Food Chemistry **12** (1983) 265–273

Preparation of Salt-Free Protein Products from Acid or Alkali-Treated Proteins

Ian L. Batey & Peter W. Gras

CSIRO Wheat Research Unit, Private Mail Bag, PO,
North Ryde, NSW, 2113, Australia

(Received: 22 April, 1983)

ABSTRACT

Wheat gluten has been hydrolysed in either alkali or acid to give a soluble protein product. Instead of using an inorganic acid or base to neutralize the hydrolysates, acidic or basic proteins have been used to neutralize the reaction mixtures after alkali or acid treatment, respectively. Both casein and a deamidated wheat gluten have been investigated as suitable acidic proteins. Salmine has been used as the basic protein for neutralization. This technique has the advantages of avoiding inorganic salt formation in the product and improving the amino acid composition of the final product. In particular, casein has been studied in some detail for its utility in neutralizing the reaction mixtures of alkali-treated proteins, mainly because it is readily available on an industrial scale and already has many applications in the food industry. Only the sulphur-containing essential amino acids are deficient in the products formed when alkali-treated gluten is neutralized with casein. The other essential amino acids are in excess of the recommended dietary intake.

INTRODUCTION

Proteins which have been solubilized by hydrolysis in either alkali or acid require neutralization before final isolation. This step inevitably results in the formation of salts in the protein product. The salt may be removed by isolating the product by precipitation at its isoelectric point, followed by

Food Chemistry 0308-8146/83/$03·00

centrifugation and washing of the precipitate before finally drying the material. Alternatively, the salt may be removed by dialysis or ultrafiltration, although the latter process may result in the loss of some low molecular weight peptides. In addition, these processes are comparatively expensive to operate on an industrial scale and consume large quantities of water.

For many purposes, the presence of some salt in the hydrolysed protein need not be a disadvantage. In some applications of the protein product, particularly in meat pastes and sausages, extra salt is often added as an ingredient. For uses in products such as coffee whiteners and milk puddings, a bland product is required and even small traces of salt could be sufficient to affect the quality.

Glutamic acid comprises almost 40% of the amino acids of wheat gluten, and about 90% of this amino acid is present as glutamine, with the side chain containing a primary amide. Some of these side chains may be deamidated by hydrolysis to the corresponding carboxylic acid and the presence of an increased number of potentially ionic functional groups should increase the solubility above pH 4. Thus, gluten has been converted into a soluble protein derivative by deamidation in either acid (Finley, 1975; McDonald & Pence, 1961; Wu *et al.*, 1976), or alkali (Batey & Gras, 1981). In the acid treatments, the soluble gluten was usually precipitated by pH adjustment and collected by centrifugation, but in the alkali reactions, the total reaction mixtures were adjusted to pH 6·8–7·0 and the neutralized product was then dried. When this pH adjustment of the alkaline solution was carried out in the usual way with mineral acid, a product containing typically 4% salt was produced. If the source of hydrogen ions for the neutralization was an acidic protein, the resulting mixture would contain a blend of proteins, free of salts of inorganic acids. In a similar way an acid hydrolysate could be neutralized with a basic protein to allow neutralization without the formation of inorganic salts.

There are a number of potentially acidic functions in proteins, including acidic phosphate mono-esters and the carboxyl group side chains of glutamic and aspartic acids. Acidic proteins containing either functional group should be satisfactory for adjusting the pH of an alkaline hydrolysate to neutrality. Casein, for example, is a readily available food grade protein containing acidic phosphate groups. The use of this material in neutralizing alkaline hydrolysates of gluten has been investigated in some detail and the results are described here.

Neutralization with acidic proteins resulting from the deamidation of gluten has also been studied. This paper describes the feasibility of using these proteins, as well as basic proteins such as salmine, for the neutralization of hydrolysates of gluten.

