Review

Molecular transformations of proteinaceous foods during extrusion processing

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Although extrusion cooking has been applied with great commercial success to the production of shaped pasta products and ready-to-eat breakfast cereals for over 50 years, its application to the texturization of vegetable proteins is a more recent innovation. However although the last 20 years or so have seen a great deal of activity in this area and textured products from protein-rich sources, especially soy flour, are now readily available, our understanding of the chemical changes involved are only now beginning to be understood. This review summarizes our current knowledge, highlighting the possible importance of electrostatic interactions in the extruder and the chemistry involved in the formation of covalent bonds that may be of importance in texture development.

To some extent extrusion processing must be considered a developing art rather than a technology. In 1986 Lillford[1] stated that chemical and physicochemical studies relevant to this complex process are few and far between and wrote 'The art is developing purely by empirical rules relating the contribution of each of the process variables and ingredients to the properties of the final products'. Even in 1986 this was deemed to be unsatisfactory and during the last eight years considerable effort has been spent in an attempt to understand the nature of the reactions occurring during extrusion.

Processing

Extrusion processing is a single-stage continuous operation that is efficient compared with traditional processes in terms of cost. It usually involves conditioning the material (e.g. de-fatted soy flour) to a moisture content of 15–40%; this is then fed through a feeder/hopper into the hollow barrel of the extruder, which is usually heated, where a rotating screw or screws force(s) the material towards the die. Generally the diameter of the screw increases towards the die and thus the material is compressed. This pressure increase is accompanied by a temperature rise as the result of both the transfer of heat from the heated barrel and the conversion of mechanical energy into heat energy. The usual residence time in the extruder is 30–60 s, after which the 'molten' material passes into the die section before it is squirted out into the atmosphere, where the spontaneous evaporation of water takes place.

The engineering aspects of extrusion processing are well understood, as to some extent are the rheological properties of the melts and the gross effects of such processing on the nutritional quality of the product[2]. Only in recent years have changes taking place at the molecular level received much attention and our knowledge

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base has vastly improved. However, our understanding is still far from complete.

In classical terms it was usually assumed that proteinaceous material denatures and then melts within the extruder barrel; this is associated with mixing and some limited alignment caused by the extrusion screws. Further alignment occurs within the die of the extruder prior to the mix being ejected, with the instantaneous evaporation of the superheated water leading to an expanded, porous product. Possible molecular structures within the shear field are shown in Fig. 1.

If we are to understand the chemical changes taking place in an extruder we must study systems at temperatures, pressures and imposed shear similar to those achieved during the process. Such changes, taking place in what is essentially an intermediate-moisture environment, will be reflected in the texture, colour, flavour and nutritive value of the product. In practice it is difficult to simulate all of these features, although model systems with some of the characteristics may be employed.

Phase distribution within the extruder

It has been argued that mixed biopolymer systems, if they are thermodynamically incompatible, will separate into two phases, each phase consisting primarily of one type of biopolymer. As reviewed earlier[3], several workers believe this may be of importance in extrusion. This undoubtedly happens at normal temperatures, but since this phase separation is thermodynamically unfavoured at the high temperatures and pressures obtained in extrusion processing the melt can be considered a relatively homogeneous system.

Chemical changes

Weak interactions

Most extrudates are virtually insoluble in water and numerous studies have concluded (on the basis of solubility work using sodium dodecyl sulphate (SDS) or urea, compounds capable of destroying hydrogen bonds and hydrophobic interactions) that these linkages contribute to the stabilization of the extrudate (Ref. 4 and Sheard, P.R., PhD thesis, University of Nottingham, UK, 1985). This is undoubtedly true, but it is highly
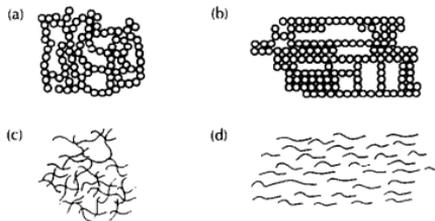
Fig. 1
Possible molecular aggregates formed during extrusion processing. Protein may form spherical molecules, either rather randomly aggregated (a) or oriented in the direction of flow within the die (b); alternatively the protein chains may aggregate as strands, either randomly (c) or oriented in the direction of flow (d). These possible configurations were suggested by Ledward and Mitchell[2].

unlikely that these types of interactions are of more than secondary importance since they will only form during cooling of the extrudate. At this stage it is assumed that the alignment of the proteins and the structure is already set and the hydrogen and hydrophobic interactions merely serve to subsequently stabilize the basic network.

