.51 ^b	6.94 ^a	6.88 ^a	6.76 ^a	6.66 ^a	6.45 ^a	6.75 ^a	6.44 ^{ab}	6.54 ^a	0.289	5.37
Gly	5.06 ^d	6.06 ^a	5.66 ^{bc}	5.93 ^{ab}	5.61 ^{bc}	5.66 ^{bc}	5.47 ^c	5.66 ^c	5.38 ^{cd}	0.050	4.40

^a Treatments as in Table 1. ^b Amino acid contents were determined in quadruplicate; lsd test only for the bean treatments and not for casein; means in a row having different letters are significantly ($P < 0.05$) different. MSE, mean square for error; casein, ANRC casein diet.

Table 3. Protein Intake and Fecal Protein Output (Grams) of Red Kidney Beans from Rat Balance Assay^a

treatment ^b	P_i^c	P_o^c
raw	3.0 ^c	2.6 ^a
home	4.4 ^a	0.9 ^b
can	4.0 ^{ab}	1.1 ^b
121-10	4.2 ^{ab}	1.0 ^b
121-20	4.4 ^a	1.1 ^b
121-40	3.4 ^{bc}	0.9 ^b
121-60	3.7 ^{bc}	1.0 ^b
121-90	3.6 ^{bc}	1.2 ^b
128-20	3.9 ^b	1.2 ^b
SEM	0.3	0.05
casein	6.9	0.4

^a Data from Wu *et al.* (1985a). P_i , protein intake; P_o , fecal protein output. The P_o from rats fed the protein-free diet was 0.13 g. ^b Treatments same as in Table 1. ^c Means based on data from four rats; lsd test only for bean diets, not casein diet. Means followed by different letters, within a column, are significantly different ($P < 0.05$).

the bean diets (Table 2) were different from the AA contents of the diets (Table 1). Unlike the AA profile of beans, which is characterized by a low level of SAAs (Met + Cys) and a relatively high level of lysine (Lys) (Sgarbieri, 1989), the AA profile of rat feces had higher SAA and lower Lys contents than beans. The contents of SAAs, alanine (Ala), proline (Pro), glycine (Gly), valine (Val), Thr, and isoleucine (Ile) in feces from rats fed beans were higher than in the beans, while arginine (Arg), glutamic acid (Glu), histidine (His), Trp, serine (Ser), Lys, aspartic acid (Asp), phenylalanine + tyrosine (Phe + Tyr), and leucine (Leu) in feces were lower than in the beans. The percent differences in AA contents between diets and feces [(AA content in diet - AA content in feces)/AA content in diet] ranged from 26.8% (Arg) to -84.4% (Met + Cys) (Figure 1). AAs such as Met and Cys were present in relatively low levels in the dietary protein and in relatively high levels in the fecal protein. High fecal Met + Cys levels were probably due to stimulatory excretion of endogenous protein, which contains relatively high levels of Met and Cys (Slup and Van Beek, 1975).

Amino Acid Availability. The thermal effect on availability of individual amino acid (AIAA) was evaluated. The raw bean diet, as expected, had the lowest AIAA values among the diets. The availability shows negative values for Met + Cys, Val, Pro, Ala, and Gly

(-18.6% for Met + Cys) and positive values for other AAs (32.5% for His) in the raw diet (Table 4). Among the thermally processed bean diets, home and 121-20 had high availability values for Arg, Glu, Ser, His, and Asp, while diets 121-90 and 128-20 had low values for Met + Cys, Ala, Thr, and Pro. The availability of Arg in the home treatment (89.1) was the highest value we ever found (Table 4).

Met and Cys are the limiting AAs and have the lowest availability among the AAs in all nine treatments (Table 4). The availability of Met + Cys (ASAA) ranged from -18.6% in the raw treatment to 46.4% in treatment 121-90 to 68.0% in the home treatment. ASAA values were not only lower than any other AIAA but also lower than TDP values in all treatments (Figure 2). Similarly, the availability of Met, determined by the rat balance method, has been reported to be 44% in canned kidney beans (Sarwar *et al.*, 1989), 29.3-40.6% in four varieties of dry beans (*Phaseolus vulgaris*) (Sgarbieri *et al.*, 1979), and 58% in dry beans (Bodwell *et al.*, 1989).

