NUTRITIONAL ASSAY

Relationship Between in Vitro Enzymatic Digestibility And in Vivo Protein Evaluation of Powdered Whey

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The flavor, physical properties, and nutritive value of food products, such as dried milk, are often affected markedly by the so-called Maillard reaction between the carbonyl groups of sugars and the amino groups of protein and/or amino acids. Animal evaluation of the damage done to the nutritive value of the protein in such products due to the Maillard reaction is costly in both time and animals. Hence, it is difficult to assess the relative influence of processing variables and storage on such products. Measurement of the microbiologically available lysine liberated from resuspended whey powders by digestion with crystalline trypsin affords a satisfactory prediction of animal growth response on the powders. The method is also applicable to milk powders, milk concentrates, and protein fractions derived from whey powders. Digestion with a series of digestive enzymes (pepsin, trypsin, and chymotrypsin) does not give a good prediction. Processing variables—in particular, drying methods and storage temperatures—affect the nutritive value of whey powders measured in vivo and by the enzymatic test.

THE PROTEIN-SUGAR REACTION [Maillard reaction (*8*)], especially as it relates to the processing and storage of dairy products, has recently aroused much interest. Thus, the elegant investigations of Henry, Kon, Lea, and White (*4*) have demonstrated that this reaction is of great importance in the preparation and storage of skim milk powder. The advent of Maillard-type changes in the powder not only changes the physical properties (dispersibility) and flavor, but also affects, in a detrimental way, the nutritive value of the protein (*3*, *5*). Analogous studies have been made by Lea *et al.* on the reaction between casein and glucose (*6*).

The reaction has been shown to involve primarily the ϵ-amino group of the lysine and the free aldehyde group of the sugar (*4*). Closely following the lysine in reactivity are arginine, histidine, methionine, and tyrosine. Tryptophan did not appear to react.

The authors have conducted a preliminary study on the influence of processing variables and of storage of powdered cheese wheys on subsequent tryptic digestibility of these powders. It has been found that in vitro enzymatic hydrolysis may be used to predict the results of animal evaluation (*11*) with a fair degree of precision. Moreover, as these studies have been on whey, they are complementary to the above-cited results on whole milk protein and casein alone. The potential and actual use of whey as a food for humans and animals, together with the high nutritive value of the whey protein, makes such studies of interest, with processing variables of particular relevance.

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Table I. Biological Value of Whey Powders

	Method of Drying	
	Spray	Roller
Lot I	91.0 (No. 2)	81.75 (No. 3)
Lot II	90.3 (No. 4)	83.5 (No. 5)
Lot III	93.9 (No. 7)	82.7 (No. 8)

Analysis of Variance

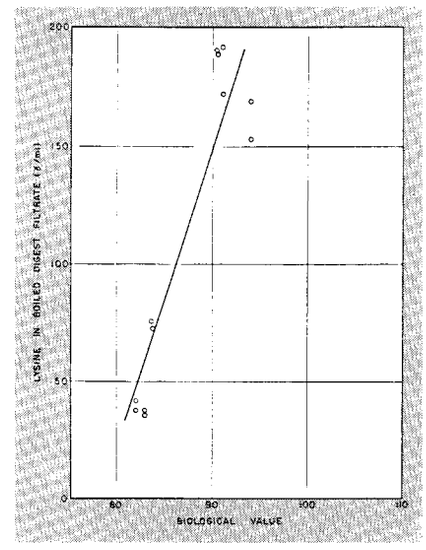
Source of variation	Degrees of freedom	Mean square
Drying method	1	1486[a]
Lots	2	25.5[b]
Interaction	2	28.5[b]
Error	66	20[c]

[a] Highly significant ($P < 0.001$).
[b] Not significant ($P > 0.05$).
[c] Estimated from ranges.

Experimental

The powdered wheys used were commercial products obtained from whey processing plants associated with cheese-

Figure 1. Relation between tryptically released lysine and biological value of whey powders



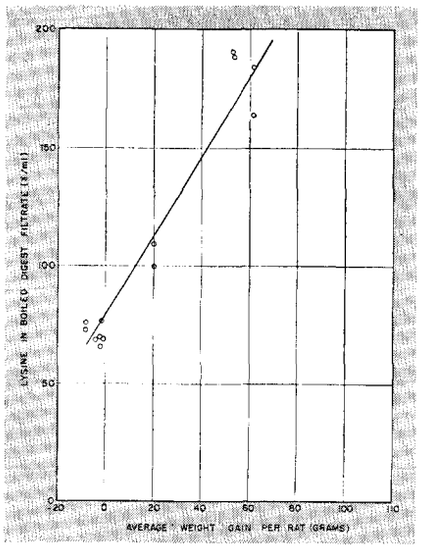


Figure 2. Relation of liberated lysine to weight gain per rat

making operations. The method was applied also to commercial milk powders and concentrates, with essentially the same results. However, only data on whey powders are reported here in detail. Crystalline proteolytic enzymes were commercial products (crystalline trypsin, Worthington Biochemical Co., Inc.; crystalline pepsin and chymotrypsin, Armour and Co.).

