ELSEVIER

Food Chemistry 51 (1994) 271–274

0308-8146/94/$7.00

The role of glutamic acid/glutamine and lysine during non-enzymic browning in heated gluten

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(Received 22 December 1993; accepted 9 February 1994)

During dry heat processing of a crude gluten preparation (*c.* 75% protein) at 160°C for 30 min, about 25% of the available lysine and 10% of the glutamine residues are lost with the evolution of water (an increase of about 10%). This represents the loss of 6–7 moles of glutamine for each mole of lysine. In the presence of a low concentration (1–5%) of fructose, glucose and maltose, the amount of lysine made unavailable increases to a maximum of about 45% of the total while the glutamine content further decreases by amounts dependent on the sugar and its concentration. The number of moles of glutamine lost per mole of lysine is in the range 5–15. On a weight basis, fructose is the most reactive sugar and maltose the least, but the amount of water generated in the systems for each reacted amino group varies. The molar ratios of additional glutamine lost in the presence of sugar to additional water produced are 4·2, 16 and 21 for glucose, fructose and maltose, respectively. The possible relevance of these reactions to the quality of extruded foods is discussed.

INTRODUCTION

It has been claimed that during high temperature processing of proteinaceous materials such as occurs in extrusion, the glutamic acid/glutamine residues of the protein undergo browning reactions involving the evolution of water and the formation of covalent linkages which may well be essential for the subsequent texturisation of the extrudate (Ledward & Mitchell, 1988). The free carboxyl group of glutamic (or aspartic) acid or amide group of glutamine (or asparagine) may cross-link directly with lysine to form glutamyl (or aspartyl) lysine isopeptides with the libration of ammonia (Mecham & Olcott, 1947; Bjarnson & Carpenter, 1970). In addition, Oates *et al.* (1987*a,b*) found that soya protein isolate (and gluten) could, when heated in the presence of alginate with a high mannuronic acid content, generate moisture with the apparent loss of glutamic acid residues. No such effect, though, was seen with alginates of low mannuronic acid content or many other polysaccharides. A possible explanation for this phenomenon is that high mannuronic acid containing alginates degrade during heat processing to yield reactive fragments that can readily undergo browning with appropriate amino and perhaps acid groups (Oates & Ledward, 1990). Further studies at 185°C, on soya isolate, dialysed soya flour and whole soya flour, confirmed the loss of glutamic acid residues (and lysine) on heating, the amount lost in the whole flour being much greater than in those samples devoid of low molecular weight material (Ledward *et al.*, 1990). Every system (flour, dialysed flour and isolate) showed massive losses of available lysine under these conditions (50–80%). Similar losses of lysine were seen with other proteins under these conditions (Rasul, 1991).

Although the ability of the acid or amide group of glutamic acid/glutamine residues to react with sugars to form brown complexes is established (Miki, 1974), most work has been concerned with the ability of the free amino groups on the protein (mainly the ϵ-amino group of lysine) to undergo such reactions. However, the proteins that are most amenable to extrusion texturisation, such as gluten and soya are relatively deficient in lysine but the rich sources of glutamic acid/glutamine residues. The present study was therefore undertaken to investigate the reactivity of the glutamic acid/amine and lysine residues in gluten with a range of reducing sugars during heat processing to assess whether such reactions could be of importance in extrusion processing.

MATERIALS AND METHODS

Materials

Crude gluten (*c.* 75% protein) was obtained from Sigma (Poole, Dorset; G-5004) and sugars (fructose,

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glucose and maltose) of 'AnalaR' quality were obtained from BDH (Poole, Dorset).

Preparation of samples

Samples of crude gluten were heated in sealed glass ampoules with glucose, fructose or maltose at 0, 1, 2·5 or 5% by weight of crude gluten, at 160°C for 30 min in a fan-assisted oven. Sample masses were usually about 0·5 g. The hot samples were allowed to cool to room temperature and analysed within 40 min. The temperature of 160°C was chosen as being typical of that used in extrusion processing and the time was extended to 30 min to exaggerate possible changes for ease of analysis.

Methods

Moisture was determined by either heating samples (~2 g) for 1 h at 130 ± 2°C (crude gluten only) or by Karl Fischer analysis. Amino nitrogen content was obtained by standard Kjeldahl analysis where protein equals $N \times 5{\cdot}7$. Starch was determined by the method of Karkalas (1985) and crude fibre by AOAC (1984). Ammonia (free and alkali-labile) was determined by cracking the ampoules into 50 ml distilled water in a Buchi tube, adding excess 40% sodium hydroxide (about 150 ml) and determining the liberated ammonia by the Kjeldahl procedure. In other samples the ampoules were opened, allowed to stand for 48 h and then analysed. This gave the content of non-volatile, alkali-labile nitrogen. Available lysine was determined by the procedure of Kakade and Liener (1969) using trinitrobenzenesulphonic acid (TNBS) to evaluate reactive (available) lysine. Glutamic acid was determined using a Boehringer Mannheim Biochemica enzymatic analysis kit (139 092).

