R56-39

Reprinted from THE JOURNAL OF NUTRITION
Vol. 60, No. 4, December 1956

MEASUREMENT OF THE NET UTILIZATION OF HEAT-PROCESSED PROTEINS BY MEANS OF THE PEPSIN DIGEST-RESIDUE (PDR) AMINO ACID INDEX [1,2]

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(Received for publication July 9, 1956)

It has long been recognized that the nutritional value of a protein is dependent primarily upon its constituent amino acids. Nonetheless, only since the publication in recent years of reliable and relatively simple methods for amino acid analysis has it been feasible to develop *in vitro* procedures for the measurement of protein quality (Mitchell and Block, '46; Kühnau, '49; Oser, '51; and Mitchell, '54). These procedures, based upon chemical analysis of proteins for their essential amino acids, yielded figures which were well correlated with biological values.[3] However, for certain proteins the calculated values did not agree with the results of animal assay. In adition, an obvious fault with these methods lay in the fact that the biological value of many proteins was considerably changed by heat processing in the absence of dis-

[1] This paper reports research undertaken by the Quartermaster Food and Container Institute for the Armed Forces and has been assigned number 644 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

[2] A preliminary report of this work was presented before the American Society of Biological Chemists at Atlantic City, New Jersey, April 16–20, 1956 (Sheffner, Adachi and Spector, '56).

[3] The term biological value is used in this paper in accordance with the formula introduced by Mitchell ('24) after Thomas.

cernible destruction of amino acids. Consequently, several procedures were developed to take into account the enzymatic availability of component amino acids (Dunn and Rockland, '47; Anderson and Williams, '51; Horn et al., '52; and Halevy and Grossowicz, '53). The results obtained with these methods generally did not correlate well with the biological value of proteins as determined by rat assay. The values reported by Horn et al. ('52) were in good agreement with the relative protein efficiency of heat processed cotton seed meals; however, no evidence was presented concerning the general applicability of the method.

A procedure for the *in vitro* estimation of the net utilization of proteins was reported by Sheffner et al. ('55, '56). This method referred to as the Pepsin Digest-Residue (PDR) index was derived by integration of the pattern of amino acids released by *in vitro* pepsin digestion with the amino acid pattern of the remainder of the protein. The new index gave excellent correlation with the net utilization value of the proteins studied. The present study demonstrates that the PDR index also measures changes in net protein utilization which occur during heat processing and storage.

METHODS AND MATERIALS

Acid and alkaline hydrolysates and enzyme digests were prepared as previously described (Sheffner et al., '56), except that pancreatin (USP) was used where trypsin was formerly indicated; also, alkaline hydrolysis for tryptophan and tyrosine was extended to 8 hours at 120°C. with 5 N NaOH. Nitrogen was measured by a macro-Kjeldahl procedure in which mercuric oxide was used as the digestion catalyst. Individual amino acid analyses were performed by the microbiological procedures of Sheffner et al. ('48) as subsequently modified ('56).

The test protein materials used in this study were: vitamin-free casein,[4] low-temperature solvent-extracted soybean meal

[4] Labco brand, The Borden Company.

and raw soybean meal.[5] The raw soybean meal was finely ground with solid CO_2 in a Wiley mill before use. For heat treatment, the finely divided casein and soybean samples were spread in a Petri dish to a depth of 0.5 inch and heated in a thermostatically controlled electric oven or in an autoclave. Steaming of samples was done in the autoclave at atmospheric pressure. Casein and soy samples which were heat treated in the autoclave were subsequently dried under vacuum for 24 hours at room temperature and then finely ground with a mortar and pestle. The preparations were then thoroughly blended in a kitchen mixer.[6]

The beef used in the study was from boneless cuts from the low end of the loin in U. S. Good beef. Steaks were cut one-half inch in thickness and trimmed of fat to three-eighths inch. Pan-fried beef was obtained by frying the steaks without added fat at 375°F. for a total of two and one-half minutes, the steaks being turned every one-half minute. Before analysis, the meat samples were finely ground in an electric meat grinder and mixed thoroughly. Beef used in the canned ground meat and spaghetti was of utility, cutter, or canner grade, trimmed and boned and carrying not more than 10% of trimmable fat. The spaghetti was a semolina farina-egg albumin blend containing not less than 12.2% ($N \times 5.7$) of protein. The ratio of meat to spaghetti in the product was 5 to one. The meat and spaghetti were pre-cooked, the meat being braised without burning. Heat processing was done at 240°F. for 140 minutes.

RESULTS AND DISCUSSION

The effect of heat treatment upon the PDR index and net utilization of casein is presented in table 1. Heating casein in the electric oven at 350°F. resulted in a progressive decrease in PDR index values from 68, initially, to 23 after 5 hours. The net utilization values of the oven-heated caseins as de-

[5] Nutrisoy 7B and raw soybean meal were obtained from The Archer-Daniels-Midland Company, through the courtesy of Dr. J. W. Hayward.

