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Aggregation States Of Cereal Endosperm Storage Proteins, With Reference To Functionality.

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Abstract

The relationship between the composition of the protein component of wheat and its functionality, and the change in gluten characteristics brought about by processing was investigated.

Buffered solutions containing 2% (w/v) of the anionic detergent sodium dodecyl sulphate, (SDS), were shown to extract maximum amounts of the endosperm storage proteins from cereal flours and wheat gluten, and to lend themselves readily to subsequent chromatographic and electrophoretic studies on the extracted proteins. It was shown that differences in protein aggregate size could not account for all of the differences in functionality observed between four cereals (wheat, barley, rye and oats) and between wheat varieties of different breadmaking quality.

Mixing of bread doughs from good quality flours caused depolymerisation of high molecular weight glutenin protein such that a greater percentage of the total protein was extractable in SDS. In contrast, protein extractability decreased during spaghetti manufacture as a result of aggregation of durum semolina proteins through sulphydryl-disulphide interchange reactions. The free SH groups in the low molecular weight glutenin fraction appear to be particularly involved. Reactions involving SH oxidation and cross-linking other than through disulphide bond formation, also occur during pasta processing. Glutenin aggregation was also observed during flour-water batter preparation using high quality flours. This aggregation process stabilised the batter and prevented separation of the gluten component. The aggregation capability of

flour proteins is, apparently related to the free SH content of the flour and the distribution of SH between high- and low molecular weight protein fractions.

Moisture uptake by commercial dry gluten was shown to increase rehydration and development times, and possibly to cause depolymerisation of glutenin proteins. However, gluten functionality in breadmaking was not affected by moisture uptake.

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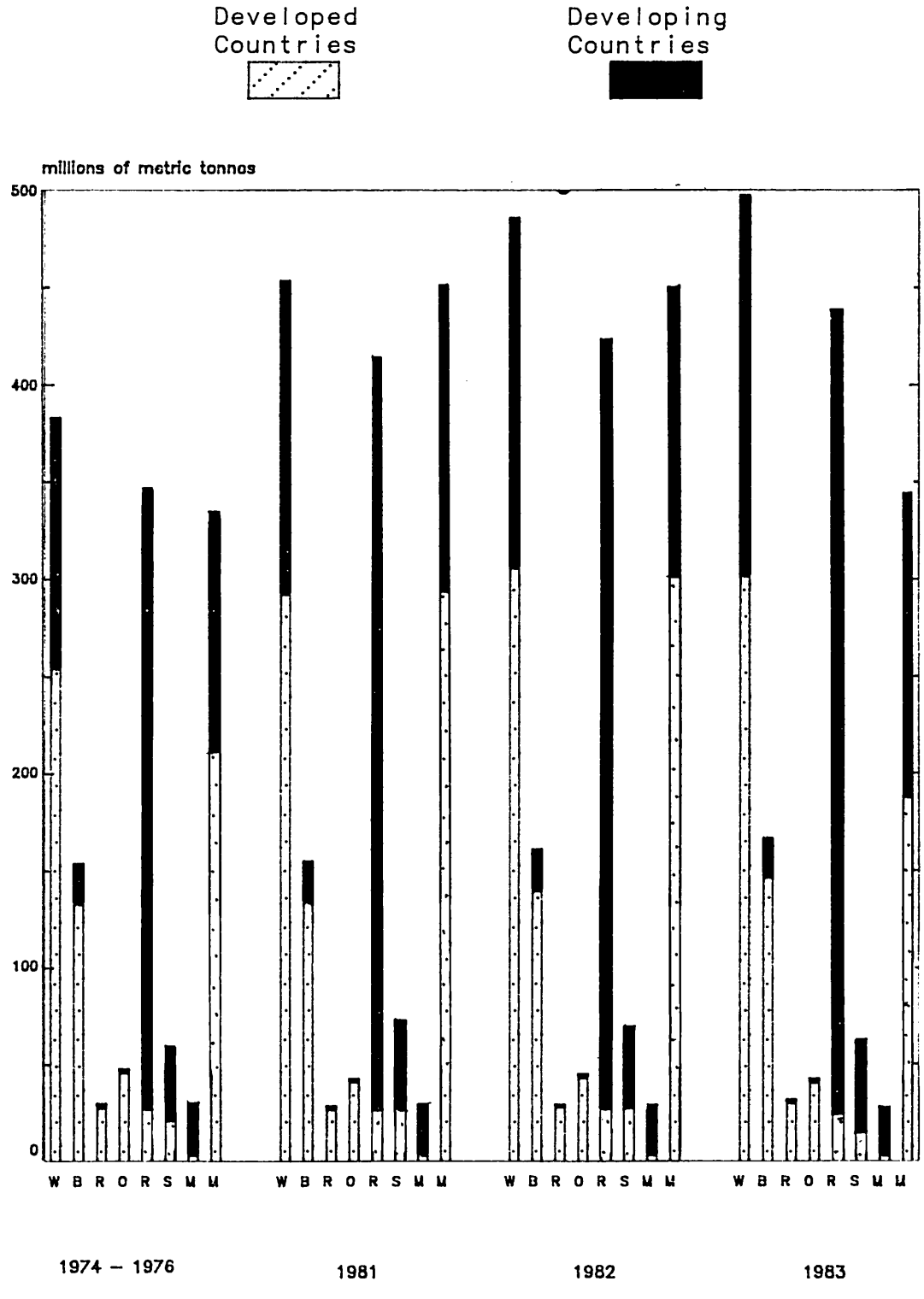
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Figure 1: World Cereal Production Between 1974 - 1983 (taken from the F.A.O. monthly bulletin of statistics, March 1984).

Cereals shown are (L -> R) wheat, barley, rye, oats rice, sorghum, millet, maize)



Chapter I: Structure and Functionality of Wheat Flour Proteins: A Review of the Literature

I.1 Introduction

Cereals are the fruits of cultivated grasses, members of the family, Graminae. The principal cereal crops are wheat, barley, rye, oats, maize, rice, sorghum and millet (Figure 1). Cereals provide approximately 50% of the world's protein supply, with wheat the single most important source. Although the nutritional value of cereal protein is limited by deficiences of certain essential amino acids, particularly lysine, their easy storage and useful functional properties have ensured that cereals, and especially wheat have remained important foods throughout history.

Wheat has many food uses. In Western cultures, most of the wheat used for human food is first milled into flour and then converted into bread. For example, in the UK, approximately 67% of all the flour produced is used in bread manufacture. This represents annual sales of about 2 million tonnes of bread products. Bread flours are prepared from hexaploid wheats, Triticum aestivum. When flour is wetted and mixed, a cohesive elastic dough is produced that can be expanded by the gases which result from yeast fermentation of sugars, and the dough can subsequently be baked into bread. The unique, cohesive properties of wheat doughs are attributed to the character of the wheat proteins, particularly the endosperm storage proteins, known collectively as gluten. If the gluten of a dough is too elastic, the structure will resist expansion by CO_2 and produce

a loaf of low volume, with a dense rubbery crumb texture. Conversely, if the gluten is insufficiently elastic, the gas cells will expand excessively, and coalesce, producing bread with a very open coarse texture. It is evident, therefore, that the gluten component of flour is of vital importance in determining wheat's breadmaking ability, and the quality of the final product.

These same viscoelastic properties also allow wheat flour to be used in a variety of other baked products, e.g. crackers, paste products such as pasta, noodles and chappatis, and to be fractionated commercially into the starch and gluten components. Pasta products are manufactured from the tetraploid wheat, Triticum durum. Pasta doughs have lower water contents than bread doughs, and gluten development occurs to a lesser extent. However, pasta quality, like bread quality is related to gluten strength.

The following chapter, reviews our knowledge of the relationship between the composition and functionality of the protein component of wheat and the changes in gluten characteristics that are brought about by processing.

I.2 Classification of Wheat Proteins

Most of the proteins of wheat flour are derived from the starchy endosperm of the grain and are made up of albumins and globulins, each of which accounts for about 10% of the total protein, and approximately equal proportions of gliadins and glutenins.

Traditionally, wheat storage proteins have been classified on the

basis of their extractabilities in various solvents. The most commonly-used procedures are based on the techniques originally devised by Osborne (1907), but since modified by numerous workers. Five fractions may be defined by such sequential extraction schemes; albumins, extractable in saline and remaining soluble on dialysis against water; globulins, extractable in saline and precipitating on dialysis against water; gliadins (wheat prolamins), extractable in concentrated aqueous alcohol solutions; glutenins (wheat glutelins) extractable in dilute aqueous acid or alkali; and a residual fraction that is not extracted, the "residue" protein. However, fractions produced by differential extractability are heterogeneous, and there is considerable overlap, particularly between the gliadins and glutenins, and between the glutenin and residue fractions (Bietz & Wall, 1975; Payne & Corfield, 1979; Jackson et al., 1983).

There is much evidence to support the proposal that gliadin and glutenin proteins should both be classified as prolamins. Glutenin polypeptides may be extracted in concentrated aqueous alcohol solutions in the presence of a reducing agent and dilute acid, particularly at elevated temperatures (up to 80°C) (Byers et al., 1983). Gliadins and glutenins are synthesised only in the developing endosperm of the wheat grain and are deposited in protein storage bodies (Shewry et al., 1984). The genes controlling the synthesis of gliadin and glutenin polypeptides are located on homoeologous group 1 and group 6 chromosomes and the genes for certain gliadin and glutenin polypeptides occur in very tightly-linked groups (Payne et al., 1984).

There are many similarities between glutenin and gliadin proteins.

Table 1: Amino acid composition of wheat protein fractions
(moles/1000)

	Albumin[a]	Globulin[a]	Gliadin[a]	Glutenin[a]	Gluten[b]
Asp	69.8	60.4	28.4	36.5	29.5
Thr	27.9	48.3	24.4	34.5	27.5
Ser	51.6	110.8	61.3	69.5	60.8
Glu	140.0	51.0	344.5	288.6	327.2
Pro	85.0	36.4	162.4	119.0	147.7
Gly	48.1	95.0	30.7	75.3	54.5
Ala	72.6	61.1	32.7	43.8	36.5
Cys	65.2	134.0	33.2	26.1	22.2
Val	80.9	23.6	47.6	48.1	46.7
Met	0.0	3.6	12.5	14.2	13.8
Ile	36.7	13.7	43.5	37.1	40.4
Leu	95.2	90.1	69.3	65.3	67.9
Tyr	21.6	16.3	18.3	24.8	26.0
Phe	35.5	24.5	42.9	36.4	40.3
Lys	87.3	106.4	5.7	19.8	12.5
His	32.1	17.7	18.6	19.0	18.0
Arg	50.1	106.5	19.9	29.8	22.2
Trp	ND	ND	4.3	12.6	6.4

a Kent, N.L. in "Protein as Human Food", Editor R.A. Lawrie, Butterworth & Co. Publ. Ltd., London, p280, (1970).

b Amino acid content of Foods and Biological Data on Proteins, F.A.O., (1970).

For example, both are rich in proline and glutamate and relatively poor in the basic amino acids, (Table 1). For both types of protein much of the glutamic acid is present in the amide form. This allows an extensive network of H-bonds to develop between the amide groups of glutamine side chains, which can act as both hydrogen donors and acceptors. This extensive H-bonding between glutamine side chains is of considerable importance in determining gluten's functional characteristics. Furthermore, the presence of relatively high levels of amino acids with hydrophobic side chains (approximately 30% of all gluten amino acid residues are apolar), means that there is considerable potential for hydrophobic interaction. Despite the similarities between gliadins and glutenins, there are, nevertheless, some important chemical and physical differences.

The gliadins are a heterogeneous group of proteins, present in wheat flour or gluten as simple proteins (i.e. comprising a single polypeptide chain) whose structures are stabilised by intramolecular disulphide bonds (Wall, 1979). In general, the gliadins contain a substantial proportion of α-helix (up to 35% in α-gliadin) (Wu & Cluskey, 1965; Cluskey & Wu, 1966; Wu *et al.*, 1967; Kasarda *et al.*, 1968) and lesser amounts ($\sim$10%) of β-pleated sheet. The gliadins mainly have molecular weights ranging from 30,000 - 45,000. One group, however, the w-gliadins have much higher molecular weights, between 75,000 - 79,000 (Booth & Ewart, 1969). These w-gliadins are not globular proteins, but are assymetric. They have been shown to possess large central domains that contain numerous β-turn conformations; these repetitive β-turns may, in turn, form a helical structure called a β-spiral (Tatham *et al.*, 1984). The w-gliadins are also noteworthy because of their deficiency of sulphur

amino acids, and, therefore, the absence of disulphide bonds.

In contrast, the glutenins are generally believed to exist as high molecular weight complexes of polypeptide subunits linked by disulphide bonds (Kasarda et al., 1977; Wall, 1979; Huebner et al., 1977). The subunit composition of glutenins is complex, with polypeptides ranging in molecular weight from 30,000 - 140,000 (Payne & Corfield, 1979; Huebner & Wall, 1980; Bottomley et al., 1982; Bietz & Wall, 1972). The disulphide-linked complexes are also extremely polydisperse with respect to molecular size. Estimates based on gel-filtration experiments indicate a range between 100,000 and several million (Payne & Corfield, 1979; Huebner & Wall, 1980; Bottomley et al., 1982). Examination of the glutenin complexes has indicated that those of lower molecular weight have a higher proportion of the lower molecular weight subunits, i.e. 30,000 - 50,000, than of subunits of 90,000 - 140,000 (Payne & Corfield, 1979). These low molecular weight glutenin complexes may be extracted to some extent by aqueous alcohol solutions and they have been termed high molecular weight gliadins by some workers (Bietz & Wall, 1972; Beckwith et al., 1966). However, sequence analysis (Bietz & Rothfus, 1971; Bietz & Wall, 1980) and 2-dimensional electrophoresis (Jackson et al., 1983) have shown that the the polypeptides that comprise the disulphide-linked complexes are different from those present in the monomolecular gliadin fraction.

I.3 Gluten Protein Structure and Dough Elasticity

The rheological properties of gluten (i.e. its combination of

viscous, elastic and cohesive properties), are generally considered to be responsible for conferring breadmaking quality on wheat flour. Wheat gluten in the hydrated state combines two physical characteristics:

1. It is capable of sustaining large deformation without rupture at low rates of shear, in which it resembles a viscous liquid.

2. It possesses the capacity to recover its original dimensions spontaneously on removal of stress, in which it resembles an elastic solid.

The unique structural feature common to all HIGH elastic substances is the presence of long polymer chains, which may be rearranged into other more highly extended configurations on the application of force (Miflin et al., 1983). An elastic material must also possess some permanence of structure, usually conferred by cross-links which join the polymer chains into a network of "infinite" extent. The strength of the network is, therefore, directly related to the number and strength of the crosslinks.

A protein network containing permanent crosslinks, can only be deformed elastically, i.e. the deformation disappears when the load is removed. Dough must be capable of deformation to allow for expansion as yeast fermentation proceeds, and also be sufficiently resistant to deformation and elastic to prevent the collapse of the dough structure. Under certain circumstances the deformation of dough is predominantly viscous or permanent, implying the breaking

of the rigid crosslinks (i.e. disulphide bonds). If the dough is to retain its cohesive elastic state, crosslinks must reform at the same rate at other sites. By reaction with sulphydryl groups, disulphide bonds can interchange and provide the required mobility.

Goldstein (1957) was the first to explain the permanent deformation in dough by the occurrence of thiol-disulphide interchange reactions. The reaction suggests the cleavage of a disulphide bond existing between two protein chains, by the action of a thiol group in a low molecular weight compound, such as glutathione. The protein chains are rearranged relative to one another, e.g. by brownian movement or mixing shear forces, and a new disulphide bond is then formed between the polypeptides at some point further along the chain. The thiol is thereby made available once again to initiate another exchange reaction. The reaction may be expressed as below:

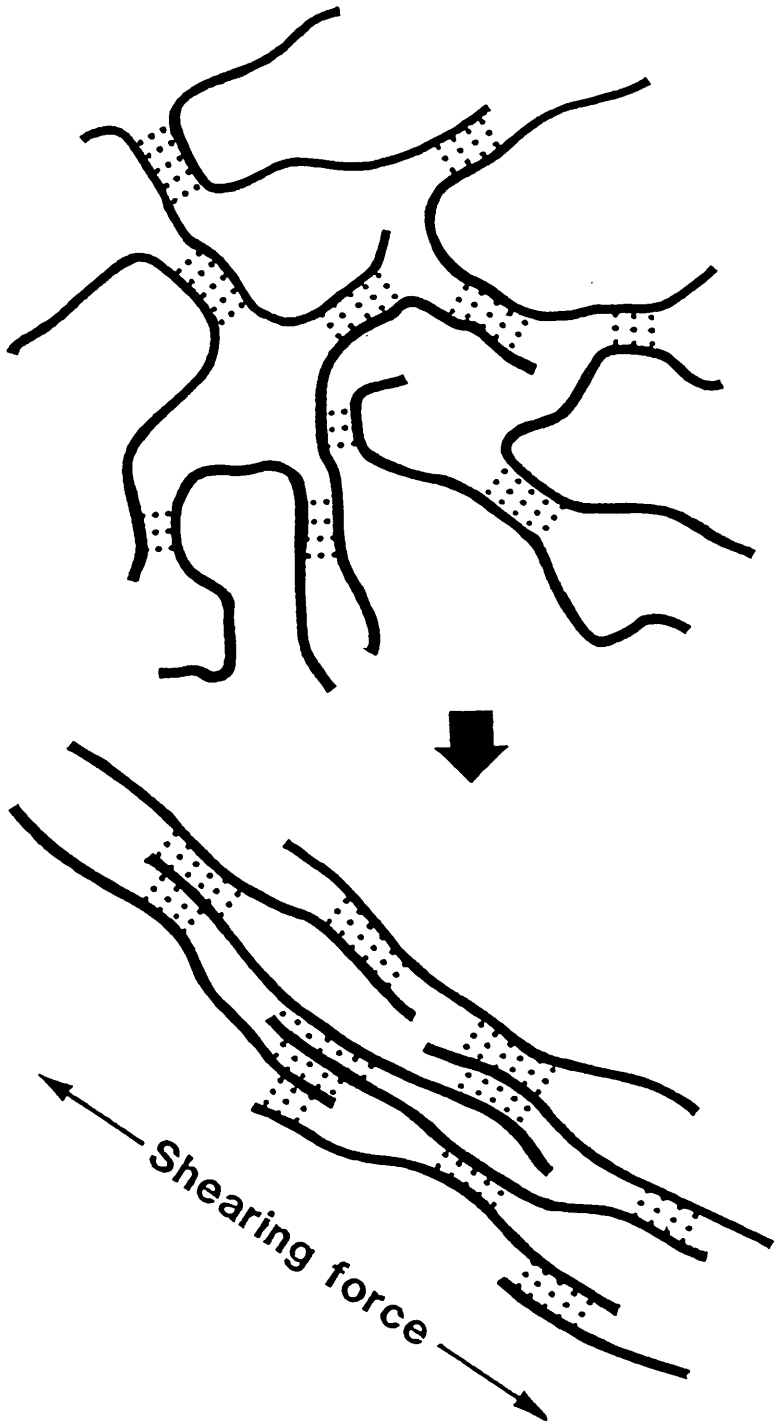
1. $R_1SSR_2 + XSH \longrightarrow R_1SH + R_2SSX$
2. $R_2SSX + R_3SH \longrightarrow R_2SSR_3 + XSH$

Overall

3. $R_1SSR_2 + R_3SH \longrightarrow R_1SH + R_2SSR_3$

The occurrence of such interchange reactions was demonstrated by McDermott & Pace (1961). These workers prepared a dough incorporating thiolated gelatin, and then isolated the gluten from it. The gluten hydrolysate was found to contain the amino acid hydroxyproline, which occurs naturally in gelatin but not in wheat proteins. Similar experiments have been reported using disulphides from other amino acids not normally found in wheat proteins

Figure 2: Schematic representation of the alignment of glutenin polymers as a result of the shear stresses imposed during dough mixing.



::: = Secondary bonds between glutenin polymers

Reproduced from Schofield, J.D. in "The Chemistry and Physics of Baking: Materials, Processes and Products". Editors J.M.V. Blanchard, P.J. Frazier & T. Galliard. Royal Society of Chemistry, London 1986. In Press.

(McDermott et al., 1969; Redman & Ewart, 1967, 1967a), using radioactively labelled amino acids (Mauritzen & Stewart, 1963; Stewart & Mauritzen, 1966) and also glutathione (Kuninori & Sullivan, 1968).

Ewart has put forward a model to explain both the structure and interactions of the gluten complex, and particularly the glutenin polymers which he considers the most important fraction in determining dough viscoelasticity (Ewart, 1972, 1977, 1979; Greenwood & Ewart, 1975). In this model, called the "linear glutenin hypothesis," the glutenin polypeptides are joined head-to-tail by an average of one interchain disulphide bond per subunit, to form linear polymers, which Ewart called "concatenations". Each subunit in the concatenation is envisaged as a coiled structure, which may be stretched out of its native conformation on the application of tension and regain its lowest energy state once the tension is removed.

According to this hypothesis, the glutenin polymers are oriented randomly with respect to one another at the onset of dough mixing (Figure 2). Although there are numerous interactions between molecules at their points of contact through weak non-covalent bonds, the overall contribution to the mechanical strength of the dough is small, because of the random arrangement of the molecules. On the application of shear in dough mixing, the non-covalent forces between adjacent glutenin polypeptides are disrupted and the polymers become aligned in the plane of the shear stress (Figure 2). New secondary bonds form as polymers come into contact, but, because of the alignment of the concatenations, these individually weak

forces now act co-operatively and as a result, dough strength is considerably increased. The monomolecular gliadins are thought to act as plasticisers in this model of dough structure, and to interact both amongst themselves and with glutenin molecules (Ewart, 1979). However, being relatively small molecules, they interfere with the ability of the glutenin polymers to form very extended co-operative networks associated through large numbers of non-covalent bonds. Gliadin does appear, therefore, to contribute to gluten's rheological characteristics.

In resting doughs, tension may be relieved without depolymerisation of the concatenations, through breaking and reformation of the disulphide bonds linking subunits together and slippage of molecules past on another. Dough breakdown, on the other hand, is explained in Ewart's model by the mechanical scission of S-S bonds when shear stresses induced by mixing cannot be relieved rapidly enough either by molecular slip or SS/SH interchange. Thus, covalent bonds holding subunits together are "pulled apart". Scission most probably occurs at disulphide bonds in the central region of the concatenations where tension is greatest, because of the probability that the largest number of effective secondary forces are distributed about the mid-point of the chain. Cleavage of SS bonds would produce thiyl radicals (Axford & Elton, 1960; Redman et al., 1966) which might be converted to SH groups by hydrogen abstraction from water, or as suggested by Sidhu et al., (1980) might react with activated double bond compounds. The overall effect, would, therefore, be reduction of the average molecular weight of the glutenin concatenations.

A basic requirement of Ewart's model, is the existence of glutenin polymers which are made up of polypeptide subunits linked together by interchain disulphide bonds. Glutenins extracted from flour or dough using strongly-dissociating solvents, have been shown by gel-filtration chromatography to comprise extremely large molecules with estimated molecular weights up to several million (Huebner & Wall, 1980; Payne & Corfield, 1979; Bottomley et al., 1982). Furthermore, addition of reducing agents to glutenin extracts reduces the molecular size dramatically, such that on gel-filtration chromatography, essentially all the protein appears as components with molecular weights of 10^5 or below (Bottomley et al., 1982). These findings have also been confirmed by viscosity measurements on solutions of glutenin. Glutenin solutions have very high intrinsic viscosities that are reduced markedly on addition of reducing agents (Ewart, 1979, 1980).

Amino acid sequence analysis of purified high molecular weight glutenin polypeptides, has demonstrated that cysteine residues are located near the N- and C- terminal ends of some subunits, but not in the central region (Shewry et al., 1984; Forde et al., 1983), Furthermore, cleavage of polypeptides specifically at cysteine residues results in only a small decrease in molecular size for certain glutenin subunits, indicating that the cysteine residues in these are located near the end(s) of the polypeptide chains (Field et al., 1982; Moonen et al., 1985). Thus, at least some glutenin subunits exist that are capable of being linked head-to-tail, as required by the Ewart model. However, not all glutenin subunits are alike in this respect. Cleavage at cysteine residues results in a marked reduction in molecular weight, indicating that in some

glutenins, one or more cysteine residues must be located towards the centre of the polypeptide chain (Moonen et al., 1985). It has been proposed that low molecular weight glutenin subunits are bound into the glutenin complex via these centrally located cysteine residues (Graveland et al., 1985).

Computer prediction of the conformational structures present in certain high molecular weight glutenin subunits, based on the known amino acid sequences, has indicated the likely presence of α-helix-rich domains at the N- and C- terminal ends of the polypeptide, and of numerous repetitive β-turn structures in the central region. Circular dichroism spectroscopy has confirmed that these high molecular weight glutenins are rich in β-turns (Tatham et al., 1984a).

The β-turn conformation appears to be a common feature in proteins possessing elasticity. In elastin, for example, it has been suggested that the numerous β-turns may result in the formation of a linear helix, the β-spiral, which may be the structure that confers elasticity (Tatham et al., 1984a). The occurrence of repetitive β-turns in some glutenins could also result in the formation of β-spirals.

Thus, the presence of repetitive β-turn structures, the fact that glutenin polypeptides may be cross-linked through interchain disulphide bonds in the N- and C- terminal regions, and that the polymers may interact through non-covalent bonds, could well account for glutenin's elasticity. Evidence appears to be accumulating, therefore, in support of the essential features of the Ewart model.

I.4 Role of Sulphydryl Groups and Disulphide Bonds in the Rheology of Doughs

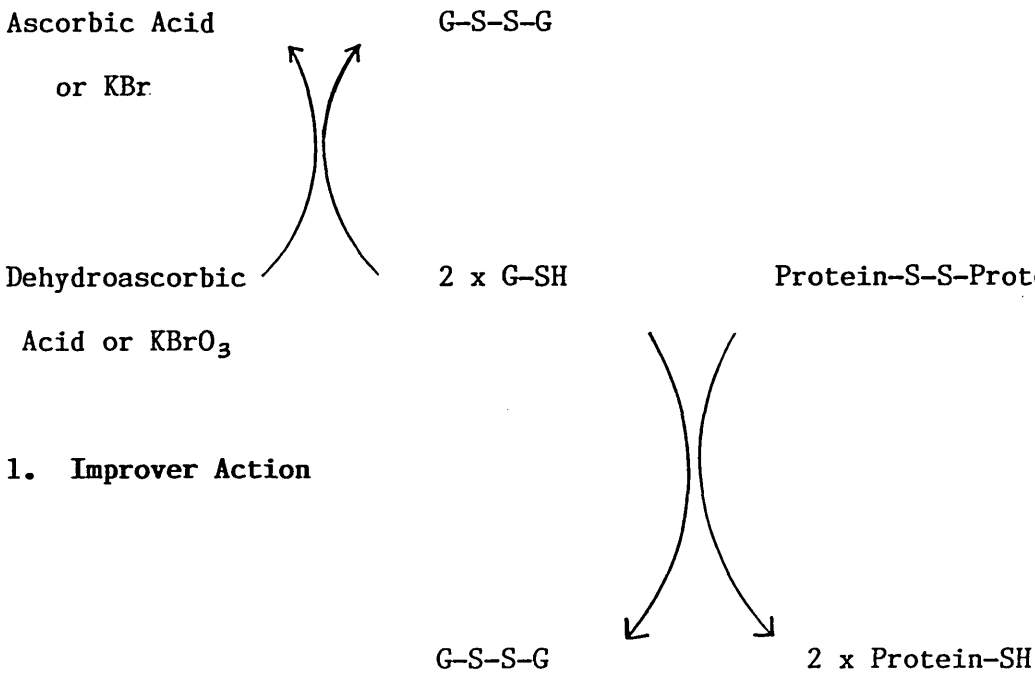
Evidence for the central role played by disulphide bonds in dough structure and rheology has accumulated from a number of observations. Addition of reducing agents such as cysteine, produces a very soft, extensible, inelastic dough, and the resultant bread has a poor open crumb structure due to coalescence of gas cells during proving and the early stages of baking. The action of reducing agents may be interpreted as both lowering the number of intermolecular SS bonds by reduction, and/or increasing the rate of SS/SH interchange. Strains induced in dough by the input of work are relieved more rapidly in the presence of cysteine. The exact location of the SH and SS groups that are rheologically important in dough mixing is unclear. Bloksma (1972) showed that the rheologically-effective fraction of SH and SS groups was only a small proportion of the total flour thiol and disulphide content. This difference in rheological effectiveness of flour thiol groups may be related to their distribution in the various protein fractions and non-protein compounds.

Thiol compounds analogous to cysteine occur naturally in flour, being derived mainly from the outer layers of the grain and the germ (Jones & Carnegie, 1969; Hird et al., 1968). Of the thiol compounds detected, only glutathione, (GSH), and its oxidised disulphide-linked dipeptide form, have demonstrable rheological activity in doughs (Jones & Carnegie, 1969a), and a negative correlation has been demonstrated between GSH content of different varieties of wheat and dough rheological properties as represented

by Farinograph mixing tolerance (Mair & Grosch, 1979), and between GSH content and flour quality tests, such as the Pelshenke fermentation and the Zeleny sedimentation tests (Archer, 1972; Coventry et al., 1972).

The available evidence suggests that SH/SS interchange reactions during dough mixing take place between reduced glutathione and protein disulphides, and between protein SH groups and oxidised glutathione (Redman & Ewart, 1967; Mauritzen & Stewart, 1963; Jones & Carnegie, 1971). The findings of McDermott & Pace (1961) suggest that similar reactions also occur between the SH and SS groups of protein molecules only.

Although some cleavage and reformation of protein disulphide bonds mediated by glutathione may be necessary to allow stress relaxation in a dough to occur, an excessive level of disulphide bond cleavage leads to deleterious effects on the dough's functional properties. The effects of oxidative dough improvers, such as bromate and ascorbate are often ascribed to reactions involving glutathione rather than protein SH groups directly, and these reactions have been regarded as controlling the levels of GSH so that excessive disulphide bond cleavage does not occur. The competing reactions that have been proposed are illustrated below:



1. Improver Action

2. Rheologically-deleterious Reaction

Oxidative dough improvers, such as bromate and ascorbate, may "control" levels of GSH **(1)** so that excessive protein disulphide bond cleavage **(2)** does not occur.

The actual improver reaction of ascorbic acid in conventional dough systems does not involve ascorbic acid itself, but rather dehydroascorbic acid. It appears that this improver reaction is enzymically catalysed involving reduced GSH as co-substrate (Sanstedt & Hites, 1945). The presence of an enzyme to catalyse this reaction, glutathione dehydrogenase, has been demonstrated in flour extracts (Sanstedt & Hites, 1945). The overall effect of reduction of dehydroascorbate and co-oxidation of glutathione, is to remove the rheologically-deleterious thiol peptide from the dough system.

The conversion of ascorbic acid to dehydroascorbate is the reaction over which there is greatest debate. Early work (Sanstedt & Hites, 1945) suggested both an enzymic and non-enzymic mechanism, and subsequent studies demonstrated the presence of an enzyme, ascorbic acid oxidase, in extracts of wheat flour (Kuninori & Matsumoto, 1963; Grant, 1974; Grant & Sood, 1980). However, some ascorbic acid analogues are known not to be substrates for ascorbic acid oxidase, and yet are oxidised in doughs (Sanstedt & Hites, 1945). Therefore, whether or not this enzyme is active in doughs is uncertain and the relative rates of the enzymic and non-enzymic reactions remains unclear. Mair and Grosch (1979) showed that GSH is rapidly oxidised on dough mixing, through the action of the enzyme glutathione dehydrogenase.

It is, therefore, clear that flour protein SH and S-S groups are of considerable importance in determining the rheological properties of dough, and that modification of reactions between these moieties may form the basis of the mechanism by which dough improvers, such as bromate and ascorbate, exert their effects.

I.5 Protein Molecular Weight Changes during Dough Mixing

In all baking procedures, the flour components (especially the proteins) are first hydrated and then by the application of shear stress are re-oriented to produce a dough with the appropriate rheological properties. Several groups of workers have demonstrated that flour protein extractability increases on dough mixing (Mecham et al., 1962; Tsen, 1967; Tanaka & Bushuk, 1973) and that the increase in extractability is related to dough mixing time and

mixing speed. Further increases in extractability are observed if SH-blocking reagents are added to doughs. These observations led to the suggestion that during dough mixing, protein agglomerates or aggregates are broken down (Mecham, 1968). Tsen (1967) observed that as mixing proceeded, increasing amounts of high molecular weight glutenin were extracted by acetic acid. When the changes were examined in relation to the mixing properties of various flours, it was found that the extractability of the high molecular weight component increased more rapidly in weak than in strong flours. Mamaril & Pomeranz (1966) also observed an increase in the proportion of high molecular weight proteins in extracts from doughs mixed in air, and additionally noted a decrease in the low molecular weight proteins. These workers suggested that the low molecular weight proteins became linked covalently to high molecular weight proteins. However, subsequent studies gave no evidence of polymerisation of low molecular weight proteins (Meredith & Wren, 1969; Tanaka & Bushuk, 1973a). In simple flour/water doughs, no change in the molecular weight distribution was observed when proteins were extracted and chromatographed in strongly-dissociating solvents, suggesting that increases in acetic acid extractability on dough mixing, were due to disruption of non-covalent bonds rather than depolymerisation due to covalent bond cleavage. However, disulphide-sulphydryl interchange reactions involving no change in molecular size cannot be ruled out.

Danno & Hoseney (1982, 1982a) reported increases in protein extractability on dough mixing, using solutions of sodium dodecyl sulphate (SDS) and short extraction times (2h). Additional material extracted, was shown to be glutenin. SDS-extracts from over-mixed

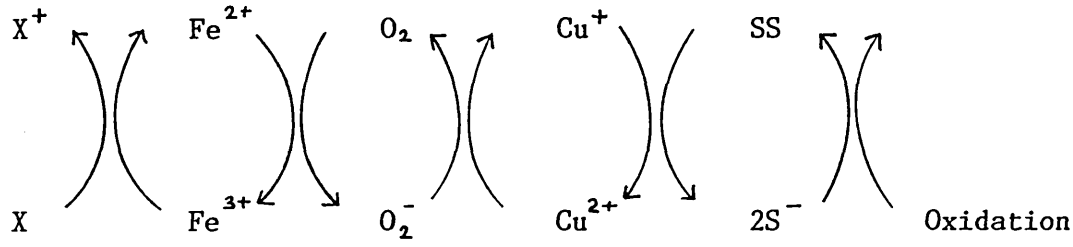
doughs had lower viscosity than those from optimally-mixed doughs, but molecular weight distributions in the extracts were essentially unchanged. They concluded that sufficient disulphide bonds were cleaved on overmixing to affect the viscosity of extracts, but that gel-filtration chromatography was insufficiently sensitive to detect changes in molecular weight.

Graveland and co-workers (1980) described an extraction procedure which produced an SDS-insoluble gel protein layer with an estimated molecular weight of >20 million. Graveland et al. (1984) subsequently demonstrated that the gel protein (Glutenin I) probably comprises a backbone of high molecular weight glutenin polypeptides (subunits 10 [allelic counterpart 11], 1 or 3 [allelic counterpart 2], 5) linked head-to-tail via disulphide bonds, to which lower molecular weight glutenin polypeptides were also linked through SS bonds. The size of the polymer was determined by the reactivity of the terminal SH groups on the subunits 1 or 3, and 5, which in Graveland's model were responsible for the polymerisation.