RESULTS AND DISCUSSION

Characterisation of gluten and sugars

The composition of the gluten is shown in Table 1. The moisture content, as determined by Karl Fischer analysis, is about 1% higher than when determined by oven drying. The glutamic acid content (of crude gluten) was found to be 24·7%, i.e. 33·6% of the protein, and the available lysine content 1·33%, i.e. 1·78% of the protein. In addition, alkali distillation liberated 3·8% ammonia, i.e. 55·2% of the protein. Assuming molecular weights of 129 for each glutamic acid residue (when present in a protein) and 17 for ammonia, leads to the conclusion that virtually all the glutamic acid exists in the amide form. The slight molar excess of ammonia (~0·03 moles) over glutamic acid is presumably because the relatively small amount of aspartic acid present (~2%) is also in the amide form. The sugars used had, as determined by Karl Fischer analysis, moisture contents of less than 5%. These differences were not considered significant to either the a_w of the system or the determination of the water contents of the mixtures.

Table 1. Composition of the gluten sample. Values are the means ± standard deviations of six (amide, moisture) or three (glutamic acid, available lysine, protein, starch and fibre) determinations

Component	Content (g/100 g material)
Protein	73·7 ± 0·55
Starch	8·68 ± 0·08
Moisture (oven)	7·67 ± 0·18
Moisture (Karl Fischer)	8·67 ± 0·82
Lipid	7·74 ± 0·22
Amide	3·85 ± 0·09
Glutamic acid	24·7 ± 1·93
Available lysine	1·33 ± 0·01

Changes induced on heating at 160°C for 30 min

The effect of heat on the moisture, total and alkali-labile (non-volatile) ammonia, glutamic acid and available lysine contents of the mixtures is shown in Table 2. All parameters measured, responded to heat indicating loss of glutamine (or acid), available lysine and increasing moisture and alkali-labile (but non-volatile) ammonia. There were no significant differences, though, between the total (mean 4·64 g/100 g) ammonia contents of the systems. Total and non-volatile (alkali-labile) ammonia contents were measured in an attempt to determine the amide loss, assuming only glutamine (and aspartamine) residues were capable of liberating ammonia on alkali treatment. This is obviously not the case and some of the products of the heat treatment must generate ammonia in the presence of alkali. Because the results show no particular trend, it is difficult to make positive claims about the amide contents of the various heated mixtures, but it would appear that free, gaseous (volatile) ammonia (0–1%) is produced in most systems. Recent work (Izzo *et al.*, 1993*a*) has shown some loss of glutamine (and perhaps aspartamine) during the extrusion processing of gluten.

More definitive statements can be made about the other reactants/products and, to aid clarification, the losses (or increases) in concentration have been corrected to moles/100g of protein in Table 3. It can be seen that, as expected, lysine becomes unavailable on heating, and in the absence of sugar this amounts to about 26% of the total available, but in the presence of sugar it rises to a maximum of about 46% of the total. This appears to be the limit to the amount amenable to react, suggesting some of the ϵ-amino groups in the gluten are protected from undergoing such condensations. It is possible that in a well mixed system, i.e. as in a screw-driven extruder, further groups may be made available for reaction. The loss of lysine will contribute to the formation of water but it seems very likely that

Table 2. The moisture, total ammonia, amide, glutamic acid and available lysine contents (g/100 g) of crude gluten and gluten–sugar mixtures heated at 160°C for 20 min. All values are the means (g/100 g material) ± standard deviation of six determinations except for the glutamic acid and the available lysine contents which are the means of three determinations

