Measuring Protein Quality

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ABSTRACT

An alternative to the time-consuming and expensive PER assay for measuring food protein quality is needed by the food industry. Many biological and chemical-based assays for measuring protein quality have been described in the literature. Most of these are still too complicated, time-consuming, or too narrow in the range of foods they will test for daily quality control use. In the past five years, rapid methods have been developed that employ chemical assays for essential amino acid composition and availability or biological assays that measure protein digestibility and growth on food proteins. Most of these assays can be completed in five days or less and are applicable to a broad range of foods. These developments have brightened the prospects for the eventual development of a rapid assay that the food industry routinely can use to monitor protein quality. This paper has discussed two assays that were tested with a wide variety of foods and that take less than 72 hr to complete. The C-PER assay, uses data on the in vitro protein digestibility and EAA composition of a food protein to predict its protein quality in terms of PER. The C-PER technique is not limited by the protein, fat, additive or spice levels in the food to be tested, and is therefore applicable to a wide range of food ingredients and processed foods. The second assay is based on the growth of the protozoan *Tetrahymena thermophila* WH_{14} on a proteolytic enzyme hydrolyzed food sample along with in vitro protein digestibility data to predict protein quality in terms of T-PER. Because the *Tetrahymena* are more difficult to control on a day to day basis, the error of the T-PER estimate is greater than that for the C-PER estimate. Also, since *Tetrahymena* growth is greatly affected by various food additives and spices, caution should be used when this assay is used to measure protein quality in foods where the composition is not definitely known. The T-PER assay is best suited for assaying protein quality in protein-containing food ingredients, such as meats, flours, protein concentrates and isolates, or on foods where the exact composition is known.

INTRODUCTION

Bodwell (1) summarized the many differing assays that are used to determine the nutritional quality of food proteins. The ultimate assay, using human subjects to determine nitrogen balance index, takes 35 to 45 days and costs $12,000 to $18,000 per sample. A direct comparison of different food proteins can be accomplished using human subjects to measure net protein utilization (NPU) or biological value (BV). These assays take 12 to 15 days and cost $4,000 to $6,000 per sample. Primarily because of the time and cost limitations, human assays cannot be used on a routine basis for food protein quality testing.

Lachance et al. (2) described two fairly rapid bioassays that will yield protein nutritional quality data within two weeks. The first is the net protein ratio (NPR) assay. This is a rat assay that requires two weeks to complete and gives data comparable to other rat assays, such as the PER and slope ratio, both of which require either longer times to perform or more test groups per determination. The NPR, as well as other rat assays, is discussed in detail in the next section of this paper.

The second rapid assay described by Lachance et al. (2) is a 10 day multipoint nitrogen balance index (NBI) using adult human subjects. The NBI is an accelerated technique which passes human subjects through a no-protein diet and three levels of a test protein in a 10 day period. This assay yields protein quality estimates which tend to be slightly higher than those values obtained using the conventional nitrogen balance assay.

Bodwell (3) recognized that the human bioassay to determine NPU was a good standard and then compared data from various rat assays to that available from human assays. Even though the data were very limited in amount, he concluded that when data from man and rat on the same proteins were compared, the results suggested that there was not a close relationship between protein nutritional quality as measured by the various rat assays and that as measured by the human using the NPU assay.

Bodwell (1) described 12 assays that have been developed for measuring protein quality, or some component of protein quality (i.e., protein digestibility, amino acid availability), that are aside from the human and rat assays for protein nutritional quality. These 12 range from determinations of kidney transaminase activity; to *Tetrahymena* growth; to modified chemical score measurements; to chemical assays for specific essential amino acids.

In general there are numerous assays available for estimating protein nutritional quality. But there is a real lack of data relating to those "secondary assays," such as the various rat, enzymatic or chemical assays, to the "primary assays," those involving the human. Secondly, since protein nutritional quality is being used in several countries as a regulatory parameter, then another need is very evident. That is the need for a "secondary assay" which can serve as a quality control function for the food processing industries.

The discussion in this paper will begin with a summary of the various "secondary assays" for measuring protein nutritional quality and end with a detailed description of two assays that may be applicable to needs of the food industry.

SECONDARY ASSAY METHODS

Rat Assays

McLaughlan (4), Samonds and Hegsted (5) and Hackler (6) have extensively reviewed the various rat assays that are being used to measure food protein nutritional quality.

PER

The protein efficiency ratio assay is widely used and is

the official assay in the U.S. and Canada. This assay (7) uses a single level of protein (10%) in the diet and measures the ratio of weight gain to protein consumed over a 28 day period. The assay is highly variable due to factors such as the strain of rats used (8) and level of food consumption (5). Limitations of the PER assay are: 1) it is a measure of protein quality as it relates to the growth requirement of the rat, and makes no allowance for the maintenance requirement of the rat; 2) the assay yields data which are not proportional (i.e., a protein having a PER of 2 does not have twice the nutritional quality of a protein having a PER of 1); and 3) the assay uses a single level of protein in the test diet (10%), a level which is biased against most plant proteins.