On dough mixing the protein gel layer disappeared (i.e. was rendered extractable in SDS solution) which Graveland et al. (1984) interpreted as depolymerisation of the Glutenin I polymer through reduction of specific SS bonds, i.e. those SS bonds linking the HMW glutenin subunits in head-to-tail configuration. On resting the dough, in conditions analogous to those employed in fermentation and proving, the gel layer reappeared, which was interpreted as repolymerisation of the Glutenin I.

Depolymerisation of Glutenin I during dough mixing required the

presence of oxygen, a reducing compound, identified as 3-methoxy-hydroquinone-β-cellotrioside, and a metal-protein complex, consisting of at least two proteins of molecular weight 60,000, which contained the metals, Iron and Copper (Graveland et al., 1984). The reduction reaction of Glutenin I is represented below:



X = (3-methoxy-hydroquinone)-β-cellotrioside.

Graveland et al. (1984) proposed that in a normal dough system, bromate might replace oxygen, that is, accelerate depolymerisation. In contrast, oxidation of ascorbic acid by the enzyme, ascorbic acid oxidase, would remove oxygen from the system, and so inhibit depolymerisation of Glutenin I.

I.6 Chemical Bonds and Hydrophobic Interactions in Doughs

Much of the preceding discussion has considered the covalent bonds which are known to be of great importance in flour/water systems, i.e. the disulphide linkages between protein chains. However, the role of the other bonds and interactions is not be under-rated. The importance of ionic bonds in dough structure may be demonstrated by the addition of salt. Ions in a dough may complex with the ionic groups of lipids (Fullington, 1967), proteins (Bennett & Ewart, 1965) and pentosans (Neukom et al., 1967). Theoretically, ions may

enhance both association and dissociation of dough components, however, in practice the former predominates and addition of salts increases dough rigidity and decreases extensibility. Preston (1981) examined the effects of neutral salts on the properties of wheat gluten proteins, particularly their solubility. Changes in extractability resulting from increasing concentrations of simple monovalent salts are attributed to their effects on electrostatic and hydrophobic bonds. At low salt concentrations, these effects are largely due to changes in the electrostatic free energy associated with the shielding of charged amino acids on the surface of the proteins. At higher salt concentrations when the electrostatic interactions between charged amino acids have effectively been neutralised, increases in salt concentration promote:

a) Salting-out of the proteins as a result of their hydrophobic character and the nature and concentration of the ion, i.e. the ability of the ion to "order" the water structure, and so, alter the free energy resulting from entropy changes when apolar amino acids are exposed to solvent.

b) Salting-in which is dependent on the ionic strength and the dipole moment of the protein, resulting from non-specific binding of salt ions to polar sites on the protein.

Preston demonstrated, that at low salt concentrations, all the ions examined (i.e. F^-, Cl^-, BrO_3^-, Br^-, ClO_4^-, I^- and SCN^-) had a salting-out effect on proteins, which was attributed to the electrostatic shielding of ionic amino acids on the protein surface.

Thus, low levels of salts diminished the electrostatic repulsion between proteins and reduced extractability. Whereas, at higher salt concentrations, when all electrostatic interactions had been neutralised the effect of the salts followed the lyotropic series in promoting extractability, i.e. chaotropic anions, increased extractability, while non-chaotropic anions decreased protein extractability. These differences in extractability and aggregation characteristics can be attributed to the hydrophobic character of the gluten proteins.

Greene and Kasarda (1971) showed that the high sensitivity of wheat proteins to salt concentration was the result of:

a) low charge density on their surface,
b) presence of hydrophobic regions,
c) presence of many amino acid residues capable of H-bonding.

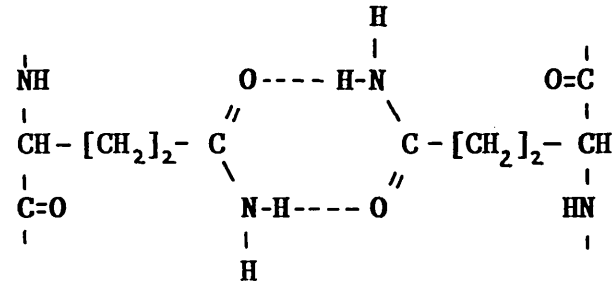
A concentration of 0.005M NaCl was sufficient to mask surface charged groups and neutralise electrostatic repulsions between two storage proteins, thereby, allowing the molecules to approach one another and develop hydrophobic and hydrophilic interactions. Bernardin (1978) calculated that in most standard dough recipes ionic compounds were present in sufficient quantities to overcome electrostatic repulsions and promote A-gliadin aggregation (below).

Concentration of ions in doughs, and potential to promote protein aggregation

Ion	Molar concentration required to promote equivalent aggregation to 0.005M KCl, pH 5.0	Molar Concentration in dough liquor
NaCl	5×10^{-3}	2% = 1.5
Na Fumarate	5×10^{-4}	2000ppm = 6.2×10^{-2}
Na Phytate	4×10^{-4}	300ppm = 1.7×10^{-3}
Na Ascorbate	1×10^{-3}	75ppm = 1.7×10^{-3}

In gluten proteins, the high levels of glutamic acid, i.e. ~30% of all amino acids, and aspartic acid and the fact that the bulk of their side chains are present in the amide form, leads to the development of an extensive system of H-bonding (Wall & Beckwith, 1969). Synthetic homopolypeptides of glutamine have been reported to form β-pleated structures that are stable under very harsh conditions. The stability of the structure results from the formation of numerous H-bonds, the amide groups of the glutamine side chains acting as both H-bond donors and acceptors (Krull *et al.*, 1965), as illustrated below:

Hydrogen bonding between glutamine residues in adjacent polypeptide chains



The viscoelastic properties of flour/gluten proteins are impaired or lost if the amide groups are converted to carboxyl groups by mild acid hydrolysis (Holme & Briggs, 1959). Beckwith and co-workers (1963) also demonstrated the important contribution of the amide group to the properties of wheat gluten, by replacing some of the amide with ester groups. Since ester groups have a smaller dipole moment than amide groups, they exhibit lower tendency to engage in H-bonds. Thus, the cohesive elastic characteristics of hydrated gluten proteins, which are in part ascribed to associations through H-bonds were progressively lost in the course of the amide -> ester conversion. When flour or gluten is solvated with deuterated water (D_2O) instead of ordinary water (H_2O), the rheological properties are altered such that the protein matrix appears mechanically stronger (Kretovich & Vakar, 1964; Tkachuk & Hlynka, 1968). This is the result of the greater strength of deuterium bonds compared with hydrogen bonds. These observations suggest, that the numerous H-bonds, though individually weak, are collectively very important in the interactions between wheat proteins.

MacRitchie (1979) demonstrated a statistically significant

correlation between the degree of gluten protein amidation and the baking performance of 37 flours milled from Australian wheats, representing 10 different varieties. However, a similar analysis of the gluten proteins from 36 UK wheats, representing 20 different varieties gave no such correlation (Ewart, 1982). Thus, it remains unclear, whether or not variation in the level of amide groups in gluten proteins is related to the breadmaking potential of a flour.

Van der Waals forces, and dipole-dipole interactions provide very weak links (0.5 kcal/mole) between proteins. Although these forces exist between all atoms, they are insignificant in the presence of stronger bonds, or at distances greater than 0.5nm. Van der Waals forces may play a role in attractions between nonpolar amino acid residues or fatty acid side chains in systems where hydrophobic bonds are not formed.

Another type of interaction that is considered to play an especially important role in the stabilisation of native conformation, and also in the aggregation and binding of proteins, is the hydrophobic interaction. Such interactions occur because in an aqueous environment there is a favourable free energy change when non-polar hydrocarbon amino acid side chains (e.g. those of valine, leucine, isoleucine and phenylalanine) come together, by the appropriate folding of a polypeptide chain, or through aggregation of two protein subunits which have non-polar side chains on their surface (Kauzmann, 1959; Tanford, 1980). Experimental evidence of the importance of hydrophobic interactions has been obtained by modifying dough properties with organic solvents (Ponte *et al.*, 1967) and hydrocarbons (Pomeranz *et al.*, 1966). Hydrophobicities of

glutenin and gliadin, calculated from data on their amino acid composition, are reported as 1016 and 1109 cal. per average amino acid residue respectively (Woychik et al., 1961). Hydrophobicities of proteins range from 400–2000 cal. per average amino acid residue, thus, on a theoretical basis, the hydrophobicities of gluten proteins are sufficient to allow inter- and intramolecular hydrophobic interactions to occur. Preston (1984) used simple neutral sodium salts of the lyotropic series to study the hydrophobic properties of wheat gluten proteins. Large increases in the extractability of gluten proteins across the lyotropic series suggested that wide differences existed in gluten protein hydrophobicities, and that glutenin-like proteins "required " the most chaotropic conditions for extraction, whilst low molecular weight gliadins and albumin-like proteins required the least.

A model for glutenin polymer structure has been proposed (Bernardin & Kasarda, 1973; Kasarda et al, 1977) in which the subunits are not covalently bonded, but are linked into very stable complexes through hydrophobic interactions. The disulphide bonds in glutenins are all envisaged to be intramolecular bonds which stabilise the three dimensional structure of each subunit. Baianu & Schofield (1982) applied methods for obtaining high resolution carbon-13 nuclear magnetic resonance (NMR) spectra from solids to the study of gluten and gluten subfractions. They demonstrated differences between the spectra of gliadin- and glutenin-enriched fractions. They suggested that the differences were related to the tightness of folding of the constituent polypeptide chains, and to the degree of mobility and interaction of the hydrophobic amino acids. However, more recent studies (Belton et al., 1985; Moonen et al., 1985) have shown that

no characteristic differences in the molecular structure of prepared wheat protein fractions can be recognised by NMR spectroscopy. These workers attributed the sharp resonances in the aromatic and aliphatic regions described by Baianu & Schofield (1982) to impurities in their materials.

Evidence in support of the model of Kasarda and co-workers was apparently provided by observations of the effect of sodium salts of fatty acids on glutenin solubility (Kobrehel & Bushuk, 1977). Molecular weight estimates of 40,000 - 50,000 were obtained for polypeptides extracted by soaps from glutenin preparations, suggesting that the glutenin had been dissociated completely to relatively low molecular weight subunits, without cleavage of disulphide bonds (Kobrehel & Bushuk, 1978). However, later work from the same laboratory showed that most of the protein extracted by sodium stearate was still in an aggregated form, and could be broken down to subunits only after reduction of disulphide bonds (Hamauzu et al., 1979).

Kobrehel & Matignon (1980) have indicated a relationship between baking quality and the level of soap required to solubilise flour proteins, particularly the high molecular weight glutenins; less soap was required to extract the maximum amount of protein from wheat varieties of poor breadmaking quality. Electrophoretic analysis of the extracts appeared to indicate that more soap was required to extract certain high molecular weight glutenin subunits from good breadmaking varieties. The implication was that protein of poor quality wheats was less strongly aggregated through hydrophobic interactions than that of good breadmaking varieties.

Popineau (1985) applied hydrophobic interaction chromatography, (HIC), to the acetic acid-soluble proteins of wheat gluten, and showed that while some glutenin subunits had hydrophobicities similar to those of the gliadins, others were significantly more hydrophobic. He also observed that glutenin aggregates differing in surface hydrophobicity differed in their subunit composition. Chung & Pomeranz (1979) indicated that acid extractable glutenins from a good breadmaking wheat were more strongly adsorbed to a phenyl-Sepharose CL-4B column, than were those from a poor breadmaking wheat; gliadins from the same flours had similar hydrophobic properties. Again the implication was that glutenins from better breadmaking wheats are more hydrophobic, and that this relates to differences in protein functionality. However, recoveries of glutenin after HIC were 50% or less, and only a small number of varieties was examined, making reliable interpretation of the data very difficult.

From the preceding discussion, it is evident that hydrophobic interactions, undoubtedly, make an important contribution to the physical properties of gluten. However, there is insufficient data available, to conclude whether or not differences in breadmaking quality can be attributed to differences in the extent of hydrophobic interactions between flour proteins.

I.7 Gluten Protein Quantity and Composition: Its Relationship with Breadmaking Performance

The desire to understand the molecular basis of baking quality in flour has prompted many studies that have demonstrated a

relationship between various wheat characteristics and breadmaking performance. Finney & Barmore (1948) showed that a positive correlation existed between protein content and loaf volume for a series of North American flours, using a traditional fermentation baking procedure. They also demonstrated differences in the slopes of the regression lines which were attributed to differences in flour protein quality. Since a large proportion (some 80-85%) of wheat flour protein comprises the gluten complex, (described earlier), which is largely responsible for the dough properties and breadmaking ability, it is not surprising, that a good positive relationship exists between the quantity of gluten present in a flour and its baking performance (Webb et al., 1971). However, flours of equivalent protein content may have gluten that is too extensible, insufficiently extensible or with optimal rheological properties. Thus, not only is the protein and/or gluten content important in terms of a flour's baking performance, but so too are the qualitative characteristics of the protein/gluten complement.

The physical properties of dough are a combination of the properties of hydrated gliadin, which is soft and extensible, and glutenin which is firm and elastic. Thus, it would be expected that the relative proportions of gliadin and glutenin would account for the overall dough properties; a strong dough which is overly-resistant to extension would be expected to contain a higher proportion of glutenin, whilst a weak, extensible dough would be the result of a higher proportion of gliadin proteins.

Although some differences in the quality and quantity of gluten components in relation to different dough making capacity of wheats

have been established, efforts to elucidate the exact nature of the relationship have produced many contradictory results.

Orth & Bushuk (1972) showed that loaf volume per unit of flour protein (ULV) was inversely correlated with the amount of acid soluble protein, in a study of 26 spring wheats, and also demonstrated a positive correlation between ULV and the proportion of insoluble residue protein, as defined by a modified Osborne fractionation procedure. Other workers have reported similar findings (Mecham & Cole, 1972; Cole & Mecham, 1973) and shown that the breadmaking potential of a flour may be improved by altering the relative proportions of high and lower molecular weight gluten proteins. Thus, weak flours could be turned into strong ones by the addition of a high molecular weight fraction of residue protein (MacRitchie, 1973), and flours which produced tough doughs made more extensible by the addition of lower molecular weight gluten protein.

Booth & Melvin (1979) evaluated the importance of gluten, starch and water-soluble proteins in determining the baking quality of flours of Maris Huntsman, a poor quality high yielding European wheat, and a mixed grist of Canadian Western Red Spring (CWRS) wheats. They showed that the difference in baking quality was associated solely with the gluten component and not with the starch or water-soluble material, and so confirmed previous findings of Hoseney et al. (1969).

CWRS contained a greater proportion of acid-insoluble residue protein than Maris Huntsman, i.e. 49% compared with 17% of total gluten protein, highlighting the importance of the quantity of this

high molecular weight material in flour. However, bread from Maris Huntsman was not significantly improved when the proportion of insoluble protein was increased to a level similar to that found in CWRS, which confirmed that qualitative factors as well as quantitative ones are important. Fractionation of the gluten into lactic acid soluble and insoluble proteins followed by reconstitution, substituting CWRS components with the corresponding fraction from Maris Huntsman showed that both the acid-soluble, and acid-insoluble fractions were "defective" in Maris Huntsman flour, and replacement of either class with the equivalent from CWRS greatly improved baking performance. In contrast, Hoseney et al. (1969a) found that the lactic acid insoluble fraction had no particular role in detemining loaf volume, only mixing requirement.

A prediction of the Ewart model of dough structure, discussed previously, is that dough strength and hence baking performance, should be related positively to the lengths of the disulphide linked glutenin polymers. Evidence for this has come from a number of studies. Glutenin molecules are of such a size that a proportion of them (the largest) is unextractable under certain conditions. The amount of this unextractable "residue" protein may vary according to the extracting solvent used, but, for any given solvent e.g. SDS solution or dilute acetic acid, the proportion has been shown to vary from one wheat to another and to correlate positively with baking performance (Pomeranz, 1965; Orth & Bushuk, 1972; Axford et al., 1978; Moonen et al., 1982).

Axford et al. (1978) used this relationship in the development of the SDS-sedimentation test, which is now much-used for screening new

breeding lines for their breadmaking potential:

Flour or wholemeal is suspended in water, and after equilibration, additions of SDS-solution, followed by aqueous lactic acid are made. The "slurry" (in a measuring cylinder) is then allowed to settle, and the volume of the sediment is recorded. This simple procedure gave correlation coefficients for "SDS-volume" with CBP loaf volume of 0.93 and 0.81 for wholemeal and flour respectively.

Lee & MacRitchie (1971) fractionated flour proteins into different molecular size classes by repeated extractions with urea and alkali, and determined the effects of these on dough mixing properties. They observed that fractions rich in high molecular weight proteins increased dough strength and stability to mixing. Consistent with these findings others have used gel-filtration chromatography to demonstrate that baking performance is related to the proportion of aggregated protein (Field et al., 1983) and to the glutenin molecular size distribution (Huebner & Wall, 1976; Bottomley et al., 1982).

The results of a recent study (Pallagi-Bànkfalvi, 1984) led to the suggestion that a flour of good breadmaking quality should have at least 50% of its gluten proteins of greater than 100,000 molecular weight, and approximately 30% above 1.5 million molecular weight.

In recent years, correlations have been identified between the presence of particular polypeptides and breadmaking quality. The high molecular weight (HMW) subunits of glutenin appear to be of particular interest in this respect.

Electrophoretic studies have shown that each wheat possesses 3 to 5 major HMW subunits (Payne et al., 1982) which appear as allelic variants from one wheat to another (Payne et al., 1984). The presence of certain HMW subunits of glutenin has been correlated with indices of breadmaking quality (i.e. SDS-sedimentation test) in several studies of European wheats (Payne et al., 1979, 1981; Moonen & Zeven, 1985; Branlard et al., 1984) and the different HMW subunits have been ranked in order of their influence on baking quality (Payne et al., 1984). Similar relationships have not been found for Australian wheat, but strong associations have been observed between glutenin subunit composition and grain hardness and dough strength, although these were, apparently, mainly pedigree associations (Wrigley et al., 1982). For Australian wheats, computer-aided analysis of gliadin patterns has indicated associations between the presence of certain gliadin electrophoretic bands and indices of breadmaking quality (Wrigley et al., 1982a). Similar associations have been observed for Italian bread wheats (Pogna et al., 1982).

In the same way, correlations have been sought between the cooking quality of pasta, and the protein subunit compostion of the durum semolina. An apparent relationship has been demonstrated by Wasik & Bushuk (1975) between the relative levels of two glutenin subunits of molecular weights 53,000 and 60,000 and spaghetti cooking quality: the better durum varieties had more of the 53,000 molecular weight subunit than of the 60,000 protein, whereas the poor-to-average varieties had less of the 53,000 relative to the 60,000 subunit.

Reversed phase high performance liquid chromatography (Burnouf & Bietz, 1984) and electrophoretic analyses (Damidaux et al., 1978; Kosmolak et al., 1980; du Cros et al., 1982) have similarly shown a relationship between the presence or absence of certain γ-gliadin bands and the pasta-making quality of durum wheats. However, in the case of durum wheats, there is evidence that the effects on pasta-making quality may be due to genetic linkage between certain low molecular weight (LMW) subunits of glutenin and particular gliadin components that are apparently associated with quality. Thus, durum varieties with strong gluten characteristics (which indicates good pasta making quality) have γ-gliadin band 45, but they also have w-gliadin band 35, and LMW-glutenin subunit 2, whereas varieties with weak gluten characteristics have γ-gliadin 42, w-gliadins 33, 35 and 38, and LMW-glutenin subunit 1 (Payne et al., 1984a). Genes coding for these two groups of proteins occur as allelic variants at the so-called Gli-B1 locus on the short arm of the 1B chromosome, and within each group, the genes are tightly linked. It seems more likely that it is the glutenin subunits rather than the gliadin proteins that affect gluten quality (Payne et al., 1984a). LMW subunits of glutenin, γ-gliadins, and w-gliadins are also encoded by genes at equivalent loci on homoeologous chromosomes 1A and 1D in bread wheats. Whether or not associations between certain gliadin bands and breadmaking quality might exist that are actually due to so-far unrecognised genetic linkage with LMW-glutenin subunits, is not yet known.

Despite these data, there is currently no explanation in chemical terms of the mechanism by which particular subunits of glutenin or particular gliadin components may influence gluten quality.

However, this genetic approach offers the possibility for resolving those constituents that are most important in determining "quality", and should provide a rationale for bringing together those proteins/polypeptides by plant breeding methods.

It is important to remember that the gluten proteins do not function against a background of inert materials. Other components of flour, such as enzymes, will have an important effect on the quality of bread produced. The added fats, and possibly endogenous flour lipids, play an important role in dough mixing and baking; of particular significance appear to be the endogenous free polar lipids especially the glycolipids. Fractionation and reconstitution experiments have shown that differences in baking performance between flours, are due not to differences in flour lipids themselves, but rather to differences in the reaction of flour proteins with these lipids (MacRitchie, 1977). Finney (1971) proposed that polar lipids bind to gliadin by hydrophilic bonds and to glutenin through hydrophobic interactions and in this way contribute to the gas-retaining complex structure in bread.

Recently, Frazier and co-workers (1981) (1983) followed the distribution of lipid in dough fractions and protein sub-fractions during dough mixing. They described a lipoprotein fraction of low molecular weight, that could be separated from gluten, and which was responsible for a major part of the lipid binding in doughs. This lipoprotein complex was named "ligolin". It was suggested (Frazier et al., 1984) that the strongly interactive nature of ligolin could be responsible for the formation of the gluten network in dough, by aggregating glutenin and gliadin chains together. On dough mixing

in air, both SS/SH interchange and hydrophobic interactions could occur, however, under anaerobic conditions disruption of inter-protein interactions by lipids competing for hydrophobic sites on ligolin, would alter the protein network markedly, and might account for the deleterious effect of high levels of bound lipid on loaf volume (Frazier et al., 1984).

I.8 "Environmental" Factors affecting Flour and Gluten Protein Functionality

The term "quality" is frequently used by cereal scientists to describe the suitability of a wheat flour to produce a specific end product, such as bread, cakes, pasta, biscuits etc. However, quality of wheat is under the control of at least three basic groups of factors:-

1. Factors which are inherent in the wheat i.e. under genetic control.

2. Environmental factors operative during wheat growth and development, e.g. soil, weather, disease, fertilisation.

3. Environmental factors imposed on wheat during processing.

Much of the preceding discussion has concentrated on the genetically controlled nature of wheat proteins with respect to functionality. However, the "environmental" factors affecting wheat production and use are also of great importance.

Nitrogen/Sulphur Imbalance

Attention has recently been focussed on the problems of Nitrogen/Sulphur (N/S) imbalance in soil, and the effect which this may have on wheat production and flour functionality. Soil sulphur levels may become limiting due, amongst other things, to reduced atmospheric sulphur deposits resulting from more stringent pollution controls, the use of "high analysis" nitrogenous fertilisers, such as ammonium nitrate, rather than sulphur-containing fertilisers, such as ammonium sulphate, and the higher demand placed on available soil sulphur supplies by greatly increased crop yields.

Sulphur (S) deficiency, i.e. low S content and a high N/S ratio, has been shown to limit yields of wheat grain, and the response to Nitrogen (N) fertilisation aimed at increasing yield (Randall et al., 1981; Moss et al., 1981). Grain hardness increases in S-deficient wheat, and the flours give rise to excessively tough doughs, with increased resistance to extension, and low extensibility (Moss et al., 1981, 1983). S-deficiency also has an adverse effect on loaf quality and indices of biscuit-making quality (Moss et al., 1983).

When post-anthesis foliar applications of urea were used to increase grain protein content, N/S ratios increased in both flour and gluten, and the proportion of the total amino acids accounted for by cyst(e)ine and methionine decreased (Timms et al., 1981). Electrophoretic analysis revealed an increase in the proportion of S-deficient proteins of gluten, the w-gliadins. The impaired functionality of the gluten was, therefore, attributed to these

changes in the protein composition of the grain. It was concluded that late foliar nitrogen induced S-deficiency in the developing grain, presumably because the S supplies from the roots were insufficient for the acute increases in available N. This N was, therefore, diverted into synthesis of those proteins which are deficient in the S amino acids, which in wheat comprises the w-gliadins. Others have observed similar effects on grain protein composition as a result of a S-deficiency (Wrigley et al., 1980, 1984; Byers & Bolton, 1979).

S-deficiency in soils is not currently regarded as a significant problem in the main wheat growing areas of the UK, although some regions of S-deficiency have been identified both at home and abroad e.g. Australia. Likewise, late foliar application of N is not a widespread practice at present. However, there has recently been some commercial interest in this method of N application for increasing grain protein contents in order that specifications for protein levels by UK millers may be achieved. It is essential, therefore, that growers and millers should continue to monitor these developments, to safeguard the quality of grain for milling.

Effects of Heat on Gluten Proteins

"Environmental" factors operative during the growth and development of the wheat plant, e.g. weather, disease, level and type of fertilisation, may affect the "quality" of grain produced, as discussed above. Similarly, conditions employed during processing of the wheat (or flour) may also have a significant effect on the

performance of a flour in a particular application, and the "quality" of the final product.

The use of wheat gluten by the milling industry to replace high protein Canadian and other imported wheats in the production of flours for breadmaking has expanded dramatically in recent years, particularly in the UK. This increase in gluten uptake, has largely been the result of a growing price differential between European and third country (non-EEC) wheats, which has dramatically reduced the relative cost/unit of protein for gluten-fortified flours. All commercial starch/gluten separation systems are wet processes and virtually all gluten is dried in pneumatic driers, operated at elevated temperatures (Schofield & Booth, 1983). Commercial gluten quality is known to be variable (Booth & Timms, 1978) and it is thought that heat denaturation during drying is the main source of this variation.

Heat denaturation of wheat gluten proteins is observed in several different contexts. Denaturation of the gluten network in fermented bread doughs takes place during the baking process, and similarly, denaturation of these proteins occurs when pasta is cooked. Grain that is harvested during wet weather may require drying, and the use of excessive drying temperatures will give rise to denaturation of the grain proteins and impair the functionality of the resultant flour. Another important example of the effects of heat on gluten protein functionality is in the drying of commercially produced gluten.

Pence et al. (1953) demonstrated that loss of gluten functionality

through heat denaturation was essentially a first order reaction having an activation energy of approximately 146 kJ/mol. However, since gluten is a complex mixture of gliadins and glutenins, it is unlikely that denaturation arises through a single step. Indeed, Pence and co-workers demonstrated the greater sensitivity to heat of the whole gluten complex as compared with gliadin alone.

The functional and chemical changes that gluten proteins undergo during heat denaturation have been studied by many groups subsequently (McCausland & Wrigley, 1976; Booth et al., 1980; Autran & Berrier, 1984; Eliasson & Hegg, 1980). Jeanjean et al. (1980) showed that heating gluten, led to decreased compressibility and increased firmness, (i.e. a tougher gluten), and that new bonds were formed, which resulted in the insolubilisation of proteins previously extractable in aqueous ethanol. Schofield et al. (1983) demonstrated that gluten baking performance in a small-scale bulk fermentation test, using reconstituted flours, declined progressively for glutens heated in the range 50 - 75°C, with most of the functionality having been lost by 75°C. Extractability of the gluten proteins in SDS buffer also diminished over this range. Gel filtration chromatography of the extracted proteins indicated that both glutenins and gliadins became less soluble on heating, and that the glutenins were affected at lower temperatures than the gliadins. The glutenins of highest molecular weight were predominantly affected by heating. Examination of the proteins that were soluble in aqueous propan-1-ol showed that only the w-gliadins were unaffected by heat. The S-rich α-, β- and γ-gliadins and alcohol-extractable low molecular weight glutenins were rendered much less soluble in the alcohol extractant after heating. The

level of total available sulphydryl (SH) groups remained essentially constant up to 100°C, but there was a transfer of SH groups from an SDS-extractable to an SDS-unextractable form.

The data suggested that loss of funtionality in heated gluten occurred through heat induced unfolding of glutenin polypeptides, that facilitated SS/SH interchange between exposed S-S and SH groups in adjacent molecules. This resulted in a tough, elastic gluten whose baking performance was diminished. Similar observations of the involvement of the S-rich proteins of gluten in the denaturation reactions have been made in other laboratories (McCausland & Wrigley, 1976; Jeanjean et al., 1980; Autran et al., 1984). The fact that functionality of heat-denatured gluten can be restored, at least partially, by dough mixing in the presence of reducing agents, also supports the involvement of reactions involving S-S bonds in the heat denaturation process (Schofield et al., 1984; Stenvert et al., 1981).

It is, therefore, evident that significant advances have been made in recent years in our understanding of the chemical basis of gluten's functionality, and the differences that exist between wheats. However, there are still many unknown factors to be identified, and many areas of confusion to be clarified.

In this thesis, the author establishes techniques for examining the aggregation states in wheat flour proteins, and applies these to the study of the relationship between protein molecular size distribution and the functional (breadmaking) characteristics of wheats of different baking quality, and to the study of four

cereals, rye, barley, oats and wheat.

Whilst the nature of the gluten proteins determines the quality of wheat, i.e. its suitability for a particular end use, processing may itself dramatically alter the characteristics of the gluten proteins.

The author, therefore, examines the effect of procedures commonly employed in commercial food manufacture, on the nature of the gluten proteins, with particular reference to changes in their aggregation states. Processes examined include the Chorleywood Bread Process, by which approximately 80% of bread is made in the UK, spaghetti manufacture from durum wheat semolina, the preparation of flour-water batters and the uptake of moisture by dried gluten, such as occurs when gluten is used to fortify bread flours.

This thesis, therefore, contributes to our understanding of the relationship between protein aggregation states in native flours and flour functionality, and the changes which are wrought on the gluten proteins during commercial food manufacturing procedures.

Chapter II: Extractability of Wheat Endosperm Storage Proteins In Buffers Containing Sodium Dodecyl Sulphate and Its Relationship with Flour Breadmaking Quality

II.1 Introduction

The structural features that give gluten proteins of wheat flour their characteristic visco-elastic properties, also make them extremely difficult to solubilise and to characterise using normal biochemical procedures. Complete dissolution of these proteins, particularly in the absence of reducing agents, thus necessitates the use of forcing conditions. Solvents employed in this role have included 2-8M urea, 6M guanidine-HCl, phenol-acetic acid-water, acetic acid-urea-cetyltrimethylammonium bromide (AUC), and sodium dodecyl sulphate (SDS) solutions.

AUC has been most frequently employed for the extraction of flour proteins (Meredith & Wren, 1966). It has also been widely used for gel filtration chromatography studies of flour protein extracts (Payne & Corfield, 1979), although more recent investigations have involved the use of guanidine hydrochloride (Huebner & Wall, 1976, 1980), urea (Huebner & Wall, 1980; Crow & Rothfus, 1968) and SDS (Huebner & Wall, 1980; Kahn & Bushuk, 1979; Danno et al., 1974, 1978; Graveland et al., 1979, 1982). Most gel filtration studies have provided at least some resolution of flour proteins into subfractions, similar to those defined by Osborne (1907) on the basis of their solubilities in water (albumins), salt solutions (globulins), alcohol (gliadins) and acid (glutelins).

Although the use of AUC has provided valuable information concerning the nature of wheat flour and gluten proteins, the solvent has a number of disadvantages. Acetic acid, at the concentration used in AUC (0.1M) can cause acid-catalysed peptide bond cleavage (Goforth, 1975). Incomplete removal of cetyltrimethylammonium bromide (CTAB) can lead to problems in subsequent analyses, such as Kjeldahl N analyses for the determination of protein recoveries and extractabilities. It is also difficult and inconvenient to change from AUC to SDS-containing buffers prior to polyacrylamide gel electrophoresis (SDS-PAGE) or gel-filtration chromatography in SDS.

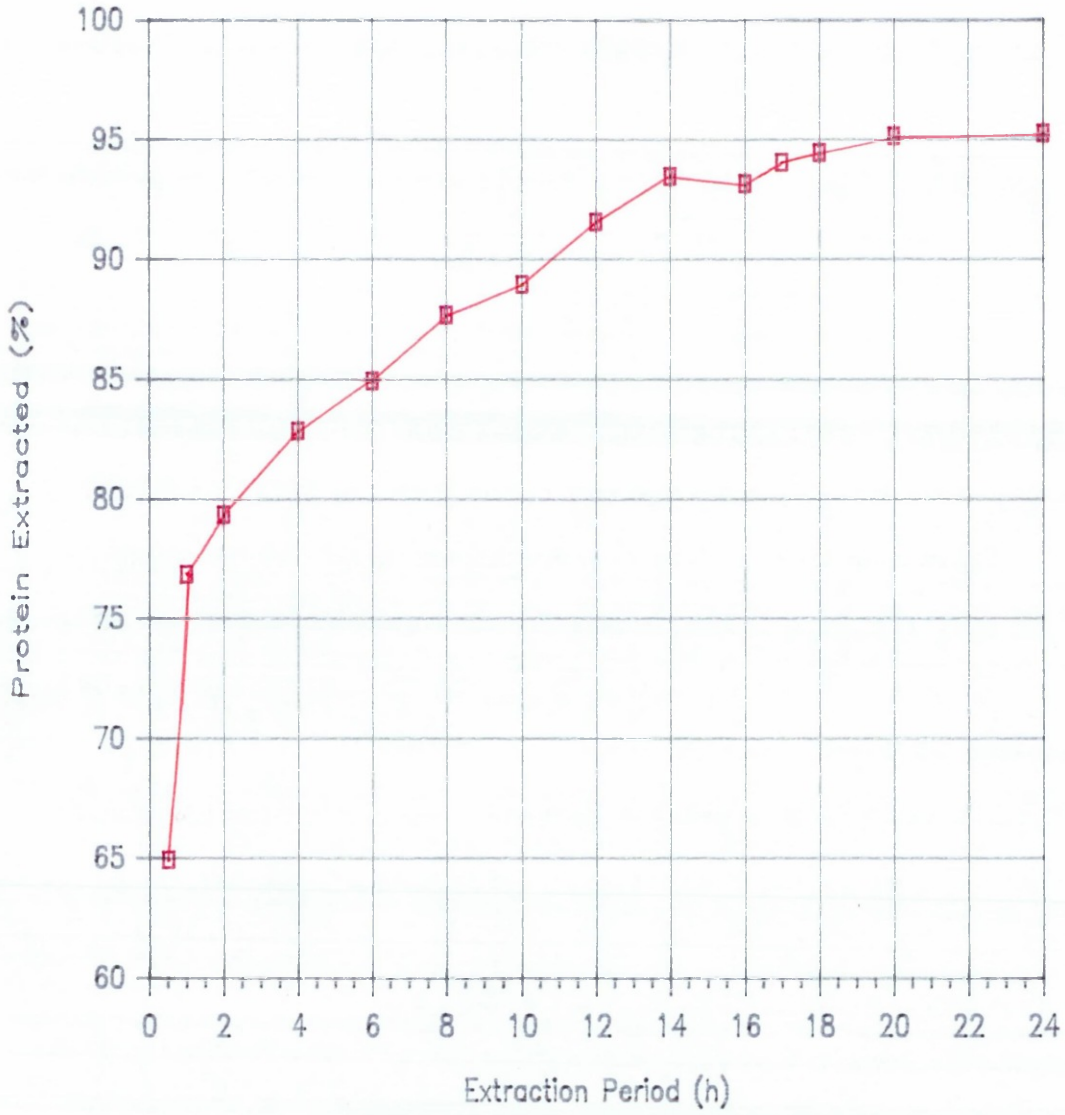
A procedure was, therefore, developed for the extraction of endosperm storage proteins from cereals using buffers containing SDS. Extractability of wheat proteins using SDS was compared with extractability in AUC and in a related urea-CTAB solution buffered to pH 5.5 with sodium citrate (CUC). The SDS extraction technique was applied to the determination of whether or not differences in protein extractability existed between flours, that could be related to differences in their functional (breadmaking) properties.