Mixture		Moisture	Non-volatile NH_3	Total NH_3	Glutamic acid	Available lysine
	Gluten	18·17 ± 0·24	3·96 ± 0·04	4·70 ± 0·66	22·5 ± 1·17	0·98 ± 0·02
+1%	Glucose	19·17 ± 0·24	3·88 ± 0·12	4·27 ± 0·32	22·1 ± 1·70	0·75 ± 0·02
+2·5%	Glucose	19·60 ± 0·19	3·94 ± 0·12	4·70 ± 0·23	20·6 ± 1·70	0·73 ± 0·04
+5·0%	Glucose	21·87 ± 0·53	3·84 ± 0·10	4·87 ± 0·39	17·7 ± 1·81	0·74 ± 0·03
+1%	Fructose	22·90 ± 0·71	4·19 ± 0·02	4·76 ± 0·39	19·9 ± 1·19	0·85 ± 0·04
+2·5%	Fructose	26·60 ± 2·43	4·21 ± 0·07	4·20 ± 0·41	18·1 ± 2·61	0·75 ± 0·07
+5·0%	Fructose	31·80 ± 1·87	4·32 ± 0·07	4·46 ± 0·35	16·4 ± 1·63	0·73 ± 0·07
+1%	Maltose	19·10 ± 0·86	3·95 ± 0·08	5·15 ± 0·51	20·5 ± 0·89	0·83 ± 0·02
+2·5%	Maltose	20·40 ± 0·56	4·22 ± 0·04	4·64 ± 0·38	20·3 ± 2·84	0·77 ± 0·02
+5·0%	Maltose	23·50 ± 0·80	4·59 ± 0·07	4·55 ± 0·43	20·0 ± 2·00	0·73 ± 0·07

reactions involving the glutamine residues are of most significance, though whether it is the amino group or the acid group formed by deamination on heating that reacts is not clear. Izzo *et al.* (1993*b*) have shown that the volatile products formed on heating deaminated gluten and native gluten differ suggesting both may (Table 3) be reactive. The lysine made unavailable in these systems is similar in all cases but it would appear that the extent and types of reactions involving the glutamine residues vary from sugar to sugar. In addition, condensation reactions involving lysine and glutamic acid are not apparently of paramount importance since, in the absence of added sugar, over 6 moles of glutamic acid/amine are lost for each mole of lysine. In the absence of added sugar each reacted mole of glutamine is associated with the production of about 30 moles of water (for glutamine plus lysine residues the value is about 27). The additional water generated in the sugar-containing systems varies with the sugar, yielding values of about 4·2, 16 and 21 moles per mole of glutamine for glucose, fructose and maltose, respectively. Though the number of moles of water produced per mole of glutamine lost is sugar-dependent it is apparent that, on a weight basis, fructose is the most reactive and maltose the least.

In all cases, the extent of reaction is far greater than could be explained by simple one-to-one condensation reactions with the reducing sugars, since the molar concentrations of the added sugar, 0·0025 moles/100g (1% maltose) to 0·027 moles/100g (5% fructose or glucose) is much less than the moles of glutamine plus lysine lost. This suggests some of the products of the reaction can react with further glutamine residues.

Table 3. The decreases in glutamic acid and available lysine and increase in water contents of gluten–sugar mixtures heated at 160°C for 30 min. Values are expressed as moles/100 g of protein

Mixture		Increase in moisture	Decrease in glutamic acid	Decrease in available lysine
	Gluten	0·71	0·023	0·0036
+1%	Glucose	0·80	0·028	0·0060
+2·5%	Glucose	0·85	0·045	0·0062
+5%	Glucose	1·05	0·081	0·0061
+1%	Fructose	1·09	0·051	0·0050
+2·5%	Fructose	1·39	0·072	0·0060
+5%	Fructose	1·84	0·096	0·0062
+1%	Maltose	0·80	0·045	0·0052
+2·5%	Maltose	0·91	0·048	0·0058
+5%	Maltose	1·18	0·055	0·0065

Numerous studies have shown that soya extrudates are primarily stabilised by disulphide bonds and hydrophobic interactions (Ledward & Mitchell, 1988) but these will only form as the extrudate cools either in the extruder die or on emerging from it. These observations are based primarily on reagents capable of breaking disulphide bonds and such weak forces as hydrogen bonds and hydrophobic interactions. However, even in these solvents some material (5–20%) is not solubilised Sheard, 1985). This suggests that other stable covalent bonds are present. These may actually form in the extruder and thus help create the initial structure which is further stabilised by the disulphide and hydrophobic interactions formed on cooling.

The authors have previously found that, on heating to 185°C for 35 min, soya isolate and a dialysed soya flour lost about 15% of their 'free' glutamic acid (or amine) (Ledward *et al.*, 1990) residues which, though slightly greater than the 10% lost on heating gluten under the milder conditions employed in the present study, is of a similar order of magnitude. However, a soya flour, presumably containing low molecular weight carbohydrate material, lost about 25% of its detectable glutamic acid (amine) residues, which is similar to the amounts lost in the present study in the presence of the sugars. Since proteins are rarely extruded in the absence of carbohydrate material, the different rates and extents of reaction of the glutamine (glutamic acid) residues with different carbohydrates (especially sugars and polysaccharide degradation products) may be of paramount importance in dictating the quality of the extrudate since they will decrease the viscosity of the mix by the production of water, from very stable intermolecular linkages, and thus contribute to the texture, produce varying degrees of browning and so modify the colour and finally generate volatiles that may affect the flavour.

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