NPR

Bender and Doell (8) proposed the net protein ratio assay to overcome the major flaw of the PER assay, in that it does not consider the protein maintenance requirements of the rat. To do this, the NPR assay includes a second group of animals on a protein free diet and assumes the protein needed to prevent weight loss of the protein free group to be a measure of the maintenance requirement of the rat. NPR is calculated using the following equation:

$$\text{NPR} = \frac{\text{Weight gain (g) + weight loss of protein free group (g)}}{\text{Protein consumed (g)}}$$

McLaughlan (4) stated that NPR is essentially equal to PER + 1.5, but has an advantage over the PER assay in that it requires only 10 to 14 days to perform.

NPU

The net protein utilization assay (9) is similar to that of the NPR, but utilizes values of body nitrogen (N) instead of body weight. NPU is calculated as follows:

$$\text{NPU} = \frac{\text{Body N (g)} - \text{Body N of protein free group (g)}}{\text{N consumed (g)}}$$

McLaughlan (4) states that NPU should be equivalent to biological value (BV) X protein digestibility.

Slope Ratio Assays

The relative nutritive value (RNV) assay (10) measures protein quality by feeding the test protein to rats at three levels (each protein limiting) plus a zero protein level. The regression lines of body weight to protein intake are then determined and compared to that of the control protein, lactalbumin. This method reports protein quality as a percentage of the control lactalbumin, and yields protein quality values that are very similar to values obtained from the NPR assay (11).

The relative protein value (RPV) assay is a modification of the RNV assay, in that the zero protein level is eliminated and the regression lines are determined solely in the region where gain in body weight and protein intake have a linear relationship. Again the slopes of the regression lines for each protein sample tested are compared to that for the control protein (lactalbumin), and protein quality is reported as a percentage of that for lactalbumin. The RNV and RPV assays require ca. three weeks for completion. Hackler (6), in summarizing the RPV assay, indicated that it tends to have low slopes for lysine deficient proteins, while having steep slopes for threonine deficient proteins. It, therefore, underestimates protein quality for lysine deficient proteins, while overestimating protein quality for threonine deficient proteins.

Assays Utilizing Microorganisms

Microorganisms which have been used to determine protein nutritional quality are *Streptococcus faccalis* and *Leuconostoc mesenteroids* (12-14) and *Streptococcus zymogenes* (15,16). These assays were used to measure the availability of selected essential amino acids (EAA) in proteins and thereby describe protein quality based upon EAA availability.

The microorganism most extensively used to measure protein quality is the protozoan *Tetrahymena pyriformis*. This organism has definite advantages over those previously listed in that it, 1) has an EAA requirement similar to that of man and the rat, and 2) can ingest particulate material, thereby not having to rely entirely on soluble nutrients for growth. By combining a proteolytic enzyme partial predigestion step along with the subsequent growth of *Tetrahymena pyriformis* W on the hydrolyzate, it has been shown (17-20) that *Tetrahymena* growth was highly correlated to rat growth (PER) on selected food proteins. Sutton (21) shortened the *Tetrahymena* assay to 66 hr by incorporating the syngen *Tetrahymena thermophila* WH_{14} and by determining growth by using a Coulter counter to determine growth. He reported protein quality as estimated by the *Tetrahymena* assay as a predicted PER or T-PER (*Tetrahymena* estimated PER).

The *Tetrahymena* assay was further improved (22) by using two powdered mixes (basal medium and enzyme + activator) for assaying *Tetrahymena thermophila* WH_{14} growth on proteinaceous foods. With this assay the *Tetrahymena* growth was found to be a direct function of the nitrogen content of the food sample being tested. When the authors (22) adjusted each food sample weight assayed with the nitrogen/weight schedule they described, the resulting predicted PERs from the *Tetrahymena* correlated very well with PERs obtained from the rat.

Assays Using Proteolytic Enzymes

Proteolytic enzymes have been used to predict one aspect of protein quality, protein digestibility. A pepsin-pancreatin enzyme system (23) and a papain system (24, 25) have been used to measure protein digestibility. Results obtained with these enzymes agreed well with in vivo rat protein digestibility data. A papain-trypsin system for determining in vitro protein digestibility (26) correlated well with in vivo digestibility. Hsu et al. (27) modified in vitro techniques (28,29) for rapid measurement of protein digestibility using a multienzyme system consisting of trypsin, chymotrypsin and peptidase.

Assays Utilizing the Amino Acid Profile

The "Chemical Score" method (30) used the first limiting essential amino acid to calculate protein quality and compared that to the essential amino acid profile of whole egg protein. Oser (31) suggested the use of total essential amino acid content instead of first limiting amino acid to determine protein quality. McLaughlan et al. (32) recommended a simplified chemical score using only three amino acids – lysine, methionine, and cysteine. The Joint FAO Expert Group on Protein Requirements (33) proposed use of a provisional reference pattern based on human amino acid requirements. This pattern was criticized by another expert committee (34) as containing excessive tryptophan and the sulfur-containing amino acids. A revised standard reference pattern for essential amino acids was proposed by FAO/WHO (35). Although the chemical score is a valuable tool for screening of protein quality, it has one real fault: it assumes all amino acids are 100% available.

Multiple regression equations based on computer-selected amino acids (36), provide estimates of PERs for foods containing meat ingredients. However, predictions from the model were found to be unreliable when examining plant-derived protein ingredients.