II.2 Experimental, results and discussion

II.2.1 Extractability of Wheat Flour Proteins into Buffers Containing Sodium Dodecyl Sulphate as a Function of Extraction Time

Extractability of wheat flour proteins into buffers containing 2% SDS solution was determined by the method described in Appendix I.2, but varying the extraction period between 0.5 - 24h.

Figure 3: Extractability of wheat flour proteins into buffers containing sodium dodecyl sulphate as a function of extraction time.



In many studies, surprisingly short extraction periods have been used, ranging from 2min to 2h, despite the recognised intractability of wheat flour proteins in solvents commonly used for protein fractionation. Results presented in Figure 3 demonstrate that under the conditions employed here,

i.e. extractant = 2% SDS, 0.1M phosphate buffer, pH 7.6,
solvent:flour ratio = 20:1,
dispersion by sample rotation, to minimise mechanical degradation of the proteins caused by shearing,

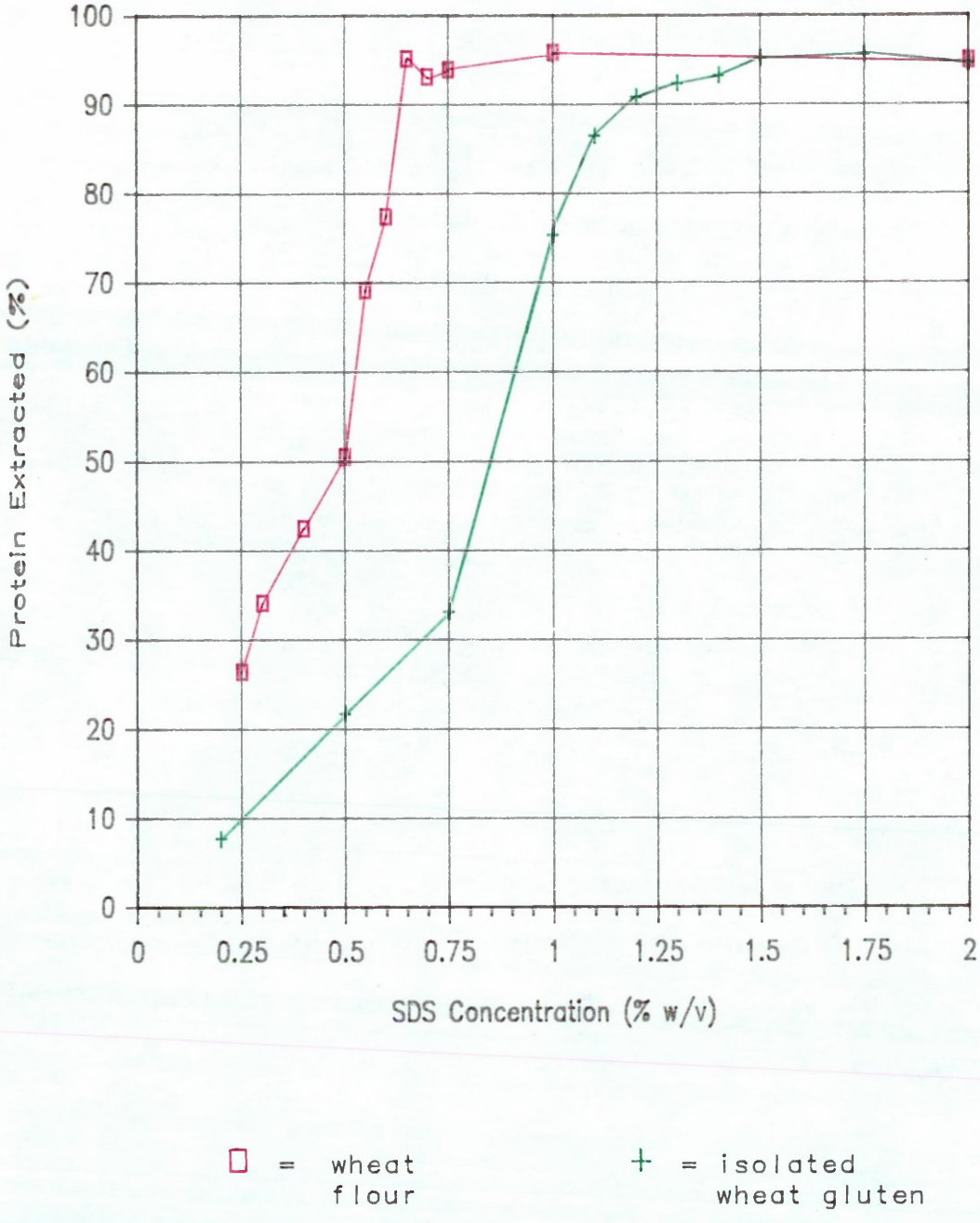
... maximum protein extraction (95%) was only achieved after a period of approximately 17h. It is probable, therefore, that many published estimates of flour protein extractability and of the proportion of residue protein remaining after extraction, suffer from the use of incomplete extraction times.

II.2.2 Optimal Concentration of Sodium Dodecyl Sulphate in Extractants for Wheat Flour and Gluten Proteins

The optimal concentration of SDS in solvents used for the extraction of wheat flour and isolated gluten, was determined by the method described in Appendix I.2, but varying the SDS concentration between 0.25% - 2.0% (w/v). Solvent:solid ratios were 20:1 for flour (12.8% protein content) and 100:1 for gluten (77.7% protein content).

Results presented in Figure 4 show that protein extractability rose with increasing SDS concentration in the extractant. Maximum protein extractability of 95% was achieved at an SDS concentration of approximately 0.65% (w/v) for flour, and approximately 1.5% (w/v) for gluten. This is equivalent to a SDS to protein ratio of

Figure 4: Relationship between protein extractability and concentration of sodium dodecyl sulphate for wheat flour and isolated gluten.



approximately 1:1 for flour, and 2:1 for gluten. The reason for the difference in the amount of SDS required to extract the maximum amount of protein from wheat flour and gluten is unclear, but may relate to the presence of non-gluten proteins in flour, which may differ markedly from the storage proteins with respect to their SDS-binding capacity.

Bietz & Wall (1972) have estimated the SDS-binding capacity of isolated fractions of gliadin, glutenin and aminoethylglutenin as 0.92, 0.84 and 0.91 respectively. Reynolds & Tanford (1970) demonstrated that many proteins hydrophobically bind identical amounts of SDS and estimated that >1.4g SDS/g protein were required to saturate all the possible sites for interaction on a protein molecule, and so effect solubilisation. The results presented above are, therefore, in general agreement with previous findings.

II.2.3 Extractability of Wheat Flour and Gluten Proteins into SDS-Tris, AUC and CUC buffers. Amino acid Composition of Extracted and Unextracted Proteins

The extractability of wheat flour and gluten proteins was determined by the method described in Appendix I.2, but employing forcing solvents as below:

1. 2% SDS, 0.1M Tris-HCl, pH 8.0 (SDS-Tris)
2. 0.04M CTAB, 4.5M urea, 0.05M sodium citrate, pH 5.5 (CUC)
3. 0.1M acetic acid, 3.0M urea, 0.01M CTAB (AUC)

After centrifugation, pellets and supernatants were dialysed against

water, and freeze-dried. The protein contents of the freeze-dried fractions were determined as Kjeldahl N x 5.7. Amino acid analyses were carried out by Dr. R.C. Bottomley by the method described in Appendix I.17.

SDS-Tris extracted approximately 95% of the protein from both flour and gluten (Table 2). Substantially less protein was extracted from flour by AUC and CUC than by SDS-Tris, although with the gluten the three solvents performed equally well. The close similarity between the data in columns 4 and 5 of Table 2 suggests that loss of nitrogenous material during processing (6% with SDS-Tris) occurred during the dialysis of the supernatant fraction. The high recovery figures for CUC extraction may be due to residual CTAB in the freeze-dried fractions. The true extraction data for CUC may, therefore, be somewhat higher than indicated.

Table 2: Extractability of Wheat Flour and Gluten Proteins in SDS-Tris, AUC, and CUC

	Sample Protein in Extract %	Protein in Residue %	Recovery Cols 1+2 %	% Protein Extracted a	b
Flour:					
SDS-Tris	87.9	6.1	94.0	93.5	93.9
AUC	79.0	11.4	90.4	87.3	88.6
CUC	83.6	14.9	98.5	84.9	85.1
Gluten:					
SDS-Tris	89.5	4.5	94.0	95.2	95.5
AUC	91.2	3.4	94.6	96.4	96.6
CUC	91.1	7.0	98.1	92.9	93.0

a Corrected for protein recovery, (column 1 x 100/column 3).

b Based on protein in residue, (100 - column 2).

Table 3: Amino Acid Composition of SDS-Tris, AUC and CUC Extracts and Residues from Flinor Flour
(mol 1000/mol recovered amino acids)

	SDS-Tris		AUC		CUC	
	Ext	Res	Ext	Res	Ext	Res
Amino Acid						
Asp	33	46	35	43	38	53
Thr	27	33	28	35	28	33
Ser	57	63	53	68	57	62
Glu	320	283	345	301	311	280
Pro	169	123	152	128	149	120
Gly	52	85	52	86	58	89
Ala	40	54	43	44	43	52
½Cys	19	17	17	14*	15	14
Val	44	51	48	45	46	52
Met	13	6	11	10	12	6
Ile	33	38	35	35	36	35
Leu	68	71	65	68	70	71
Tyr	22	24	21	23	21	23
Phe	38	37	38	35	39	37
His	20	23	20	23	20	20
Lys	18	25	18	21	22	22
Arg	26	20	22	23	28	27

* Not determined as such, but estimated to allow more precise calculation of the values for the remaining amino acids.

SDS-Tris = 2% SDS, 0.1M Tris-HCl, pH8.0
AUC = 0.1M acetic acid, 3.0M urea, 0.01M CTAB
CUC = 0.04M CTAB, 4.5M urea, 0.05M sodium citrate, pH 5.5

Ext = Extract, Res = Residue

The SDS-Tris extraction buffer used in the present study was consistently more effective than AUC in solubilising flour protein; the chemical basis of this observation is not clear but may indicate that SDS is more able to penetrate the complex flour system than is CTAB. Recently, almost complete solubilisation of wheat gluten proteins was reported using a modified AUC solvent, i.e. 6M urea, 0.055M CTAB and 0.01M acetic acid at 40°C (Field & Miflin, 1980; Miflin et al., 1980). However, others (Danno et al., 1974; Payne & Corfield, 1979) have also observed that AUC is a less efficient solvent than earlier work suggested (Meredith & Wren, 1966), although the precise proportion of flour protein solubilised appears to be very dependant on the particular extraction conditions used.

Amino acid analysis (Table 3) demonstrated that the proteins extracted by the three solvents were of very similar amino acid composition. The amino acid compositions of the residue fractions were generally similar to those of the extracts. Nevertheless, there were some notable differences between the two fractions. The levels of glutamic acid (glutamic acid plus glutamine prior to acid hydrolysis) and proline, regarded as characteristic of gluten protein, were substantially lower, (by approximately 10%) and the levels of glycine were much higher in the residues than in the extracts. Similar observations were made on extracts and residues for both flour and gluten.

II.2.4 Characterisation of SDS-extractable Proteins from Wheat Flour

The identity of proteins extracted by SDS was determined by gel-

filtration chromatography, SDS-PAGE and amino acid analysis.

1. The molecular weight distribution of SDS-extractable proteins from wheat flour (cv. Flinor) was determined by gel-filtration chromatography on columns of Sepharose CL-4B, according to the method described in Appendix I.3. Protein extracts for chromatography were prepared using the optimum conditions derived above. The gel-filtration chromatography profile (below) showed four distinct regions, designated A, B, C and D.

Figure 5: Gel-filtration chromatography of SDS-Tris extractable proteins of Flinor flour on Sepharose CL-4B.

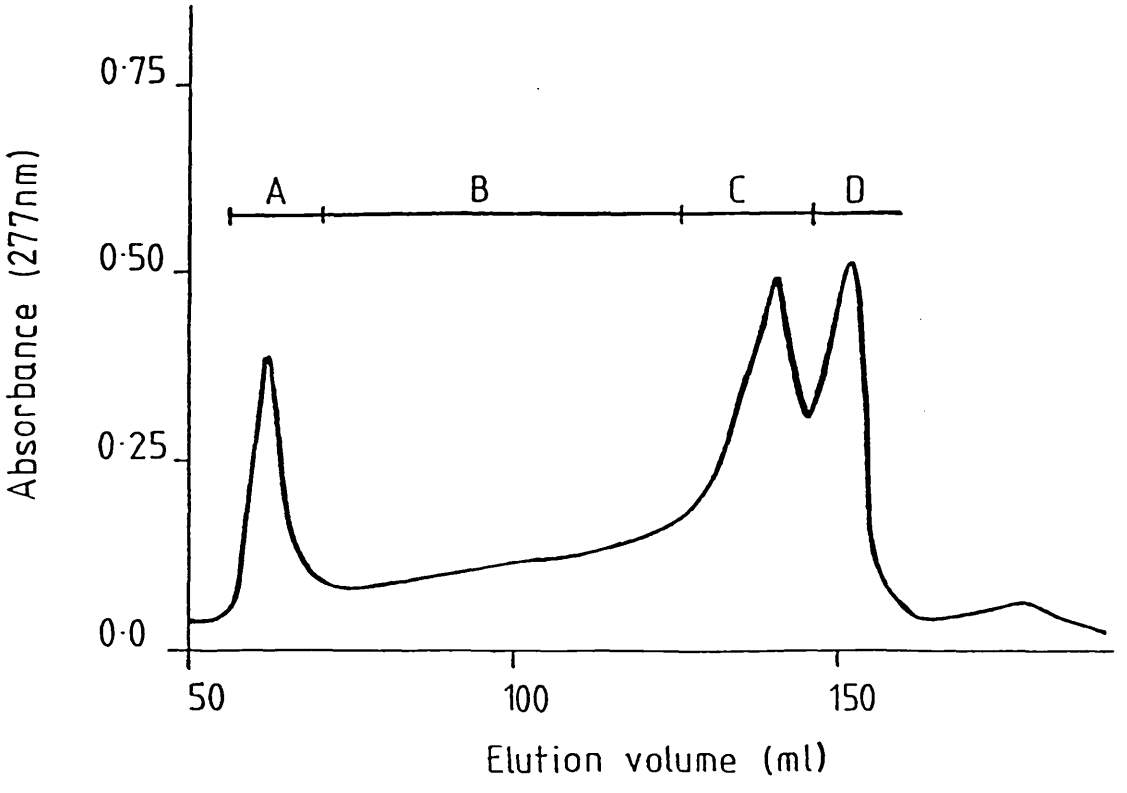
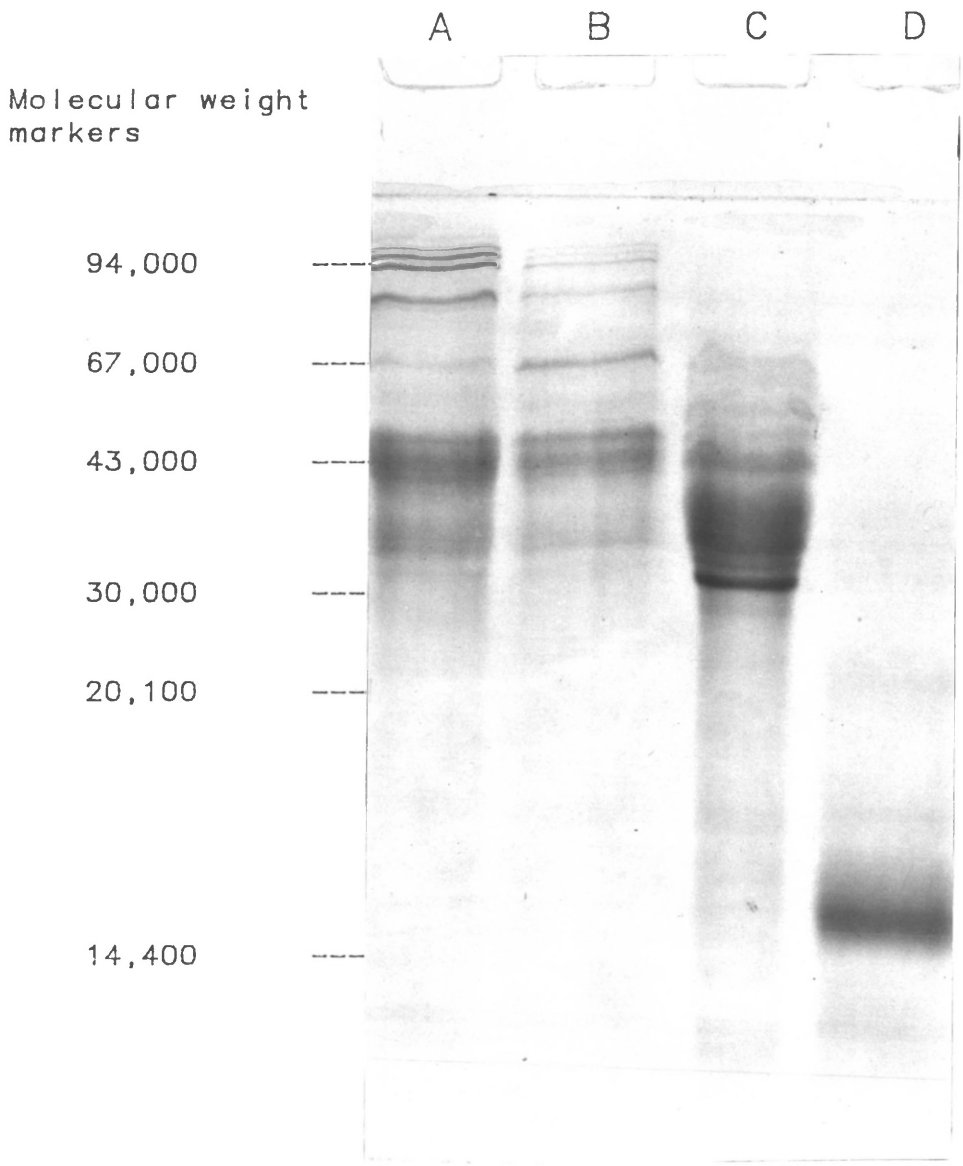


Figure 6: Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate of the polypeptides present in different regions of Sepharose CL-4B chromatograms.



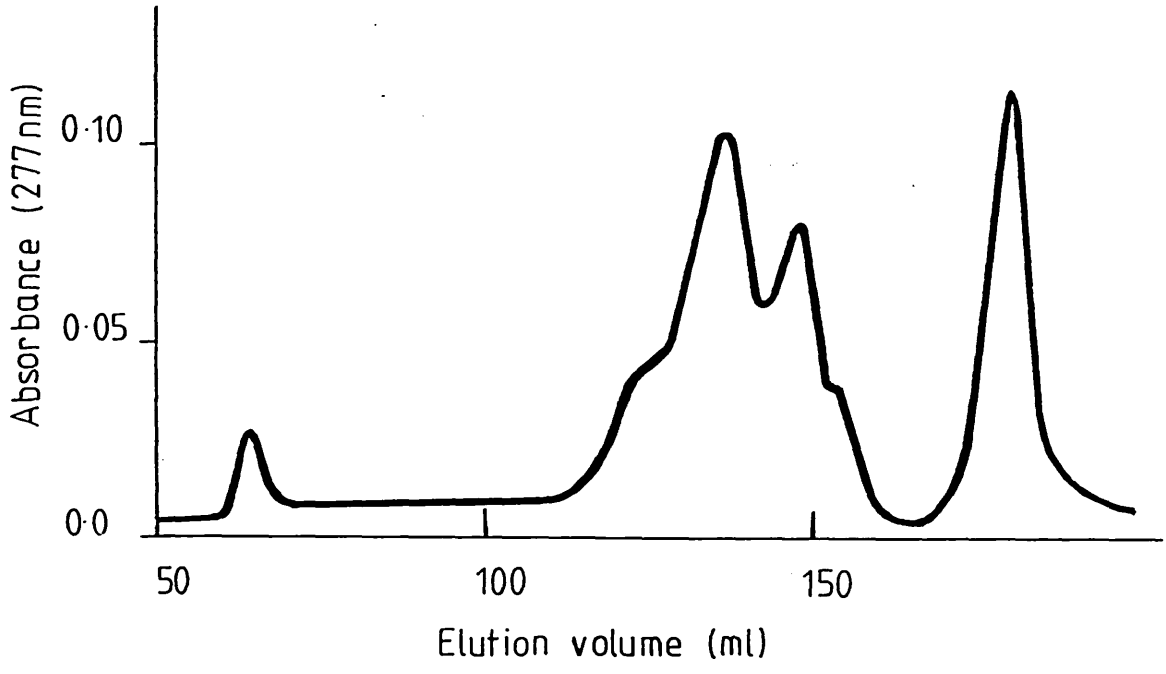
The identities of the protein pools, A - D, are shown in Figure 5.

2. The polypeptide subunit composition of proteins eluting in each of the regions of the chromatography profile was determined by SDS-PAGE of pooled column effluents according to the method described in Appendix I.4.

The results presented in Figure 6 demonstrate the differences in polypeptide composition of the four regions of the chromatography profile. There was virtually no overlap between proteins in regions C and D; the polypeptides in region D were clearly of much lower molecular weight than those in region C. The spectrum of polypeptides in region B was quite different from that of region C, although there was not only molecular size overlap between the two regions, but also, at least in terms of molecular weight, apparently some identity of a number of polypeptides. This observation probably implies that region C includes very low molecular weight aggregates of glutenin (although notably not including the high molecular weight [HMW] glutenin polypeptides) as well as gliadins. All of the polypeptides present in region A were also present in region B. As applied to Sepharose CL-4B columns, these polypeptides were present as high molecular weight complexes, generally recognised as glutenins.

When the extracts were reduced with mercaptoethanol prior to chromatography, regions A and B were almost completely removed to elution positions corresponding to much lower molecular weights (Figure 7). These data suggest that the high molecular weight complexes are maintained by disulphide bonds. The small breakthrough peak (Figure 7) did not contain protein, as determined by amino acid analysis of the void volume material.

Figure 7: Gel-filtration chromatography of a mercaptoethanol reduced SDS-Tris extract of Flinor flour



In the "standard" column profile (Figure 5), the two regions containing glutenin aggregates can be attributed approximate molecular sizes. Region A, at the column void volume, contains only very high molecular weight protein complexes, probably of molecular weight 5×10^6 upwards. Region B evidently contains a very wide range of glutenin complexes from about 10^5 up to about 5×10^6. There is, however, a lack of standard proteins of known molecular weights covering this size range and these data are, therefore, based on the manufacturers data for polysaccharides, whose

properties probably correlate more closely with SDS-protein complexes than do those of globular proteins. Regardless of the precise molecular weights, it is clear that the glutenin complexes are extremely polydisperse with respect to molecular size.

Column profile regions C and D contained monomolecular proteins of molecular weight values below 10^5, corresponding to gliadins, and water- or salt-soluble materials respectively.

It should be noted that the chromatographic profile is obtained by continuous monitoring of the optical density of column effluents, and is dependent on the ultraviolet absorption of the amino acids tyrosine, tryptophan and phenylalanine. The absorption at 277nm of the two former amino acids is greater than that of the latter. This method of protein assay, although convenient may lead to incorrect quantification when the amounts of these amino acids varies greatly in different types of protein. For wheat proteins the content of the aromatic amino acids and tryptophan is shown below.

	Albumin	Globulin	Gliadin	Glutenin
Tyrosine (moles/1000)	21.6	16.3	18.3	24.8
Tryptophan (moles/1000)	ND	ND	4.3	12.6
Phenylalanine (moles/1000)	35.5	24.5	42.9	36.4

Reproduced from: "Proteins as Human Food", (1970), N.L. Kent, p280. Editor, R.R. Lawrie. Butterworth and Co. (Publ.) Ltd., London.

Table 4: Amino acid composition of Sepharose CL-4B column pools of an SDS-Tris extract of Flinor flour
(mol 1000/mol recovered amino acids)

	Pool from column region			
	A	B	C	D
Asp	31	27	36	61
Thr	35	37	31	37
Ser	84	96	76	58
Glu	317	286	332	224
Pro	134	144	136	98
Gly	86	79	46	74
Ala	36	49	45	60
½Cys	25	21	22	32
Val	35	36	39	61
Met	4	10	10	15
Ile	21	24	27	38
Leu	67	64	65	68
Tyr	25	25	23	34
Phe	33	35	41	33
His	19	24	23	28
Lys	16	16	17	35
Arg	33	28	32	42

The identity of the four regions of the chromatography profile, A, B, C and D, are shown in Figure 5.

Therefore, estimates of the proportion of protein in each of the molecular weight regions, determined by continuous UV monitoring of column effluents may not accurately represent the actual composition of the SDS-extracts.

3. Further evidence for the present classification of the four major regions of the gel-filtration chromatography profile was provided by amino acid analysis of the pooled column effluents. (Table 4). The amino acid profile of region D was quite different from the other regions, as would be expected for a comparison of albumin/globulin proteins with gluten proteins. The two glutenin pools (regions A and B) had very similar amino acid compositions, in addition to the close similarity of their polypeptide composition as determined by SDS-PAGE (Figure 6). The amino acid profile of the gliadin pool (region C) was generally quite similar to those of the glutenin pools, the only major difference being in the proportion of glycine, which was much lower than the glutenin pools.

For satisfactory investigations of the chemical properties of most proteins, it is important that an efficient and convenient solvent system is chosen. The data presented here demonstrates that, for wheat proteins, 2% SDS, 0.1M Tris-HCl (pH 8.0) solutions, not only extract as much as possible of the total protein, but also lend themselves readily to chromatographic and electrophoretic studies of the extracted proteins.

The reasons why SDS-containing buffers have been less favoured than AUC as extractant and chromatography solvents for wheat proteins are not clear. The absence to date of any systematic study of SDS

buffers as applied to the extraction, chromatography and electrophoresis of wheat proteins might be one explanation. Another might be the observation by Meredith & Wren (1966) that SDS caused precipitation of acetic acid/urea-extracted proteins, even though the reasons for this precipitation were not investigated and may have been due simply to the low pH or the complexity of the system.

The methods described here are applied throughout this thesis in the investigation of the relationship between protein molecular weight distributions and functional (breadmaking) properties, and the study of the effect of processing on the protein components of wheat under a variety of different processing regimes.

II.2.5 Relationship between Flour Protein Extactability at Different Concentrations of SDS and Baking Quality

Kobrehel & Matignon (1980) have proposed that flours of good breadmaking quality have strong hydrophobic interactions between their proteins, and hence, higher levels of soap are required to disrupt these secondary forces and solubilise the protein from good breadmaking varieties, than from poor breadmaking flours.

The extraction procedure developed here using solutions of the anionic detergent SDS was, therefore, applied to determination of whether or not differences in hydrophobic character, (as measured by protein extractability at different concentrations of SDS) existed which could be related to the functional (breadmaking) properties of a flour.

Nine monovarietal samples of wheat from the 1979-1980 harvest, were milled on the experimental Buhler mill, and assessed for baking performance by the bulk fermentation procedure described in Appendix I.5. Baking data are presented below.

Baking performance of nine monovarietal wheat samples (6 varieties) of the 1979-1980 harvest

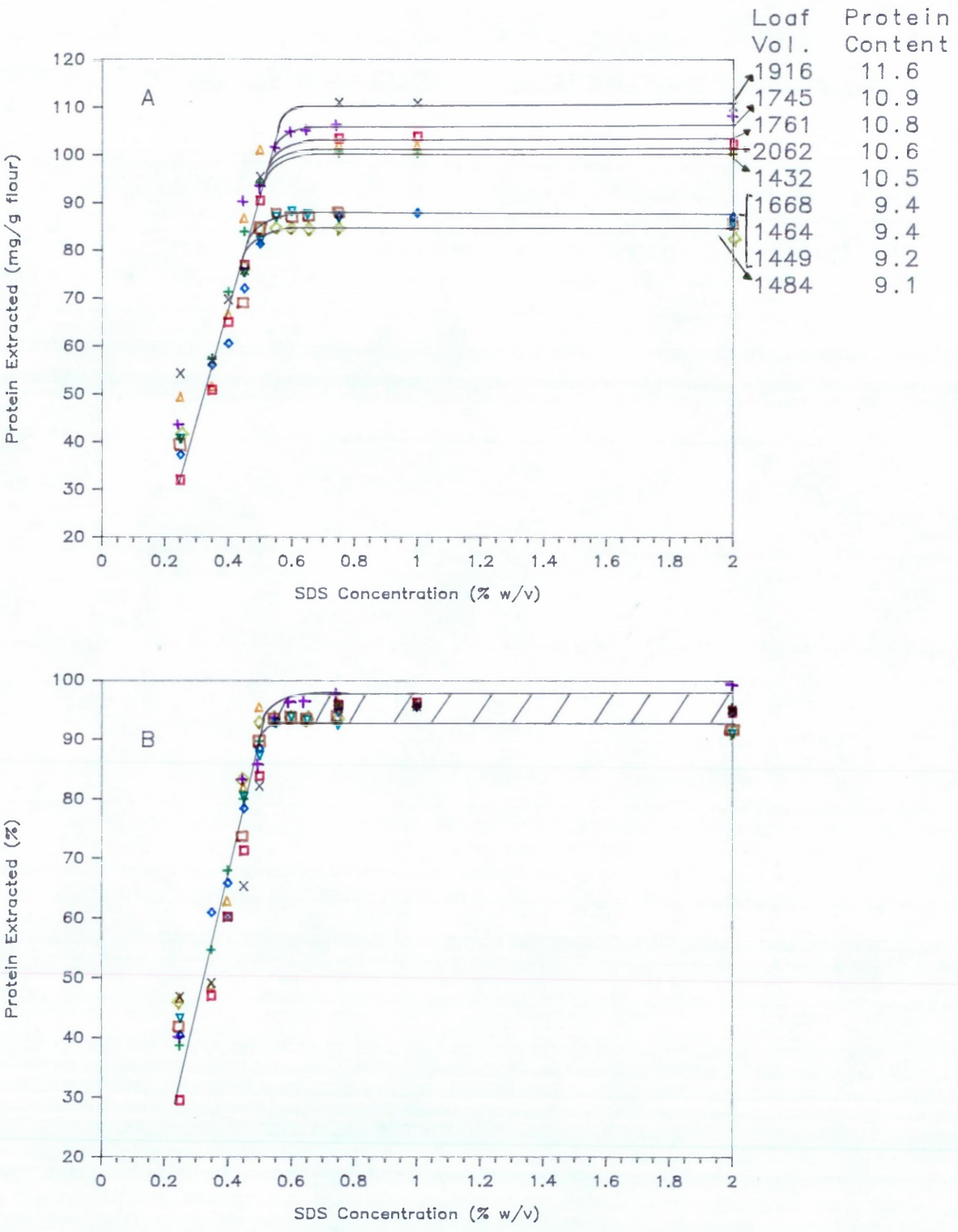
Sample		Protein Content (%)	Loaf Volume (ml)	Texture Score	Site Grown
Copain	AB624	9.4	1464	4	Essex
Copain	AB552	10.9	1745	4	Oxon
Flanders	AB594	9.4	1668	4	Essex
Flanders	AB523	11.6	1916	2	Kent
Avalon	AB543	10.8	1761	5	Leics
Avalon	AB575	9.2	1448	3	Oxon
Armada	AB522	10.5	1432	2	Kent
Timmo	AB623	10.6	2062	5	Essex
Bounty	AB580	9.1	1484	4	Oxon

Protein contents are Kjeldahl N x 5.7 at 14.5% moisture content.

Loaf volumes were determined by seed displacement.

Texture score was assessed by expert panel on a scale 0 (poor) to 7 (good)

Figure 8: Relationship between the amount of protein extracted by SDS from flours differing in breadmaking quality, and the concentration of SDS in the extractant.



Results are expressed in terms of (A) protein extracted per unit weight of flour, and (B) proportion of total flour protein extracted.

The protein extractability of each flour was determined according to the method in Appendix I.2, but varying the concentration of SDS in the extractant between 0.25% and 2.0% (w/v).

Extractability of protein from flour increased sharply for all varieties between 0% and 0.6% SDS, and then reached a plateau such that further increases in SDS concentration did not lead to increased protein extractability. (Figure 8A). When the amount of protein extracted per g flour was plotted against SDS concentration, there appeared to be a trend towards greater amounts of protein in the SDS-extracts in this plateau region, from those flours of better baking quality (Figure 8A). The regions of the curves between 0% and 0.6% SDS were essentially superimposable and no differentiation could be made between varieties of good or poor breadmaking performance. However, when the extraction curves were plotted as the proportion of total flour protein extracted (%) versus SDS concentration, all of the curves were now superimposable, including the plateau regions (Figure 8B), indicating that differentation amongst varieties in the plateau regions was due to differences in flour protein content rather than to differences in extractability.

Thus, whereas Kobrehel & Matignon (1980) have suggested that better breadmaking flours require higher concentrations of soap to extract the maximum amount of protein, than do poor breadmaking flours, these data indicate that there is no difference in the proportion of total protein extractable into SDS solutions from flours of different baking quality.

Although hydrophobic interactions between proteins, undoubtedly make

an important contribution to dough/gluten physical properties, the available data are insufficient to conclude whether or not differences in breadmaking quality can be attributed to differences in the hydrophobic interactions between the flour proteins.

Chapter III: Comparison of the Molecular Size Distribution of the Endosperm Storage Proteins of Wheat, Barley, Rye, and Oats, with reference to Differences in their Functional Properties

III.1 Introduction

When water is added to wheat flour and mixed, the water-insoluble proteins hydrate and form gluten, a complex, coherent mass, in which starch, added yeast and other dough components are embedded. Gluten, therefore, is the skeleton, or framework of wheat flour dough, and is responsible for gas retention, which makes the production of light, leavened products possible.

Of the cereals, only wheat and to a lesser extent rye, have the ability to be turned into bread. The structural features of wheat that are responsible for these functional properties have been reviewed in Chapter I. In the present investigation, the aggregation states of the endosperm storage proteins of four cereals, wheat, barley, rye and oats are compared, in order to identify any differences in their protein components that could account for their different functional characteristics.

The anatomical structure of all cereal grains is basically similar and has been reviewed previously by Angold (1979). Three of the four cereals considered in this study, i.e. barley, rye and wheat, are thought to share a common genetic origin (Kasarda, 1976) and

recent studies have revealed considerable structural and genetic homology between their protein components (Autran et al., 1979; Shewry et al., 1980, 1982). Despite this, these species differ considerably in many of their functional properties.