Diet home had higher ASAA than other diets. Autoclaving at 121 °C for 10-90 min gradually reduced ASAA from 60.1% to 46.4%, though a significant difference was not observed (Table 4). Similarly, Antunes and Sgarbieri (1980) studied the influence of storing and cooking time on common bean (*P. vulgaris* var. Rosinha G2). When cooking time was increased from 60 to 95, 116, and 300 min for three storage conditions, A, B, and C, respectively, the bioavailability of Met decreased from 46.3% to 43.1%, 38.2%, and 27.6%, and the bioavailability of Cys decreased from 51.6% to 45.8%, 43.0%, and 30.0%, respectively. Low ASAA in legumes may be due to the presence of less digestible (containing SAA) protein fractions or peptides (Sarwar, 1984), the hypersecretion of endogenous proteins (Liener and Kakade, 1980), antiphenological factors (Stein, 1976), and/or strong microbial growth in the large intestine (Eggum, 1985). With the lowest AA content and the lowest availability value, Met + Cys negatively affects the protein quality of beans. How to improve the content and availability of SAAs in legumes is an important area of research for plant geneticists and nutritionists.

Though lysine is relatively high in legume seeds, improper thermal processing can cause it to become one of the limiting amino acids in beans (Wu *et al.*, 1994).

Table 4. Availability (Percent) of Individual Amino Acids of Thermally Processed Red Kidney Beans

treatment ^a	raw	home	can	121-10	121-20	121-40	121-60	121-90	128-20	MSE	casein
Essential Amino Acids ^b											
Lys	27.0 ^e	85.0 ^a	75.4 ^c	82.3 ^{ab}	77.1 ^{bc}	76.9 ^{bc}	77.2 ^{bc}	69.4 ^d	73.1 ^{cd}	12.1	97.1
Met + Cys	-18.6 ^d	68.0 ^a	39.8 ^c	56.4 ^{abc}	58.4 ^{abc}	60.1 ^{ab}	49.1 ^{bc}	46.4 ^{bc}	55.2 ^{abc}	21.1	92.6
Thr	10.6 ^c	78.4 ^a	72.6 ^a	75.9 ^a	71.0 ^a	69.2 ^{ab}	74.4 ^a	58.9 ^b	67.6 ^{ab}	27.6	94.9
Ile	12.5 ^c	82.6 ^a	75.9 ^{ab}	81.7 ^a	75.1 ^{ab}	74.4 ^{ab}	76.3 ^{ab}	64.5 ^b	68.1 ^b	30.9	93.8
Leu	4.5 ^e	85.6 ^a	74.3 ^{cd}	80.7 ^{ab}	75.5 ^{bc}	76.1 ^{bc}	75.5 ^{bcd}	70.1 ^{cd}	69.2 ^d	13.2	97.2
Val	-8.3 ^e	82.1 ^a	68.0 ^{bcd}	75.6 ^{ab}	68.2 ^{bcd}	71.9 ^{bc}	68.2 ^{bcd}	63.4 ^d	64.5 ^{cd}	22.8	95.9
Phe + Tyr	8.5 ^c	85.3 ^a	78.9 ^{abc}	83.0 ^{ab}	76.5 ^{cd}	77.3 ^{cd}	77.6 ^{bcd}	71.8 ^d	73.6 ^{cd}	14.1	98.0
Trp	12.7 ^c	84.3 ^a	63.3 ^b	83.0 ^a	76.5 ^a	80.7 ^a	86.3 ^a	77.7 ^a	79.7 ^a	27.8	97.3
Nonessential Amino Acids ^b											
Asp	12.9 ^e	85.7 ^a	74.9 ^{cd}	83.2 ^{ab}	78.6 ^{bc}	77.9 ^c	78.7 ^{bc}	69.2 ^d	74.7 ^{cd}	12.1	93.8
Glu	22.9 ^d	87.5 ^a	78.8 ^c	85.1 ^{ab}	80.6 ^{bc}	81.6 ^{bc}	81.9 ^{bc}	77.0 ^c	78.0 ^c	10.8	95.3
His	32.5 ^e	86.5 ^a	79.7 ^{bcd}	84.2 ^{ab}	79.3 ^{bcd}	80.7 ^{bc}	80.1 ^{bc}	74.6 ^d	77.9 ^{cd}	12.9	97.8
Arg	28.4 [§]	88.1 ^a	77.8 ^f	85.5 ^{ab}	81.9 ^{cd}	84.2 ^{bc}	81.7 ^{cde}	78.5 ^{ef}	79.9 ^{def}	3.8	96.5
Ser	9.6 [§]	86.8 ^a	74.5 ^c	82.6 ^{ab}	78.3 ^{bc}	80.5 ^{abc}	77.5 ^{bc}	74.2 ^c	74.7 ^c	19.0	90.5
Pro	-12.4 ^b	72.2 ^a	65.1 ^a	70.6 ^a	68.1 ^a	65.5 ^a	75.1 ^a	61.2 ^a	64.3 ^a	10.6	98.1
Ala	-16.6 ^e	73.1 ^a	56.4 ^d	69.7 ^{ab}	62.8 ^{bcd}	65.5 ^{abc}	64.7 ^{bc}	56.4 ^d	57.8 ^{cd}	24.3	92.3
Gly	-9.3 ^f	75.2 ^a	62.8 ^{de}	71.8 ^{ab}	69.2 ^{bc}	68.2 ^{bcd}	69.5 ^{bc}	61.1 ^e	63.9 ^{cde}	12.2	90.0

^a Treatments same as in Table 1. ^b Means same as in Table 1. LSD test only for the bean treatments and not for casein; means in a row having different letters are significantly ($P < 0.05$) different.