An enzymatic-ultrafiltrate digest (EUD) assay (37) predicts food protein nutritional quality. This assay involves digesting a protein sample with pepsin-trypsin-pan-

creatin and then determining the available amino acids by analyzing the ultrafiltrate of the enzyme digest. A high correlation was obtained (37) between the EUD index of 10 food samples and their respective rat-based biological values.

The PER was predicted (38,39) for a variety of food proteins by expressing the essential amino acid profile of each sample as a percentage of a reference casein essential amino acid profile, after each was corrected for protein digestibility (27). A C-PER (computed PER) was then predicted and based on the actual PER for casein.

THE T-PER AND C-PER ASSAYS

This section of the paper discusses development and subsequent testing of two rapid assays for protein quality. The computed PER (C-PER) assay utilizes EAA profile and in vitro protein digestibility data to predict protein quality. The *Tetrahymena* bioassay utilizes the syngen *Tetrahymena thermophila* WH_{14}.

MATERIALS AND METHODS

Food Sample Ingredients and Additives Tested

The protein-based foods and food ingredients listed in this section were derived from both commercial and laboratory sources. Various speices were tested for their effect on the *Tetrahymena* assay (21). Spices were added at the 0.01, 0.10 and 1% levels to 50 ml of a solution containing 364.9 mg of ANRC sodium caseinate (protein base for the assay). Spices tested were ginger, cinnamon, clove, red pepper, black pepper, white pepper, oregano, thyme, sage and onion.

Other food additives were also tested for their effect on the *Tetrahymena* assay. These were sodium nitrate, nitrite, erythrobate, ascorbate, sorbate, benzoate and propionate.

Protein Content

The Kjeldahl method (7) was used to determine total protein contents. Factors of 5.7, 6.25, and 6.38 were used for cereal-based proteins, soy, meat and combination protein blends, and milk-based proteins, respectively.

Rat Bioassay for PER

The protein efficiency ratios (PER) of various experimental and commercial food ingredients and products were determined using the official method for PER as described in AOAC (7).

The C-PER Assay

In vitro protein digestibility. The in vitro apparent protein digestibilities of various protein samples were measured using a modification of the multienzyme automatic recording technique (27). The modifications were: (a.) at exactly 10 min from the time the enzymes trypsin-chymotrypsin-peptidase were added to the protein sample, stirring in a 37 C water bath, 1 ml of a bacterial protease (purchased from Sigma Chemical Co., St. Louis, MO., USA. It is catalogue No. P-5130, Type VI from *Streptomyces griseus*) solution (7.95 mg enzyme/ml) is added to the sample; (b.) immediately, the solution is transferred to a 55 C water bath; (c.) nine min after adding the bacterial protease solution to the sample, the sample is removed from the 55 C water bath and returned to the 37 C water bath; (d.) at exactly 10 min after the sample had received the bacterial protease (1 min back in the 37 C water bath), the pH of the enzyme hydrolyzate is recorded; (e.) the pH measured in Step 4 is recorded as the 20 min pH; (f.) in vitro protein digestibility of the sample is then calculated using the following equation: % Digestibility = 234.84 − 22.56 (X), where X is the pH recorded in Step 5.

Amino acid composition. Protein samples were hydrolyzed with 6 N HCl, under vacuum, for 24 hr at 110 C. Tryptophan was released using a $Ba(OH)_2$ hydrolysis (40); the sulfur-containing amino acids were obtained using a performic acid pretreatment of samples and then hydrolyzing the sample with 6 N HCl (41). All hydrolyzates were analyzed using a Beckman 120 C amino acid analyzer.

Calculation of the C-PER. This C-PER model was constructed from samples having corrected rat PERs ranging from 0.67 and 3.22. The model is not designed to operate outside this range.

The standard error of the estimate (Sx) of the C-PER is about ± 0.36 PER units for the samples we have tested.

Essential amino acids (EAA) used in this assay are lysine, methionine + cystine (M + C), threonine, isoleucine, leucine, valine, phenylalanine + tyrosine (P + T), and tryptophan.

Computation procedure

Step 1. Determine the in vitro digestibility of the protein as per Hsu et al. (27).

Step 2. Determine the g/100 g protein (P) of each EAA in the sample and reference ANRC casein.

Step 3. Express each EAA as a percentage of the FAO/WHO standard using the following equation:

$$\% \text{ FAO} = \frac{\text{g EAA/100 g P}}{\text{FAO/WHO Std. for that EAA}} \text{X in vitro digestibility}$$

where the FAO/WHO (35) standard is assumed to be:

EAA	g/100 g P
Lysine	5.5
M + C	3.5
Threonine	4.0
Isoleucine	4.0
Leucine	7.0
Valine	5.0
P + T	6.0
Tryptophan	1.0

Step 4. Examine each percentage of the FAO/WHO standard and adjust as follows: (a.) if all EAA percentages are above ⩽100% of the FAO/WHO standard proceed to Step 5, otherwise; (b.) if any EAA percentage is greater than 100%, *reduce* to 100% and proceed to Step 5.