Barley, Rye and Oats: Distribution and Uses

Barley (Hordeum sativum or H. vulgare) is grown in temperate climates as a spring or a winter crop, and has a geographical distribution similar to that of wheat. The cultivation of barley reaches far back into history being known in pre-dynastic times in Egypt. Historically, barley was used in bread meal and cakes and formed a large part of the staple diet of the peasants in England up to the 15th century. However, as wheat and oats became more generally available, and with the introduction of potatoes, the importance of barley as a human food declined in Western Europe.

Although the breadmaking quality of barley is poor, during the Second World War, when supplies of imported wheat were restricted in Britain, the addition of variable quantities (up to 10% in 1943) of barley, or barley and rye or oats to a breadmaking grist was permitted. For this purpose the barley was generally "blocked", which removes most of the husk, or "pearled", which removes all the husk and some endosperm. Today, the principal uses of barley are for animal feed in the form of barley meal, for malting and brewing in the manufacture of beer and for distilling in whisky manufacture. Barley finds little use as human food in Europe and North America, although it is still widely consumed in Asia.

Some 20% of the barley grown in the UK is used in malting and brewing, the two major steps in the manufacture of beer. Malting is the controlled germination of the grain in order to promote enzymic "modification" or liberation of the protein and carbohydrate reserves of the endosperm. Brewing is the process of converting the starch to an alcoholic solution, first by hydrolysis of the starch to sugar and then by yeast fermentation of sugar to alcohol. About 75% of the starch is generally fermented to alcohol. Although barley is the grain malted most frequently, there does not appear to be any biochemical limitation to the use of other cereals. Wheat, is used to a small extent in Europe, but its application is limited by the fact that the growing root/shoot emerges from the seed coat at a very early stage of germination, and is damaged during turning of the "malted" grain. Sorghum is used in brewing in South Africa, and presumably in other African countries also.

Good malting barley should have a high starch content, since it is the starch component - converted to maltose and dextrins - that makes up the most important fermentable constituent of the malt extract. In addition, a low nitrogen level is desirable. Baxter (1980, 1981) suggested that at high protein levels, large disulphide-linked aggregates were formed which resisted modification during malting. Furthermore, these aggregated hordeins formed a matrix around the starch granules which restricted amylolytic activity during mashing and so reduced yields of malt extract.

Rye, (Secale cereale), is generally a less profitable crop than wheat when grown on good soil, but on light acid soil, it gives a more satisfactory yield than wheat. Rye is more resistant than

wheat to pests and diseases and is better able to withstand cold. Therefore, rye tends to be grown on land just outside the wheat belt, such as areas of northern and eastern Europe.

Rye is used for making soft bread and also for crispbreads. The former are usually prepared by a sour dough process, from a blend of rye and wheat flours. In the sour dough process, a rye dough is left to ferment for several hours at 75 - 80°F to induce lactic acid formation by fermentation of starch by naturally occurring grain micro-organisms. Alternatively, the dough may be inoculated with sour milk and rested for a few hours, after which a mixture of acids (acetic, lactic, fumaric) is added to simulate the flavour of a normally soured dough. This starter dough is then mixed with a strong flour and yeast to make a mild sour dough. Although nutritious and palatable to some people, rye bread is dark and dense and inferior to wheat bread as regards crumb quality and loaf volume potential.

Rye crispbread is usually made from wholemeal or flaked rye, and may be fermented or unfermented. The traditional method in Sweden, is to mix rye meal with snow or powdered ice. On baking, the small air bubbles in the ice-cold foam raise the dough. Rye crispbreads are frequently incorporated into weight-reducing diets. This is because rye contains higher levels of pentosans than any other cereal. These contribute to a feeling of satiety, and are hydrolysed slowly, thereby preventing wide fluctuations in blood sugar level, and helping to control appetite. Rye flour is characteristically weak, but is greatly improved in strength and mixing tolerance by the addition of 25% wheat flour.

Rye is also used in the manufacture of rye whiskey in North America for which 100% rye malt is required, in pancake flours, breakfast cereals, and as a thickener in soups and sauces.

Oats (Avena sativa) are widely cultivated in temperate regions and are more successful than wheat or barley in wet climates, although they tolerate the cold poorly. In the UK, oats are grown extensively in Scotland and the north of England, where a better quality crop is produced than in the south. Although a small proportion of the oat crop is milled for human consumption, most is used as an animal foodstuff.

A recent classification of the graminae (Clayton, 1978) recognised six subfamilies and over 50 different tribes. The tribes in which cereals are found are shown below:

Classification of Grasses (Adapted from Clayton, 1978).

Subfamily	Tribe	Cereals
Bambusoideae	Oryzeae	Rice
Centostecoideae	-	-
Arundinoideae	Eragrostideae	Finger millet, Tef
Panicoideae	Paniceae	Foxtail millet, Fundi
		Pearl millet, Proso
	Andropogoneae	Maize, Sorghum
Pooideae	Aveneae	Oat
	Triticeae	Wheat, Barley, Rye

The structure of the endosperm starch granules has been used for classification of the grasses since 1880. Tateoka (1962) recognises four types of starch granule. He has shown that the more primitive grasses have Type 4 starch, i.e. compound granules, composed of many granuli. Oats contain Type 4 starch granules.

In contrast, the triticoid Type 1 starches comprise two populations of granules: large lenticular, and small spherical. The Type 1 granules are restricted to a relatively small number of grasses, which includes wheat, barley and rye.

Thus, although wheat, barley, rye and oats are all graminae of the Pooideae subfamily, there is much evidence to suggest that the three former cereals are more closely related to one another than they are to oats.

The processing of oats in the mill differs from that of wheat because of differences in the anatomical structure and chemical composition of the grains. The adherent husk of the oat grain is tough and fibrous and quite inedible to humans. If edible products are required from a milling, the husk must first be removed by a special shelling process. In contrast, the chaff of wheat, which corresponds morphologically to the oat husk, separates easily from the kernel during threshing.

Groats (the oat kernel) differ from the wheat grain in two major repects which affect processing, storage and applications of this cereal. Firstly, the fat content of the groat is 2-5 times higher than that of wheat, and secondly an active lipase is located in the

pericarp of the kernel. Lipases hydrolyse fats into glycerol and free fatty acids, which in oat are principally oleic, linoleic and palmitic acids. Oat lipids are distributed throughout the endosperm, germ and aleurone, particularly the latter two regions, thus, in the intact kernel the fat and lipase of oat do not come into contact. When oats are milled, the lipase is released and there as a gradual increase in the levels of free fatty acids and glycerol. This is accompanied by the development of bitterness, i.e. the milled products become rancid.

Utilisation of oats is dependent on inactivation of the lipase before or during milling. This may be accomplished by heat treatment under suitable conditions (Kent, 1978) or by treatment with acids (Martin, 1954). After inactivation, the oats are dried, shelled and then broken into a coarse "pinhead meal", or ground on stones and sifted to give finer cuts of oatmeal flour. Adopting these procedures allows oats to be used in a variety of foodstuffs, e.g. in oat flakes, porridge, black puddings and haggis.

The second major difference between oats and wheat lies in their protein component. Similar amounts of protein are found in both cereals, but that from oats does not form a gluten when mixed with water, thus, oats and oatmeal flour are unsuitable for breadmaking. In oats, the major storage proteins are a salt-soluble globulin fraction, and not the alcohol-soluble prolamins (the avenins). This constitutes a major difference with the other cereals such as wheat, barley and rye (Peterson & Smith, 1976).

Oat globulins are nutritionally superior to the low-lysine prolamins

of the other cereals (Draper, 1973) making this cereal potentially a source of low cost, high quality protein. However, oats have received little attention as a human food source, partly through lack of information regarding their physicochemical and functional properties.

Endosperm Storage Proteins of Barley, Rye and Oats

Like wheat, most of the proteins of barley, rye and oats are located in the starchy endosperm of the grain, and these storage proteins have been characterised by workers according to size, solubility and amino acid composition.

	% Total Protein in each Fraction			
	Wheat	**Barley**	**Rye**	**Oats**
	a	b	c	d
Water solubles	6 - 10	16	29	13
Salt solubles	6 - 10	16	8	42
Alcohol solubles	35	32	17	15
Acid solubles	35	36*	15	15
Residue protein	10 - 40		31	15

a Huebner & Wall, 1976; Orth & Bushuk, 1972.

b Kent, 1978.

c Preston & Woodbury, 1975.

d Ching-Yung Ma & Harwalker, 1984.

* Total glutelin fraction

Electrophoretic analyses of barley and rye proteins (the hordeins and secalins respectively) reveal many points of similarity between these cereals, and wheat.

Both barley and rye contain high molecular weight proteins which are poorly extracted from the seed, except with denaturing or reducing solvents. This low extractability is due to extensive disulphide cross-linking between polypeptide subunits. SDS-PAGE under reducing conditions reveals that the high molecular weight protein of rye consists of polypeptides in excess of 90,000 and in barley is a disulphide-linked aggregate of a single polypeptide, D-hordein, of about 100,000 molecular weight. The amino acid composition of these polypeptides is similar to the glutenin subunits of wheat, having a high glycine content (>12%), and somewhat lower levels of proline than are found in the prolamin fraction (Miflin et al., 1983).

Within the prolamins of rye and barley are groups of sulphur-poor proteins, the C- or w-secalins (Miflin et al., 1983) and C-hordeins respectively. These have molecular weights between 40,000 and 80,000 and, like the w-gliadins of wheat, are characterised by very low levels of sulphur amino acids, (e.g. cysteine 0-0.4% and methionine 0-0.2%) and low levels of lysine. These proteins also have high levels of glycine (40% - 50%), proline (20% - 35%) and phenylalanine (7% - 9%). The very low levels of sulphur amino acids precludes formation of disulphide bonds between these protein chains, and their relatively low molecular weights permits easy extraction in alcoholic solution in the absence of reducing agents.

Also extractable in alcohol solutions, but containing higher levels

of sulphur amino acids, are the B-hordeins of barley and the B- or γ-secalins of rye. These proteins have a range of molecular weights similar to the α-, β- and γ-gliadins of wheat, i.e. 30,000 - 44,000, and characteristic amino acid composition, i.e. 37% - 42% glutamate and glutamine, 15% - 19% proline and 2% cysteine. The higher content of cysteine probably results in the formation of intramolecular disulphide bonds and, to a lesser extent, some intermolecular disulphide bonds. Shewry et al. (1982) suggested that the secalins of rye also contained some polypeptides that had higher molecular weights of ~75,000, but were otherwise similar to the bulk of prolamins of this group.

A group of low molecular weight proteins (i.e. <20,000) is also present in this solubility fraction. These A-hordeins and A-secalins have high levels of tyrosine and sulphur amino acids, but are low in lysine and histidine.

The differences in the proportions that each of these fractions represents of the total storage proteins in barley, rye and wheat may go some way to explaining differences in the functional properties of these cereals. For example, the S-poor prolamins, comprise approximately 10% of the total gliadin of wheat, whereas in barley they may constitute up to 30% of the prolamin fraction and frequently occur at levels between 15% - 20% (Miflin et al., 1983). A high proportion of S-poor prolamins in a flour, may be expected to reduce the potential for development of a stable gluten matrix through intermolecular disulphide bond formation, and hence, reduce breadmaking quality. Nevertheless, these proteins may still participate in non-covalent interactions between proteins, which are

also important in dough structure.

SDS-PAGE analysis of oat globulins under non-reducing conditions gave estimates of molecular weights between 52,000 - 70,000. Within this range, two major bands were always present with variable amounts of other minor components of both higher and lower molecular weights. Upon reduction of disulphide bonds, the oat globulins produced two discrete groups of polypeptides known as the α-subunit group, and the β-subunit group (Robert et al., 1983). The α-group consisted of 2 major and up to 4 minor subunits ranging from 33,000 - 43,000 molecular weight, while the β-group consisted of 4 subunits between 20,000 - 25,000, of which 3 were usually present as major bands. The behaviour of the oat globulins on reduction suggests that the proteins of the 52,000 - 70,000 family result from the association of the α- and β-subunits alone through disulphide crosslinks.

Despite their low levels in oat, the prolamins (avenins) are similar to other cereal prolamins in many respects. For example, amino acid analysis reveals that the avenins have high levels of glutamate and proline, but low levels of lysine. Most avenins have molecular weights between 22,000 - 40,000. SDS-PAGE of extracted oat prolamin under reducing and non-reducing conditions showed little or no variation in the protein band pattern and estimated molecular weight, suggesting these proteins are not linked through disulphide bonds to form aggregates, but are present as simple monomolecular proteins (Robert et al., 1983a).

From the preceding discussion, it is evident that there are many

similarities amongst the protein components of wheat, barley and rye and oats. However, there must also be significant differences to account for the viscoelastic properties which only wheat, and to a lesser extent rye, possess. The following study compares the extractability characteristics and protein aggregation states of barley, rye, oats and wheat, to determine whether or not wheat differs from the other cereals and if so whether these differences may explain variations in their functional properties.

III.2 Experimental, results and discussion

III.2.1 Preparation of materials

Single cultivar samples were taken to represent four of the cereals:-

Triticum aestivum var. Bounty	(wheat)
Hordeum vulgare var. Athene	(barley)
Secale cereale var. Greenfold	(rye)
Avena sativa L. var. Peniarth	(oat)

Grain was milled to pass a 0.5mm screen in a laboratory Retsch centrifugal mill. Protein contents were determined on the wholemeal flours, as Kjeldahl N x 5.7 at 14% moisture content, (below). All samples were stored at -20°C.

Cereal	Protein Content
T.aestivum var. Bounty	11.7 %
H.vulgare var. Athene	9.2 %
S.cereale var. Greenfold	7.6 %
A.sativa L. var. Peniarth	10.1 %

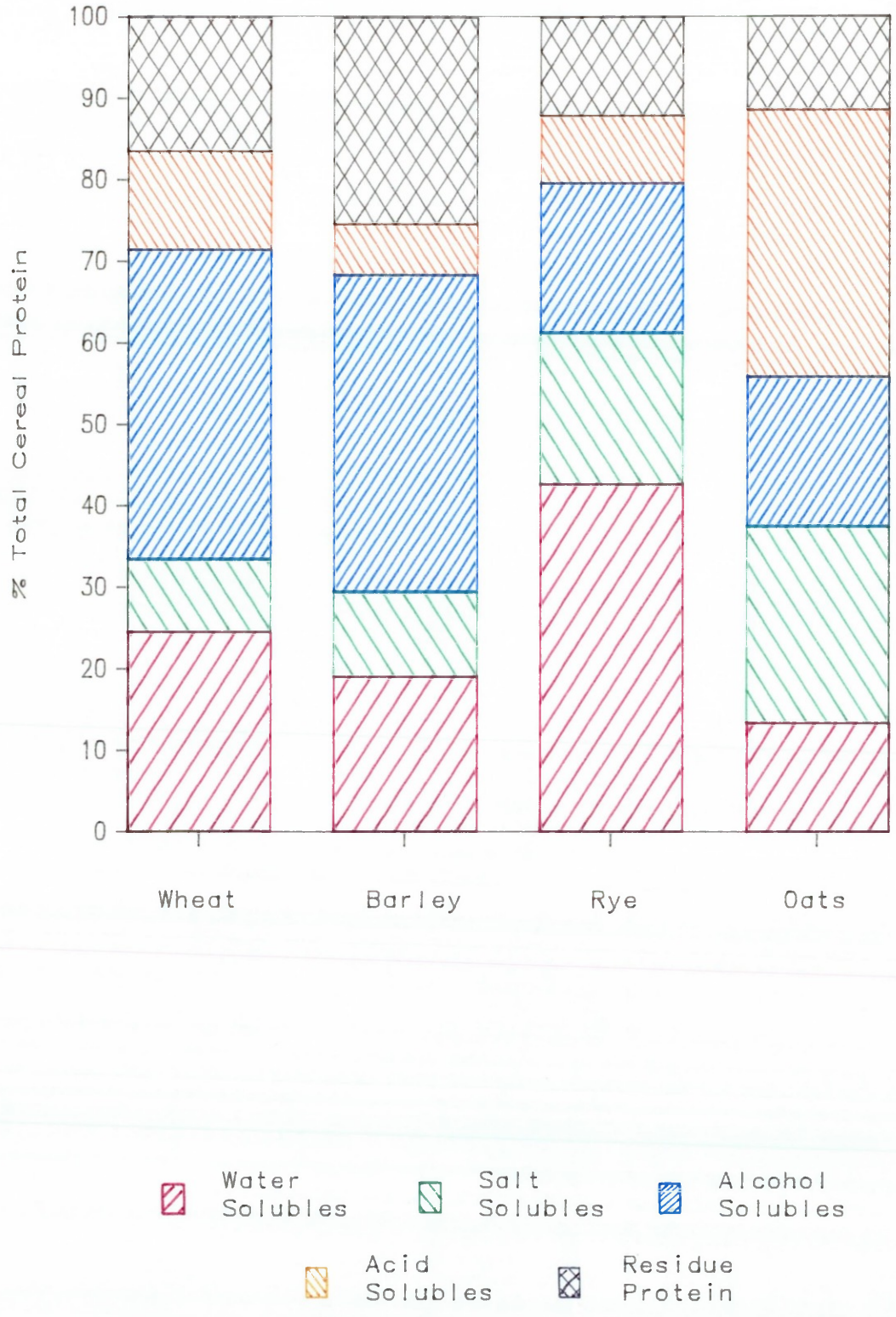
The protein content of cereal grains varies widely and depends on both inherited characteristics and environmental conditions during growth. In wheat, for example, the protein content may vary between 7% and 22%, but is generally higher than the other cereals. A protein content of ~11% or more is usually preferred for flours used in the manufacture of yeast-leavened bread, the actual level depending on the bread and process type. Thus, only wheat, of the four cereal GRAIN SAMPLES examined would be acceptable for breadmaking on the basis of protein quantity, irrespective of protein quality parameters.

III.2.2 Extractability characteristics of wheat, barley, rye and oat endosperm proteins by a modified Osborne fractionation

The fractionation procedure of Osborne (1907) as modified by Chen and Bushuk (1970) was applied to determination of the extractability characteristics of wheat, barley, rye and oat endosperm proteins (Appendix I.1). The proportion of total protein of each cereal, in the five Osborne solubility fractions is presented in Figure 9.

Such fractionation procedures suffer from two main disadvantages,

Figure 9: Distribution of protein of wheat, barley, rye and oats into different solubility fractions. (modified Osborne procedure)



Results are expressed as the proportion of total protein in each solubility fraction.

(a) different classes of proteins overlap somewhat in their solubility characteristics and (b) such schemes fail to solubilise all the protein present.

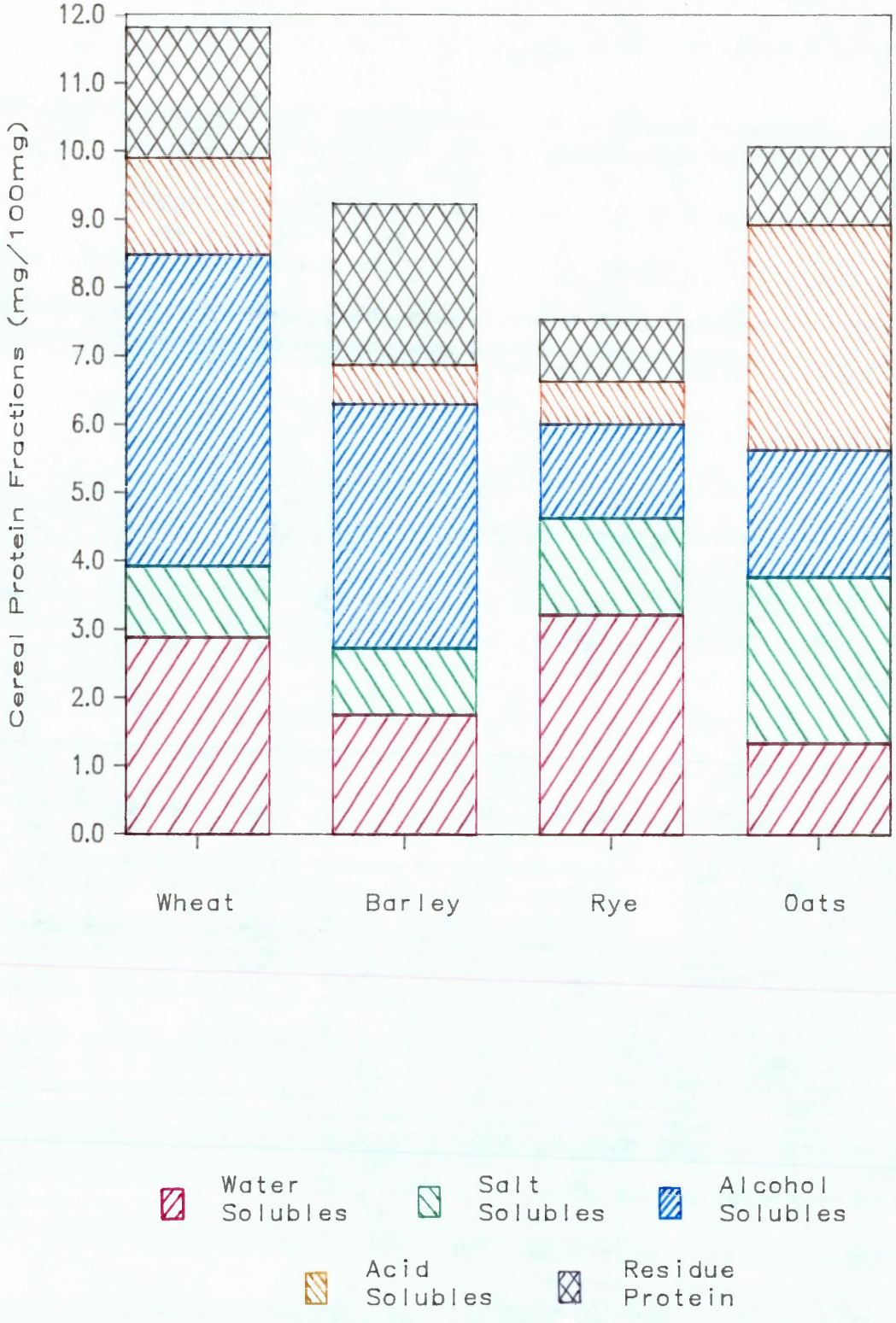
The cereal endosperm proteins, excepting those of oats, may be divided according to their solubility into two principal groups:

i) The cytoplasmic proteins which are soluble either in water (albumins) or dilute salt solutions (globulins). These comprise the enzymes and related proteins that are largely responsible for the metabolic activity of the developing cell, and may be used as an energy source during germination.

ii) The storage proteins, that are generally insoluble in water and salt solutions. These comprise the alcohol-soluble prolamins and the acid-soluble glutelins. The insoluble protein remaining in the residue fraction is also glutelin in character.

Each of the cereals examined, had a "distinctive" distribution of its total protein into the different solubility fractions (Figure 9). The sample of wheat examined had higher levels of albumin (25%) and lower levels of glutelin (i.e. acid soluble protein = 12%, residue protein = 16%) than are usually observed in this cereal, although the values lie within the wide range observed between varieties by Orth & Bushuk (1972). Barley most closely resembled wheat in profile, but contained a higher proportion of residue protein. In contrast, rye had higher levels of the water- and salt-soluble proteins and lower amounts of the prolamin and glutelin

Figure 10: Distribution of protein of wheat, barley, rye and oats into different solubility fractions. (modified Osborne procedure)



Results are expressed as the cereal protein present in each solubility fraction per unit weight of cereal flour

proteins than wheat.

The globulins (salt-soluble fraction) comprise a greater proportion of the total protein in oat than in wheat, barley or rye. However, whereas Ching-Yung Ma & Harwalker (1984) found levels of approximately 42% and 15% for the oat globulins and glutelins respectively, the present investigation observed levels of 24% and 33% for the same fractions. The fractionation procedure of Chen & Bushuk (1970) employs extraction times of 2 x 1h with 0.5M salt solution. This period was, apparently, inadequate for the extraction of all the oat globulins. The low levels of globulin and high levels of glutelin protein, observed here, may, thus, represent an experimental artifact, ALTHOUGH EXPERIMENTS SHOWED THAT NO MORE PROTEIN COULD BE EXTRACTED WITH SALT.

In Figure 10, the amount of protein in each solubility class is represented as mg/100mg flour. Thus, account is made of variations in the protein content of the different cereal grains.

These data suggest that major quantitative differences exist in the protein component of the cereals with respect to:

1. The total protein content
2. The quantity of each "Osborne" solubility class
3. The proportion that each solubility class represents of the total cereal protein content.

Thus, irrespective of the protein "quality" characteristics, some or all of these quantitative differences may significantly affect the functional properties of barley, rye and oats relative to wheat, but

with the limited data at present available it cannot be concluded which factor(s) is(are) the most important.

III.2.3 Extractability of wheat, barley, rye and oat endosperm proteins in buffers containing SDS

Cereal protein extractability into buffered solutions of SDS was determined by the method described in Appendix I.2. A prediction of the Ewart model for glutenin structure is that dough strength, and hence baking performance, is related positively to the lengths of the disulphide-linked polymers. Glutenin molecules are of such a size, that a proportion of them is unextractable under certain conditions. The amount of this "residue" protein may vary according to the solvent used, but for any given solvent the proportion will vary from one wheat to another and correlate positively with baking quality (Pomeranz, 1965; Orth & Bushuk, 1972; Axford et al., 1978; Moonen et al., 1982).

Figures presented below show that a greater proportion of the cereal protein was extracted by SDS, than by sequential extraction with saline, alcohol and acid solutions. However, there was an approximately linear relationship between the amount of residue protein remaining after extraction by the two schemes, for the four cereal samples examined.

Proportion of Unextractable Residue Protein in Wheat, Barley, Rye and Oats, after SDS-extraction or Sequential Extraction with Salt, Alcohol and Acid Solutions

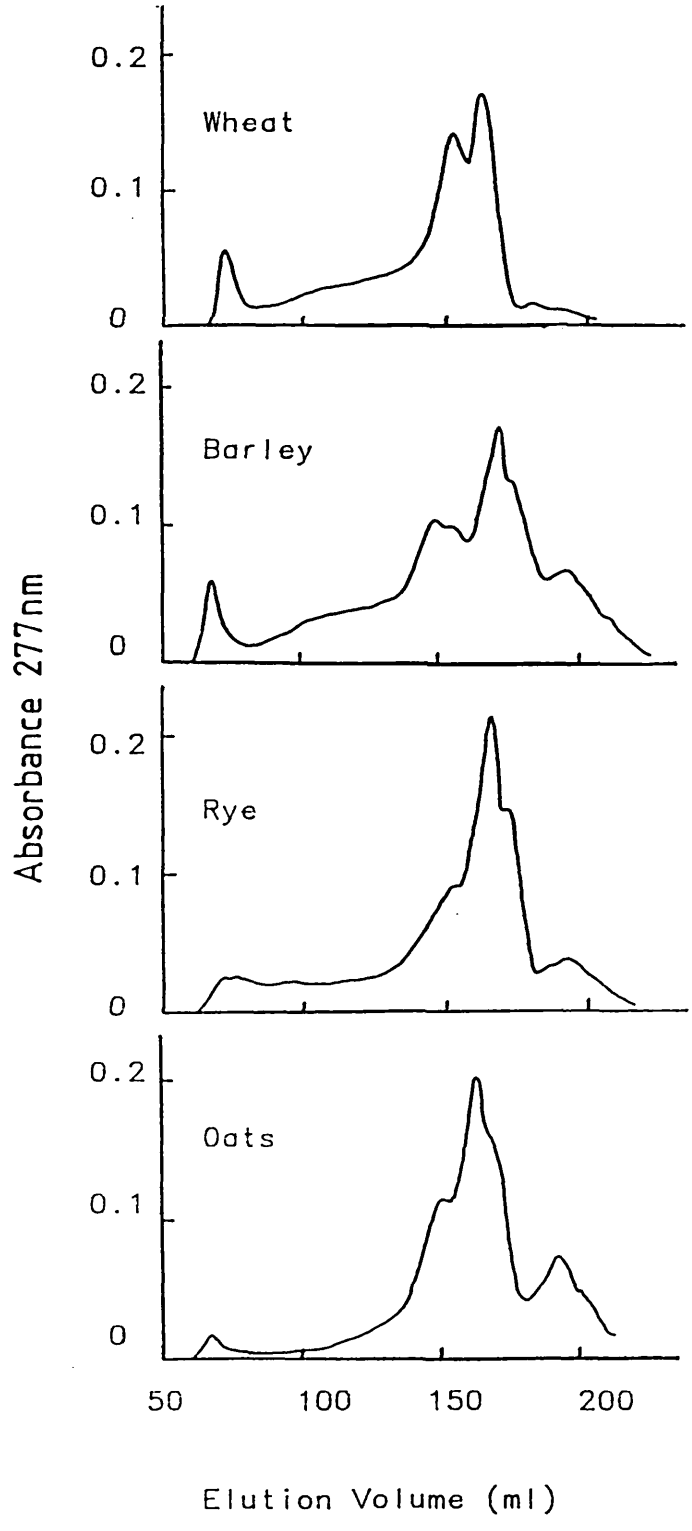
Cereal	Unextractable Residue (% Total Protein)	
	SDS	Modified Osborne
Wheat	7.0	16.4
Barley	19.1	25.4
Rye	8.3	12.1
Oats	8.6	11.4

It has been reported that the amount of "residue" protein in different wheat samples correlates with breadmaking quality (Orth & Bushuk, 1972). Oats and rye have levels of residue protein similar to that of wheat by both extraction procedures, and barley has considerably larger amounts of unextractable protein, (above). However, none of these cereals possess the functional properties of wheat. Presumably, therefore, the "quality" of wheat residue protein is superior to that occurring in barley, rye or oat.

III.2.4 Molecular weight distribution of SDS-extractable proteins of wheat, barley, rye and oats

Gel-filtration chromatography of extracted wheat flour and gluten proteins has shown that baking performance is related to the proportion of aggregated protein present (Field et al., 1983) and to glutenin molecular size distribution (Huebner & Wall, 1976;

Figure 11: Gel-filtration chromatography on Sepharose CL-4B of SDS-extracted proteins from wheat, barley, rye and oat flours.



Bottomley et al., 1982).

Therefore, the molecular size distribution of SDS-extractable proteins from wheat, barley, rye and oats was determined by gel-filtration chromatography, according to the method described in Appendix I.3, in order to define more precisely differences between the cereals that may relate to their different functional properties. The chromatography profiles are shown in Figure 11.

It is again evident that, although the molecular size distribution of the disulphide-linked glutenin polymers appears to be related to breadmaking quality in wheat, protein aggregate size cannot be the only factor of importance in baking. Endosperm proteins of barley, rye and oats have the same range of molecular weights as wheat proteins, and, in the case of barley, the distribution of proteins of different molecular sizes is quite similar to wheat, yet barley does not possess breadmaking ability. The SDS-extracts of all cereals contained very high molecular weight protein that was eluted at the void volume of the Sepharose column. In wheat this material consists of glutenin polymers, in which subunits are linked via disulphide bonds (Bottomley et al., 1982). A similar situation is envisaged in the other cereals. Although high levels of acid-soluble protein were observed previously in oat, these evidently do not comprise significant amounts of high molecular weight glutelin polymers. This further suggests that extraction of globulin protein with salt solution was incomplete.

Barley, rye and oat also contain larger proportions of protein with molecular weights below ~50,000 than wheat. The presence of

elevated levels of low molecular weight material may reduce the cohesiveness of the barley, rye or oat "gluten" protein, and so reduce their ability to be used in breadmaking.

III.3 Conclusions

The proteins of wheat flour are crucial in determining a flour's breadmaking quality. The differences between wheat, barley, rye and oat proteins which make the last three cereals unsuitable for conventional breadmaking have yet to be resolved.

This study has shown quantitative differences in the molecular size distribution of the SDS-extractable endosperm storage proteins of these four cereals. In particular, there are apparently greater amounts of the lower molecular weight species in rye, barley and oats. However, it is unlikely that these differences alone could account for the wide variations observed in cereal functionalities.

Both quantitative and qualitative factors are responsible for variations in breadmaking quality of different wheats. It is, therefore, suggested that qualitative differences in the proteins also play a vital role in determining the physicochemical properties of the other cereals.

Future comparative studies on isolated proteins and polypeptides (amino acid analyses, computer-prediction of conformation, estimates of protein hydrophobicity etc.) will contribute to our understanding of the chemical composition of cereal proteins and perhaps, provide the clue to their different technological behaviour.

Chapter IV: Protein Molecular Weight Distributions in Wheat Flours of Different Baking Quality

IV.1 Introduction

Wheats and their flours are often classified broadly into two groups: strong and weak, on the basis of their suitability for the manufacture of yeast-leavened bread.

Strong wheat flours contain a relatively high percentage of protein that forms a tenacious, elastic gluten with good gas-retaining properties, and they are capable of being baked into well-risen shapely loaves possessing good crumb grain and texture. The flours require a considerable amount of water to make a dough of proper consistency and so give a high yield of bread. The doughs have excellent handling properties, and are not critical in their mixing and fermentation requirements. For this reason, they yield good quality bread over a wide range of baking conditions and have a good fermentation tolerance.

In contrast, weak flours have a relatively low protein content and form a soft, weak, relatively inelastic gluten with poor gas-retaining properties. The flours have relatively low water absorbing capacity and yield doughs with inferior handling properties. The doughs are very critical in their baking and fermentation requirements and so are more likely to fail in baking. Weak flours require less mixing and fermentation than strong flours to give optimum baking results.

The desire to understand the molecular basis of baking quality in wheat flour has prompted many studies, which have been reviewed in Chapter I. These have demonstrated a relationship between several wheat characteristics and breadmaking performance, but whilst a complete description of the structure and interactions of flour proteins is lacking, an explanation of functionality will be similarly incomplete.