EXPERIMENTAL

Vital wheat gluten was obtained from the following commercial suppliers: Allied Mills Starches, Homebush, New South Wales; Fielders Starches, Leichhardt, New South Wales, and Manildra Starches, Auburn, New South Wales. Hydrochloric acid casein was obtained from the Colac Dairy Company, Colac, Victoria. Deamidated gluten was prepared as described below. Salmine sulphate was purchased from Sigma Chemical Corp., St. Louis, Mo., USA, and was converted to the free base form by passage through a column of Dowex 1-X8 (OH form). For amino acid analysis, samples were hydrolysed for 24 h in 6M hydrochloric acid containing *nor*-leucine as an internal standard, and were analysed on a Technicon AA-1 instrument with a 140 cm column and a total elution time of 18 h.

Protein solubilities (defined as the fraction which does not sediment at 6000 × *g* after dispersion in water) were determined by the biuret method. Biuret reagent was purchased from Ajax Chemicals (Auburn, New South Wales). Alternatively, the reagent may be prepared by the method of Simmonds & Ronalds (1975). Solutions of the dry, modified glutens were prepared at concentrations of 10% and 15% w/v in distilled water. After centrifugation at 6000 × *g* for 30 min, the supernatant fractions were diluted, 1:10 in the case of the 10% solution and 1:15 in the case of the 15% solution, in order to have solutions with soluble protein within the range of 0–10 mg/ml. A 1-ml aliquot of the sample was incubated with 6 ml of the biuret reagent at 37 °C for 30 min. The mixtures were filtered through Whatman GFA filter papers and the absorbance was measured at 550 nm. A graph of absorbance at 550 nm versus protein concentrations was drawn using standard solutions containing 0, 1, 2, 4, 6, 8 and 10 mg/ml, respectively of bovine serum albumin (Calbiochem, Carlingford, New South Wales) and the protein concentrations of the test solutions were read from the graph and multiplied by the dilution factor to give the concentration of soluble protein in the original solution.

Preparation of deamidated gluten

Gluten (20 g) was reacted with 0·2M sodium hydroxide for 6 h and the reaction mixture was adjusted to pH 4·5 by the addition of 2M hydrochloric acid. The precipitate was collected by centrifugation at 5000 × *g* for 20 min. In order to remove sodium chloride, the pellet was washed once by resuspending it in water, recentrifuged and then lyophilized. This product is referred to as 'acidic gluten'.

Preparation of salt-free proteins by alkaline hydrolysis

Gluten was reacted with 0·2M sodium hydroxide as described above. An acidic protein was added as a solid to bring the reaction mixture to pH 6·8–7·0. The total reaction mixture was dried by lyophilization.

Preparation of soluble proteins by acid hydrolysis

Gluten was heated under reflux in 0·1M hydrochloric acid. After a reaction time of 15 min, the mixture was cooled and neutralized with either sodium hydroxide or salmine, which had been converted to the free base form. The product was isolated by lyophilization.

RESULTS AND DISCUSSION

Acidic proteins were found to be very effective for the neutralization of alkali-treated gluten. At the same time, the solubility characteristics introduced into the gluten by the alkaline treatment were retained, and, in some cases, enhanced (Table 1). There were a number of advantages in using casein for the neutralization. The final products in this case were more soluble, had a nutritionally more desirable amino acid content (Table 2) and had a better flavour than those derived from neutralization with acidic gluten. The solubility of the product neutralized with acidic gluten was found to be almost the same as the solubility of the product neutralized with hydrochloric acid (Batey & Gras, 1981). The slightly increased solubility may result from the absence of salt which is known to decrease the solubilities of some proteins, but the increase is too small to be of any real significance. The casein-neutralized product also had a high solubility, with the total protein content dissolving in a 10% w/v solution in distilled water. A 20% solution also appeared to be completely soluble,

TABLE 1
Solubilities of Wheat Gluten Solubilized by Acid or Alkali Treatment

Reaction conditions	*Neutralization conditions*	*Protein content** (%)	*Protein solubility (mg/ml)*	
			10% solution	*15% solution*
Alkali 5 h	HCl	67	61	93
Alkali 6 h	HCl	67	66	114
Alkali 6 h	Acidic gluten	66	67	120
Alkali 5 h	Casein	79	81	120
Alkali 6 h	Casein	79	82	129
Alkali 6 h	Casein + HCl (Casein:gluten = 2:3)	76	76	117
Alkali 6 h	Casein + HCl (Casein:gluten = 1:4)	70	68	97
Acid 15 min	NaOH	68	62	94
Acid 15 min	Salmine	76	74	107

* The protein contents were determined by multiplying the Kjeldahl nitrogen by 6·25 for the casein neutralized samples, and by 5·7 for all other samples.

but the solubility was difficult to measure quantitatively because of the high viscosity of the solution. A similar problem was observed with other products and solubilities were tested in 10% and 15% solutions.