Step 5. Compute the following:

$$X = \left\{\left[\frac{1}{(\%\text{ FAO/WHO for each aa})\ (\text{associated weight})}\right]\right.$$

$$Y = \left\{\text{weights}\right.$$

Weights to be used in Step 5 computations:

% FAO/WHO[a]	Weight
100	1
91-99	2
81-90	2.83
71-80	4
61-70	5.66
51-60	8
41-50	11.31
31-40	16
21-30	22.63
11-20	32
0-10	45.25

[a]Round to nearest integer.

Step 6. Divide the sum of the weights (Y) by the sum of the reciprocals (X) for the casein reference and the protein sample. The results are termed the essential aa scores for the casein and sample.

Step 7. Divide the essential aa score of the sample by

TABLE I

Calculation of a C-PER for a Protein Sample

A. Amino acid profile of the sample and ANRC sodium caseinate.

aa	aa Profile of sample g aa 100 g protein	aa Profile of reference casein g aa 100 g protein
Lysine	5.28	7.51
M + C	2.46	2.96
Threonine	5.17	3.43
Isoleucine	4.97	5.01
Leucine	7.73	9.20
Valine	5.97	5.42
P + T	7.75	9.81
Tryptophan	0.51	1.21

% protein digestibility as determined by the method described previously.

Sample = 79.81%
Casein = 90.03%

B. Computation of lysine as a percentage of FAO/WHO standard for the sample:

$$\% \text{ FAO/WHO} = \frac{5.28 \text{ g lysine}/100 \text{ g P}}{5.5 \text{ g lysine}/100 \text{ g P}} \times 79.81\% = 76\%$$

Do this for all amino acids.

C. aa profiles expressed as a percentage of the FAO/WHO standards as per above procedure with associated weights.

	Sample		Reference casein	
aa	% FAO/WHO	Wt.	% FAO/WHO	Wt.
Lysine	76	4	124	1
M + C	56	8	76	4
Threonine	103	1	77	4
Isoleucine	99	2	113	1
Leucine	88	2.83	118	1
Valine	96	2	98	2
P + T	102	1	145	1
Tryptophan	42	11.31	113	1
	X = .5573 Y = 32.14		X = .1750 Y = 15.00	

D. Essential amino acid score = 57.67 = 85.72

E. SPC = 57.67/85.72
= .6728

F. Z = [(.6728)(2.94)] [2.5/2.94]
= 1.6820

G. C-PER = -2.1074 + 2.8528 (1.682) - .4030 (2.829)
= 1.6

the essential aa score of the casein. This expresses the sample protein as a ratio of the casein standard (SPC).

Step 8. Compute the C-PER as follows: (a.) z = ((SPC) 2.94) (2.5/2.94); (b.) C-PER = -2.1074 + 2.8525 (Z) - 0.4030 (Z^2).

The C-PER model is programmed for the Hewlett-Packard 67 or 97. Program code and program cards are available upon request.

An example showing the actual calculation of a C-PER is given in Table I.

The T-PER Assay

Prediction of protein quality using the T-PER assay involves use of the modified in vitro protein digestibility assay (27) described in the previous C-PER section, along with the growth of *Tetrahymena thermophila* WH_{14} on the partially hydrolyzed protein (21). The T-PER assay (21) is outlined in Fig. 1.

Calculation of T-PER

The T-PER assay incorporates the following data into its prediction of protein quality (PER): (a.) X_1 = in vitro protein digestibility; (b.) X_2 = 66 hr growth X 10^{-4} on the control (ANRC sodium caseinate); (c.) X_3 = (66 hr - 24 hr) growth X 10^{-4} on the sample.

This data is then entered into the following equation to yield the T-PER: T-PER = 7.1116 + 0.0152 (X_1) - 0.2501 (X_2) + 0.0325 (X_3).

DISCUSSION OF THE C-PER AND T-PER ASSAYS

The C-PER Assay

The C-PER assay is capable of providing a rapid estimate of protein quality easily within 72 hr. As is evidenced by the comparison of C-PER to actual rat PER data, the C-PERs are very close to PER values obtained from the rat assays (Tables II and III). In general, it can be stated that the C-PER assay will give reasonably accurate prediction of PER, regardless of the food being tested. Secondly, the level of protein and/or fat in the food being tested seems to have no effect on the C-PER assay. The C-PER assay reported here is an improvement over the same assay reported by Hsu et al. (27), which is due primarily to the incorporation of the four enzyme in vitro digestibility