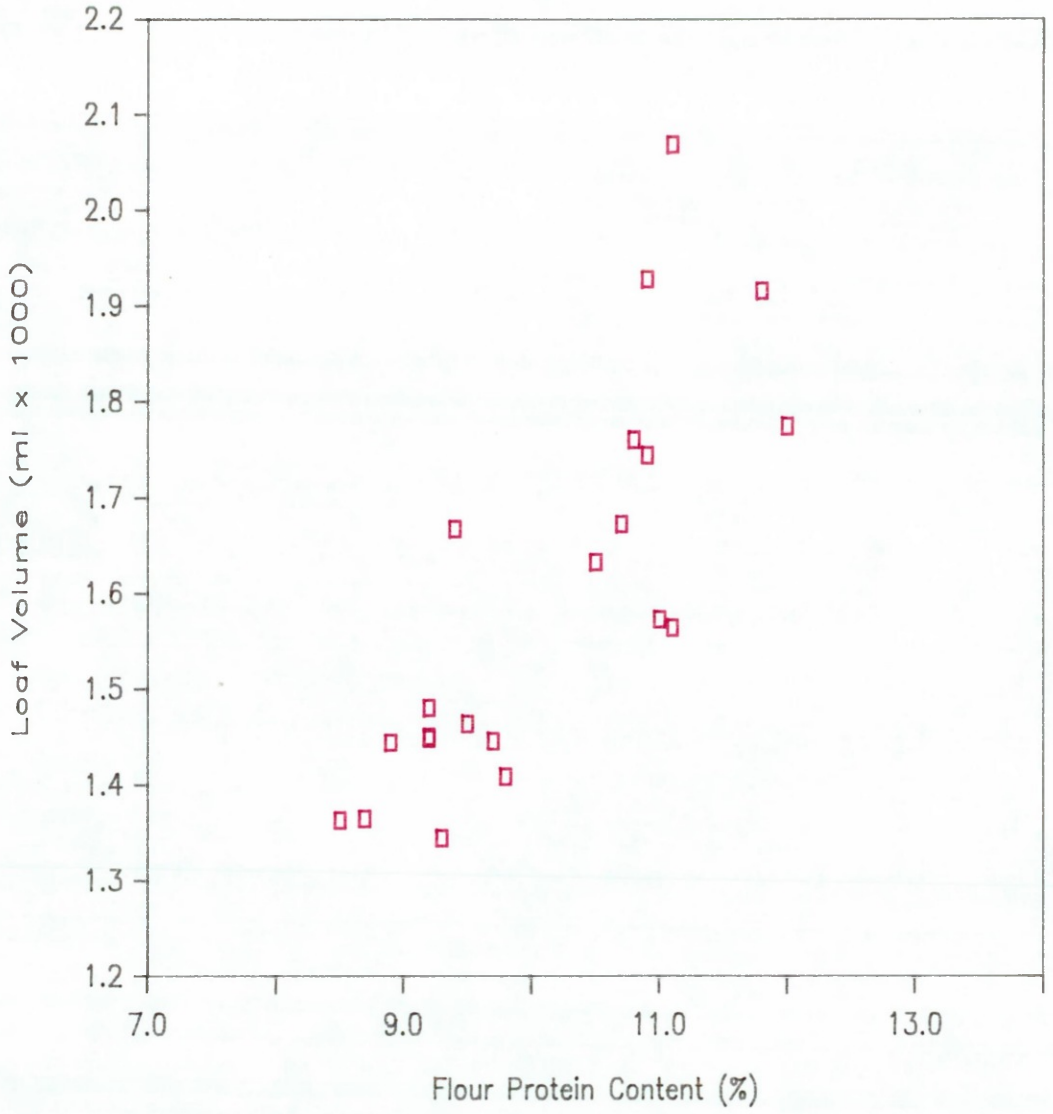
As previously discussed, Ewart proposed that dough strength, and hence baking performance, was related to the length of the disulphide-linked glutenin polymers. This investigation, therefore, examines the molecular size profile of proteins extracted from wheats of different breadmaking ability, in order to determine whether or not a relationship exists between the proportion of high molecular weight glutenin in a flour and its functional properties.

IV.2 Experimental, results and discussion

IV.2.1 Baking performance of 21 Buhler-milled flours from wheats of the 1980 harvest

11 wheat varieties, chosen to represent a range of baking qualities, were grown at 5 different UK sites during 1979-1980. Several varieties were grown at more than one site, thus, in total 21 samples of wheat were harvested in 1980. The wheats were milled into flours on an experimental Buhler mill after overnight conditioning. Resultant flours were analysed for protein content (Kjeldahl N x 5.7), α-amylase activity, starch damage, water absorption and moisture content. The breadmaking quality of the

Figure 12: Variation in loaf volume with flour protein content.



Loaf volume = 157.1 x Protein content (%) + 12.6

r = 0.81

Buhler-milled flours was assessed by the bulk-fermentation procedure, described in Appendix I.5. Analytical and baking data for the 21 samples of wheat flour are presented in Table 5, overleaf.

There is much evidence to suggest that the major factor accounting for variation in loaf volume within a variety is protein content. For the samples examined here, the relationship between loaf volume and protein content was essentially linear between 8.5% - 12.0% protein (Figure 12), however, there was insufficient data to determine the regression lines for each variety.

Whereas the quality of the flour protein is largely a heritable characteristic, the quantity depends on variety and class, but mainly on environmental factors during growth. For example, cold, cloudy, dull weather conditions during the period of kernel development usually result in a low protein content, whereas warm, dry weather during the same period, but with adequate soil moisture to ensure availability of nutrients, particularly N, to the crop favours high protein content. Protein content is also considerably influenced by the available soil nitrogen, and rises with increasing levels of fertilisation. Thus, some variation in protein content between the samples would be expected due to their different sites of growth and the available soil nutrient supplies. Comparing samples of a single variety grown at several sites, e.g. Copain, Armada or Flanders, protein levels were generally highest in samples grown at the Leicestershire or Kent sites, and lowest in those obtained from Essex.

Table 5: Flour, Dough and Bread Characteristics of 21 Wheat Samples of the 1980 Harvest

Code	Variety	Growing Site	P %	α-Amylase F.U.	Starch Damage %	Farinograph S 1 min.	Farinograph S 2 B.u.	W.A. %	Loaf Volume ml
AB592	Armada	Essex	8.9	7	41	1.00	125	64.6	1445
AB619		Hants.	9.7	< 1	42	1.25	110	66.0	1446
AB542	"	Leics.	11.1	4	32	2.25	90	62.6	1564
AB576	"	Oxon.	9.2	3	31	1.75	100	60.4	1451
AB543	Avalon	Leics.	10.8	1	27	5.25	50	58.6	1761
AB575	"	Oxon.	9.2	< 1	43	2.25	90	66.4	1449
AB540	Bounty	Leics.	10.7	7	31	1.75	85	60.4	1673
AB624	Copain	Essex	9.5	2	46	1.75	85	65.2	1464
AB621		Hants.	10.5	< 1	46	2.75	85	65.5	1633
AB499	"	Kent	11.0	1	41	2.25	100	69.0	1573
AB550	"	Leics.	12.0	39	32	2.50	120	62.0	1775
AB552	"	Oxon.	10.9	1	38	3.00	70	65.6	1745
AB594	* Flanders	Essex	9.4	3	17	1.00	140	57.0	1668
AB523		Kent	11.8	2	12	1.50	120	67.4	1916
AB549	"	Leics.	10.9	6	15	2.50	95	54.0	1928
AB513	* Norman	Kent	9.2	8	20	0.75	120	58.0	1481
AB505	* Rapier	Kent	9.3	2	22	0.50	160	60.4	1345
AB541	Timmo	Leics.	11.1	5	17	1.50	90	54.3	2069
AB578	* Abele	Oxon.	8.7	7	30	1.00	110	60.4	1366
AB626	* Baron	Essex	8.5	212	27	0.75	230	59.6	1364
AB500	* Mithras	Kent	9.8	4	23	1.25	110	60.8	1409

* = Soft wheat varieties. Unmarked samples are hard-milling wheats.
P = Protein content = Kjeldahl N x 5.7
W.A. = Water absorption capacity
Farinograph S 1 = Dough stability . AACC Method 54-21 (1962)
Farinograph S 2 = Dough softening AACC Method 54-21 (1962)
F.U. = Farrand units
B.U. = Brabender units

The range of protein contents varied as:

Copain	12.0% (Leics)	- 9.5% (Essex)
Armada	11.1% (Leics)	- 8.9% (Essex)
Flanders	11.8% (Kent)	- 9.4% (Essex)

The source of this site-to-site variation in protein content was not investigated.

It is generally accepted that the mechanical or rheological properties of doughs are related to the quality of the baked products and are determined partly by protein quality factors (primarily the gluten portion of flour protein). Quality is often appraised by subjecting the flour to physical tests, using devices such as the Brabender Farinograph or Extensograph or Chopin Alveograph, which measure various rheological characteristics of the dough.

The tests are usually performed on flour-water doughs and characterise the gluten portion of the protein by measuring factors such as extensibility and resistance to extension, dough development time, dough stability at a predetermined consistency and degree of softening at a fixed time after maximum dough development.

Values for the Farinograph characteristics of the test doughs (Table 5) indicate considerable variations in gluten strength that are generally parallelled by variations in loaf volume, i.e. doughs with a short period of stability and a high degree of softening produce loaves of lower volume than doughs that are more resilient during

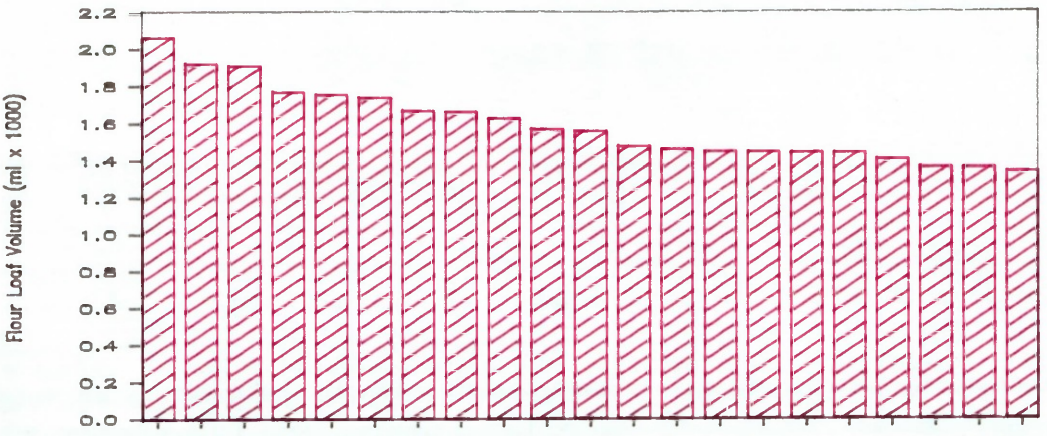
mixing.

Amylolytic modification of starch during breadmaking depends on both the quantity of enzyme present and the state of the substrate, since hydrolysis of damaged starch proceeds more rapidly than hydrolysis of intact granules. Normal flour from sound wheat contains ample levels of β-amylase, (a saccharogenic enzyme), but levels of α-amylase, (a dextrinogenic enzyme), are often supplemented by the addition of malt or fungal α-amylase, usually to a level of 8 Farrand Units (F.U.). The level of damaged starch in a flour depends on the severity of the milling process and the hardness of the wheat endosperm and averages about 9% in bread flours.

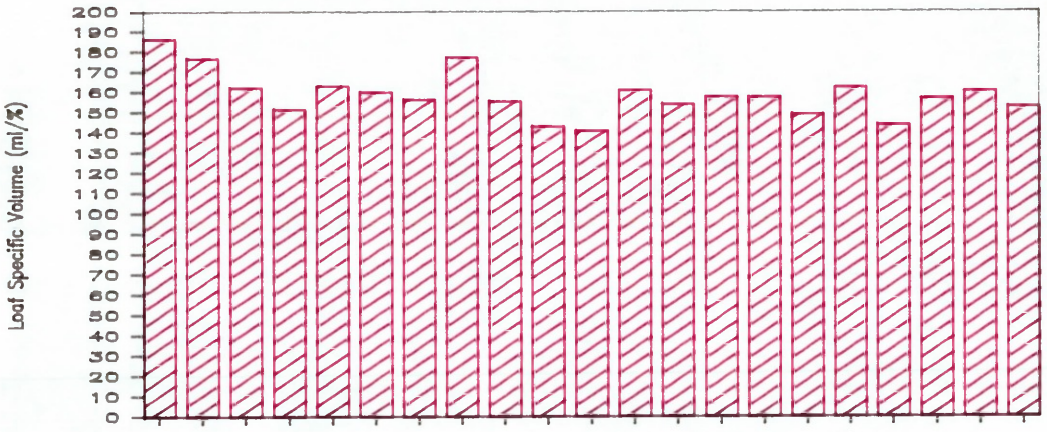
Water added in dough making is largely absorbed by the protein and starch fractions. The gluten portion of flour has a relatively constant water-inbibing capacity ($\sim$ 2.0 times the dry gluten content). Intact starch granules take up $\sim$35% of the starch weight at normal dough temperatures (Sandstedt, 1955) but have greater water-inbibing capacity when damaged. Flour water absorption capacity is an important parameter in the production of all types of baked goods. In "No-time" dough systems, high water absorption values are usually desirable since they tend to increase the unit yield of products.

The gluten matrix is in reality the skeleton or framework of dough that is responsible for gas retention and makes the production of light, leavened goods possible. However, the foregoing discussion clearly indicates that the quality of the bread produced is the result of complex interactions between protein, starch, enzymes,

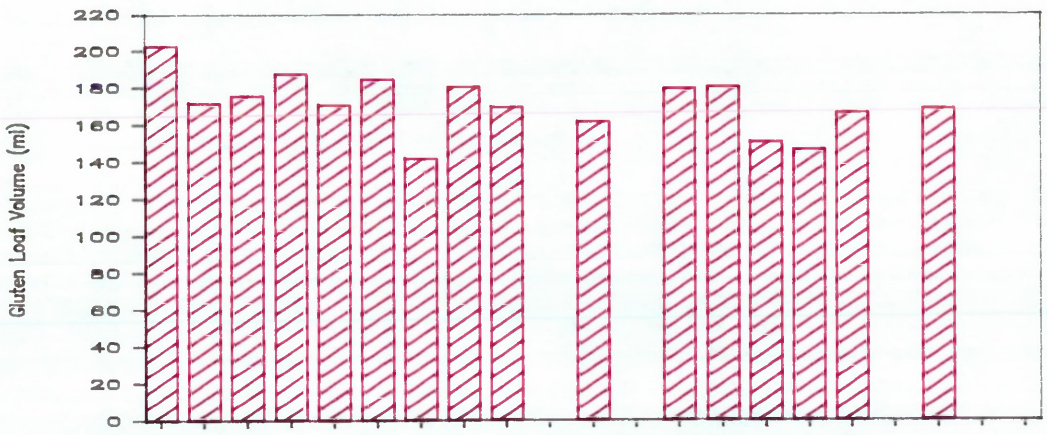
Figure 13: Quantitative and qualitative protein factors and their influence on loaf volume.



A: Range of loaf volumes (ml) for 21 flours differing in breadmaking potential.



B: Loaf specific volume (ml/%) for 21 flours differing in breadmaking potential



C: Loaf volumes of 16 reconstituted "flours" prepared from glutens isolated from flours differing in breadmaking potential, and a standard starch and water-soluble fraction.

Samples are ranked in the same order in A, B and C

yeast and other dough constituents.

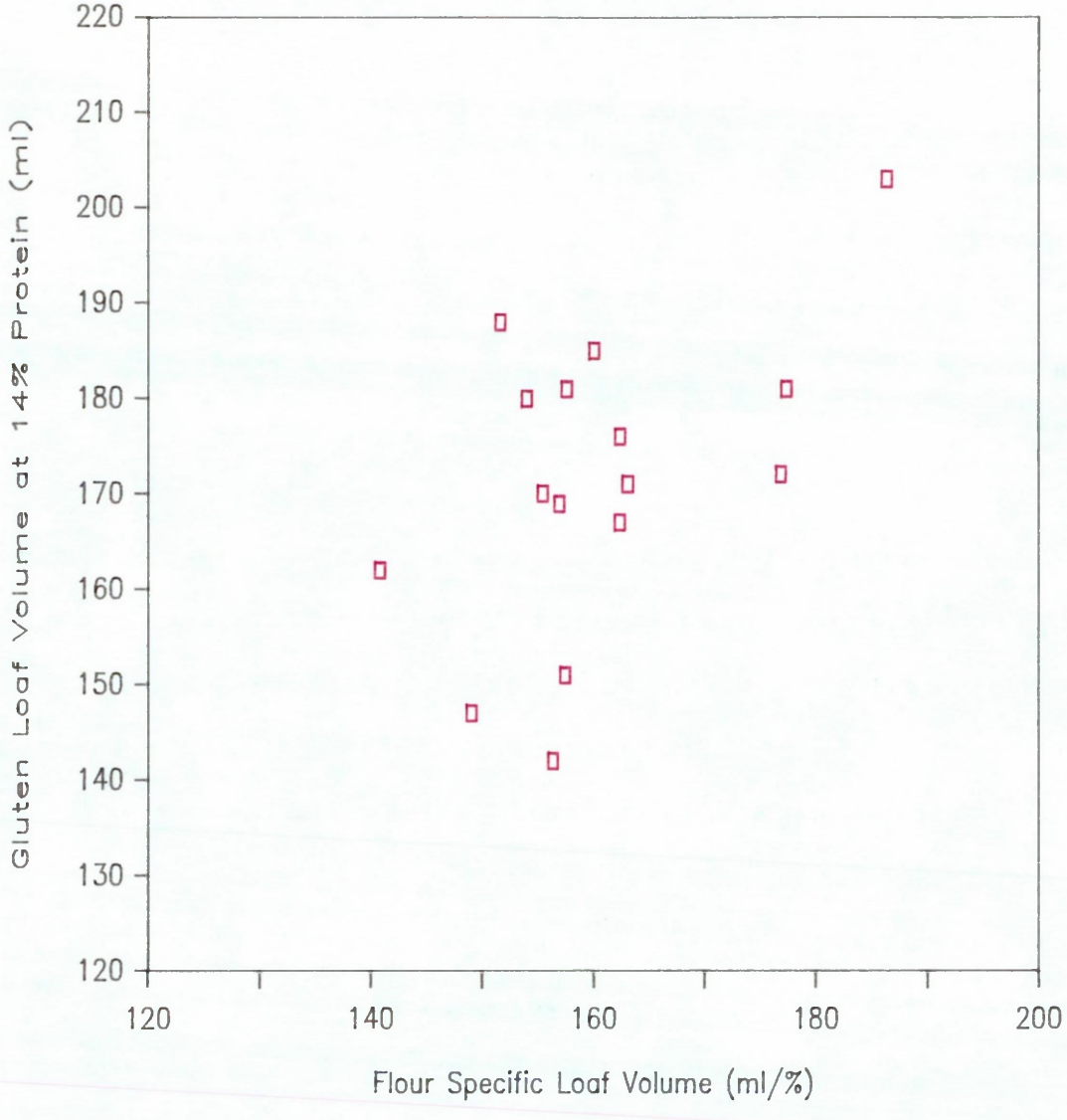
IV.2.2 Baking performance of glutens isolated from flours of different breadmaking quality, as measured by a reconstitution baking procedure

Variations in baking performance of the native flour may be the result of differences in protein level, enzymic activity, damaged starch levels etc. Baking of glutens isolated from flours, by reconstitution with CWRS starch and CWRS water-solubles permits evaluation of the gluten protein component against a constant and common background.

Therefore, vital wheat gluten was isolated from 16 of the flour samples by the procedure described in Appendix I.6. Insufficient flour was available for gluten isolation from AB499 Copain, AB500 Mithras, AB626 Baron, AB505 Rapier and AB515 Norman. The baking performance of each of the dried glutens was assessed in a reconstitution baking procedure as detailed in Appendix I.7.

The samples are presented in Figure 13A ranked in order of the loaf volume. A continuous spectrum of loaf volume was observed for the native flours. This variation is due to differences both in protein quantity and in protein quality in the flours. When these data were expressed as loaf specific volume (i.e. volume per unit of flour protein, Figure 13B), thereby eliminating the effects of flour protein content, it was evident that some samples that gave loaves of relatively low volume, e.g. AB578 Abele, AB592 Armada, contained protein, apparently, with functionality equivalent to that

Figure 14: Comparison of flour and gluten baking performance in the absence of the effects of protein quantity on loaf volume.



of samples that gave higher loaf volumes, e.g. AB621 Copain, AB543 Avalon.

Reconstitution baking of the freeze-dried glutens at 14% protein content, with CWRS starch and water-soluble material, also allows evaluation of protein quality in the absence of variations in loaf volume due to protein quantity (Figure 13C). However, the profile of gluten loaf volumes did not correlate well with the profile of flour loaf specific volumes for the same samples. (Figure 13B). This is demonstrated more clearly in Figure 14.

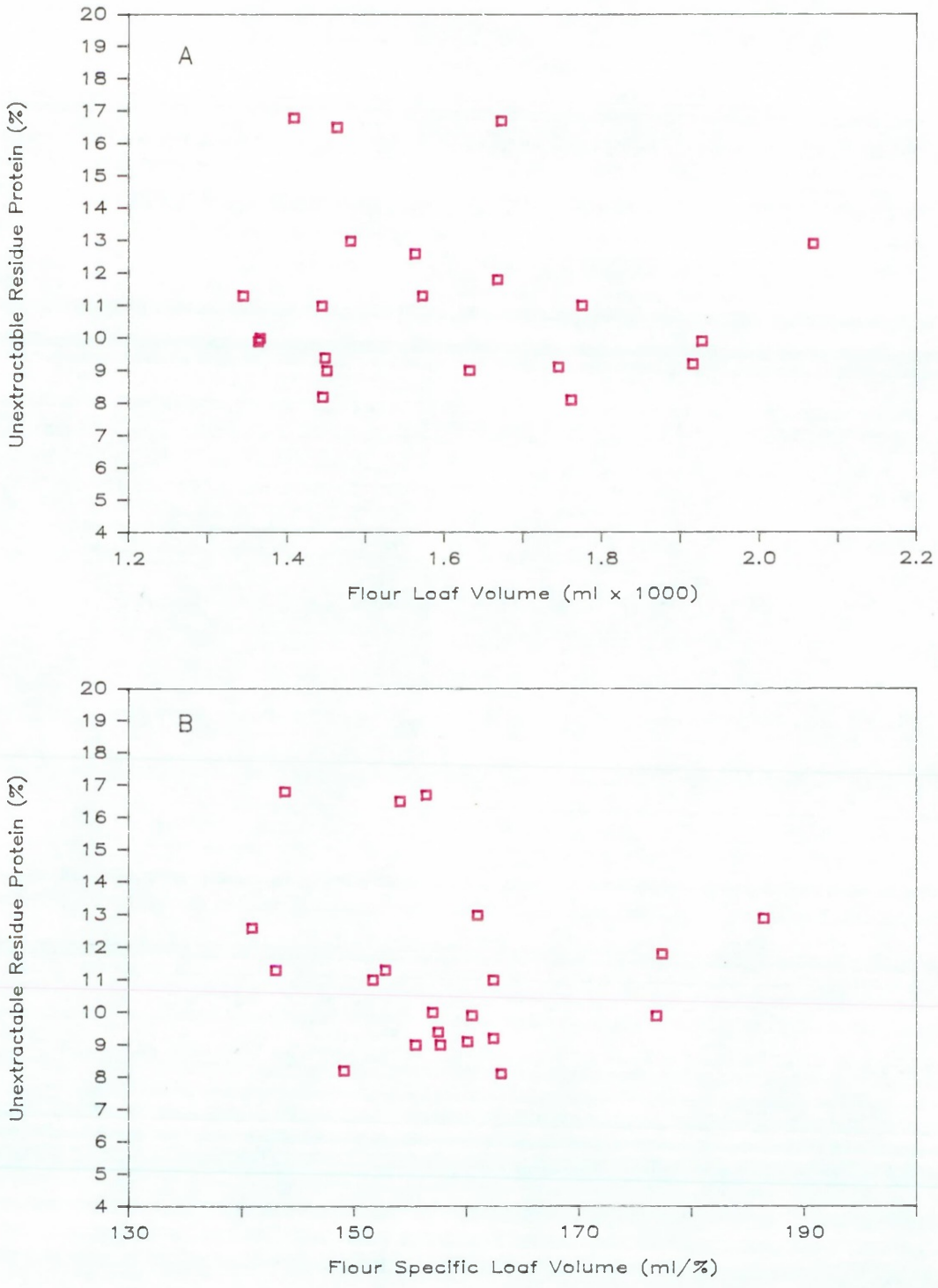
Since protein content is constant, and gluten protein components are common in both estimates of loaf volume, the residual scatter in Figure 14, is presumably due to differences between samples in the starch and water-soluble fractions, which, in the reconstitution baking procedure, are derived from a CWRS "standard" flour.

IV.2.3 Extractability of wheat flour proteins into buffers containing SDS

Other workers have demonstrated a positive correlation between the amount of unextractable residue protein and baking performance of different wheat samples. To determine whether or not such a relationship existed here, protein extractability into buffered solutions containing SDS was determined for the 21 samples of flour, by the method described in Appendix I.2.

On average, 90% of the total protein was extracted from the flours, although the protein extractability of the individual samples

Figure 15: Relationship between the proportion of SDS-unextractable protein and baking performance for flours of 21 wheats differing in bread-making potential.



Results are expressed as the proportion of SDS-unextractable protein in the flour as a function of (A) flour loaf volume, and (B) flour loaf specific volume.

covered a wide range, i.e. 83.2% - 91.9%. Bottomley et al. (1982) have demonstrated that extraction buffers containing SDS are consistently more effective than AUC in extracting flour protein. These workers demonstrated that SDS extracted approximately 94% of the total protein in a sample of Flinor flour. The high solvent efficiency of SDS may be the result of its ability to penetrate the complex flour system and dissociate and extract proteins that are organised into macromolecules through non-covalent bonds. In the present study, such high levels of extractability were not achieved but, since experimental conditions for determining protein extractability were similar in both studies, the higher proportion of unextractable protein seen here was probably due to variations in raw materials.

No correlation was observed between the proportion of unextractable residue protein and either loaf volume (Figure 15A) or loaf specific volume (Figure 15B).

IV.2.4 Molecular weight distributions of SDS-extractable proteins of flours of different baking quality

In order to examine more precisely the relationship between protein molecular size distribution and breadmaking quality, SDS-extracts of the 21 samples of wheat flour were chromatographed on Sepharose columns according to the method described in Appendix I.3. The protein molecular size distributions for the 21 samples are summarised in Table 6.

Table 6: Protein molecular weight distributions of 21 samples of wheat flour of different baking quality

Code	Loaf Volume (ml)	LSV * (ml/%)	A	B	C	D	Residue
			<----% of Total Flour Protein---->				
AB592	1445	162.4	8.1	28.3	30.4	22.1	11.0
AB619	1446	149.1	7.6	29.3	32.7	22.6	8.2
AB542	1564	140.9	4.5	28.8	36.2	17.9	12.6
AB576	1451	157.7	8.2	28.6	34.3	20.1	9.0
AB543	1761	163.1	6.0	32.8	30.2	21.2	8.1
AB575	1449	157.5	6.9	29.8	29.9	24.0	9.4
AB540	1673	156.4	6.4	28.9	27.0	21.0	16.7
AB624	1464	154.1	6.8	27.1	26.6	23.0	16.5
AB621	1633	155.5	6.3	30.3	30.8	23.7	9.0
AB499	1573	143.0	4.3	23.5	32.5	28.4	11.3
AB550	1775	151.7	6.1	29.7	31.8	21.5	11.0
AB552	1745	160.1	7.8	29.1	30.2	23.9	9.1
AB594	1668	177.4	7.0	27.1	30.1	24.1	11.8
AB523	1916	162.4	7.9	29.1	32.1	21.8	9.2
AB549	1928	176.9	7.1	28.3	34.1	20.7	9.9
AB513	1481	161.0	5.5	27.8	27.7	26.2	13.0
AB505	1345	152.8	5.1	27.8	27.6	26.3	11.3
AB541	2069	186.4	7.9	29.9	30.6	18.7	12.9
AB578	1366	157.0	8.6	26.7	30.7	24.0	10.0
AB626	1364	160.5	7.3	26.9	28.7	26.0	9.9
AB500	1409	143.8	5.2	25.9	25.8	26.5	16.8

* LSV = Loaf specific volume = loaf volume (ml) / flour protein content (%)

A = High molecular weight glutenin proteins, eluted at the void volume of a Sepharose CL-4B column.

B = Lower molecular weight glutenin proteins, included within the Sepharose CL-4B matrix.

C = Gliadin proteins.

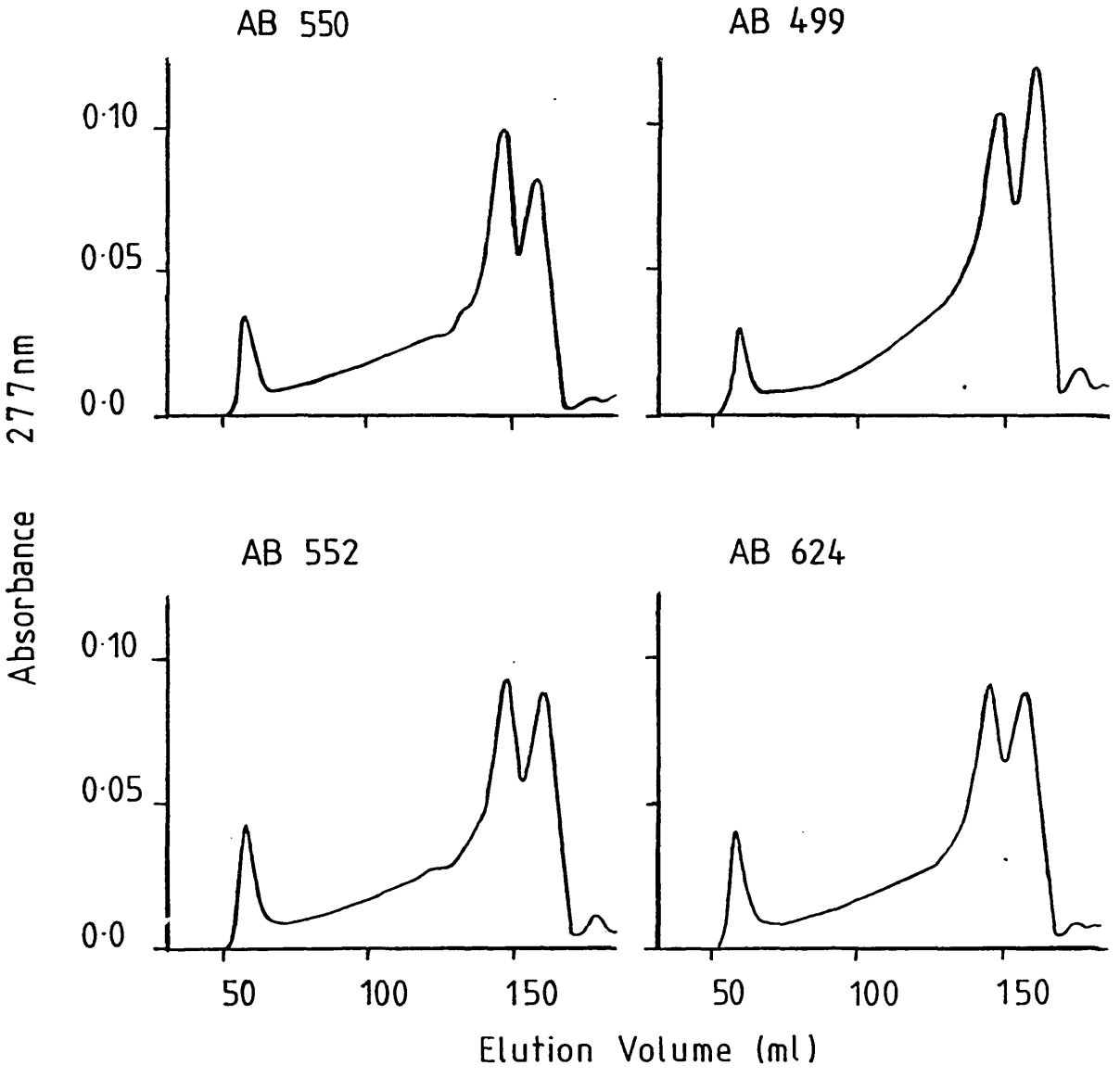
D = Albumin and globulin proteins.

Residue = Protein not extracted by 2% SDS, 0.1M Tris-HCl buffer, pH 8.0.

The elution profiles of protein extracts of flours of different baking quality showed marked variation with respect to the proportion of protein in each of the regions A, B, C and D (defined in Chapter II.2.4.). These differences appear to be the result of both genetic and environmental factors: samples of a single variety, e.g. Copain, showed as much variation in the molecular weight distribution of their proteins (Figure 16, overleaf) as samples of different varieties, (Figure 17, overleaf).

The genetic complement of the Copain samples is essentially the same, thus, variation in the amounts of glutenins, gliadins, albumins and globulins is presumably the result of variation in environmental and agronomic conditions during growth and development. In flours of different varieties, differences in protein molecular weight distribution due to environmental factors will be superimposed on differences due to their individual genetic complements. In most instances the relative importance of these two considerations is difficult to quantify, and "variety characteristics", (i.e. genetically determined) with respect to molecular weight distribution of their proteins, may be obscured. There are indications that Armada, for example, generally has high levels of gliadins (peak C) and low levels of albumin + globulin (peak D), but this is based on a limited study and more data is necessary before this observation can be confirmed. Other workers have observed, similarly, that protein ratios are affected by climate and soil conditions as well as by genetic factors (Bushuk et al., 1969; Koenig et al., 1964).

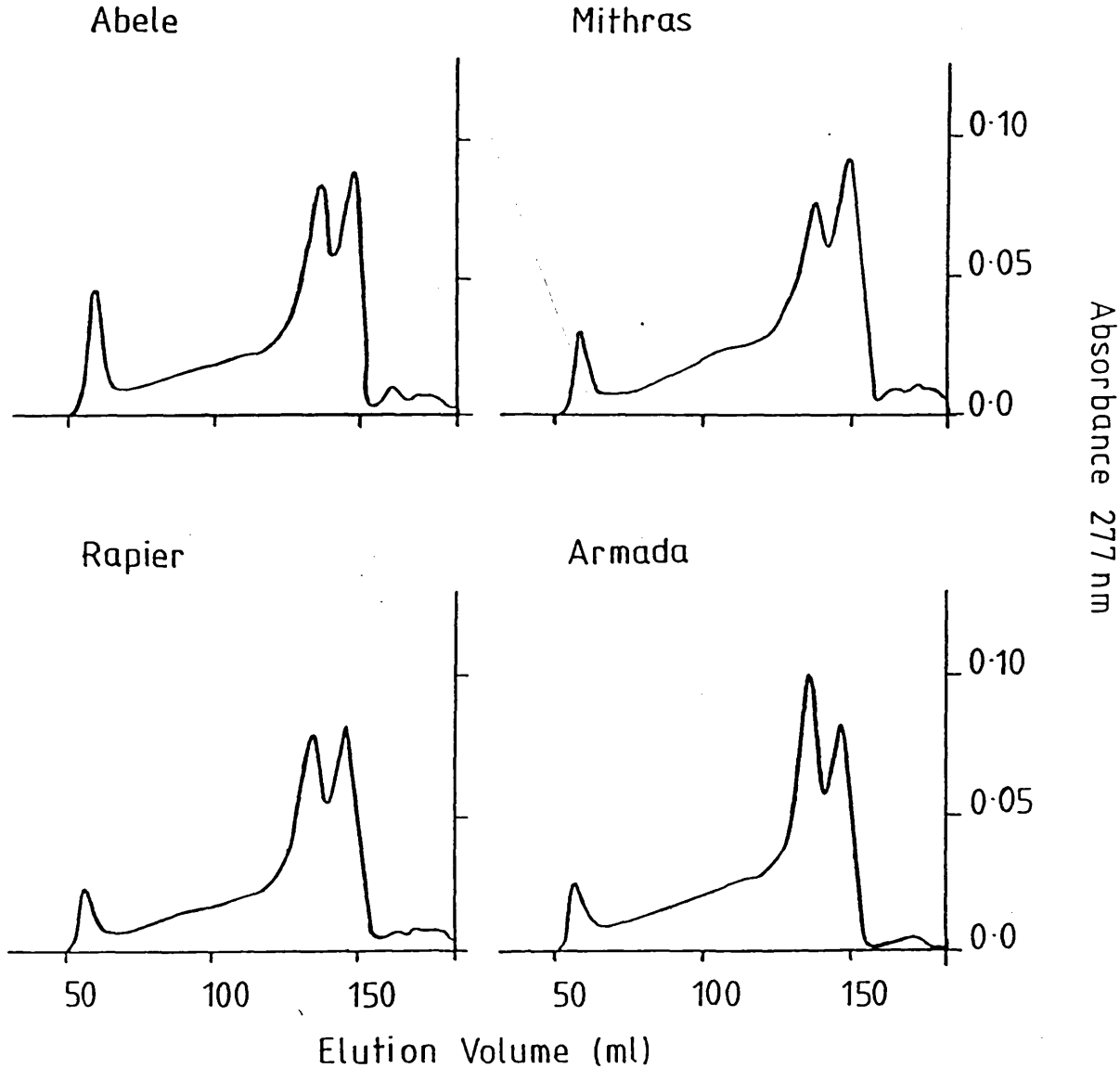
Figure 16: Variations in molecular weight distribution of proteins extracted by SDS from four samples T. aestivum var. Copain.