The neutralization of acid-solubilized gluten could also be achieved without the formation of inorganic salts in the product. In this case, a solution of salmine was added to the hydrolysis mixture to adjust it to pH 6·8–7·0. This procedure did cause some problems in that, during the neutralization of the mixture, the solution passed through the isoelectric point of the deamidated proteins. Some precipitation was observed around pH 4·8–5·5, but this precipitate redissolved when the solution was

TABLE 2
Amino Acid Contents of Gluten and Alkali-Treated Gluten Products

	Gluten (*g/100 g protein*)	*Acid neutralized* (*g/100 g protein*)	*Casein neutralized* (*g/100 g protein*)
Aspartic acid	4·8	4·2	6·4
Threonine	3·1	3·7	4·4
Serine	5·6	5·7	6·5
Glutamic acid	39·0	39·5	31·1
Proline	14·6	15·7	14·0
Glycine	4·6	4·8	3·2
Alanine	3·0	3·2	3·4
Valine	4·6	4·8	6·0
Cystine	2·6	0	0
Methionine	2·1	2·2	3·1
Iso-leucine	4·4	4·5	4·9
Leucine	8·4	8·5	9·6
Tyrosine	4·3	4·5	5·5
Phenylalanine	7·3	6·6	6·2
Lysine	2·2	2·3	6·2
Histidine	2·7	2·8	3·0
Arginine	4·3	4·4	4·0

stirred at pH 7 for 10–20 min. The solubilities of the acid-treated products and their amino acid contents are given in Tables 1 and 3, respectively.

The amino acid content of the various protein products is somewhat different. The original gluten, the products from neutralization of the alkali treatment with either acidic gluten or hydrochloric acid, as well as the acid-treated gluten neutralized with sodium hydroxide, had approximately the same amount of each of the essential amino acids (Table 4). The casein-neutralized gluten had an improved content of lysine, and only methionine was still below the value recommended by WHO (WHO, 1973). The salmine-neutralized gluten was deficient in all essential amino acids, except phenylalanine. The use of casein and gluten mixtures has been described previously by Schmandke *et al.* (1976*a*,*b*) in the context of spinning fibres for meat substitutes and analogues. In this instance, the purpose of the mixture is to improve the nutritional quality of the gluten protein, which is the major component of the mixture.

TABLE 3
Amino Acid Contents of Acid-Treated Gluten Products

	Gluten (*g/100 g protein*)	*Alkali neutralized* (*g/100 g protein*)	*Salmine neutralized* (*g/100 g protein*)
Aspartic acid	4·8	4·4	3·6
Threonine	3·1	3·3	2·6
Serine	5·6	5·7	7·5
Glutamic acid	39·0	38·6	31·3
Proline	14·6	15·1	15·1
Glycine	4·6	4·9	3·8
Alanine	3·0	3·0	2·5
Valine	4·6	4·5	4·6
Cystine	2·6	1·4	1·1
Methionine	2·1	2·0	1·5
Iso-leucine	4·4	4·5	3·7
Leucine	8·4	8·1	6·3
Tyrosine	4·3	4·0	3·2
Phenylalanine	7·3	6·5	5·0
Lysine	2·2	2·1	1·8
Histidine	2·7	2·9	2·4
Arginine	4·3	4·7	17·4

When the presence of some salt is of no consequence, it is possible to combine the use of casein and hydrochloric acid to effect the neutralization. These products retain the high solubilities of the products which were completely neutralized with casein (Table 1), but their solutions have viscosities greater than those of the products in which casein was not used. It would therefore seem possible to prepare products with solutions of a particular viscosity (within the feasible range) provided that the presence of some salt is of little importance. The nutritional quality may also be improved to meet the WHO recommendations for essential amino acids without effecting the neutralization completely with casein. When the amount of casein used is approximately half of the weight of gluten, the amount of lysine is in excess of the recommended level.