Disulphide bonds

If extruded soy products are mixed with β-mercapto-ethanol, ~20–40% of the exudate usually becomes soluble, indicating that disulphide linkages make a contribution to the perceived or measured texture. Recent studies on whey have confirmed the occurrence of disulphide bonds in extruded products[5]. Earlier workers[4,6,7] assumed that it was primarily these disulphide bonds plus contributions from hydrophobic interactions and hydrogen bonds that were responsible for the texture of protein extrudates. This observation was primarily based on the fact that mixtures of SDS plus β-mercapto-ethanol normally led to almost complete solubilization of soy extrudates. About 10 years ago Sheard (PhD thesis) found that this solvent mix (1% SDS plus 1% β-mercaptoethanol) solubilized 69–92% of the extrudate, depending on the concentration of soy protein (Table 1); solubility was 100% with the unextruded flour at all concentrations. This suggests, since even at high β-mercaptoethanol : extrudate ratios some protein is insoluble, that other stable covalent linkages are formed, which although small in number make a significant contribution to stabilizing and setting up the extrudate texture. Again, since disulphide bonds break at high temperature, one would expect them to form only during cooling – i.e. in the die section of the extruder or even later – although they will undoubtedly form at a higher temperature than the weaker hydrogen bonds and even perhaps the hydrophobic interactions. It seems likely, therefore, that although disulphide bonds are numerous and important in stabilizing extrudate texture, other linkages are the key to setting up the initial structure.

Electrostatic interactions

Unlike most synthetic polymers, proteins carry either a net positive or negative charge, depending on their environment; only at their isoelectric point do they carry a net charge of zero. However, even if the net charge is zero the total charge on the protein will still be significant and this charge will not be distributed uniformly along the protein chain. In dilute solutions where the molecules are fully hydrated, repulsive and attractive electrostatic forces between molecules, although of significance, are only one of many possible interactions.

However, electrostatic forces are often assumed to be virtually athermal (their strength varies little with temperature) and thus it is tempting to suggest that it is primarily the charge and its distribution on the proteins and other macromolecules that dominate the interactions in the environment within the extruder barrel, where hydrogen, hydrophobic and disulphide linkages are unlikely to form. Although the shear imposed in the system will make long-range alignment difficult, short-range intermolecular forces should survive. Unfortunately, our knowledge of how the charge distribution on a protein varies with temperature, pressure and water content, i.e. under the conditions applying in the extruder barrel, is limited.

Pressure is known to increase the ionization of groups, such as the carboxylate and amino groups on a protein. Thus if temperature has little effect on ionization then it is likely that the total charge on a protein will increase, as it travels along the barrel of the extruder, in parallel with the increase in pressure. As the small amount of moisture in the system will limit the degree of hydration the ionized groups will be free to interact or repulse one another depending on whether they are of the opposite or the same charge.

Studies have looked at the effect of pH on the texture and colour of extruded products and although the results are complex there appears to be a marked dependence of both the diameter and the strength of the extrudate on pH. This work has been reviewed by Ledward and Mitchell[2]. Most studies suggest quite strongly that as the net charge on the protein (as assessed at room temperature and pressure) increases so the textural strength of the extrudate decreases while the ability of the extrudate to absorb water is increased, i.e. hydration occurs more

Table 1. Solubility in 1% SDS plus 1% β-mercaptoethanol of soy protein in an extruded flour[a]

Concentration of protein (%)	Solubility (%)
1	91
2	92
5	85
10	69

[a] The protein in the flour was totally soluble at all concentrations, up to 10%, in this solvent. Data taken from Sheard, PhD thesis.

rapidly and extensively. This is as would be expected if electrostatic forces are important since repulsion between like charges should lead to a more expanded and easily hydrated structure.

However, in view of the difficulty in modifying an extrudate in such a way that only the charge on the protein is changed, the importance of electrostatic forces during extrusion must still be viewed as speculative.

Loss of amino groups

The environment inside an extruder is ideal for Maillard or nonenzymic browning reactions to take place. These reactions, which involve the condensation of an amino group with a free or potentially free aldehyde or ketone group, are favoured by high temperatures and intermediate moisture contents. The chemistry involved in Maillard browning is complex but the final products are brown insoluble pigments. As the melt within the extruder often contains many free amino groups and also sources of potential carbonyl groups it is not surprising that extruded products are invariably brown, though the degree of browning varies quite markedly with both the processing conditions and the nature of the ingredients.