	A	B	C	D	Residue Protein
AB 550	6.1	29.7	31.8	21.5	11.0
AB 499	4.3	23.5	32.5	28.4	11.3
AB 552	7.8	29.1	30.2	23.9	9.1
AB 624	6.8	27.1	26.6	23.0	16.5

Results are expressed as the proportion of the total flour protein present in each of the regions of the Sepharose CL-4B chromatogram, or not extracted by SDS

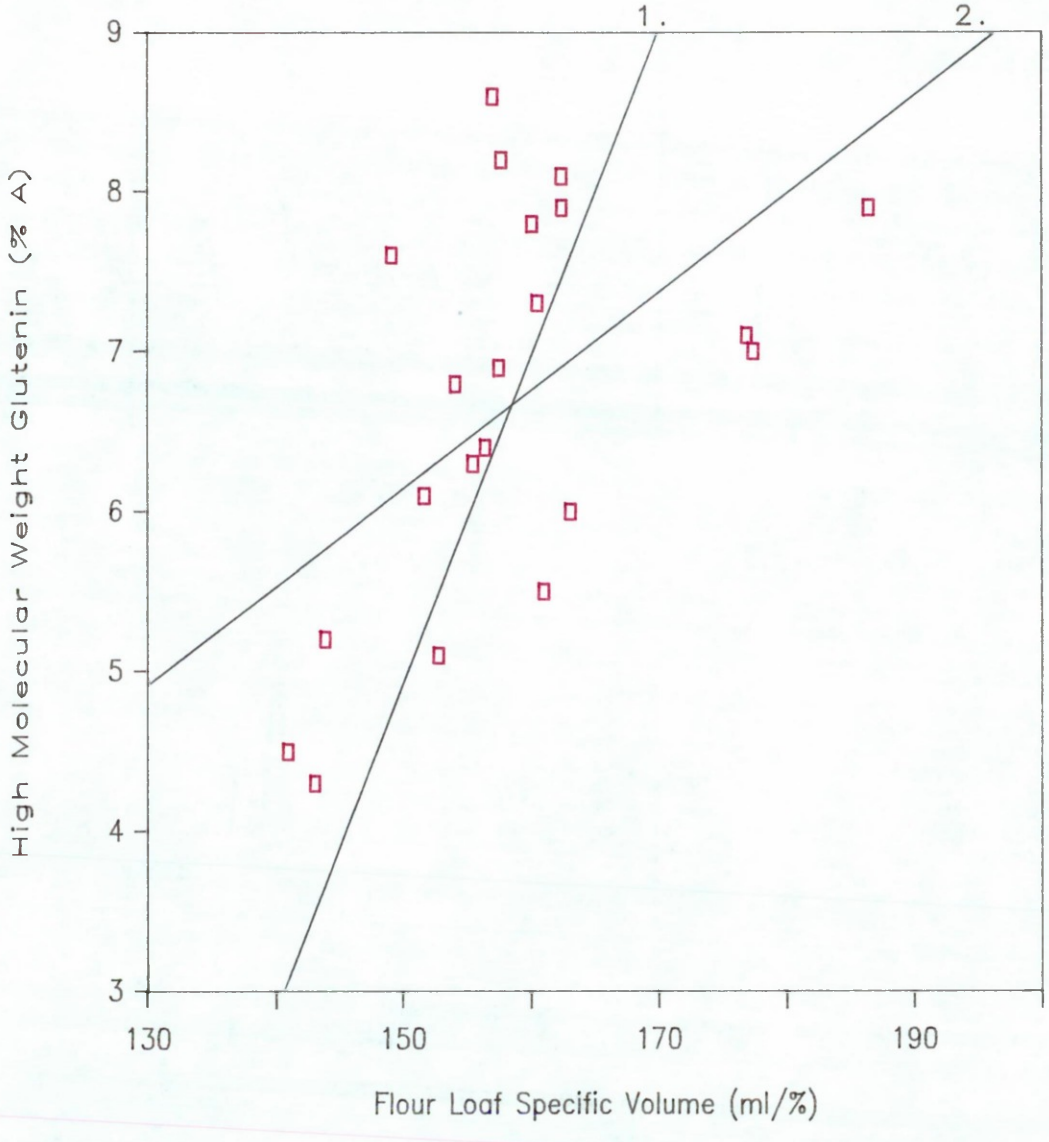
Figure 17: Variations in molecular weight distribution of proteins extracted by SDS from four samples T. aestivum of different varieties.



	A	B	C	D	Residue Protein
Abele	8.6	26.7	30.7	24.0	10.0
Mithras	5.2	25.9	25.8	26.5	16.8
Rapier	5.1	27.8	27.6	26.3	11.3
Armada	4.5	28.8	36.2	17.9	12.6

Results are expressed as the proportion of the total flour protein present in each of the regions of the Sepharose CL-4B chromatogram, or not extracted by SDS

Figure 18: Relationship between the proportion of SDS-extractable high molecular weight glutenin protein and loaf specific volume for 21 flours differing in breadmaking potential.



High molecular weight glutenin (%A) is defined as the proportion of the total flour protein that is extracted by SDS and eluted at the void volume of a Sepharose CL-4B chromatography column.

Equations of the straight lines:

1. Loaf specific volume = 4.89 x %A + 125.81
   ± (2.1 x 9.62) 95% confidence limit

2. %A = 0.06 x Loaf specific volume − 2.99
   ± (2.1 x 1.08) 95% confidence limit

$r^2 = 0.303$

Since loaf volume is known to be dependent on both protein quantity and quality, it was considered that loaf specific volume, i.e. Loaf Volume/Flour Protein Content, was a better index of intrinsic protein quality than actual loaf volume. Many workers have indicated that sufficient glutenin content, especially high molecular weight glutenin, is essential for the formation of strong doughs (Booth & Melvin, 1979; Huebner & Wall, 1976; Pallagi-Bànkfalvi, 1984). The present study also points to the importance of large glutenin aggregates in determining breadmaking performance, (Figure 18).

Analysis of the data revealed a correlation between the proportion of high molecular weight glutenin protein and the loaf specific volume, although this correlation was not statistically significant. $r^2 = 0.303$, or, approximately 30% of the variation in loaf specific volume could be explained by variations in the amount of aggregated glutenin protein present in the flours.

The equations of the straight lines through the data points are:

1. Loaf specific volume = $4.89 \times \%A + 125.81 \pm (2.1 \times 9.62)$

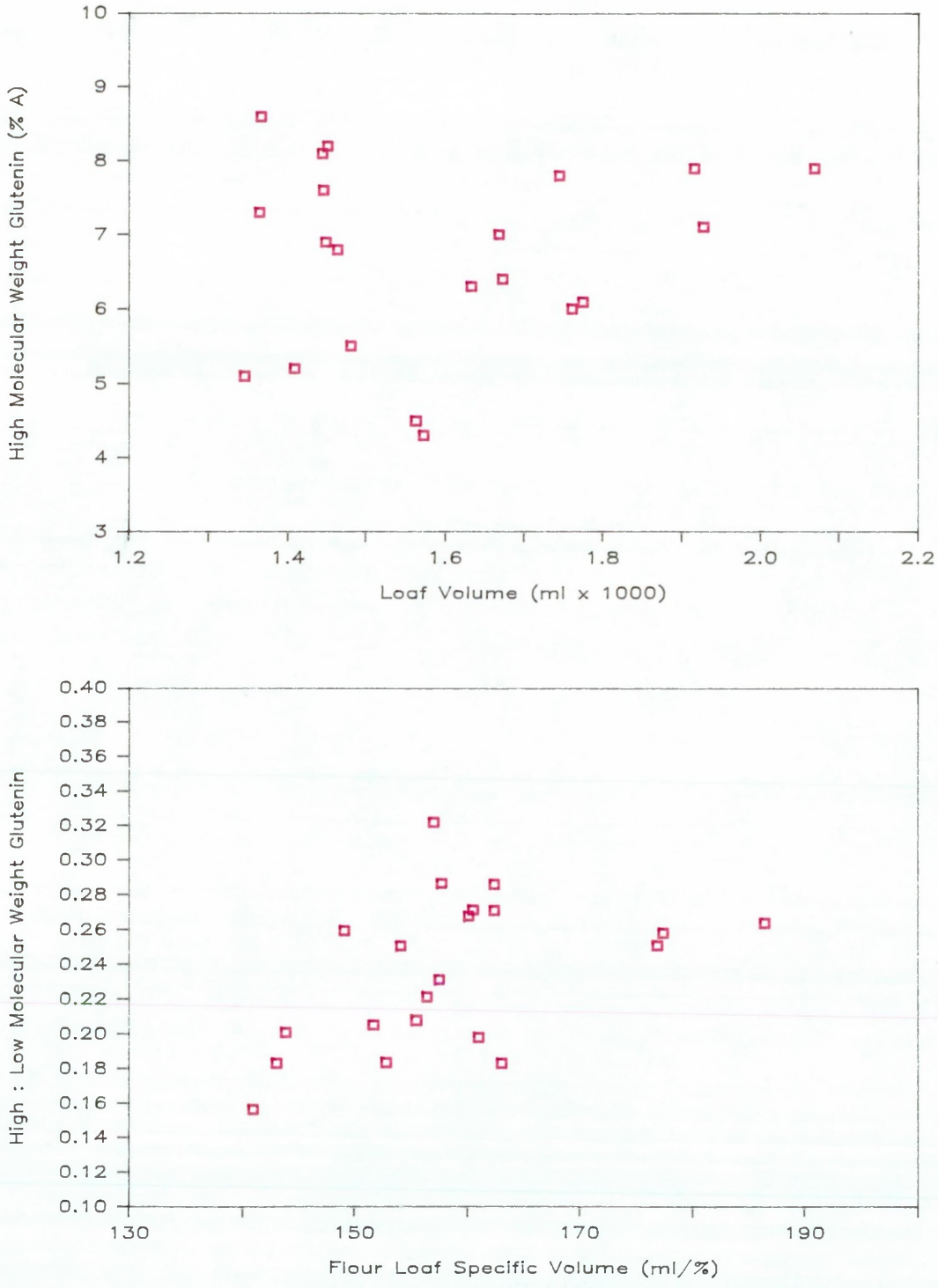
95% confidence limit

2. $\%A = 0.06 \times$ Loaf specific volume $- 2.99 \pm (2.1 \times 1.08)$

95% confidence limit

No other component(s), or ratio of components of the flour protein correlated with baking performance significantly (Figure 19).

Figure 19: Relationship between the molecular weight distribution of SDS-extractable glutenins and breadmaking performance.



SDS-extractable flour proteins are chromatographed on Sepharose CL-4B: high molecular weight glutenins (%A) appear at the column void volume; low molecular weight glutenins (%B) are included within the column volume.

IV.3 Conclusions

Cereal chemists use the term "quality" to describe the suitability of a wheat flour for a particular end use, e.g. breadmaking, pasta production etc., but "quality" cannot be expressed in terms of a single property, and is dependent on many characteristics of the raw material.

The data presented here showed that a major factor accounting for variation in loaf volume was flour protein content, the relationship being essentially linear. Reconstitution baking of the isolated gluten components of flour showed that protein quality varied between the samples also. Loaf volume per unit of flour protein did not correlate perfectly with loaf volumes for glutens reconstituted at constant protein content. Since both measurements eliminate the effect of variations in gluten protein quantity, and have common gluten components for a given sample, these data suggest that components of flour, other than the gluten proteins, influence breadmaking quality.

There was no correlation between the amount of SDS-unextractable residue protein and either loaf volume or loaf specific volume. Determination of the proportion of different molecular weight species in SDS-extracts of the different flours indicated that a high level of very high molecular weight glutenin was related positively to loaf volume. These findings do not necessarily contradict the work of others, who have demonstrated a relationship between the amounts or proportions of protein insoluble in 3M urea (Pomeranz, 1965), in dilute acetic acid (Orth & Bushuk, 1972; Mecham

et al., 1972; Orth et al., 1976), or in AUC (Huebner & Wall, 1976) and baking performance. The relationship between the amount of insoluble glutenin protein and quality is very dependant on the efficiency of the extraction procedure, and where a poor extraction is achieved, a negative correlation may be obtained (Orth & Bushuk, 1972). However, the use of a better solvent gives a positive correlation, particularly when the amount of high molecular weight aggregated glutenin is considered. SDS is a better extractant of flour proteins than 3M urea, acetic acid or AUC, thus, some of the protein that is not extracted by these other solvents may be extracted by SDS, and appear in the very high molecular weight glutenin protein fraction, that is excluded from the matrix of the chromatography resin owing to its molecular size (Region A).

Chapter V: Changes in Wheat Flour Proteins during Bread Manufacture by a Mechanical Dough Development Procedure

V.I Introduction

Changes in the type of bread we eat, from the simple unleavened product made by ancient civilisations, to bread as we know it today, have been the result of tremendous changes in the technology of milling and baking.

Traditionally, bread in the UK was made from a dough of flour, yeast, salt, water and usually a little fat. The dough was mixed for 15 - 20 min at a low speed, in a mixer designed to imitate the action of the human hand, and the dough was then left to ferment for 2 - 3h at 27°C. After fermentation, the dough was divided into pieces of the required weight, moulded and then rested for a further 15 - 20 min; the first proof. The doughpiece was then remoulded, and placed in a baking tin before fermentation for 45-60 min at a temperature between 38 - 48°C, and high relative humidity. Finally the doughpiece was baked for 30 min at 220 - 230°C.

There are many variations on this basic procedure, the characteristics of the end-product being largely determined by the bread recipe, the shape and structure of the loaf and the baking conditions employed. Procedures that involve long fermentation times, obviously occupy a great deal of time and floor space, and make the bakery production very vulnerable to plant breakdown.

However, in such procedures, the dough cannot be processed immediately after mixing since this produces bread that is of inferior quality, characterised by low volume, coarse crumb and poor keeping properties. The bulk fermentation effects some beneficial change(s) on the properties of the dough, which is/are reflected in the quality of the bread. The most important changes due to bulk fermentation are observed in the improved rheological properties of the dough. Directly after mixing the dough is short and elastic, and cannot be expanded by gas. As fermentation proceeds, the dough mellows and is able to hold entrapped CO_2 in the form of finely-dispersed, discrete bubbles. Excessively long fermentation periods cause the dough to lose its gas-retaining properties.

Thus the "ripe" or "mature" or "developed" dough, as it is frequently described, is essential to the production of a bold, well-risen, fine-textured resilient, loaf.

Dough, however, may be "developed" by means other than prolonged yeast fermentation. Swanson and Working (1926) showed that intense mechanical energy could, to a large degree, replace the bulk fermentation of a dough, without loss of bread quality. However, it was 30 years before this concept was sucessfully implemented in a commercial process. The "Do-Maker" process, described by Baker (1954), involved the principles of continuous dough production by mechanical development, under pressure of a continuous feed of ingredients. This process was subsequently investigated at the British Baking Industries Research Association (BBIRA), now the Flour Milling and Baking Research Association (FMBRA). Here, the principles of mechanical dough development were defined more

precisely, and a batch procedure was developed that gave bread that was acceptable to the British palate and was indistinguishable from that produced by the traditional bulk-fermentation procedures used at that time in UK bakeries (Chamberlain et al., 1962, 1965; Elton, 1965; Axford et al., 1963). Today, some 75% of all bread in Britain is made by this "Chorleywood Bread Process" (CBP).

The characteristics of the CBP may be summarised as below:

1. **Work Level**

The optimum work input has been established at 40 kJ/kg, although some variation in energy requirements may result from flour differences or from the type of bread required. The prescribed energy must be imparted within 5 min of mixing for best results. (2 - 4 min mixing are usual). The energy requirement is approximately 5 - 8 times that consumed when mixing a dough for a bulk-fermentation procedure, and is applied in one fifth of the time.

2. **Oxidation**

Oxidants are traditionally used in breadmaking to replace the ageing and maturing of flour brought about by prolonged storage, which results in enhanced gas-retaining properties in the dough, and thus, better baking performance. A mandatory requirement of the CBP is the use of relatively high levels of oxidising agents, as a result of the reduced time in which oxidation of flour components may take place. Originally 75 ppm of ascorbic acid were added, but this has been superseded by the use of ascorbic acid in combination with

other improvers, such as potassium bromate. Ascorbic acid is converted to the active oxidising agent, dehydroascorbic acid in a non-enzymic, metal-ion-catalysed oxidation reaction and also by the action of an endogenous flour enzyme, ascorbic acid oxidase.

3. Fat

The inclusion of approximately 0.7% fat (flour weight basis) in the CBP recipe improves gas retention during baking, and the volume, crumb softness and keeping properties of the bread produced.

More important for improving quality than total fat level is the proportion of solid fat present in the fat added, (i.e. the proportion of fat with a melting point higher than the temperature of the dough).

4. Water Addition

An increased water addition of 2% - 4% is necessary in the CBP in order to maintain dough consistency. The absence of a bulk fermentation stage diminishes the loss of flour solids (sugars) ordinarily fermented by yeast, and minimises softening of the dough due to enzymic degradation of starch with subsequent release of absorbed water.

The additional water is largely compensated for by the increased retention of solids so that CBP bread contains only 0.5% more water than bread produced by bulk fermentation.

5. Yeast

In order to maintain "normal" proof times, yeast level must be increased by 50% - 100%. This is a result of the yeast failing to reach the level of activity that is attained during a lengthy bulk-fermentation and the reduced length of time for gas accumulation.

6. Flour

It has been shown (Axford et al., 1963; Chamberlain et al., 1965) that flours of lower protein content may be used for the production of bread by mechanical dough development procedures than may be used for production of bread by bulk fermentation, without perceivable loss of bread quality. The reason for this is not clear, but may be due to the reduced time available for the deleterious action of proteases and thiol compounds etc. (Balls & Hale, 1936). In practice, bread flours for CBP now contain about 11% protein or less, whereas flours for bulk fermentation breadmaking contain 12.0% - 12.5% protein. This has substantially reduced the need for imports of high protein, strong North American wheats, and allowed a greater proportion of lower protein, home-grown wheats to be used in breadmaking grists.

It is, therefore, obvious that the implementation of the CBP has many advantages in the commercial bakery:

1. 60% reduction in processing time in the conversion of raw materials to bread, compared with fermentation procedures.

2. Reduction of space required in the dough room.

3. Reduction of the quantity of dough being processed at any one time, and therefore, a reduced risk of loss in the event of plant machinery breakdown.

4. Increased bread yield of ~4% due to retention of flour solids, higher water and yeast addition, and greater accuracy in dividing a denser and more uniform dough.

5. Facility for using lower protein flours without loss of bread quality.

6. Reduced staling rate.

7. Simplicity of operation, which allows dependency on craftsmanship to be replaced by precise automatic control.

8. Versatility of the process, which allows the whole range of typical British breads to be produced, with only small modifications to the basic procedure.

The disadvantages of the CBP are a requirement for:

1. Higher level of yeast addition.

2. Higher level of work input.

The reasons are clear, therefore, why the Chorleywood Bread Process

has been so readily adopted by the baking industry since it was first demonstrated in 1961.

Several workers have described the changes that occur in flour protein extractability and aggregation states during dough mixing. These findings were reviewed in Chapter I. However, previous investigations have all examined the effect of low energy mixing conditions. Since the majority of bread consumed in the UK is produced by the CBP, it was considered more relevant, here, to examine the effects of higher energy mixing on flour proteins.

Heaps et al. (1967) suggested that for any one flour there exists a definite level of work input which gives maximum dough development. However, little change was observed in loaf volume and other bread characteristics when work levels and mixer speeds, significantly in excess of the minimum requirements were used during dough mixing (Kilborn & Tipples, 1972). Kilborn & Tipples (1972) demonstrated that strong flours were more tolerant to overmixing than weak flours, and that deterioration, in the latter case, mainly related to dough handling properties and bread crumb texture.

Therefore, the effect of different levels of work input on protein extractability and protein aggregation states in doughs was determined for:

1. A filler wheat (i.e. a wheat that may be included in bread-making grists, but which is not of the highest baking quality): the variety chosen was Armada.

2. A wheat grown mainly for animal feed purposes; variety Mardler.

3. A commercial bread flour.

Comparison was, therefore, made of the effect of high energy mixing on proteins of flours of different "strength".

In addition, a second commercial flour was processed, but in this instance all improvers were omitted from the flour, and all oxidants excluded from the bread recipe. The action of oxidative dough improvers on protein SH groups, mediated through reactions involving glutathione, were described in Chapter I. Improvers, such as bromate, ascorbate and azodicarbonamide, help to improve the grain and texture of the end product, and have a similar action to that of "natural" aging or maturing of flour. The omission of oxidants and improvers from the bread recipe, therefore, allowed their role in the modification of flour proteins during high energy mixing to be examined.

V.2 Experimental, results and discussion

V.2.1 Flour Characteristics and CBP Breadmaking Performance

Monovarietal wheat samples were grown at Model Farm, Watlington, Oxon. during 1978-1979. These were T. aestivum cv. Armada (a filler grade wheat), and T. aestivum cv. Mardler (a feed grade wheat). All grain was conditioned to 15.5% moisture, and milled on an experimental Buhler mill to 72% extraction. A commercial bread

flour (CF), typical of that used for the production of bread by the Chorleywood process, and an equivalent, but unbleached, untreated commercial flour (UCF), were also used in the investigation. All flours were assessed for moisture and protein content, starch damage (SD), α-amylase activity, flour colour grade (FCG) and water absorption (WA). Data are presented below.

Table 7: Characteristics of the flours used for bread manufacture by the Chorleywood Bread Process

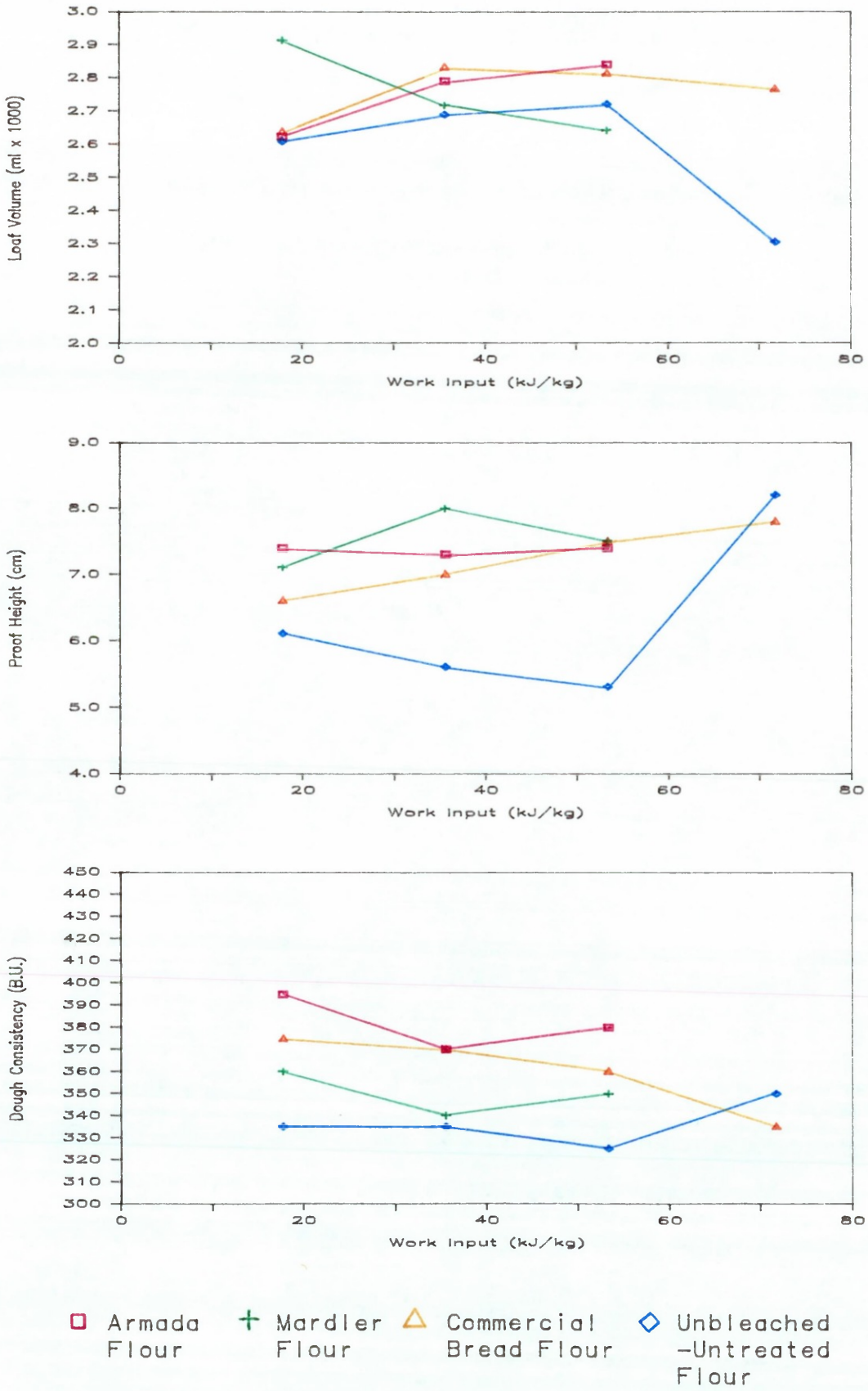
	Moisture %	Protein %	SD %	α-Amylase FU	FCG	WA BU
Armada	14.3	10.8	26.5	14	1.4	61.5
Mardler	13.3	9.4	29.5	710	3.1	59.7
CF	14.5	11.0	24.0	7	2.5	63.4
UCF	14.5	11.0	24.0	7	2.5	63.7

FU = Farrand Units

BU = Brabender Units

Each flour was assessed for baking performance in a CBP procedure similar to that used in commercial practice, employing various levels of work input between 17.8 - 53.4 kJ/kg for Mardler and Armada flours, and 17.8 - 71.8 kJ/kg for the two commercial flours. Details of the test-baking procedure are given in Appendix I.8. All oxidants (i.e. ascorbic acid and potassium bromate) were omitted from the recipe during baking of the UCF. Small samples of each

Figure 20: Baking performance of four flours mixed to different levels of work input in a Chorley-Wood Bread Process



dough were taken immediately after mixing, frozen in liquid N, freeze-dried and then milled to a fine powder in a coffee grinder. These samples were used in the analyses of dough proteins, described later.

Baking characteristics (dough consistency immediately after mixing, height of the doughpiece after the second proof and loaf volume) of each flour at the different levels of work input are presented graphically in Figure 20. The crumb texture scores of the loaves produced are shown below.

	Crumb Texture Scores			
Work Level (kJ/kg)	17.8	35.6	53.4	71.8
Armada	3.5	3.0	3.0	-
Mardler	1.5	1.0	1.0	-
CF	4.0	5.0	5.0	3.0
UCF	2.0	2.0	3.0	1.0

Texture score was assessed by a trained panel on a scale of 0 (poor) to 7 (good).

For the production of bread by the CBP, flour with a protein content of 11% is usually preferred. Both of the commercial flours and the Armada flour met this requirement, but the flour from the feed wheat Mardler, was of 9.4% protein only (Table 7). Since protein quantity (as well as protein quality) is a primary factor in determining breadmaking potential, loaves from Mardler flour might be expected

to have reduced volumes.

As the internal loaf temperature rises during baking, the solubility of dissolved gases, predominantly CO_2, is reduced, and liquids in the dough (water and ethanol) vaporise, exerting an internal pressure on the dough. Loaf volume, thus, increases rapidly during the early stages of baking. This rapid expansion is generally known as "oven spring". At low levels of work input, the Mardler loaf volume was high; the dough matrix was evidently capable of supporting the oven spring.

The Mardler flour had very high levels of α-amylase activity (710 FU) which may be indicative of sprouting of the wheat kernels before harvesting took place. Excessive amounts of α-amylase cause extensive liquefaction and dextrinisation in the dough during breadmaking and make large amounts of sugars available for yeast fermentation. The high levels of enzymic activity may, thus, account for the initially high loaf volume of the Mardler loaves, and the poor crumb texture scores.

At higher levels of work input, i.e. >17.8 kJ/kg, the membranous gluten structure of the Mardler dough was apparently broken down and it was unable to withstand the stress of expansion by gases. The loaf thus collapsed and volume decreased with increasing work input. Paredes-Lopez & Bushuk (1983) used the scanning electron microscope to examine the microstructure of gluten at different stages of mixing. They observed, that overmixing promoted the conversion of the continuous membranous structure, which is apparent in an optimally mixed dough, to a discontinuous fibrillar and globular

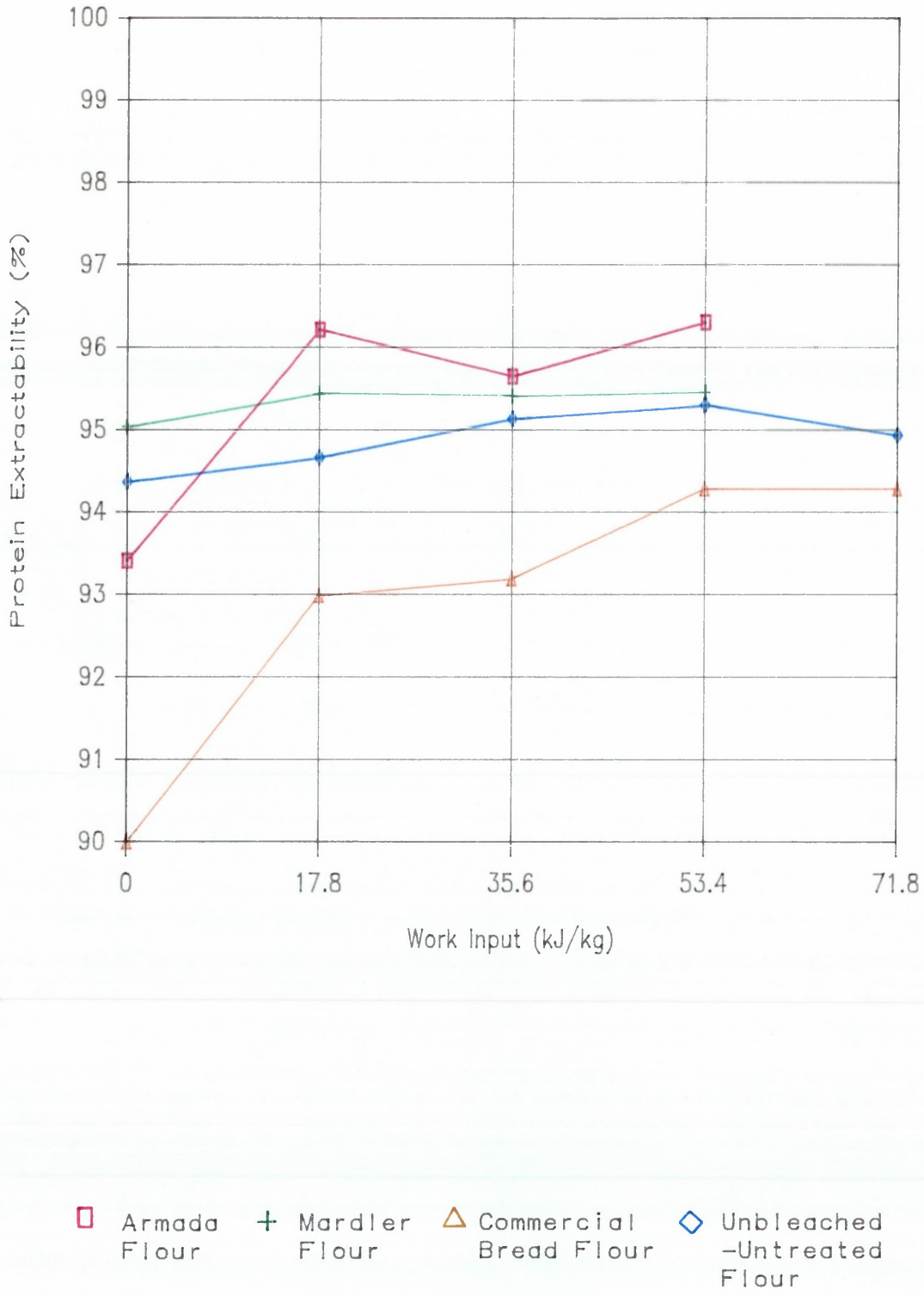
matrix. This change was thought to be the result of further aggregation of the hydrated flour proteins. They also observed a discontinuous matrix at all stages of mixing in doughs prepared from weak flours.

Loaf volumes for the other flours in this study, increased with increasing work levels. Loaf volumes and crumb textures for the CF were relatively constant between 35.6 - 71.8 kJ/kg. In commercial practice, there is little opportunity to experiment or make adjustments to accomodate variations in flour performance. It is, therefore, essential that all the ingredients, particularly the flour, perform well under a standard set of plant operating conditions. The "tolerance" of the CF to under- or over-mixing is, thus, a quality essential for its use in the commercial bakery.

These findings generally agree with those of others who have shown that flours with different mixing characteristics require different levels of work input to achieve optimum dough development (Heaps et al., 1967), but that satisfactory bread may be produced at work levels in excess of that indicated by peak dough consistency, and that for some flours, an increase in loaf volume may be achieved by the use of "excessive" levels of work (Kilborn & Tipples, 1972).

Loaf volume and crumb texture showed a marked deterioration in the bread produced from the 71.8 kJ/kg UCF dough. A large increase in the ex-mixer dough consistency and proof height suggested that work in excess of 53.4 kJ/kg resulted in "work hardening" of the UCF dough. However, whilst this hardening of the dough produced an increase in proof height, it subsequently prevented the expansion of

Figure 21: Extractability of wheat flour and dough proteins in buffers containing sodium dodecyl sulphate.



the dough in the oven, resulting in a small (2303 ml) and coarse textured loaf (score 1).

For the CF doughs, proof heights increased and ex-mixer dough consistencies decreased, with increasing levels of work input. Changes in proof height and ex-mixer dough consistency of the Armada and Mardler doughs were small and probably not significant.

V.2.2 Extractability of flour and dough proteins in buffers containing sodium dodecyl sulphate

As described above, the four flours used in this study were assessed for baking performance in a Chorleywood Bread Process, at various levels of work input and small samples of each dough taken ex-mixer. The extractability of each flour and freeze-dried dough sample into buffered solutions containing 2% SDS was determined by the method in Appendix I.2. Results are presented in Figure 21.