Digestions were carried out at 35° C. for 24 hours on 2% (protein basis) suspensions of the whey powders. The pH was adjusted to 7.4 with phosphate buffer (0.076*M* sodium monohydrogen phosphate). The concentration of trypsin is indicated in the protocol for separate experiments, as are other deviations from the above procedure. At the end of the digestion period, the samples were heated in a boiling water bath for 20 minutes and filtered. Amino acid assays were made on these filtrates by microbiological assay procedures (*2, 12*) using ATCC strain 9790 of *Streptococcus faecalis* and are reported as micrograms per milliliter of digest filtrate. A standard sample of lactalbumin was run on all occasions to serve as an internal control. Nonprotein nitrogen (not precipitable by metaphosphoric acid) and formol nitrogen determinations were also made on enzyme digests.

Results and Discussion

In Figure 1, it is shown that an approximately linear relationship exists between tryptically released lysine and the biological value (*9*) of several whey powders. The equation for this line is $Y = -1120 + 13.0\ x$ where the slope has a 95% confidence interval of ±8.9. The six whey powders tested fell naturally into two groups, as recorded in Table I.

In another experiment, the lysine liberated on tryptic digestion was compared to the weight gain per rat on the several diets, containing different whey powders. An approximately linear relation is observed if the lysine liberated is plotted against weight gain per animal (*10*) (Figure 2). However, if the biological criterion used is average weight gain per gram of protein consumed (Figure 3), a curved relationship becomes apparent. This is also shown in Table II. The same is true if the biological criterion is the weight gain per gram of protein consumed (Figure 4), corrected again for food consumption by a covariance analysis (*1*). Thus the enzyme assay predicts accurately only the absolute weight gain, which is composed of a number of factors whose intercorrelation is somewhat complex. Thus, the roller powders, which are lower in biological value, are consumed to a lesser extent. When correction is made for protein consumption, the enzyme assay overestimates the biological differences between the powders.

This is also shown in tests made on some protein fractions separated from roller- and spray-dried powders and offered for enzymatic testing. These data, recorded in Table III, show also that enzyme digestion with pepsin, trypsin, and chymotrypsin does not predict the animal performance at all, whereas the good agreement of tryptic digestion with animal data is shown again. As it seems reasonable to believe that the amino-aldehyde reaction takes place in its earlier stages, mainly at the ε-amino group of the protein, it is altogether possible that the result of the Maillard reaction in this case, both in vivo and in vitro, is to prevent tryptic proteolysis, trypsin being known to be specific for peptide bonds on the carboxyl side of residues of the basic amino acids. As pepsin, trypsin, and chymotrypsin all have different specificities, it is possible that preliminary peptic digestion might render some bonds in the resulting fragments resistant to in vitro tryptic or chymotryptic hydrolysis which would have been split by these enzymes in the original protein. However, it is also possible that some other mechanism is operative.

The influence of alcohol extraction is essentially the same upon the performances of both roller- and spray-dried wheys. However, although the heat-coagulable fraction of spray-dried whey

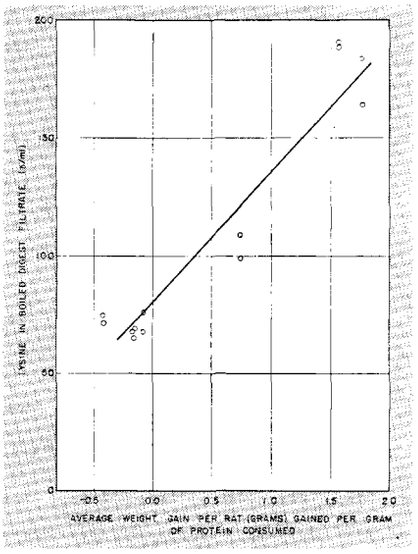


Figure 3. Relation of liberated lysine to protein consumed

shows better response in both enzyme and animal tests than does the parent spray-dried whey, the corresponding fraction from roller-dried whey shows very much more favorable response than does the original roller-dried whey. This would not be the case if a lactose-lysine reaction were the chief factor influencing the results. Lowry and Thiessen (*7*) found that hydroxymethylfurfural inhibited tryptic digestion of casein. It may be that the browned wheys con-

Table II. Agreement of Enzyme and Animal Tests on Roller-Dried Whey Powders

Powder	Lysine, γ/Ml.	Gain per Animal, Grams	Gain/G. Protein Consumed
21	176	40	1.44
22	280	49	1.76
23	125	25	1.05
24	130	− 1	−0.04
25	121	− 4	−0.16
26	278	49	1.78
Lactalbumin	439	105, 105	2.63, 2.49

Analysis of Variance (Lysine Values, Coded × 1/5)

Source of variation	D.F.	S.S.	M.S.	F
Linear regression of lysine values on gain per animal	1	2965.9	2965.9	35.0[a]
Error	5	423.5	84.7	

[a] Highly significant, $P < 0.01$.

All substrates in 4% (protein basis) suspension digested 24 hours at pH 7.4 and 35.5° C. with crystalline trypsin 10 mg./100 ml. digest, boiled, filtered, and assayed.