Most studies of the changes that protein undergoes during extrusion processing have concentrated on the free amino groups of lysine, and it has been established that the concentration of these groups decreases following extrusion[8]. The amount of available lysine, i.e. that which possesses the free amino group, decreases by up to 50%, depending on the severity of the processing and on the other ingredients. As well as markedly affecting the colour of the product this reaction is also of importance nutritionally since with cereal-based products lysine is the limiting amino acid; any decrease in the content of available lysine further decreases the nutritive value of cereal proteins. It has been argued that this loss of lysine is also important in developing the structure of the product, since covalent linkages so formed would be very strong[9]. The problem with this hypothesis is that lysine is usually found at low concentrations in proteins that are amenable to texturization by extrusion processing. Thus, even if lysine residues are reactive they are unlikely to account for all the covalent bonds required for effective texturization.

It is readily apparent that proteins that can be easily extruded (e.g. gluten and soy) contain very high concentrations of glutamic acid residues. These residues represent ~33% of the total amino acid residues in gluten and ~20% of those in soy protein. In addition, in the native proteins these residues invariably exist in the amide form so that they do possess a free amino group. It has been shown that deamidation does occur during the extrusion processing of gluten[10]. However, it is not clear whether the loss of amide is due to the evolution of ammonia from the residue, or whether the amino group itself takes part in nonenzymic browning reactions, or both.

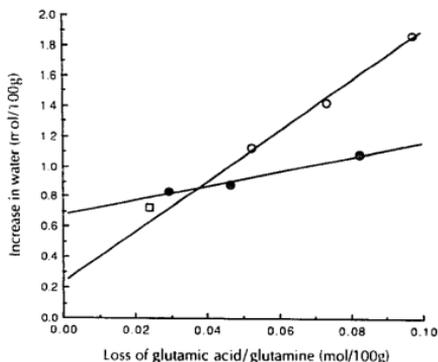We have recently shown that heating model closed systems to temperatures equivalent to those obtained in

Fig. 2

The relationship between the loss of glutamic acid/glutamine residues and the formation of water, after heating at 160°C for 30 min, in: (□), gluten (75% protein); (○), mixtures of gluten and fructose; and (●), mixtures of gluten and glucose. The sugar concentrations were 1, 2 and 5% (w/v) of the mixture; water generation (and glutamic acid loss) increased with increasing sugar content. (The data are calculated from the results of Yoong et al.[12])

the extruder gives rise to some loss of glutamic acid or glutamine residues[11]. For example, after heating a crude gluten preparation containing ~75% protein (the residue being water, starch, lipid and other carbohydrate material) at 160°C for 30 min there is a marked decrease in the content of glutamic acid residues. In the presence of small amounts of reducing sugars the decrease in glutamic acid levels becomes even more marked. There is an increase in the concentration of moisture (as determined by Karl Fisher analysis) in the system; moisture is one of the products of Maillard browning reactions. Not unexpectedly there is a relationship between moisture generated and glutamic acid/glutamine loss but this is dependent on the type and concentration of sugar present (Fig. 2). In soy systems similar decreases are observed and the decrease is more marked in native flour than in dialysed flour containing little soluble low molecular weight carbohydrate material[12].

Most of this work has been published very recently, but there is increasing evidence that glutamine or the acid derived by deamidation can undergo browning reactions during the extrusion of proteinaceous material, although extrapolating work from model systems to the environment in an extruder must be viewed with caution. However, such reactions may contribute to the texture as well as the colour and perhaps the flavour of the product. Such linkages will be very stable and certainly form at higher temperatures than disulphide bonds or hydrophobic interactions; they may well be the first permanent linkages set up during extrusion processing.

Conclusions

Though admittedly very speculative we suggest that for the successful extrusion processing of proteinaceous material the following mechanism may operate.

- At the high temperatures in the barrel of the extruder all the molecules and lower molecular weight components, including water, become mixed into a homogeneous semi-liquid/viscous phase.

- Within that phase, which is being subjected to shear, the large macromolecules align (interact) in such a way as to minimize electrostatic repulsion between them. When so aligned and/or at the same time potentially reactive groups on the protein and other material in the melt will undergo browning reactions. These will involve certain lysine residues and some glutamine/glutamic acid residues.

- Some fragmentation of the macromolecules may also occur as heat-labile bonds rupture. For example, disulphide bonds present in the proteins will rupture in the extruder but no new disulphide bonds will form until the system cools.

- On emerging from the high shear field of the extruder longer-range alignment occurs in the streamline flow in the die and further linkages, primarily disulphide bonds and some hydrophobic interactions, are then superimposed upon the structure already established and stabilized by glutamic acid/glutamine-derived crosslinks and electrostatic interactions.

The above proposed mechanism for developing texture and subsequently colour and flavour in an extruded proteinaceous material is speculative but could form the basis of fruitful research in coming years. However, major advances will depend on a full understanding of how the chemistry of such systems is affected by the imposed shears and pressures developed in extrusion processing.

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