Protein hydrolysates from Bovine lung

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1. Introduction

In recent years various attempts have been made to recover the protein from abattoir waste (e.g. Young & Lawrie, 1974) and to texturise it with appetising meat analogues either by spinning alkaline dopes of the proteins into acetic acid/salt baths (Young & Lawrie, 1975; Swingler & Lawrie, 1978) or by spinning mixtures of protein and alginate into coagulating baths of calcium chloride (Imeson et al, 1979).

Although these processes may be technologically and economically feasible, there is also a growing market for protein hydrolysates. These hydrolysates may be used as flavour enhancers, functional ingredients or simply as nutritional additives in foods of low protein quality.

The production of protein hydrolysates from legumes, such as soya bean, and from meat wastes has already been studied (Wolf & Cowan, 1971; Criswell et al, 1964; Konyushenko et al, 1976; Gault, 1977). These processes usually involve digestion with strong acids. Due to these extreme processing conditions, such acid hydrolysates generally possess poor functional and nutritional qualities and are thus restricted to use as flavour enhancers. In addition, following neutralisation of the digest, the hydrolysates contain large amounts of salt.

The search for a process milder than acid digestion led to the investigation of proteolytic enzymes (proteases) to hydrolyse unaesthetic protein sources. Thus, Roozen & Pilnik (1974) prepared an enzyme hydrolysate from soya bean which had properties making it suitable for the fortification of orange juice hydrolysates with good foaming properties, and hence possible replacers for egg white, have been prepared from wheat gluten, soya and casein (Gunther 1979). Several groups of workers (McBride et al, 1961; Sen et al, 1976; Hevia, 1976) solubilised protein concentrates with enzymes, making them suitable for use in soups, beverages and infant feeds.

Preliminary studies on the preparation of hydrolysates from several meat industry by-products have recently been reported (Webster et al, 1982). Those studies, and the present investigation describe the use of enzymes of differing specificity to produce protein hydrolysates having different characteristics and thus functional properties. Four enzymes were used. The composition, yield and molecular weight distributions of the hydrolysates were determined.

2. Materials and Methods

2.1. Hydrolysis procedure

Beef lungs were obtained from a local slaughterhouse. The tracheae were removed and the lobes minced and stored at -25°C until required. Before hydrolysis the lung was processed at 121° in a steam retort for 1 hr Hydrolysates were prepared from processed lung by batch hydrolysis (Webster, 1981). A 2l hydrolysate flask with outlets for stirrer, pH electrode, thermometer and burette was used. Batches of lung (1kg) were hydrolysed for 4 hr at the appropriate pH and temperature (Webster *et al*, 1982). The enzymes used were Pepsin, Papain (Sigma Chemicals Ltd.), Neutrase and Alcalase (Novo enzymes Ltd.). Their properties have been described elsewhere by Webster *et al* (1982). An enzyme:substrate ratio of 2:100 and a substrate concentration of 8% (Protein Concentration) were used. The degree of hydrolysis was determined by the pH-stat method (Novo, 1978) and/or the Trinitrobenzenesolphonic acid assay (Alder-Nissen, 1979). After 4 hr. hydrolysis the enzyme was inactivated by heat (90°C) for 30 minutes. The mixture was centrifuged for 45 min at 22,000 x g and a temperature of 6°C. After removal of the fat layer by filtration the soluble phase was freeze-dried and stored at room temperature.

2.2. Analysis

The hydrolysates were analysed for protein content by the kjeldahl method (Joslyn, 1970), fat content was determined by Soxhlet extraction (Pearson 1976) and hydroxyproline by the Stegemann-Stalder method (1967).

2.3. Estimation of molecular weight by gel chromatography

Freeze-dried defatted hydrolysate samples (70mg/ml) were dissolved in distilled water (1-1.5ml), applied to the top of a Sephadex G-50 column (68cm height x 2.5cm diameter) and eluted with Tris-HCl (0.05M, pH7.5). Effluent fractions (3ml) were collected. The protein content of the eluted fractions was estimated by the absorbance at 280nm and by reaction with ninhydrin (Moore and Stein, 1954).

The column was calibrated using proteins ranging from 25,000 to 3,500 Daltons in molecular weight.

3. Results

3.1. Yield and composition of hydrolysates

From Table 1 it can be seen that yield and degree of hydrolysis do not necessarily correlate. Peptic digestion resulted in the highest degree of hydrolysis. The differing degrees of hydrolysis produced over 4hr reflect the differing specifications of enzymes used. Neutrase and Pepsin hydrolysates have a higher collagen content than Alcalase and Papain hydrolysates suggesting that Neutrase and Pepsin have a greater capacity to attack the collagen present in lung (Webster, 1982).