[6] Hobart Kitchen Aid.

termined by the Mitchell ('24) method of nitrogen balance in rats were similarly lowered. The PDR index of casein was not appreciably changed when the casein was autoclaved at 250°F. for 30 minutes or for 20 hours. Net utilization values were not obtained for these samples; however, Chick et al. ('35) reported little, if any, change in the biological value or digestibility of casein heated at 250°F. for as long as 72 hours.

The effect of processing and storage upon beef and a beef with spaghetti mixture is presented in table 2. For fresh raw beef, a PDR index value of 76 was obtained. This checks closely with the net utilization values obtained by Mitchell

TABLE 1

The effect of heat treatment upon the PDR index and net utilization of casein

TREATMENT	PDR INDEX	NET UTILIZATION
None	68	82
Oven, 350°F., 40 min.	60	
Oven, 350°F., 1 hr.	39	44
Oven, 350°F., 5 hrs.	23	24
Autoclave, 250°F., 30 min.	71	
Autoclave, 250°F., 20 hrs.	66	

and co-workers ('49) and Mayfield and Hedrick ('49). Pan-fried beef was not significantly different from the control. In this respect, both Mitchell et al. and Mayfield and Hedrick have reported that roasting does not reduce the net utilization of beef protein. In the case of the mixed beef and spaghetti, there was a decrease in the PDR index from 72 to 66 following processing and a further decrease to 60 after storage for 6 months at 118°F. These changes in PDR index reflected the drop in net utilization as measured by rat assay.

The PDR index and net utilization value of raw and heated soybean meals are presented in table 3. The PDR index of soybean meal steamed for 30 minutes was the same as the net utilization value. Soybean meal autoclaved for 8 hours showed an equivalent decrease in both the PDR index and the net

utilization value. The PDR index of low-temperature solvent-extracted soybean meal also was very close to the net utilization value. However, contrary to the PDR index results, the rat assays indicated that the raw soybean meal had a net protein utilization value which was significantly below that of the steamed soybean meal. Since, in the calculation of the

TABLE 2

Effect of processing and storage upon the PDR index and net utilization of beef products

PRODUCT	TREATMENT	PDR INDEX	NET UTILIZATION
Beef	None (raw)	76	74,[1] 76[2]
Beef	Roasted, 5 hrs., 300°F.	..	74[1]
Beef	Roasted, open pan, 325°F. to internal temp. of 176°F.	..	77[2]
Beef	Pan fried, 2½ min., 375°F.	77	
Beef with spaghetti	Precooked	72	77
Beef with spaghetti	Precooked, processed, 240°F., 140 min.	66	66
Beef with spaghetti	Processed, 240°F., 140 min., stored 6 mos., 118°F.	60	58

[1] Mitchell, Hamilton and Beadles ('49).
[2] Mayfield and Hedrick ('49).

TABLE 3

The effect of heat treatment upon the PDR index and net protein utilization of soybean meal

TREATMENT	PDR INDEX	NET UTILIZATION
Raw	70	58
Steamed, 212°F., 30 min.	69	69
Autoclaved, 250°F., 8 hours	44	47
Low temp. solvent extracted	65	63
Low temp. solvent extracted, steamed, 212°F., 30 min.	70	..
Low temp. solvent extracted, autoclaved, 250°F., 8 hours	42	..

PDR index, only the pepsin digest and total amino acid results are used, the question arose as to whether a correction for trypsin digestion should be introduced into the PDR index to account for the effects of anti-tryptic factors in raw soybean meal.

In an attempt to answer this question, the soybean samples were treated with pepsin as usual, then adjusted to pH 8.2 and incubated with pancreatin for 24 hours at 37°C. The

TABLE 4

Effect of optimal heating upon the enzymatic release of amino acids from soybean meal

AMINO ACID	COMPLETE HYDROLYSIS		PEPSIN		PEPSIN PLUS PANCREATIN	
	Raw	Steamed [1]	Raw	Steamed	Raw	Steamed
	mg/gm	*mg/gm*	*per cent liberation*		*per cent liberation*	
Cystine	12.8	12.4	2.3	1.6	4.7	21.0
Lysine	59.1	59.5	2.0	1.7	20.6	68.9
Histidine	29.2	30.8	2.4	2.0	17.1	33.8
Valine	57.8	56.5	16.3	15.4	36.7	56.8
Methionine	13.2	13.5	15.9	14.1	36.4	51.1
Isoleucine	54.0	54.5	47.6	47.5	68.2	89.9
Leucine	77.3	78.0	57.6	60.3	77.8	96.4
Tyrosine	31.0	30.9	13.9	13.9	66.8	81.6
Tryptophan	16.8	17.0	22.6	22.4	43.4	51.2
Phenylalanine	57.0	59.7	17.7	16.8	44.9	50.2
Threonine	37.9	39.0	53.8	48.7	74.9	84.1