Many workers have demonstrated that protein extractability increases on mixing a flour to a dough, using a variety of solvent systems such as acetic acid (Tanaka & Bushuk, 1973), AUC (Meredith & Wren, 1969; Tsen, 1967) and SDS (Danno & Hoseney, 1982; Graveland et al., 1984). These workers all showed that the additional protein extracted as a result of dough mixing was glutenin. Several mechanisms have been suggested to explain the increased extractability of glutenin on mixing:

(a) The mixing action breaks and separates aggregates to form a continuous gluten film, and the glutenin proteins are

rendered more accessible to solvent.

(b) Once the continuous gluten matrix has been formed, further mixing may result in dough breakdown through "scission" of non-covalent bonds, i.e. H-bonds, hydrophobic interactions, salt linkages.

(c) Free thiols on water soluble proteins and peptides may initiate SH/SS interchange reactions with the insoluble flour proteins, leading to depolymerisation of large aggregates, rendering them extractable.

Graveland and coworkers (1980, 1984) have described a high molecular weight, SDS-insoluble glutenin protein fraction (Glutenin I) that, apparently, comprises a backbone of HMW glutenin polypeptides linked head-to-tail via disulphide bonds. Graveland proposed (1984) that on dough mixing specific SS bonds, i.e. those linking the glutenin subunits in head-to-tail configuration, were reduced, resulting in depolymerisation of the Glutenin I. The reduction reaction required the presence of oxygen, a reducing hydroquinone compound and a metal-protein complex. These workers proposed that in normal dough systems, bromate might replace oxygen and so accelerate depolymerisation. In contrast, oxidation of ascorbic acid by the enzyme, ascorbic acid oxidase, would remove oxygen from the system and so inhibit depolymerisation of Glutenin I.

It has, also, been shown that more glutenin is depolymerised if a dough is mixed in the presence of activated double-bond compounds (fumaric acid, N-ethylmaleimide or ferulic acid), or fast-acting

oxidants, such as potassium iodate (Hoseney et al., 1980; Schroeder & Hoseney, 1978; Sidhu et al., 1980; Weal et al, 1977). This has been attributed to reaction of these substances with thiyl radicals that arise from disulphide bond cleavage during dough mixing.

Mardler, Armada and UCF flours had greater extractability in SDS (93% - 95%) than the CF flour (90%). On mixing, Mardler and UCF doughs showed a very small increase in protein extractability (0.5%), at any level of work input. Mixing the Armada and CF flours to a work level of 17.8 kJ/kg, produced larger increases in protein extractability, i.e. an additional 3% of the total protein was extracted. Further mixing produced no overall change in the protein extractability of Armada doughs, but rendered an additional 1.5% of the total protein in CF dough extractable at a work input of 71.8 kJ/kg.

Paredez-Lopez & Bushuk (1983a) suggested that the amount of residue protein remaining after acetic acid extraction was related inversely to the energy input during dough mixing, i.e. the amount of protein extractable in acetic acid was proportional to the work input. A similar relationship was observed here, for the doughs prepared from CF flour, but not for doughs of the Armada, Mardler and UCF flours.

The two commercial flours used in this investigation differed in that bleach and oxidants were added to CF at the mill, whereas UCF was unbleached and untreated. These additions may result in oxidation of sulphydryl groups in flour, some of which may be located on polypeptide chains. Thus, crosslinks may be formed between gluten proteins, increasing the average molecular size, and

rendering a greater percentage of the protein unextractable. No oxidants were added to the Armada and Mardler flours, which were prepared on the experimental Buhler mill. The absence of oxidants in the UCF, Armada and Mardler flours may thus, explain their greater extractability in SDS, relative to CF.

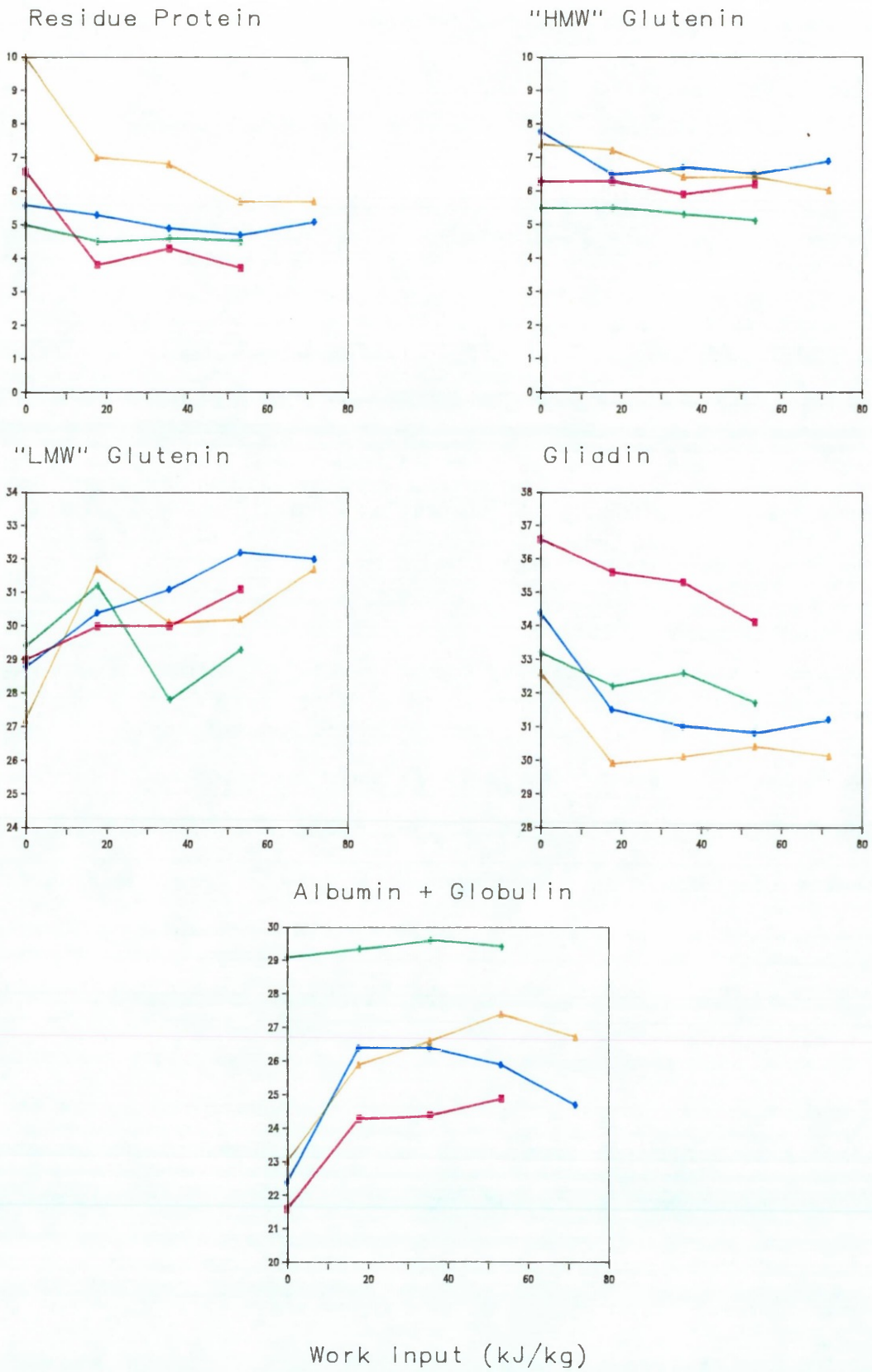
V.2.3 Molecular weight distributions of SDS-extractable proteins from flours and freeze-dried doughs

In order to define more precisely the changes that occur in flour proteins during dough mixing, SDS-extracts of the flours and their freeze-dried doughs, prepared by mixing to different levels of work input, were examined by gel-filtration chromatography according to the method described in Appendix I.3.

Several workers have examined the stability of the molecular weight distributions in wheat proteins during dough mixing. Tsen (1967) separated the protein components in acetic acid extracts of flours and doughs by gel-filtration chromatography on BioGel P-150. He showed that mixing increased the amount of a high molecular weight component (component I) that was eluted at the void volume of the BioGel column. Other components of the extract did not change significantly.

Similarly, Meredith & Wren (1969) showed that dough mixing increased protein extractability in AUC, the increase occuring in the amount of high molecular weight glutenin that was eluted at the void volume of a Sephadex G-200 column. They also noted the appearance of a peak at an elution volume corresponding to a molecular weight of

Figure 22: Changes in protein molecular weight distributions during dough mixing by a Chorleywood Bread Process



Figures show the percentage of the total sample protein in the each molecular size fraction.

□ Armada + Mardler △ CF ◇ UCF

80,000 - 100,000.

In contrast, Tanaka & Bushuk (1973) extracted flour and dough proteins, also with AUC, but revealed a decrease in the amount of high molecular weight glutenin and an increase in the amount of low molecular weight glutenin and gliadin by chromatography on Sephadex G-150.

Whilst providing useful information, all of these studies suffer from limitations in the resolving power of the gel-filtration media employed. The void volume of the Biogel P-150 column, for example, is 150,000 and therefore, relatively insensitive to changes in molecular weight distribution of the glutenin proteins, whose molecular weights range from 100,000 - 5 million or greater. The void volume of the Sepharose CL-4B columns used in these studies corresponds to a molecular weight of approximately 5×10^6. Thus, considerably more information can be obtained about the stability of the protein molecular weight distributions, particularly of the glutenin proteins, where the greatest changes may be expected.

The molecular weight distributions of the SDS-extractable proteins of the flour and ex-mixer dough samples, as determined by gel-filtration chromatography are presented graphically in Figure 22.

Bottomley *et al.* (1982) demonstrated that proteins in the residue fraction and areas A and B, of the gel-filtration chromatogram correspond to glutenin protein, while areas C and D correspond to gliadins and to albumins plus globulins respectively.

In general, there were only very small differences in the total amount of glutenin (i.e. residue protein + peak A + peak B) in the flour and dough samples of each variety examined.

e.g. Residue + A + B for Armada Flour = 41.9%

Residue + A + B for Armada 53.4 kJ/kg dough = 41.0%

However, the relative proportions of each of these fractions varied considerably on mixing the flour into a dough. The greatest change occurred in the initial stages of mixing, i.e. between the flour and the dough mixed at the lowest work level. For CF and Armada flours, there was a large decrease in the amount of insoluble glutenin protein, and a corresponding increase in the amount of low molecular weight glutenin. For UCF and Mardler flours, there were negligible changes in the amount of residue protein, although the amount of low molecular weight glutenin increased with increasing levels of work input in the UCF dough. For all flours, the percentage of high molecular weight glutenin in the SDS extract remained approximately constant in the flours and at each stage of mixing.

It is probable, therefore, that the rate of disaggregation of residue protein, rendering it extractable in SDS, is approximately equal to the rate of disaggregation of high molecular weight glutenin to yield lower molecular weight aggregates. Hence, there is no net change in the amount of void volume material.

Similarly, there was a relatively large decrease in peak C on initial mixing of the flour into the dough and an approximately

equal increase in the area of peak D, but only small changes in the amounts of these on further mixing to 53.4 or 71.8 kJ/kg. Since peak C largely comprises gliadin proteins, which are monomolecular proteins containing only intramolecular disulphide bonds, SS/SH interchange reactions, resulting in reduction of molecular size cannot explain the decrease in the proportion of peak C on dough mixing. Gliadins have been envisaged as "plasticisers" in doughs, which interact both amongst themselves and with glutenin molecules (Ewart, 1979). The fall in gliadin levels on dough mixing may, therefore, be the result of changes in gliadin interactions with other protein species, possibly through non-covalent bonds.

Very small changes in the molecular weight distribution of Mardler proteins were observed on mixing the flour into a dough at any level of work input. Arakawa and coworkers (1974) showed that the quality of a flour is closely related to the aggregation behaviour of gluten separated from it, and is due mainly to the aggregation properties of the glutenins. The apparent "inability" of Mardler proteins to associate/dissociate may, therefore, be responsible for the poor breadmaking performance of Mardler flour demonstrated here, although protein rearrangements involving no overall change in molecular weight cannot be discounted.

For CF and Armada flours, increased work input during dough mixing resulted in a progressive reduction in molecular size of the gluten proteins, which thereby increased extractability in SDS and the proportion of low molecular weight material eluting from columns of Sepharose. These changes were initially associated with improved loaf volumes and crumb texture scores, however, "over-mixing"

resulted in decreased bread quality.

In molecular terms, over-mixing or dough breakdown has been proposed as occurring when the tension in glutenin polymers (concatenations), brought about by mixing cannot be relieved solely through molecular slippage, and disulphide bond cleavage and reformation. As excess work is imparted to the dough, covalent bonds holding subunits together are "pulled apart". The continuity of the protein matrix is, therefore, disrupted and the dough is unable to support expansion by gases during proving and baking. The loaf quality thus deteriorates. The level of work input that constitutes over-mixing varies according to the flour used. Marked deterioration in bread quality was not observed in Armada or CF flours up to a work level of 53.4 kJ/kg. However, further mixing of the CF flour to 71.8 kJ/kg produced smaller (2766ml) loaves with a coarse crumb texture score (3).

The most significant difference between UCF and CF flours with respect to their protein molecular weight distributions, related to the initial levels of the different protein components in the flours and the breakdown of residue protein that was observed on mixing CF, but not observed on mixing UCF. Other changes during dough mixing were parallelled by the two flours.

The techniques employed were unable to detect any significant difference in the molecular weight distribution of the 71.8 kJ/kg UCF dough compared with the 53.4 kJ/kg dough that could account for the large decrease in loaf volume (2720 -> 2303 ml) and poor crumb texture score (3 -> 1). CF was apparently more tolerant to over-

mixing than UCF in terms of the quality of the bread produced.

Principal component analysis of the extractability and chromatography data revealed that for each of the four flours, the changes in protein molecular weight distribution due to increasing levels of work input during dough mixing were not statistically significant, although the trends described may be "real" nonetheless.

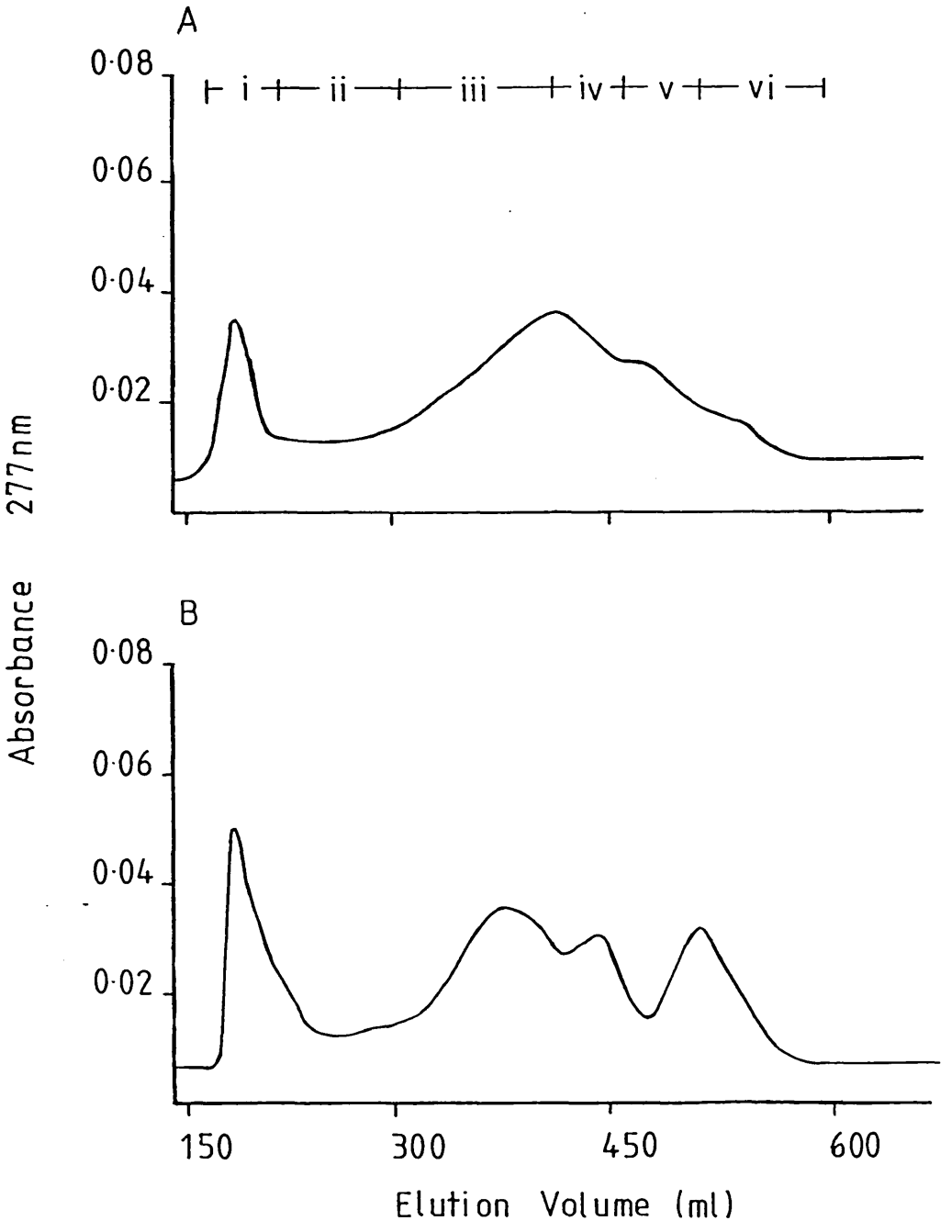
V.2.4 Fractionation of acetic-acid extractable proteins of Armada flour and freeze-dried doughs by gel filtration chromatography

In order further to examine changes in the glutenin components of flour due to CBP mixing, acetic acid extracts of Armada flour and the Armada doughs mixed to different levels of work input, were fractionated by gel-filtration chromatography according to the method described in Appendix I.9. The amount of the total protein extracted from each sample with acetic acid is shown below.

Acetic acid extractability of proteins of Armada flour, and doughs prepared by mixing to different levels of work input

	Protein Extracted of Total Recovered
Armada: flour	70.6 %
17.8 kJ/kg dough	72.2 %
35.6 kJ/kg dough	75.6 %
53.4 kJ/kg dough	78.6 %

Figure 23: Molecular size distribution of acetic acid-extractable proteins of wheat flour and dough



Wheat flour (Armada) was mixed to a work input of 53.4kJ/kg in the CBP. The figure shows the molecular size distribution of acetic acid-extractable proteins on Sephadex G-150, of (A) flour and (B) freeze-dried dough. Column effluents were pooled as indicated.

Many workers have reported increases in protein extractability on dough mixing (Mecham et al., 1962; Tsen, 1967, 1969; Tanaka & Bushuk, 1973), although most have demonstrated much larger changes than were observed here. Tanaka & Bushuk (1973) observed increases in extractability of up to 20% for a weak flour, but of only 3% for a strong flour when mixed in air.

Molecular weight profiles for the acetic acid extracts of Armada flour and 53.4 kJ/kg dough are shown in Figure 23. Fraction (i) represents 12.0% of the total area of the A277 profile of Armada flour, compared with 18.7% of the total area for the 53.4 kJ/kg dough. This increase in the amount of protein that was eluted at the void volume of the G-150 column, could result either from aggregation/polymerisation of lower molecular weight components and/or disaggregation of unextracted glutenin protein due to mixing. Tsen (1967) showed that the additional protein extracted by acetic acid on dough mixing occurred primarily in the high molecular weight glutenin fraction, with little or no change apparent in either the gliadin or low molecular weight albumin and globulin fractions. He concluded, therefore, that the increased protein extractability resulted from disaggregation of very high molecular weight glutenin complexes, and was not at the expense of other protein components. The results presented here also support this conclusion.

Column effluents of the chromatographed flour and dough extracts were pooled as shown, (Figure 23), and the polypeptide composition of the different molecular weight fractions was examined by two-dimensional electrophoresis.

Figure 24:
Armada flour

Armada 17.8 kJ/kg dough

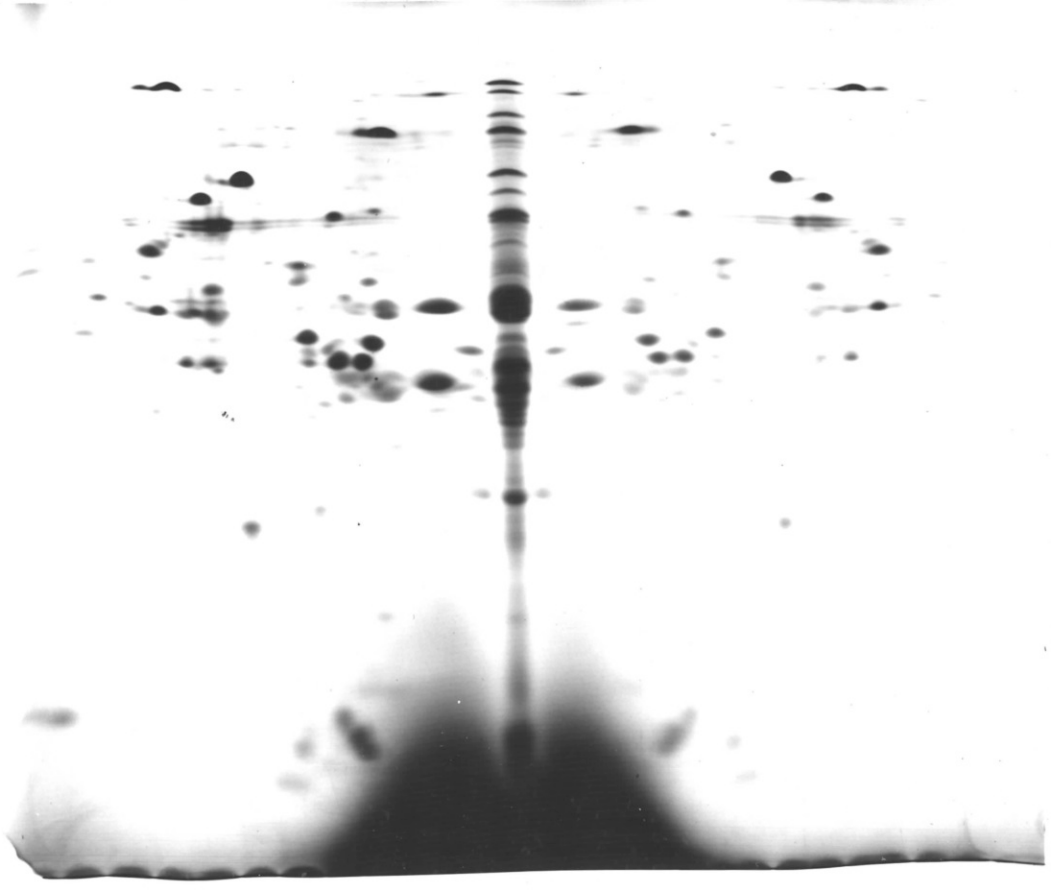


Figure 25:
Armada 35.6 kJ/kg dough

Armada 53.4 kJ/kg dough

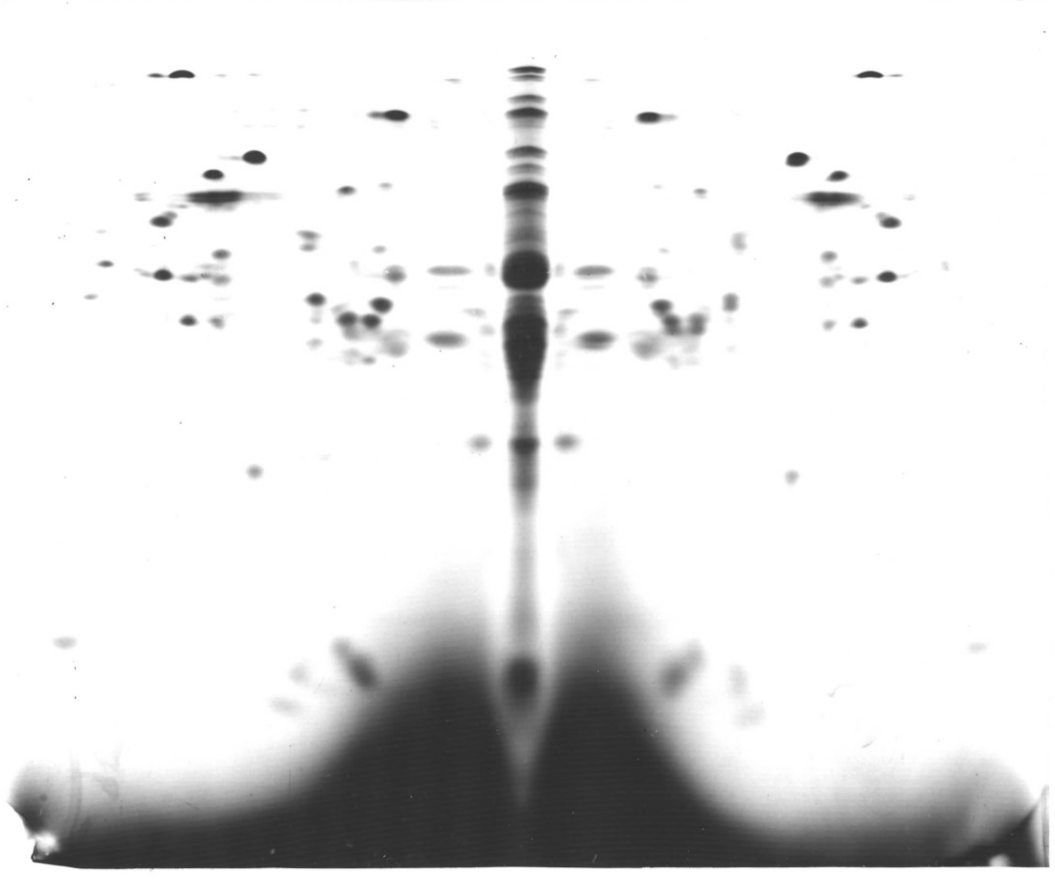


Figure 24:
Armada flour

Armada 17.8 kJ/kg dough

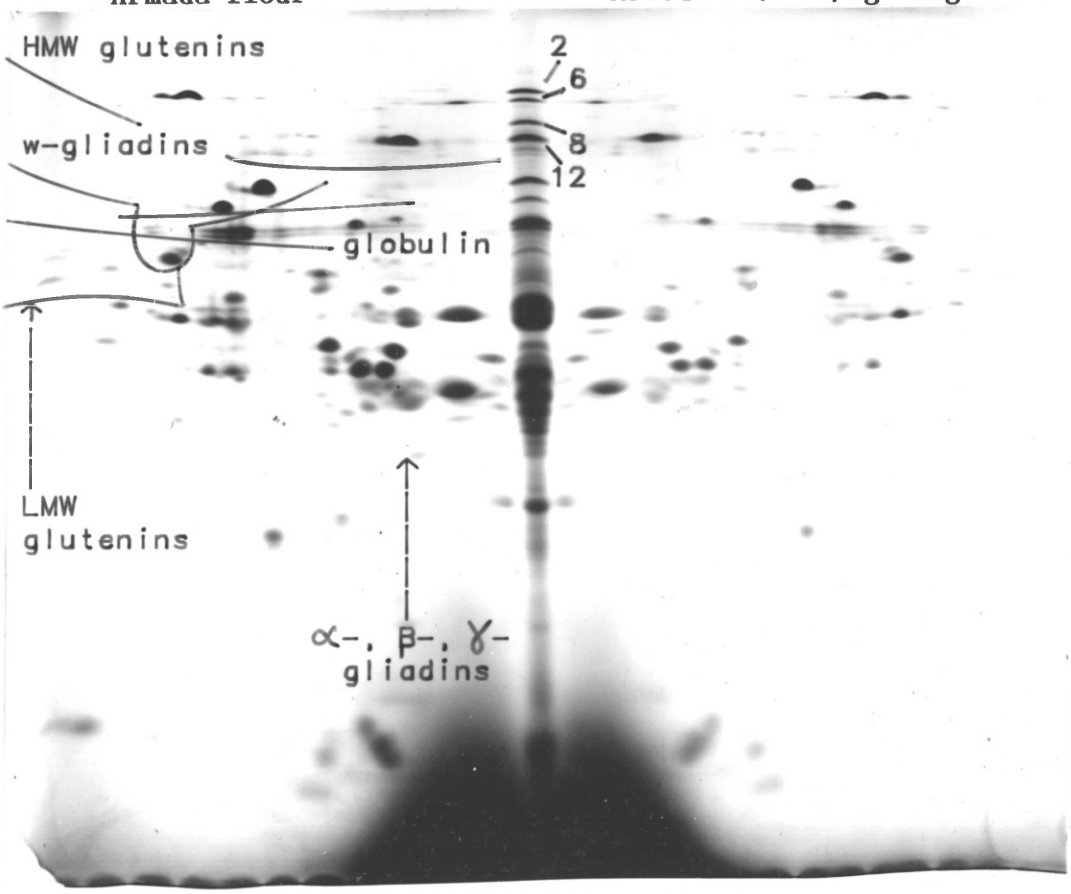
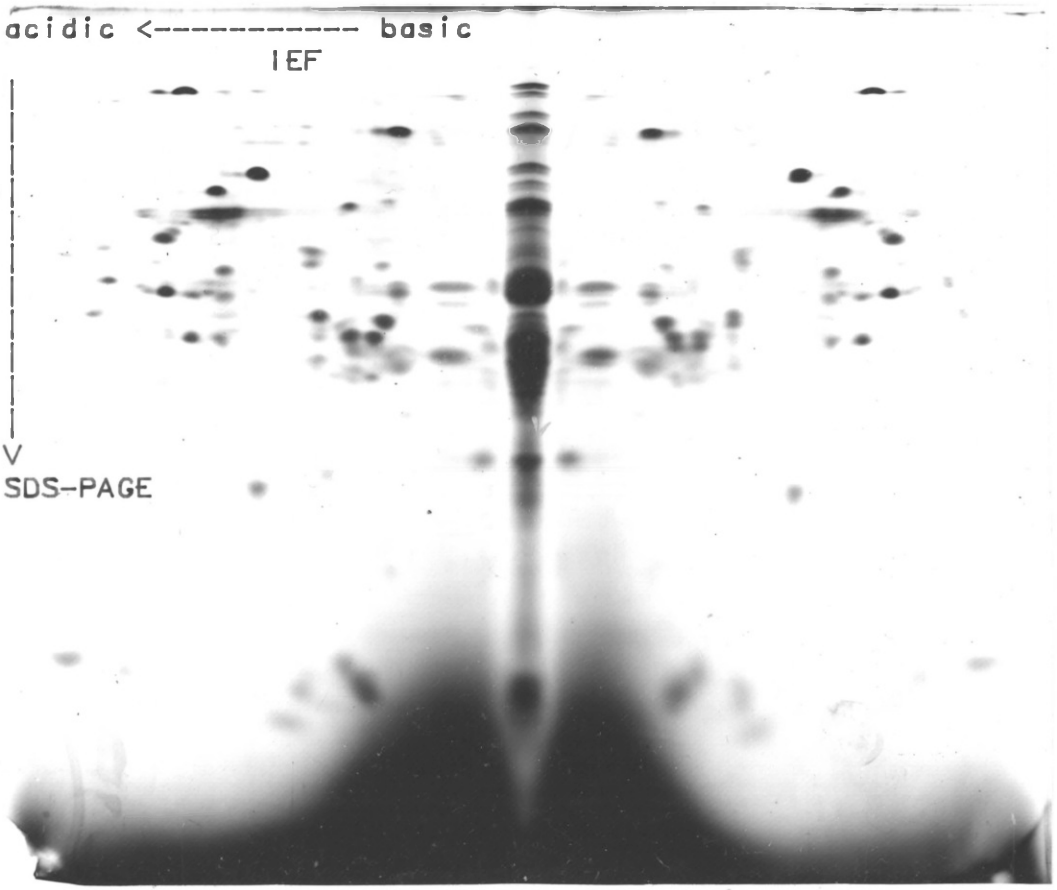


Figure 25:
Armada 35.6 kJ/kg dough

Armada 53.4 kJ/kg dough



V.2.5 Polypeptide composition of acetic acid extracts of Armada flour and doughs, mixed to different levels of work input, by two-dimensional electrophoresis

In order to examine the subunit composition of different molecular size fractions of glutenin, and any changes that occurred as a result of CBP mixing, proteins of Armada flour and freeze-dried doughs were extracted with acetic acid and fractionated according to their molecular sizes as described in Appendix I.9. The identity of the fractions (i) - (vi) is shown in Figure 23. The polypeptide composition of different molecular weight pools was determined by two-dimensional electrophoresis according to the method described in Appendix I.10.

Figures 24 and 25: No changes were observed in the 2D polypeptide/protein patterns of the whole unfractionated samples as a result of dough mixing, i.e. there is no apparent polypeptide crosslinking or cleavage other than through disulphide bonds.

Protein components other than glutenins were extracted by the acetic acid solution. In particular, α-, β-, γ- and w-gliadin components were also present in extracts, making subsequent interpretation of the 2D electropherograms more difficult.

Negligible differences were observed in electropherograms of fractionated proteins of Armada flour and the 17.8 kJ/kg and 35.6 kJ/kg doughs (data not shown). More significant differences were observed in electropherograms of G-150 fractions of the Armada flour and 53.4 kJ/kg dough. Figures 26-31.