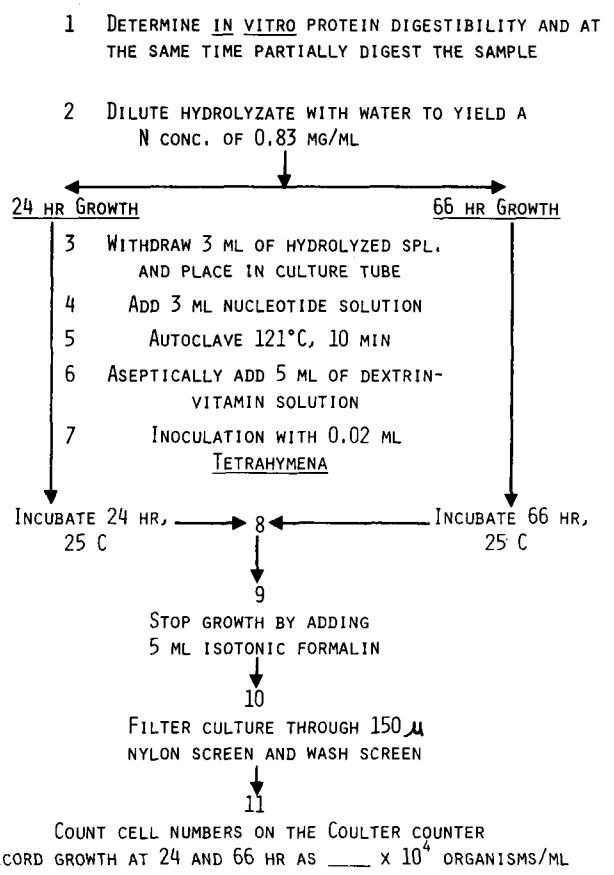

FIG. 1. Procedure for the inoculation and growth of *Tetrahymena thermophila* WH_{14} on a food sample.

TABLE II

Comparison of C-PER and Rat-PER of Various Food Proteins-Ingredient Proteins

Sample	Rat-PER	C-PER
Cornmeal	0.7	1.1
White wheat flour	0.7	0.8
Durum wheat flour	0.9	0.7
High protein flour	1.2	1.3
Soy isolate	1.3	1.3
Whole corn	1.4	1.4
Soy flour (20 PDI)	1.6	1.3
Soy flour (70 PDI)	1.6	1.8
Yeast protein concentrate	1.8	2.0
Textured soy protein	1.9	2.0
Lactalbumin	2.4	2.4
Egg white	2.5	2.3
Mechanically deboned turkey meat	2.6	2.7

assay. The four enzyme assay gives a better estimate of protein digestibility than does the previous method (27).

As a rapid assay, the C-PER has several real advantages over the rat PER assay in that it: (a.) can yield a predicted PER in 72 hr or less and at a much lower cost than can the rat PER assay; (b.) is biologically explainable in that the factors used to predict the protein quality are its degree of protein digestibility as well as its EAA profile; (c.) provides information as to why the PER is high or low by indicating the degree of protein digestibility and by quantitating the degree each EAA is limiting in the protein; (d.) and, is sensitive to trypsin inhibitors and changes in protein structure, which occur during processing (27) and that affect in vitro protein digestibility and ultimately protein quality.

TABLE III

Comparison of C-PER and Rat-PER of Various Proteins-Finished Foods

Sample	Rat-PER	C-PER
Fortified pasta No. 1	1.6	1.9
Fortified pasta No. 2	1.8	1.8
Fortified cookie	1.5	1.6
Extruded corn-soy blend	2.0	1.8
Breakfast bars	1.8	1.9
Fortified breakfast cereal	2.2	2.1
Oat breakfast cereal	1.2	1.7
Whole wheat breakfast cereal	1.1	1.6
Sausage analogue	2.0	2.0
Pizza product No. 1	2.2	2.2
Pizza ingredients No. 2	2.1	2.3
Pizza product No. 2	2.2	2.2
Macaroni and cheese dinner	1.8	1.7
Beef and noodle dinner	2.1	1.9
Turkey pot pie	2.4	2.0
Lean beef	2.5	2.2
Cooked whole egg	3.2	2.7

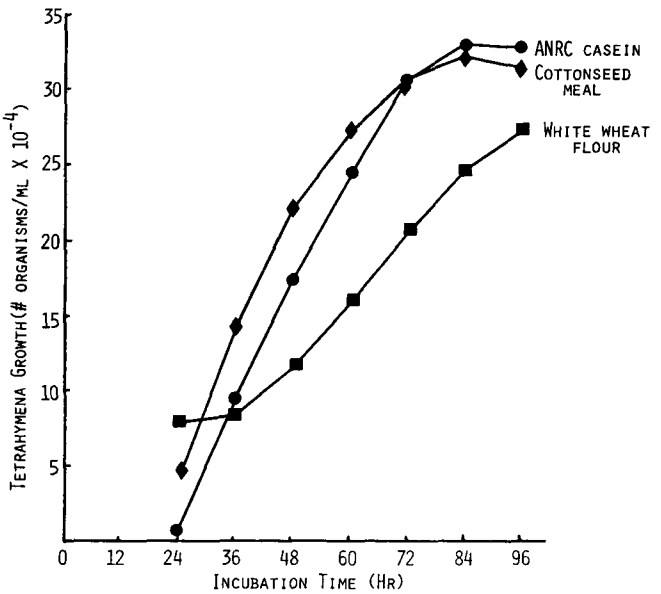

FIG. 2. Growth curves of *Tetrahymena thermophila* WH_{14} on ANRC casein, cottonseed meal and white wheat flour (Sutton, 1978).

The T-PER Assay

Research (17-19) has shown that the protozoan *Tetrahymena*, when grown on a protein hydrolyzate, can give an indication of the protein quality of that hydrolyzate by measuring the rate and extent of growth during a specified incubation period.

Dryden et al. (20) incorporated the in vitro protein digestion assay (27) into the *Tetrahymena* assay and used both protein digestibility and *Tetrahymena* growth to predict a T-PER.