[1] Steamed at atmospheric pressure (212°F.).

total amino acid composition of the proteins and the percentage liberation of amino acids from the raw and steamed soybean meals by the pepsin and the pepsin plus pancreatin treatments are presented in table 4. Whereas there is no change in the quantity of amino acids in the completely hydrolyzed protein nor in the amount of amino acids released by pepsin, there is a considerable increase in the amount of amino acids released from the steamed soybean meal by the pepsin plus pancreatin treatment. The results also show that this increased liberation following pancreatin treatment varies with the individual amino acids, and in this respect

they are in agreement with the work of Melnick, Oser and Weiss ('46). Thus, while the increase in geometric mean for the 11 amino acids was only 42.3%, the increase for cystine was 347% and for lysine 234%. However, contrary to the results obtained by Melnick et al. with pancreatin alone, the increased liberation of methionine from the steamed soybean meal was found to be no greater than that of the mean increase.

The non-uniform suppression of the pancreatic release of amino acids by anti-tryptic factors has been suggested as a major cause of the lower biological value of raw soybean meal (Melnick et al., '46). If this were true, supplementation of optimally heated soy meal with the amino acids limiting in

TABLE 5

Effect of optimal heating and amino acid supplementation upon the biological value of raw soybean meal

SUPPLEMENTATION	TREATMENT	BIOLOGICAL VALUE
None	None	68
None	Steamed, 30 min.	75
Methionine and lysine	None	79
Methionine and lysine	Steamed, 30 min.	86

the trypsin digest, namely lysine and cystine (or methionine), should not result in a biological value greater than that of the supplemented raw meal. Consequently, in the present study, raw soybean meal was supplemented to correct for the deficiency of these amino acids both at the tryptic stage of digestion and in the total protein. Another sample of the raw soy meal was similarly supplemented after optimal heat treatment. The biological values of these preparations as determined by rat assay are presented in table 5. The results show that an equivalent increase in biological value due to heating occurred whether or not the raw soybean meal was supplemented with lysine and methionine. Since lysine and methionine (or cystine) were the amino acids most affected by the anti-tryptic factors, the data suggest that although the

anti-tryptic factors of raw soybean meal introduce differences in the rate of release of individual amino acids, these differences do not significantly influence the biological value of the raw protein. On the basis of the results reported here, a correction for tryptic digestion would not be expected to improve the accuracy of the PDR index for predicting the net utilization value of raw soybean meal.

Overestimation by the PDR index of the net utilization value of raw soybean meal is probably best explained as being due to the presence in the meal of a toxic factor or factors. Such toxic factors have been experimentally demonstrated and shown to be sensitive to heat. (Liener et al., '49; Liener, '53; Desikachar and De, '47; Klose et al., '48; Borchers et al., '48; Westfall et al., '48). Consequently, the PDR index should also be an accurate indicator of the net protein utilization in soybean preparations in which the toxic factor has been destroyed by heat treatment.

The particular advantage of the nitrogen balance method for measuring biological value (Thomas, '09; Mitchell, '24) over other biological assay methods is that it determines directly the storage of protein in growth rather than assuming that this storage is proportional to body weight gains. The procedure also distinguishes between loss of nitrogen in the digestive process, i.e., undigested plus secretory protein, and losses due to the remaining metabolic processes of the animal body. However, for purposes of appraising the value of a food as a source of dietary protein, a single figure for the net protein utilization has distinct advantages (Mitchell, '44). For most food proteins the distinction between biological value and net utilization is academic since their coefficients of digestibility are very high. However, in the case of heat-processed foods in which protein digestibilities are significantly reduced it is important for practical nutritional considerations to measure the net utilization rather than the biological value. The PDR index which measures the net utilization of proteins directly is a useful procedure for estimation of the nutritional quality of both natural and processed proteins.

SUMMARY

The pepsin digest-residue (PDR) amino acid index was found to reflect the effects of heat processing upon the net protein utilization of proteins and mixed protein foods.

Data are also presented which indicate that the discrepancy between the PDR index and the net utilization value of raw soybean meal is due to the presence in raw soybeans of substances which exert effects apparently unrelated to the enzymatic release of amino acids. The PDR index can be used to predict the net utilization of soybean meals accurately if these "toxic" factors are inactivated.

ACKNOWLEDGMENT

The authors wish to thank Dr. D. H. Calloway for determining the biological value and net utilization of the proteins used in this study. We also express our appreciation to Mr. John McMullen for supplying the proximate analyses and Mr. Lawrence Wills for aiding in the amino acid analyses.

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