The fat content of Pepsin hydrolysates is much lower than that of the other hydrolysates. This was because the soluble phase of the Pepsin hydrolysate gelled on centrifugation and allowed easy removal of the fat layer.

The high ash content is due to some extent, to the presence of NaOH/HCl used to adjust/maintain the pH before or during hydrolysis.

3.2. Estimation of molecular weight of hydrolysates

Fig 1 shows the elution profiles of the hydrolysates determined by the absorbance of the fractions at 280nm. The degree of hydrolysis produced by the different enzymes correlates with elution profiles. Neutrase hydrolysis produced the lowest DH% and appears to be the least hydrolysed (Fig. 1). The profile exhibits two major peaks one representing peptides of molecular weight above 30,000 daltons and a smaller peak representing peptides of molecular weight above 3,000 daltons.

Pepsin hydrolysis produced the highest DH% and the elution profile at 280nm shows that the proportion of large peptides present was low. Two peaks are seen representing peptides of molecular wieght between 9,000 and 3,000 daltons and less than 1,500 daltons.

Hydrolysis using Alcalase resulted in a hydrolysate with low, intermediate and high molecular weight peptides (Fig. 1). The profile shows that there is a significant number of peptides present of molecular weight 30,000 daltons and above and also low molecular weight peptides of molecular weight 4,000 daltons and under. A "plateau" present at an elution volume of 180 ml represents intermediate peptides of molecular weight between 15,000 and 4,000 daltons.

Papain hydrolysis resulted in a degree of hydrolysis of 14%. The elution profiles shows that the hydrolysate is composed of peptides over 30,000 daltons and lower molecular weight peptides of above 7,000 and 2,000 daltons.

The elution profiles of hydrolysates determined by reaction with ninhydrin are shown in Figure 2. As it is the free amino groups that react with ninhydrin the assay is more sensitive for lower molecular weight polypeptides The ninhydrin profiles for Alcalase and Neutrase hydrolysates exhibit all the peaks present in chromatograms determined by absorbance at 280nm. The ninhydrin assay also detected the major fractions present in the 280nm chromatograms of Papain and Pepsin hydrolysates. However, the smaller peaks detected by the absorbance at 280nm (Papain elution volume 240-250ml, Pepsin elution volume 280-300ml) were only weakly detected or not detected at all. This suggests that these absorbance peaks were due to non-amino acid containing compounds or to low molecular weight peptides containing high contents of aromatic amino acids as it is established that the elution of aromatic compounds is delayed on sephadex columns (Gelotte, 1960).

4. Conclusions

When Enzyme:Substrate ratio, time of hydrolysis and substrate concentration are fixed, enzyme specificity has an important effect on yield, composition and molecular weight distribution of the protein hydrolysates prepared from beef lung. The hydrolysates produced by the enzymes in this study had varying proportions of small and larger polypeptides. This has important consequences for functionality of the product since such properties as gelling ability, emulsifying and foaming capacity are dependent on the molecular weight of the protein. In this respect it is interesting to note that although the average molecular weight of the polypeptides in Pepsin hydrolysates was low, only these hydrolysates gelled on standing. This suggests that the type of polypeptide in these hydrolysates is different from those in the other preparations.

As the type and molecular weight distribution of each of these hydrolysates is different it suggests that controlled hydrolysis with various enzymes may well enable products with widely differing functional properties to be produced from such underutilised tissues as beef lung.

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Table 1. Yield and composition of bovine lung hydrolysates

Enzyme	Degree of Hydrolysis (% of total bonds broken)	Yield (Dry weight of soluble protein as % total dry weight of substrate)	Crude Protein [N x 6.25] (% dry weight of sample)	Collagen (Hydroxyproline x 7.25) (% dry weight of sample)	Ash (% dry weight of sample)	Fat (% dry weight of sample)
Alcalase	11.7	55	91	17.0	6	5.75
Neutrase	6.4	35	80	24.6	7.6	5.8
Papain	14.7	60	80	17.3	5	3.8
Pepsin	16.2	46	89	23.5	9	0.86