Sutton (21) accelerated the *Tetrahymena* assay using the syngen *Tetrahymena thermophila* WH_{14} and measured growth with a Coulter counter. This procedure was able to predict protein quality in 72 hr or less. Figure 2 shows that the growth of *Tetrahymena* up to 72 hr is fairly linear with foods having widely varying protein qualities. Sutton (21) chose to use a 66 hr incubation period, which followed proteolytic predigestion of the food protein (27) and subsequent inoculation of the hydrolyzate with *Tetrahymena*.

Figure 2 also illustrates the problem that arises when a Coulter counter is used to measure *Tetrahymena* growth on foods. The Coulter counter counts all particles which lie in

TABLE IV

Observed and Predicted PERs for Selected Protein Sources as Determined by the *Tetrahymena pyriformis* WH_{14} Bioassay

Protein source	Rat-PER	Predicted T-PER
Durum wheat flour	0.9	0.9
Bean protein concentrate	1.0	1.5
Oat breakfast cereal	1.2	1.4
High protein flour	1.2	1.1
Soy isolate	1.2	1.5
Soy flour (20 PDI)	1.6	1.6
Macaroni and cheese dinner	1.8	1.4
Extruded corn-soy blend	2.0	1.6
Wheat protein concentrate	2.1	2.2
Fortified breakfast cereal	2.2	2.2
Steak analogue	2.2	1.9
Whey	2.3	1.8
Cottonseed meal	2.3	1.9
Turkey pot pie	2.4	2.2
Egg white	2.5	2.9
Casein (Sigma Chemical Co.)	2.5	2.4
Sodium caseinate (ANRC)	2.5	2.5
Fish fillets	2.6	2.4
Nonfat dry milk	2.7	2.3
Lean beef	2.8	2.4
Whole egg	3.2	3.0

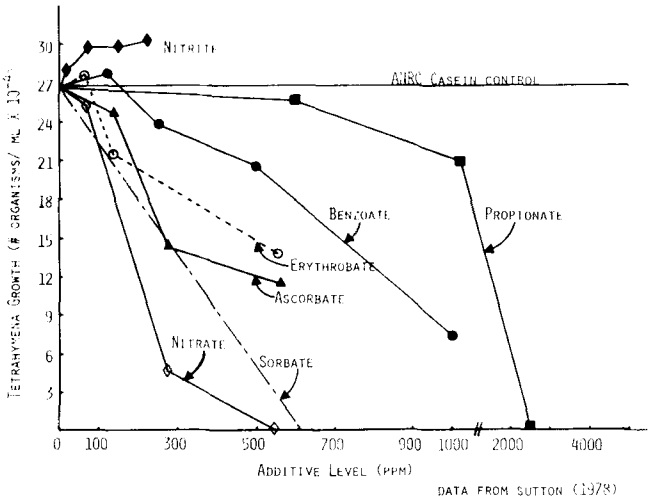

FIG. 3. The effects of selected food additives on the growth of *Tetrahymena thermophila* WH_{14} in an ANRC casein medium (Sutton, 1978).

the 20 to 70 μ range, and is unable to differentiate between food particles and *Tetrahymena* cells.

Preliminary work (21) indicated that at 24 hr after inoculation, the *Tetrahymena* growth was essentially zero. Therefore, counting the sample 24 hr after inoculation with *Tetrahymena* gave a Coulter count which reflected the amount of particulate material in the sample culture. This then could be subtracted from the 66 hr count to give a better estimate of the actual number of cells present after growing for 66 hr on the enzyme-hydrolyzed food protein sample. Figure 2 shows the high 24 hr *Tetrahymena* count for white wheat flour and cottonseed meal, which is actually due to particulate material in the culture media.

The equation given in the Materials and Methods section uses a corrected count (66 hr count – 24 hr count) to estimate the actual growth of *Tetrahymena* on a food protein. Since ANRC sodium caseinate is a completely soluble sample, it has a 24 hr count of essentially zero. The count at 66 hr is believed to be entirely due to the *Tetrahymena* cells + insoluble food particles, as is true for many foods and food ingredients.

Table IV gives a comparison of the T-PER and rat PER values for 17 protein foods and food ingredients. The

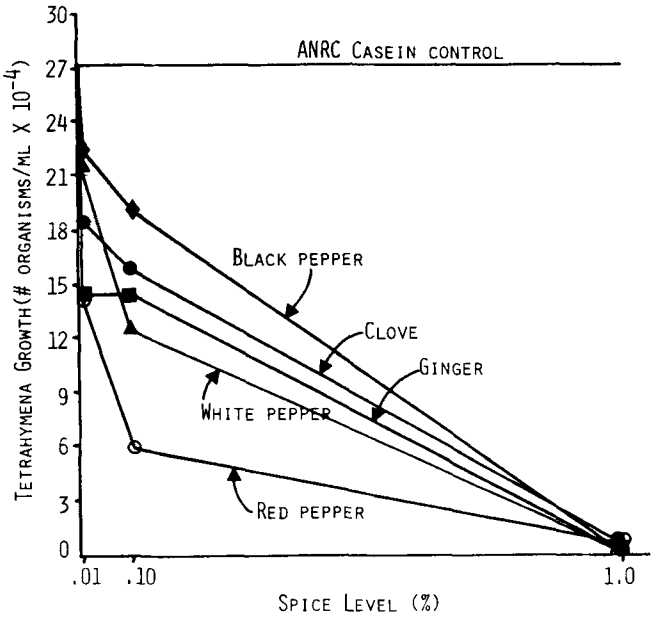

FIG. 4. The effects of black, white and red pepper, clove and giner on the growth of *Tetrahymena thermophila* WH_{14} in an ANRC casein medium (Hsu et al., 1978).