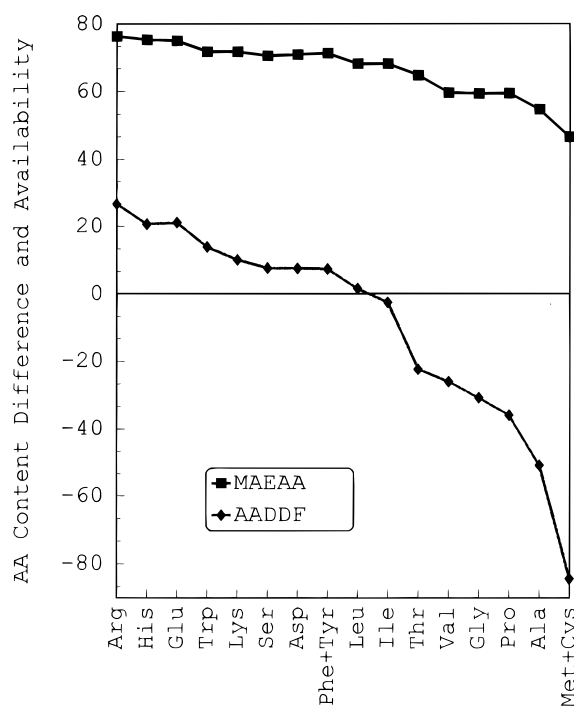


Figure 1. Comparison of amino acid content difference between diets and feces (AADDF) and mean availability for each amino acid (MAEAA).

Available lysine is a good predictor of the quality of thermally processed protein. Estimating available lysine in foods has been explored by several methods. One of the common chemical methods is the estimation of available lysine by the trinitrobenzenesulfonic acid (TNBS) method (Eklund, 1976). An excellent relationship between available lysine values (from 39.6% to 74.4%) measured by the TNBS method (Wu *et al.*, 1994) and availability of lysine (from 27.0% to 85.0%) measured by the rat balance assay (Table 4) in the same diets was observed. Values for available lysine *in vitro* appeared to be 4–11% lower than for availability of lysine *in vivo*.

The 16 AIAA values in each treatment are averaged, and the result is called the mean availability in each treatment (MAET). The difference between MAET and TDP was less than 7%. However, the differences

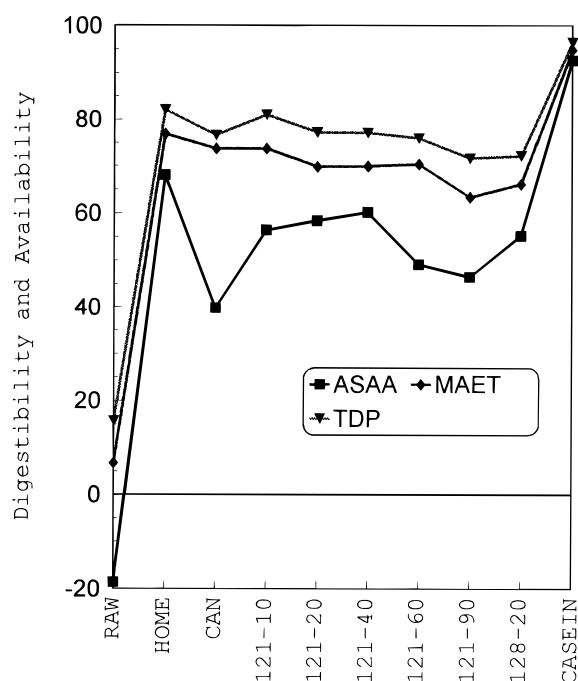


Figure 2. Comparison of availability of sulfur amino acids (ASAA), mean availability in each treatment (MAET), and true digestibility of protein (TDP) of thermally processed red kidney beans and casein.

between ASAA and TDP (from 16% to 37%) and between ASAA and MAET (from 14% to 30%) were large (Figure 2).

The nine AIAA values for each individual AA are averaged and the result is called the mean availability for each AA (MAEAA). Arg has the highest MAEAA value (82.1%), whereas Met + Cys has the lowest one (42.9%). The thermal processing effect on MAEAA was similar to the percent AA content difference between diets and feces since the AA_{mf} (0.13 g) was small (Figure 1). The higher the amino acid content excreted in the feces, the lower was the availability of the amino acid expected.

