Effect of Buffering Capacity on a Commonly Used Assay of Protein Digestibility

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ABSTRACT

A commonly used in vitro assay of protein digestibility depends upon the measurement of pH change as the protein is digested by a multi-enzyme preparation. This report presents evidence demonstrating that the buffering capacity of the protein under assay can have a major effect on the digestibility value assigned using this method. A modification of the assay using an automatic titrator has overcome this problem.

INTRODUCTION

MEASURING PROTEIN QUALITY in vivo is slow and expensive, and the value of results obtained using the rat has been called into question (Mariani and Spadoni, 1979; Bodwell and Marable, 1981). Thus there has been for many years a search for an in vitro assay of protein digestibility (Akeson and Stahmann, 1964; Ford and Salter, 1966; Hahn et al. 1982). Hsu et al. (1977) have described a rapid technique which depends upon measuring the pH-drop as peptide bonds are hydrolyzed when protein solutions are incubated with proteolytic enzymes. It is easily carried out on any protein forming a homogenous suspension in water. The original method has been widely used and one modification has been incorporated into the 'Calculated Protein Efficiency Ratio' (C-PER) protein quality assay (Hsu et al. 1978; Satterlee et al. 1979). C-PER is regarded as a promising approach to in vitro protein quality measurement (Bodwell, 1979) and has been published in the J. Assoc. Off. Anal. Chem. (Satterlee et al., 1982). Bodwell and Marable (1981) have noted, however, that the ability of the C-PER to predict actual PER values for a series of soy products was quite poor. It has therefore been suggested, both by Bodwell and Marable (1981) and elsewhere (Satterlee et al., 1982) that the application of the method may be limited to the measurement of a single product or group of similar products for quality control.

The end point of the procedure is a pH change, and as this may be affected by buffering in the assay mixture, it was felt that the extent of any such effect should be investigated. Although buffering capacity of the protein was considered unimportant by the original authors (Hsu et al., 1977), evidence presented here shows that it may alter considerably the values of digestibility obtained using this type of assay.

MATERIALS & METHODS

Soluble casein was obtained from B.D.H. Ltd, Poole, Dorset, U.K., soy protein (PS90E) from Putna Protein Application Ltd., St. Albans, Hertfordshire, U.K., whey and dried milk from Cow and Gate Ltd., Trowbridge, Wilts, U.K., and gluten, lactalbumin and all enzymes from Sigma London Ltd. Co., Ltd, Poole, Dorset, U.K. Nonfat dried milk (NFDM) was a commercial brand obtained from a supermarket.

‘pH-drop’ method

The method followed was essentially that of Hsu et al., (1977). Proteins were ground to pass through an 80 mesh sieve, and suspensions prepared in water and adjusted to pH 8. A multi enzyme solution containing 1.6 mg ml⁻¹ trypsin (Bovine, type 1, 16590 BAEE units mg⁻¹), and 3.1 mg ml⁻¹ chymotrypsin (Bovine, type II 50 units mg⁻¹) and 1.5 mg ml⁻¹ peptidase (Hog, grade III, 0.05 units mg⁻¹) was prepared in water and adjusted to pH 8. This solution (5 ml) was added to 50 ml of protein suspension, stirred and maintained at 37°C and the drop in pH recorded after 10 min.

Results & Discussion

RESULTS from the ‘pH-stat’ method (Fig. 1) demonstrated increased peptide bond cleavage at higher substrate concentrations. In comparison, the ‘pH-drop’ method demonstrated an apparent decrease in peptide bond cleavage at higher substrate concentrations for most of the proteins tested (Fig. 2). The probable explanation is that the detection of bond cleavage is masked in the ‘pH-drop’ method due to increased buffering capacity at higher protein concentrations.

Prediction of digestibility from the pH-stat data would require comparison of the results from a large number of proteins with in vivo values for the same proteins, thus in order to make a direct comparison between the two in vitro assays, digestibility (calculated according to Hsu et al. (1977)) and ‘ml NaOH added’ in the ‘pH-stat’ assay were...
BUFFERING CAPACITY AND DIGESTIBILITY...

![Graph showing pH-drop assay of digestibility](image)

**Table 1—Comparison of digestibility (as calculated by procedure of Hsu et al. [1977]) with values obtained in the pH-stat assay for various proteins**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Digestibility</th>
<th>ml Alkali added in 10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy</td>
<td>89.91</td>
<td>0.227</td>
</tr>
<tr>
<td>Whey</td>
<td>88.28</td>
<td>0.327</td>
</tr>
<tr>
<td>Casein</td>
<td>86.10</td>
<td>0.400</td>
</tr>
<tr>
<td>Gluten</td>
<td>85.57</td>
<td>0.160</td>
</tr>
<tr>
<td>NFDM</td>
<td>84.45</td>
<td>0.484</td>
</tr>
<tr>
<td>Lactalbumin</td>
<td>85.75</td>
<td>0.196</td>
</tr>
<tr>
<td>Dried milk</td>
<td>84.3</td>
<td>0.412</td>
</tr>
<tr>
<td>Defatted milk</td>
<td>97.2</td>
<td>0.410</td>
</tr>
</tbody>
</table>

Recent adaptation of the assay, which is used in the C-PER procedure, also depends upon a pH-change as the end point and therefore will probably also be susceptible to these errors.

Other assays similar to the PPD of Akeson and Stahmann (1964) may be more suitable for the requirements of the food industry and have been used with satisfactory results by a number of authors (Craig and Broderick, 1981; Li-Chan and Nakai, 1981; Satterlee, 1981). A 'pH-stat' type assay has also been used successfully by other authors (Friedman et al., 1981).

**REFERENCES**


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