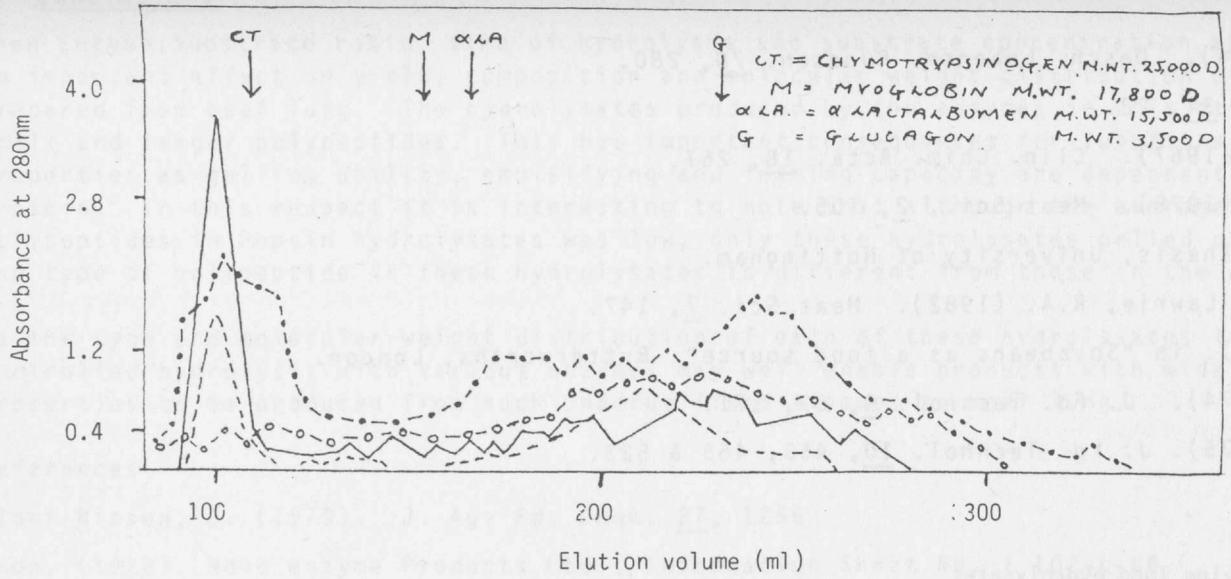

Fig. 1. Elution profiles of Alcalase (•—•—), Neutrase (——), Papain (— — —) and Pepsin (o—o—) Hydrolysates determined by absorbance at 280nm.

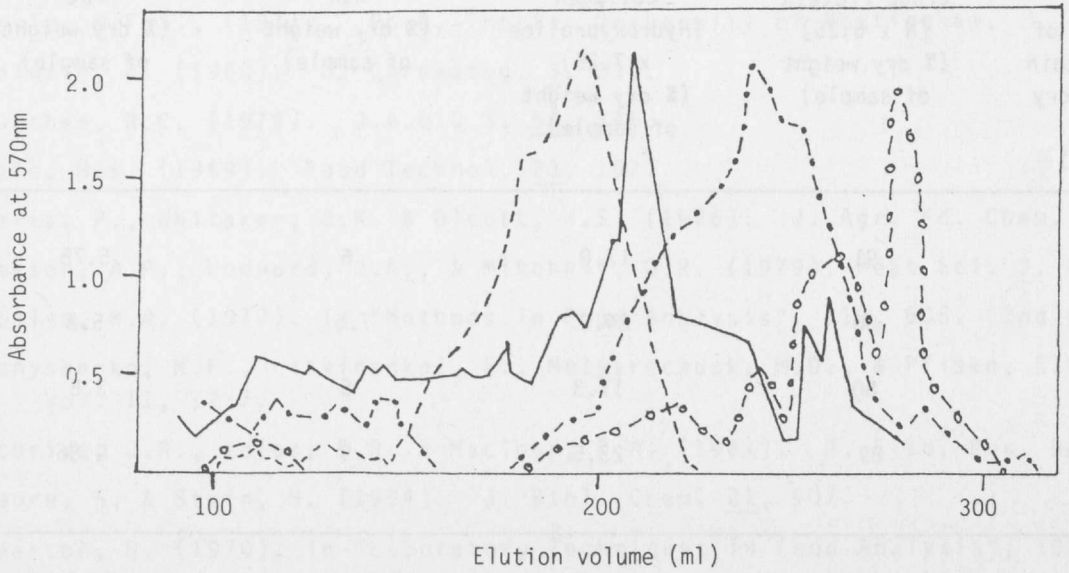

Fig. 2. Elution profiles of Alcalase (•—•—), Neutrase (——), Papain (— —) and Pepsin (o—o—) hydrolysates determined by ninhydrin reaction