Table III. Comparison of in Vitro and in Vivo Tests on Whey Powder Protein Fractions

Sample	Mean Weight Gain/Animal	Gain/G. Protein Consumed	Combined Enzymes[a] Lysine	Combined Enzymes[a] NPN	Combined Enzymes[a] Formol N	Trypsin Alone[b] Lysine	Trypsin Alone[b] NPN	Trypsin Alone[b] Formol N
Lactalbumin	96.3	2.42	260	0.83	0.23	344	1.57	0.61
Spray-dried whey (No. 9)	50.8	1.74	732	2.24	0.48	260	1.57	0.76
Roller-dried whey (No. 10)	1.0	0.04	377	2.24	0.60	87	1.69	0.74
Spray-dried whey (No. 9) alcohol-extd.	87.5	2.48	841	2.05	0.56	379	1.58	0.74
Roller-dried whey (No. 10) alcohol-extd.	21.9	0.86	424	2.35	0.59	141	1.94	0.68
Spray-dried whey (No. 9) heat-coagulated	91.2	2.49	182	2.41	0.16	422	1.83	0.47
Roller-dried whey (No. 10) heat-coagulated	69.6	2.11	175	0.72	0.24	258	2.12	0.59

[a] 100 ml. of suspension (2% in protein) at pH 2 digested 25 hours at 35.5° C. with 5 ml. pepsin solution (37 mg. crystalline pepsin/44 ml.), pH adjusted to 8.0 and 5 ml. tryptic enzymes (40 mg. crystalline trypsin, 80 mg. crystalline chymotrypsin in 40 ml.) added. After 24 hours enzymes heat inactivated, made to 150 ml., and filtered.

[b] 100 ml. of 2% protein in suspension at pH 7.3 digested 24 hours at 35.5° C. with 0.50 mg. crystalline trypsin per ml. digest. Samples heat inactivated and filtered.

Table IV. Tryptic Release of Amino Acids from Whey Powders

(Expressed as % of yield from lactalbumin)

	Lysine	Arginine	Histidine	Tryptophan
Fresh				
Spray-dried	105	119	85	100
Roller-dried	36	91	45	82
Stored at 40° F. (6 mo.)				
Spray-dried	106	69	73	..
Roller-dried	40	48	51	..
Stored at room temp. (6 mo.)				
Spray-dried	48	..	47	..
Roller-dried	29	36	30	..

All substrates digested 24 hours at pH 7.4 and 35° C., in 2% (protein basis), with 100 $\gamma\mu$/ml. crystalline trypsin, boiled, filtered, and filtrates assayed for amino acids. Each figure is average of at least three samples.

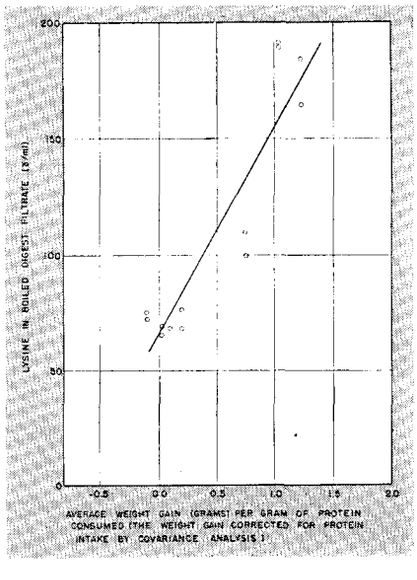
tain some proteolysis inhibitor which is not coagulated in the heat treatment. It was gratifying that the enzyme assay gave results in good agreement with the animal test, even though some qualitative fractionation of the proteins had taken place. A given type of fraction was the same in composition and yield, whether prepared from roller- or spray-dried powder. However, the heat-coagulated and alcohol-extracted samples were very different from each other in composition, particularly with respect to ash and lactose content (*11*).

It can also be seen in Table III that the nonprotein nitrogen and formol nitrogen of the whey digest do not predict animal growth response. This is due to widely varying blank values of the different fractions. Even upon correcting for blank values, data on nonprotein nitrogen and formol nitrogen digestion do not correlate as well with animal growth response as do data on lysine digestion (correlation coefficients 0.6 to 0.8 *vs.* 0.90 to 0.95, respectively, for a series of data not reported here).

As another example of the use of this method, the data of Table II may be presented, where the enzyme results predicted the animal performances in a satisfactory manner on a series of roller-dried powders. Roller-dried whey powders 22 and 26 gave as good response as did the previously tested spray-dried powders. This indicates that process conditions can be adjusted to optimum levels for the product, as revealed by both enzyme and in vivo tests.

The production of other amino acids from the various powders, both fresh and after storage, was also determined. These data, calculated as percentages for the appropriate amino acid released from fresh lactalbumin, are recorded in Table IV. Arginine does not lose its capacity for tryptic liberation on roller drying as do the other amino acids,

Figure 4. Relation between liberated lysine and weight gain



but it disappears on storage at 40° F. Contrariwise, lysine and histidine are markedly affected by roller drying, but are stable if stored at 40° F. The high heat of roller drying may induce a different mechanism of protein-sugar reaction than prolonged storage at lower temperatures.

Acknowledgment

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