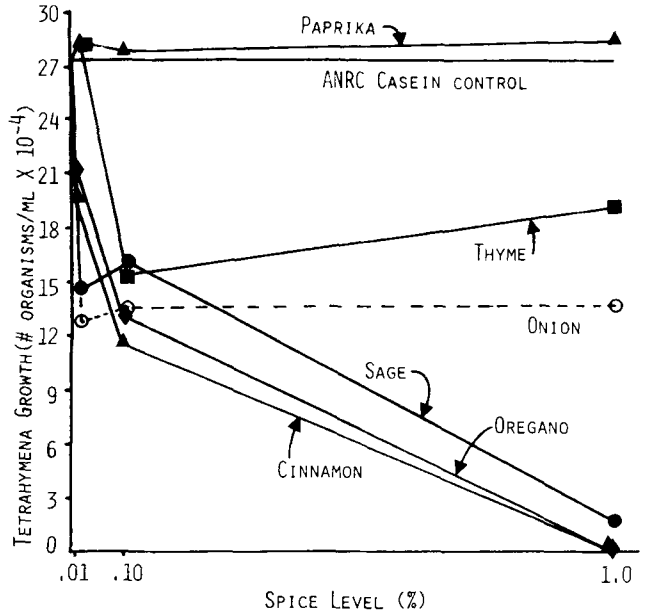

FIG. 5. The effect of paprika, thyme, onion, sage, oregano and cinnamon on the growth of *Tetrahymena thermophila* WH_{14} in an ANRC casein medium (Hsu et al., 1978).

T–PER assay is not as accurate in its ability to predict PER as is the C-PER assay, since its standard error of the estimate (Sx) is 0.52, as compared to 0.36 for the C–PER assay.

Since the T-PER assay utilizes a living organism, there is concern that nonprotein food ingredients could affect its growth and subsequently lead to an erroneous prediction of protein quality.

Smith and Pena (42) measured protein quality in leaf protein concentrates using a *Tetrahymena* assay and reported relatively low protein qualities. It is well known that leaf protein contains bound chlorogenic acid, which causes a small reduction in protein quality as measured by the rat (43), but a large reduction in protein quality as measured by *Tetrahymena* (44), which is unable to utilize protein, peptides and amino acids containing bound chlorogenic acid.

Figure 3 illustrates the effects that selected food additives have on *Tetrahymena* growth. All additives were tested at levels extending from the top level either allowed by law, or commonly used by the food industry, down to levels at the threshold of its effectiveness in foods. When looking at the effects of each additive and now it affects *Tetrahymena* growth and its ability to predict protein quality, the following was observed: (a.) nitrite has no effect at concentrations up to 35 ppm, but causes an enhancement in *Tetrahymena* growth at concentrations from 75 ppm to 200 ppm; (b.) nitrate is nontoxic at concentrations up to 67.5 ppm, but severely toxic at higher concentrations; (c.) ascorbate and erythrobate are nontoxic at concentrations up to 67.5 ppm and moderately toxic at higher concentrations; (d.) benzoate is nontoxic at concentrations up to 120 ppm and moderately toxic at higher concentrations; (e.) propionate is nontoxic at concentrations up to 600 ppm and very toxic at higher concentrations; (f.) sorbate is extremely toxic at all concentrations tested (600 ppm up to 5,000 ppm).

Effects of various spices on *Tetrahymena* growth (Fig. 4 and 5) were: (a.) paparika is nontoxic at concentrations up to 1% in the culture medium; (b.) thyme is nontoxic at the 0.01% concentration, but is toxic at higher concentrations; (c.) onion is moderately toxic at all concentrations tested; (d.) sage, ginger, clove, oregano, cinnamon and all three peppers (white, red and black) are very toxic at the levels tested.

It should be noted that the food additive and spice levels tested and reported above are levels that were present in the enzyme-hydrolyzed media at the time of inoculation with *Tetrahymena*. The *Tetrahymena* assay of Sutton (21), as well as other assays, use a medium containing ca. 0.25 mg nitrogen per ml. Therefore, most food samples need to be diluted to give that nitrogen content per ml of medium. This dilution may then be enough to lower the concentration of additives or spices present in the food below the levels that are toxic to *Tetrahymena*.

Yet, when analyzing a finished food which may contain one or more of the additives and/or spices that are toxic to *Tetrahymena*, it is difficult, if not often times impossible, to find the levels of additives and/or spices in the food.

Since food ingredients (additives, spices, and naturally occurring compounds (chlorogenic acid)) other than the protein in the food can affect *Tetrahymena* growth, caution must be exercised when using *Tetrahymena* to predict protein quality on finished foods or food ingredients, as these foods may contain compounds potentially toxic to *Tetrahymena*.