Availability-Corrected Amino Acid Score. Differences between AIAA and TDP in the heat processed beans were less than 10% in most cases. However, wide differences existed between ASAA and TDP. A similar result from canned kidney bean was reported by Sarwar

Table 5. Amino Acid Score Indices and Corrected Relative Net Protein Ratio of Thermally Processed Red Kidney Beans^a

treatment ^b	AAS ^c	AAS _{IVPD} ^c	AAS _{TDP} ^c	AAS _{MAET} ^c	AAS _{ASAA} ^c	CRNPR ^c
raw	92.8 ^a	41.3 ^c	13.9 ^d	8.3 ^d	-29.4 ^d	-44.5 ^d
home	90.9 ^a	71.5 ^{ab}	74.6 ^a	71.6 ^a	61.8 ^a	66.3 ^a
can	82.2 ^a	69.9 ^{ab}	62.7 ^{bc}	58.6 ^c	40.9 ^c	67.6 ^a
121-10	87.0 ^a	70.6 ^{ab}	70.1 ^{ab}	67.2 ^{ab}	52.3 ^b	61.7 ^{ab}
121-20	88.6 ^a	72.3 ^a	66.3 ^{abc}	66.4 ^{abc}	51.6 ^b	56.1 ^{abc}
121-40	88.8 ^a	70.0 ^{ab}	68.4 ^{abc}	66.6 ^{abc}	50.0 ^b	58.8 ^{ab}
121-60	88.4 ^a	68.5 ^{ab}	67.3 ^{abc}	64.0 ^{bc}	43.6 ^c	52.0 ^{bc}
121-90	91.5 ^a	68.1 ^{ab}	65.5 ^{bc}	62.4 ^c	42.5 ^c	49.1 ^{bc}
128-20	84.0 ^a	67.3 ^b	60.5 ^c	58.5 ^c	42.1 ^c	43.2 ^c
MSE	9.6	8.8	35.7	29.6	28.3	12.9
casein ^d	100.0	100.0	100.0	100.0	100.0	100.0

^a AAS, amino acid score; AAS_{IVPD}, *in vitro* protein digestibility-corrected amino acid score (Wu *et al.*, 1994); AAS_{TDP}, true digestibility of protein-corrected amino acid score (Wu *et al.*, 1995a); AAS_{MAET}, mean availability for each treatment-corrected amino acid score; AAS_{ASAA}, availability of sulfur amino acids - corrected amino acid score; CRNPR, corrected relative net protein ratio (unpublished data). ^b Treatments same as in Table 1. ^c Means followed by different superscripts, within a column, are significantly different ($P < 0.05$). Values larger than 100 are equal to 100.

(1987): availability of Met was 37% lower than TDP. Because ASAA had the lowest availability, the AAS should be corrected for ASAA rather than for TDP to evaluate the protein quality of the beans. The AAS_{ASAA} value was negative (-18.6%) in the raw diet, and positive (39.8-68.0%) in heat-processed-bean diets (Table 5).

Sarwar (1984) introduced the term available amino acid score (AAAS). The lowest availability of limiting AA was used to calculate AAAS. In legumes, Met and Cys are the most limiting AAs. Availability of Met and availability of Cys were not always the same in most diets. Antunes and Sgarbieri (1980) showed that the availability of Met was 3-5% lower than the availability of Cys in common beans in different conditions. Cys can spare Met in the body. Therefore, use of the average availability of Met and Cys (ASAA) to correct AAS for protein quality of the beans is better than the use of availability of Met or availability of Cys alone.

AAS had the highest values among several AAS indices (Table 5). AA does not take into account digestibility, or availability substantially overestimates the protein quality of beans. When AAS was further corrected (multiplied) for IVPD (AAS_{IVPD}), for TDP (AAS_{TDP}), for MAET (AAS_{MAET}), and for ASAA (AAS_{ASAA}), the indices of protein quality were gradually reduced and close to the value of CRNPR (Table 5).

The CRNPR method has practical application for protein products in which the SAAs are first limiting for rat growth (Sarwar, 1987). CRNPR values of processed beans were considered to be acceptable parameters for evaluating the protein quality of plant protein. In general, AAS, AAS_{IVPD}, AAS_{TDP}, and AAS_{MAET} were higher than CRNPR values. AAS_{ASAA} values were more similar to CRNPR than others, especially for the raw diet. Values for AAS_{ASAA} and CRNPR were negative in treatment raw, which reflected the weight loss of rats when fed the raw bean diet (Table 5). Comparisons between CRNPR and the AAS indices were made. The correlation coefficient was 0.995 ($P < 0.001$) between CRNPR and AAS_{ASAA}. Methods of AAS_{ASAA} and CRNPR appear to be the best ones with which to evaluate protein quality of beans.

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