In practical terms, casein has many advantages as the acidic protein of choice to effect neutralization of alkaline hydrolysates of gluten. It is readily available on a large scale and is easily stored and handled in the

TABLE 4

Comparison of the Essential Amino Acid Content of Gluten, Alkali-Treated Gluten Neutralized with Hydrochloric Acid and Alkali-Treated Gluten Neutralized with Casein with the Suggested Human Requirements

	Gluten (*g/100 g protein*)	*Acid neutralized* (*g/100 g protein*)	*Casein neutralized* (*g/100 g protein*)	*Dietary recommendation** (*g/100 g protein*)
Threonine	3·1	3·7	4·4	4·0
Valine	4·6	4·8	6·0	5·0
Cystine + Methionine	4·7	2·2	3·1	3·5
Iso-leucine	4·4	4·5	4·9	4·0
Leucine	8·4	8·5	9·6	7·0
Tyrosine + Phenylalanine	11·6	11·1	11·7	6·1
Lysine	2·2	2·3	6·2	5·5
Tryptophan	1·2	—†	—†	1·0

* WHO (1973). These values have been converted to the units of g/100 g protein.

† The tryptophan content was not determined in either of the alkali-treated products, but would be expected to be above the suggested requirement.

dry form in which it is supplied. After neutralization with casein, the amino acid content of the product shows a substantial nutritional improvement over the composition of gluten which is deficient in both lysine and methionine. In addition, a variety of products containing differing proportions of gluten and casein and having a range of viscosities in solution may be prepared. This is not possible when acidic gluten is used for the neutralization. Finally, the product neutralized with either hydrochloric acid or acidic gluten has a slightly unpleasant taste, arising, perhaps, from the hydrolysis of its lipid content. When alkali-treated gluten is neutralized with casein, this taint is diluted and the flavour of the product is much more acceptable.

Casein has been used for neutralizing the alkaline hydrolysates of a number of other vegetable proteins, including soy protein, maize protein and lupin protein (I. L. Batey, unpublished results). These products had a lower protein content than the gluten products and the larger proportion of non-proteinaceous materials caused some problems with the determination of the protein solubility, but the use of casein in the

neutralization step proved just as useful as with gluten. The method, therefore, seems generally applicable for the neutralization of alkaline protein hydrolysates. The neutralization of acid hydrolysates of gluten with salmine has been shown to be feasible but is not practical on a commercial scale. The availability of a food-grade basic protein on a large scale would allow neutralization of acid hydrolysates with other proteins to become as practical as those of alkaline hydrolysates with casein.

REFERENCES

Batey, I. L. & Gras, P. W. (1981). Solubilization of wheat gluten with sodium hydroxide. *J. Fd. Technol.*, **16**, 561–6.

Finley, J. W. (1975). Deamidated gluten: A potential fortifier for fruit juices. *J. Fd. Sci.*, **40**, 1283–5.

McDonald, C. E. & Pence, J. W. (1961). Wheat gliadin in foams for food products. *Food Technol., Champaign*, **15**, 141–4.

Schmandke, H., Paul, D., Schmidt, G., Gensrich, H. J., Luther, H., Maune, R. & Bartsch, D. (1976*a*). Characteristics and spinning of alkaline solutions of wheat protein and casein. *Nahrung*, **20**, 737–42.

Schmandke, H., Schmidt, G., Kormann, I. & Maune, R. (1976*b*). Studies on modification of the shear strength of wheat protein/casein fibres. *Nahrung*, **20**, 831–4.

Simmonds, D. H. & Ronalds, J. A. (1975). Rapid protein determination in cereal grains using the biuret reaction. *Bakers Digest*, **49**(4), 36–40.

WHO (1973). *Energy and protein requirements.* Report of a Joint WHO/FAO *Ad Hoc* Expert Committee. WHO, Geneva, p. 63.

Wu, C. H., Nakai, S. & Powrie, W. D. (1976). Preparation and properties of acid solubilized gluten. *J. Agric. Fd. Chem.*, **24**, 504–10.