REFERENCES

1. Bodwell, C.E., Food Tech. 31(6):73 (1977).
2. Lachance, P.A., R. Bressani, and L.G. Elias, Ibid. 31(6):82.
3. Bodwell, C.E., Cereal Chem. 54:958 (1977).
4. McLaughlan, J.M., Cereal Sci. Today 17(17):162 (1972).
5. Samonds, K.W., and D.M. Hegsted, in "Evaluation of Protein for Humans," Edited by C.E. Bodwell, AVI Publishing Co., Westport, CT, 1977, p. 68.
6. Hackler, L.R., Cereal Chem. 54:984 (1977).
7. "AOAC Official Method of Analysis," Twelfth Edition, Association of Official Analytical Chemists, Washington, DC.
8. Bender, A.E., and B.H. Doell, Brit. J. Nutr. 11:140 (1957).
9. Bender, A.E., and D.S. Miller, Biochem. J. 53:vii (1953).
10. Hegsted, D.M., R. Neff, and J. Worcester, J. Agric. Food Chem. 16:190 (1968).
11. Yanez, E., and J.M. McLaughlan, Can. J. Physiol. Pharmacol. 48:188 (1970).
12. Horn, M.J., A.E. Blum, M. Womack, and C.E.F. Gersdorff, J. Nutr. 48:231 (1952).
13. Horn, M.J., A.E. Blum, and M. Womack, Ibid. 52:375 (1954).
14. Terri, A.E., W. Virchow, and M.E. Loughlin, Ibid. 59:589 (1956).
15. Ford, J.E. Brit. J. Nutr. 14:485 (1960).
16. Leleji, O.I., M.H. Dickson, and L.R. Hackler, Hort. Sci. 30:277 (1972).
17. Landers, R.E., in "Protein Nutritional Quality of Food and Feeds," Part I, Edited by M. Friedman, Marcel Dekker Inc., New York, 1975, p. 185.
18. Frank, D., H. Baker, S.H. Hutner, I.I. Rusoff, and R.A. Morck, Ibid. 14:203.
19. Evancho, G.M., H.D. Hurt, P.A. Devlin, R.E. Landers, and D.H. Ashton, J. Food Sci. 42:444 (1977).
20. Dryden, M.J., J.G. Kendrick, L.D. Satterlee, L.J. Schroeder, and R.G. Block, Ibid., 43:650 (1977).
21. Sutton, N.E., "Protein Efficiency Ratio as Estimated by *Tetrahymena thermophila* WH_{14} and Its Limitations," M.S. Thesis, University of Nebraska, Lincoln, NB, 1978.
22. Baker, H., O. Frank, I.I. Rusoff, R.A. Morck, and S.H. Hutner, Nutr. Rep. Int. 17:525 (1978).
23. Akeson, W.R., and M.A. Stahmann, J. Nutr. 83:257 (1964).
24. Buchanan, R.A., Brit. J. Nutr. 23:253 (1969).
25. Buchanan, R.A., and M. Byers, J. Sci. Food Agric. 20:364 (1969).
26. Saunders, R.M., M.A. O'Connor, A.N. Booth, E.M. Bickoff, and G.O. Kohler, J. Nutr. 103:530 (1973).
27. Hsu, H.W., D.L. Vavak, L.D. Satterlee, and G.A. Miller, J. Food Sci. 42:1269 (1977).
28. Maga, J.A., K. Lorenz, and O. Oneyemi, Ibid. 38:173 (1973).
29. Rhinehart, D., "A Nutritional Characterization of the Distiller's Grain Protein Concentrates," M.S. Thesis, University of Nebraska, Lincoln, 1975.
30. Mitchell, H.H., and R.J. Block, J. Biol. Chem. 163:599 (1946).
31. Oser, B.L., J. Am. Diet Assoc. 27:396 (1951).
32. McLaughlan, H.H., and R.J. Block, J. Biol. Chem. 163:599 (1946).
33. Report of the FAO Committee, Protein Requirements, FAO Nutritional Studies, Series No. 16, Rome, Italy, 1957.
34. FAO/WHO Report of a Joint FAO/WHO Expert Group, Protein Requirements, World Health Organization Technical Report Series No. 301, Geneva, 1965.
35. FAO/WHO Ad Hoc Expert Committee, Energy and Protein Requirements, World Health Organization Technical Report, Series No. 522, Rome, Italy, 1973.
36. Alsmeyer, R.H., A.E. Cunningham, and M.L. Happich, Food Tech. 28:34 (1974).
37. Floridi, A., and F. Fidanza, Riv. Sci. Tech. Alim. Nutr. Um. 5:13 (1975).
38. Satterlee, L.D., J.G. Hendrick, and G.A. Miller, Food Technol. 31(6):78 (1977).
39. Hsu, H.W., N.E. Sutton, M.O. Banjo, L.D. Satterlee, and J.G. Kendrick, J. Food Sci. (In press).
40. Pataki, G., "Techniques of TLC in Amino Acid and Peptide Chemistry," Ann Arbor Science Publishers, Inc., Ann Arbor, MI, 1968, p. 115.
41. Moore, S., J. Biol. Chem. 238:235 (1963).
42. Smith, E.B., and P.M. Pena, J. Food Sci. 42:674 (1977).
43. Horigome, T., and M. Kandatsu, Agric. Biol. Chem. 32:1093 (1968).
44. Dryden, M.J., and L.D. Satterlee, J. Food Sci. 43:650 (1977).