Figure 26: Armada flour
Fraction (i) Fraction (ii)

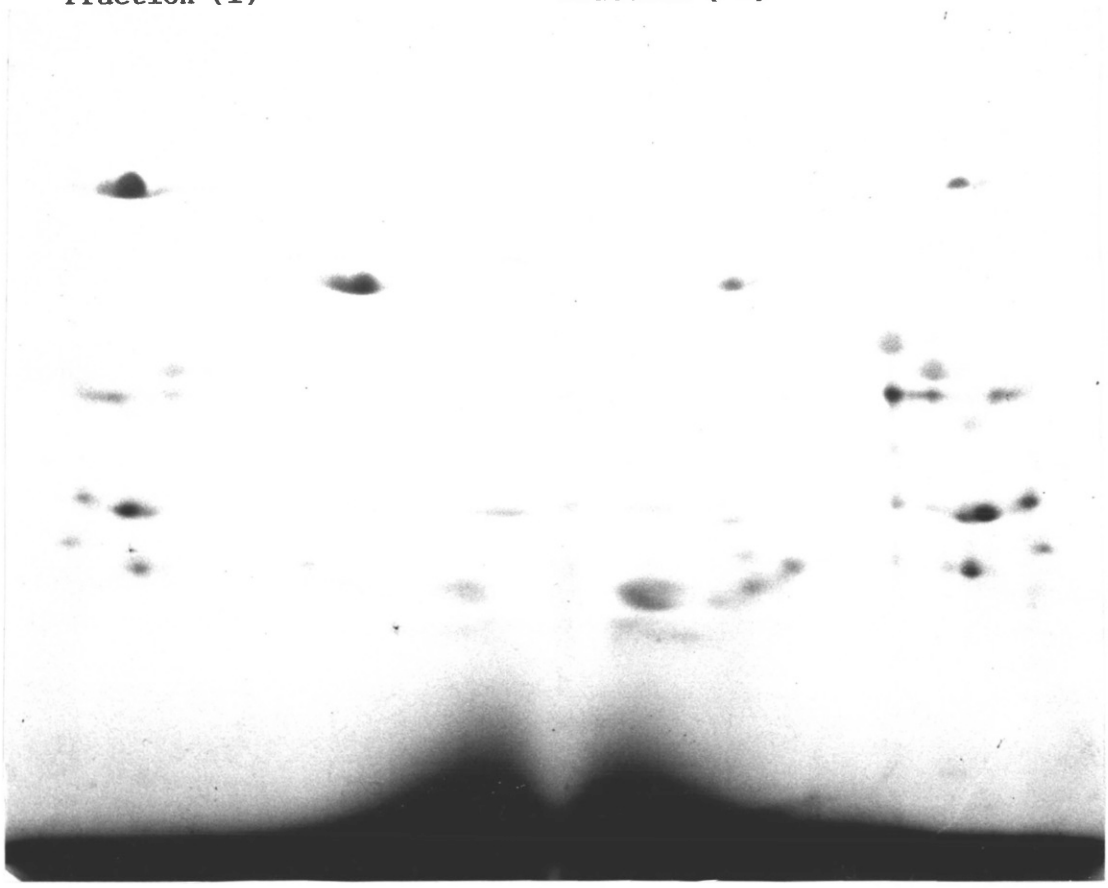
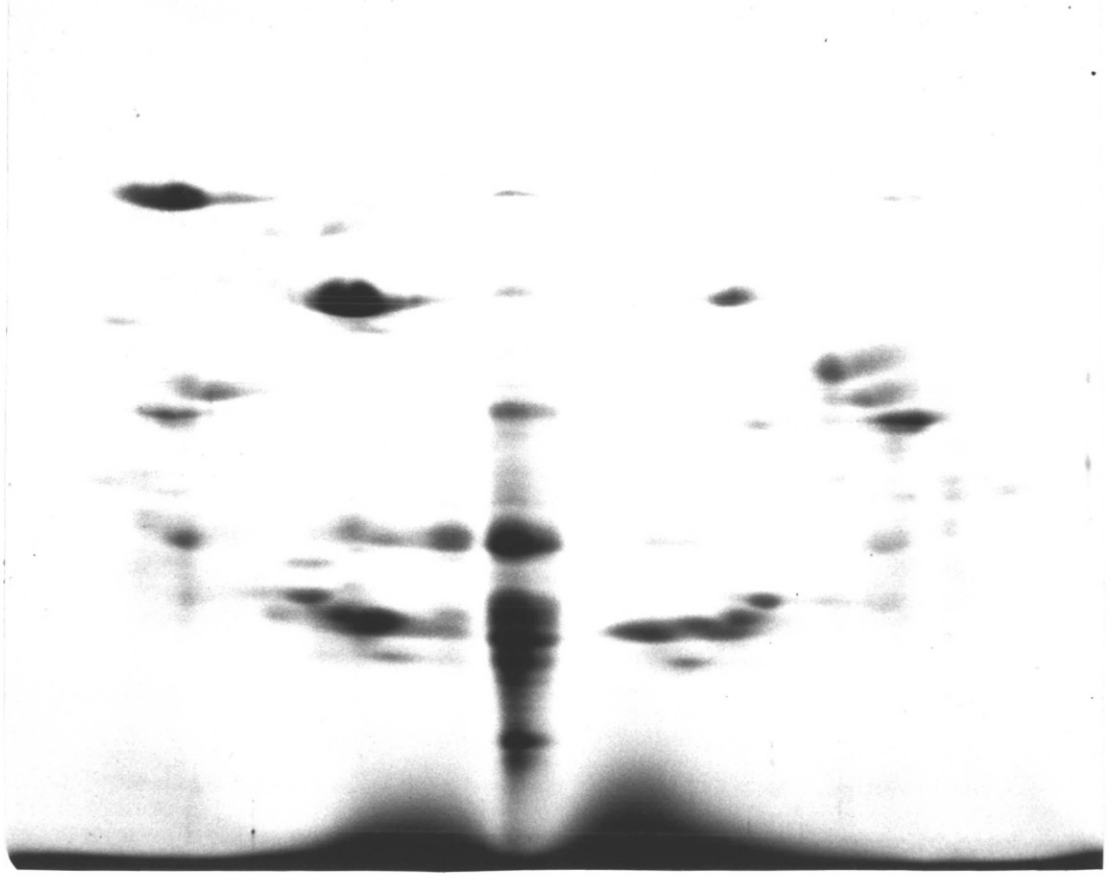


Figure 27: Armada 53.4 kJ/kg dough
Fraction (i) Fraction (ii)



Figures 26 and 27:

Fractions labelled (i) represent protein that was eluted at the void volume of the G-150 column. The HMW glutenin subunits 2, 12 and 6 were readily identified as components of the highest molecular weight glutenin proteins. Subunit 8 was less readily observed. Gliadin components were also present in this fraction, and may be associated with the glutenin subunits through non-covalent bonds. There were also traces of the LMW glutenin subunits in fraction (i) of the Armada dough (Figure 27). These LMW subunits may have been present also in fraction (i) of the flour extract, but were not apparent, possibly due to differences in sample loading.

No major differences were observed in proteins/polypeptides present in fraction (ii) of the flour and dough extracts. However, there may have been some differences between the samples in the relative amounts of some components, particularly the gliadin proteins.

Figure 28: Armada flour
Fraction (iii) Fraction (iv)

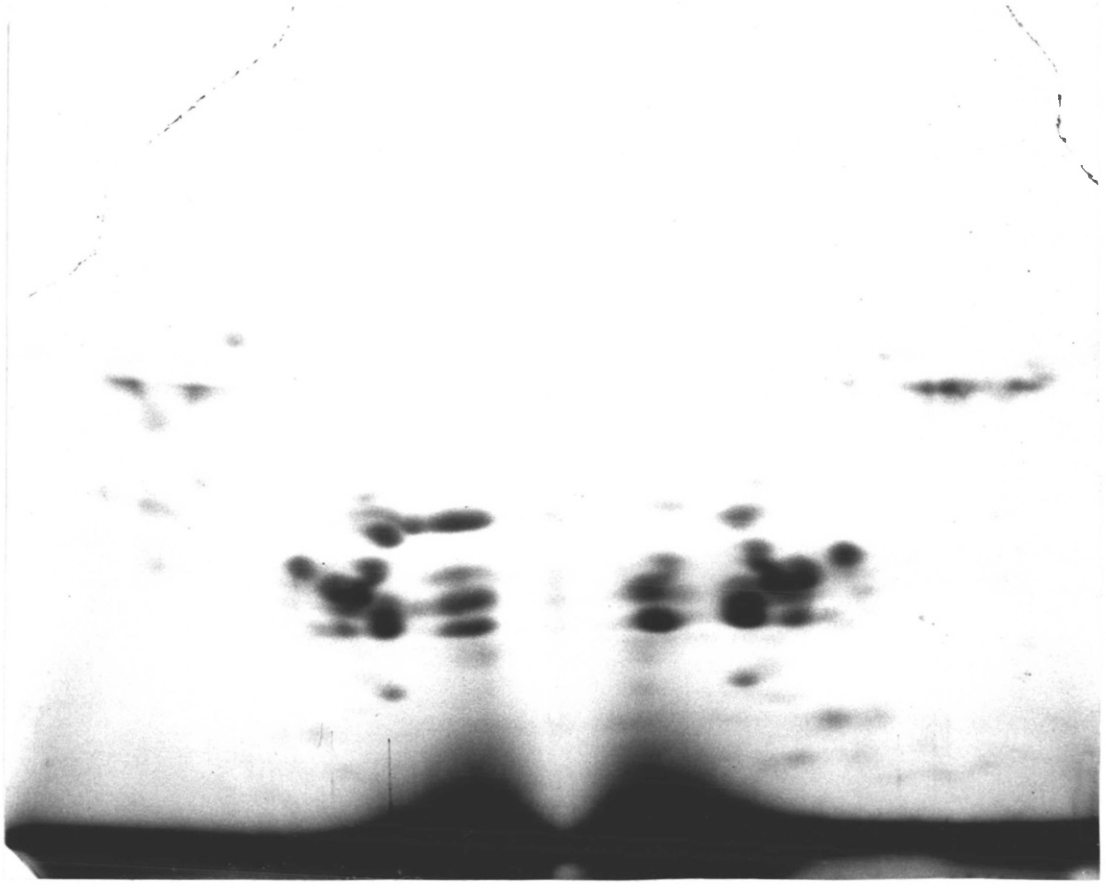
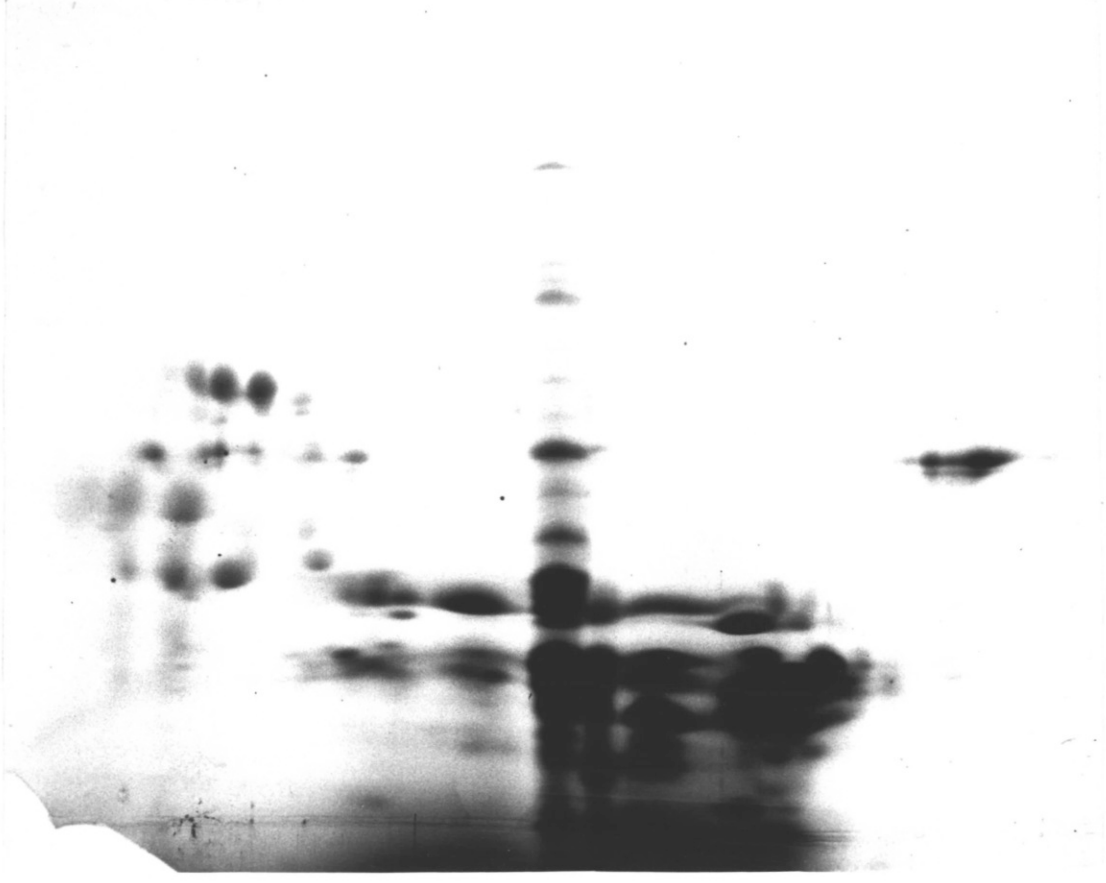


Figure 29: Armada 53.4 kJ/kg dough
Fraction (iii) Fraction (iv)



Figures 28 and 29:

Certain basic α-, β- and γ-gliadin components that were present in fraction (iii) of the flour (Figure 28), were apparently present in fraction (iv) of the over-mixed dough (Figure 29). Fraction (iii) of the dough also showed a marked increase in the amount of globulin and w-gliadin proteins, relative to those observed in fraction (iii) of the flour.

Figure 30: Armada flour
Fraction (v) Fraction (vi)

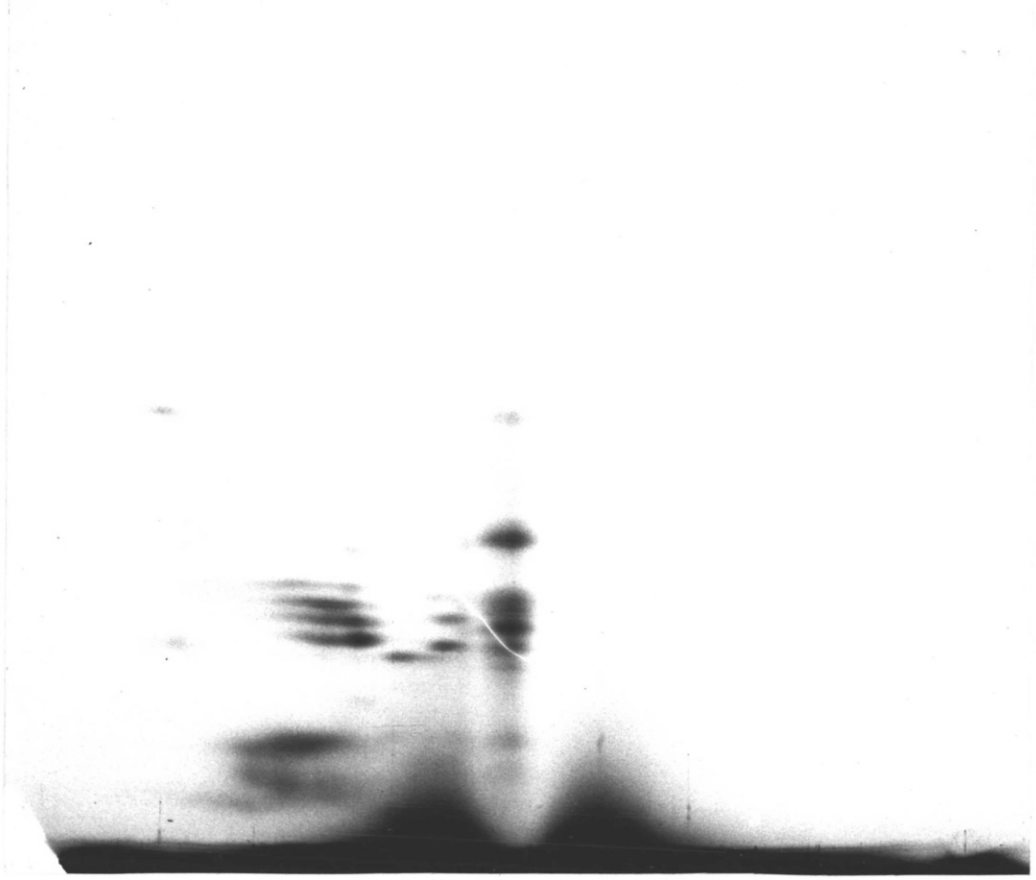
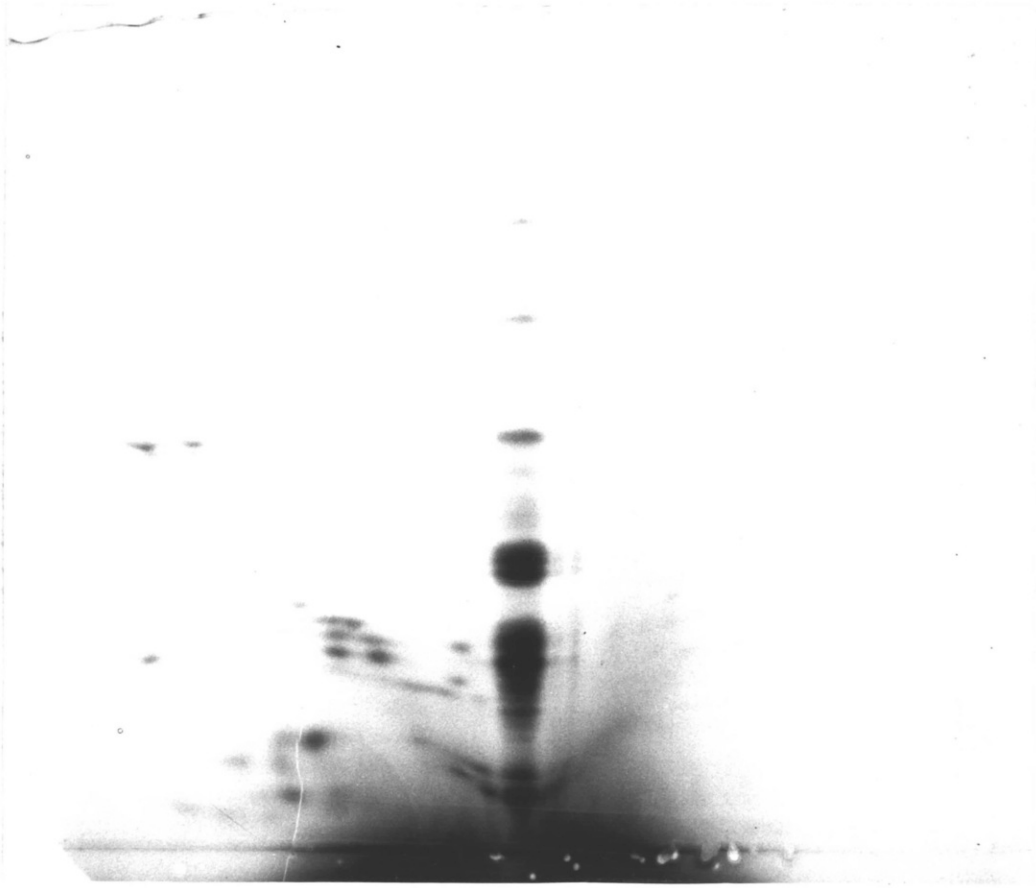


Figure 31: Armada 53.4 kJ/kg dough
Fraction (v) Fraction (vi)



Figures 30 and 31:

No significant differences were observed between fractions (v) of the Armada flour and dough (Figures 30 and 31 respectively). The UV absorbing material in fraction (vi) of column effluents was, presumably, low molecular weight peptides or protein fragments that had electrophoresed from the 2D-gels. Hence, Fraction (vi) in Figures 30 and 31 show no protein staining bands.

There was, apparently, no significant rearrangement of glutenin polypeptides during dough mixing that could be detected by the methods used here. An apparent "movement" of gliadin proteins from higher to lower molecular weight protein pools was observed between the flour and dough samples respectively. This may have been the result of protein rearrangements through non-covalent bonding. However, it may also have been due to small variations in pooling of the column effluents, i.e. an operational artifact.

V.3 Conclusions

CBP mixing resulted in depolymerisation of glutenin molecules in the better quality CF and Armada flours, such that a greater percentage of the total protein was extractable in SDS (i.e. there was a decrease in the proportion of residue protein) and there was an increase in the proportion of low molecular weight glutenins, as measured by gel-filtration chromatography of SDS-extracts. The greatest change occurred in the initial stages of mixing, with only small decreases in glutenin molecular size being observed on further mixing.

A "weak" flour (Mardler) did not show any significant change in the molecular size distribution of the gluten proteins on CBP mixing, which may be related to its poor baking performance. These findings contradict the results of other workers, who have reported the greatest increases in protein extractability on mixing weak flours (Tanaka & Bushuk, 1973).

There was no detectable change in the subunit composition of different molecular weight fractions of acetic acid extracts as a result of CBP mixing of Armada flour, i.e. no detectable rearrangement of polypeptides through SS/SH interchange reactions.

Two commercial flours, with and without added flour improvers, differed in the amount of SDS-unextractable residue protein which they contained, i.e. 10.0% of CF flour protein was unextractable compared with 5.6% of UCF flour protein. CBP mixing of the UCF flour without addition of improvers or oxidants in the recipe, did not affect SDS-extractability of the total flour protein, but resulted in a reduction in molecular size of the SDS-extractable proteins. The absence of oxidants apparently decreased the tolerance of the dough to overmixing and the quality of all bread produced.

Chapter VI: Changes in Durum Wheat Semolina Proteins during Pasta Processing

VI.1 Introduction

The preparation of pastes, is one of the oldest methods devised for the consumption of wheat, and takes place in virtually every corner of the world. This is due largely to the simplicity of the process and the advantages offered by being able to dry and store paste products for relatively long periods without significant deterioration.

Paste products are prepared from a basic mixture of wheat endosperm and water, although other ingredients such as egg may be included. This mixture is then formed into some convenient shape, and either cooked and eaten immediately or dried for consumption at some later date.

In Europe and the West, paste products are largely confined to macaroni, spaghetti, lasagne and sundry other products that are basically similar, differing principally in shape. In the Eastern world, where the manufacturing capabilities are more limited, such pastas are relatively rare. The more common forms of paste product in India and Pakistan are chappatis (thin unleavened "pancakes") and in S.E. Asia, China and Japan are noodles of various types.

The raw material of choice for the manufacture of pasta (i.e. spaghetti-type) products is the hard wheat Triticum durum. Durum

wheat belongs to the same genus as bread wheat, Triticum aestivum, but the two differ in that the former is a tetraploid whereas the latter is hexaploid. Durum wheat has a tough horny endosperm, which lends itself readily to the production of semolina, a granular form of the endosperm that is preferred to flour for pasta manufacture, mainly because it requires less water to form a dough. Since any added water must eventually be removed in the drying process, which is both time-consuming (up to 36h depending on the drier and the product [Irvin, 1978]) and costly, it is desirable to add the minimum water during dough preparation.

The manufacture of pasta products is one of the most highly automated of the food industries. In a modern plant, semolina and water are mixed to form a stiff dough with a moisture content of about 30%. The dough is kneaded under vacuum to remove occluded air bubbles and then extruded through a die, under a pressure of about 80 - 130 kg/cm^2, to produce the particular shape of product desired. As the product is extruded from the die, it is surface dried by hot air and then further dried in a two stage process to a final moisture content of about 12.5% or less. A more detailed account of pasta manufacture, and of uses of pasta as a food ingredient has been given by Antognelli (1980).

Semolina protein characteristics and pasta quality

Pasta made from good quality durum wheat should resist breaking and cracking (checking) during and after processing since this reduces the yield of saleable material, and should be bright and yellow in its dry form. On cooking, the pasta should swell but retain its

shape and coherence, so that its texture is firm and resilient. Whereas the colour characteristics of pasta are determined largely by carotenoid pigments and the activity of the enzyme, lipoxygenase, which may bleach these pigments (Irvine & Winkler, 1950) the textural properties of the dried and cooked product are governed mainly by the proteins of durum wheat (Irvine, 1978).

Effect of protein content on pasta quality

In the same way that the breadmaking ability of a flour is determined by the quantity of its protein component, so the mechanical properties of the dried pasta and its cooking quality are governed by the protein content and gluten strength of the durum semolina. To produce spaghetti with the required mechanical properties and acceptable cooking quality, a minimum of 10% protein is desired in the durum semolina, although a protein content of 12% is more usual (Matweef, 1966).

Walsh (1973) suggested that protein content was less important in determining durum wheat quality than it was in determining bread wheat quality, but that an "adequate" amount was needed for good cooking performance and nutritional value of pasta products.

The relationship between spaghetti cooking quality and protein content has been demonstrated by examining the quality of cooked spaghetti prepared from semolinas covering a wide range of protein contents (Matweef, 1966). Matsuo *et al.* (1972) investigated the effect of protein content on the cooking quality of spaghetti, by blending high- and low-protein semolinas, and by back addition of

crude gluten, water soluble and starch fractions derived from three separate durum varieties. In all cases the high protein samples were firmer, less compressible and more elastic than the low protein samples, which were soft and showed little elasticity.

Similar conclusions were reached in a study of two series of samples of Canadian durum wheats, which were grown under similar environmental conditions but had a range of protein contents (Dexter & Matsuo, 1977). A marked decrease in mixing time (Farinograph) was observed concomitant with an increase in consistency and mixing tolerance, for a moderate increase in protein content. Furthermore, the cooking quality and tolerance to overcooking continued to improve as the protein content increased in both cultivars over the whole range of protein levels examined (approximately 10% - 15%). Thus, protein content was a significant factor influencing both rheological and cooking properties of the pasta.

Effect of protein "quality" on pasta properties

It is well known that pasta cooking quality and mechanical properties depend upon qualitative factors associated with durum wheat proteins, as well as overall protein content. Thus, some durum varieties yield pasta of much better quality than other varieties at equivalent protein contents. Considering only durum wheats, the "stronger" the gluten (i.e. the greater the resistance to extension under a given load) the better the quality of the cooked spaghetti (Matsuo & Irvine, 1970). This relationship does not hold if one also considers hexaploid wheats, some of which have

"stronger" glutens but yield spaghetti of inferior quality.

The biochemical features of durum wheat proteins that are responsible for pasta quality are not well established. Wasik and Bushuk (1975) investigated the relationship between different protein fractions and spaghetti-making quality of 15 samples that varied widely in gluten strength. They found that the proportion of glutenin was significantly correlated with spaghetti firmness. Similar results have been reported by others (Dexter & Matsuo, 1977; Walsh & Gilles, 1971).

Dexter & Matsuo (1978) demonstrated that fortification with lactic acid soluble proteins from semolina, led to improved cooking quality, whereas the lactic acid insoluble residue imparted only limited improvement. In contrast, Matsuo et al. (1978) suggested that high levels of residue protein were important in determining pasta cooking quality, and were responsible for the greatest variations in gluten strength and Farinograph properties (Dexter & Matsuo, 1980).

More recently, correlations between the presence of certain low molecular weight glutenin subunits and gliadin proteins and pasta-making quality have been identified. These associations, which are discussed in greater detail in Chapter I, provide no explanation in chemical terms as to the mechanism by which particular glutenin subunits or gliadin components might influence gluten quality. However, concerted efforts are now being made by several groups to characterise individual gluten polypeptides chemically, which will hopefully throw some light on this problem.

Changes in Semolina Proteins on Pasta Manufacture

When pasta dough is prepared under standard manufacturing conditions, (i.e. 30% - 35% moisture content), a decrease in the proportion of semolina protein extractable in acetic acid, is observed. A large amount of mechanical work is imparted to the premixed pasta dough in passing from the mixing chamber to the extrusion die. Thus, it is surprising that gluten breakdown, with a resultant increase in protein solubility is not observed during these stages of processing (Dexter & Matsuo, 1977a). Application of a modified Osborne fractionation procedure has indicated that the proportion of globulins decreased during dough mixing, whilst the amount of insoluble residue increased (Dexter & Matsuo, 1979; Wasik, 1978; Schofield et al., 1981). Wasik (1978) also noted a decrease in the proportion of proteins in the soluble glutenin fraction.

However, when a pasta dough was mixed at higher moisture levels, similar to those used in bread doughs ($\sim$50%), extractability in acid solution increased, and the proportion of high molecular weight protein in acetic acid extracts increased significantly. From these data Dexter & Matsuo (1979) concluded that at moisture levels below 45%, gluten development during dough mixing was limited. These workers observed that mixing a dough at higher temperatures (50°C) and higher moisture levels, led to reduced solubility in acid solutions, which was presumed to be the result of protein denaturation. Denaturation, presumably offset any increase in solubility that resulted from gluten breakdown.

Wasik (1978) observed a decrease in the proportion of glutenins amongst AUC-extractable proteins in finished pasta compared with the semolina. Other workers have demonstrated a decrease in the total number of sulphydryl groups during dough mixing and extrusion (Dexter & Matsuo, 1977a) although a concomitant increase in the total number of disulphide bonds was not detected. These data suggest that mixing of a pasta dough results in the formation of glutenin complexes whose molecular size renders them insoluble even in highly dissociating solvents, and this insolubilisation probably occurs through disulphide bond formation.

The present work was undertaken to establish the effect of commercial manufacturing procedures on durum semolina proteins. The semolina was examined at each stage of pasta processing:

1. Semolina
2. Dough
3. Freshly-extruded spaghetti
4. Spaghetti ex-predrier
5. Spaghetti ex-main drier = final product

...using the previously established techniques for determination of protein extractability, molecular size distribution of proteins in SDS-extracts and polypetide/protein composition in whole and fractionated samples. The distribution of free sulphydryl groups in unextractable proteins and different molecular weight species of the extractable protein fraction was determined at each stage of the pasta-making process. Thus, the nature of the reactions between wheat proteins, and the mechanism by which they occurred, were

monitored for an industrial food manufacturing process.

VI.2 Materials

Semolina (milled from Canadian Amber durum wheat) and pasta samples were taken from a long goods (i.e. spaghetti) processing line at Pasta Foods, Great Yarmouth, Norfolk, at various stages during pasta manufacture. The processing line consisted of a Braibanti Kobra-1300 press, feeding a Braibanti Kibra spaghetti drier; the drier system comprised a short pre-drier and a long main-drier with operating conditions as specified below.

Operating conditions of the pasta pre-drier

Pasta moisture into the drier	29 %
Pasta moisture leaving the drier	18 %
Temperature	44°C
Residence time	1 h

Operating conditions of the pasta main drier

Pasta moisture into the drier	18 %
Pasta moisture leaving the drier	11 %
Temperature	55°C
Residence time	22 h

The following stages in the spaghetti manufacturing process were selected for sampling:

1. Semolina
2. Dough from the vacuum mixing chamber, immediately before its transfer to the extrusion press
3. Freshly-extruded spaghetti
4. Spaghetti emerging from the pre-drier
5. Dried spaghetti emerging from the main-drier = final product

Samples were collected into polythene bags, frozen in liquid nitrogen and transported in liquid nitrogen to the laboratory. After freeze drying the samples were ground in a Retsch centrifugal mill (0.5mm screen), and then stored in a dessicator over NaOH pellets at room temperature.

VI.3 Experimental, results and discussion

VI.3.1 Protein extractability of durum semolina and pasta at different stages of spaghetti manufacture

Protein extractabilities into water and salt solution, acetic acid, and SDS solution were determined for durum semolina and pasta at different stages during spaghetti manufacture, according to the methods described in Appendices I.11, I.12 and I.2 respectively. The results are presented below:

Extractability of durum semolina at different stages of commercial spaghetti manufacture

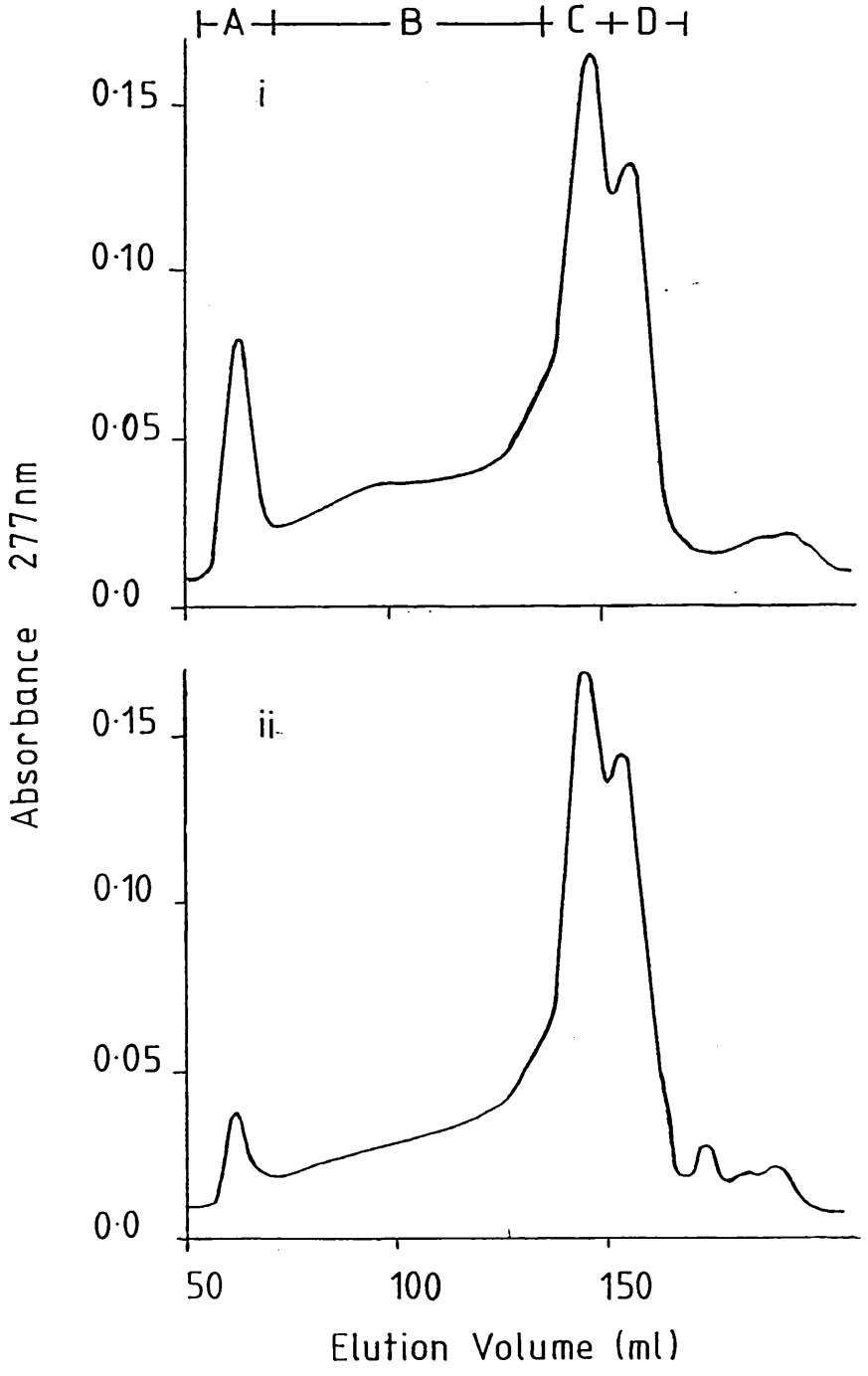
	NaCl Extracted			
	H_2O-soluble	H_2O-insoluble	Acetic Acid	SDS-Tris
Semolina	11.3	6.1	61	94.1
Dough	10.1	4.0	54	88.9
Freshly-Extruded Spaghetti	9.7	3.0	56	85.8
Ex-Predrier	9.6	2.6	55	81.2
Ex-Main Drier	9.3	2.0	54	78.6

Values are expressed as a percentage of the total protein in the sample that is extracted or soluble in each solvent. Protein extractabilities into water, salt and acetic acid solutions are reported courtesy of Mr. M.F. Timms.

For each extractant, a marked reduction in extractability was observed during pasta processing, the most significant changes occurring during dough mixing and extrusion; changes during the drying stage were comparatively small. The changes in protein extractability into saline, water and acetic acid are similar to those described previously (Dexter & Matsuo, 1977a).

Changes in protein extractability were also observed with SDS-Tris solvent. The largest decreases again occurred during the mixing of semolina into a dough, on extrusion of the dough to form spaghetti

Figure 32: Molecular size distribution of SDS-extractable proteins of durum semolina and dried spaghetti by gel-filtration chromatography on Sepharose CL-4B.



i = Durum semolina, ii = Spaghetti ex-main drier

A = high molecular weight (excluded) glutenin
B = lower molecular weight (included) glutenin
C = gliadin
D = albumin + globulin

and during the pre-drying stage. Only a small change in SDS extractability was observed during the main drying stage. The spaghetti, in fact, makes five passes through the main drier, but SDS extractability at the end of the first four passes was similar to that at the end of the fifth pass (data not shown).

These observations indicate that durum semolina proteins are altered during the manufacture of spaghetti, and that the major changes occur prior to the main drying stage when water activity is highest.

VI.3.2 Molecular size distribution of SDS-extractable protein from durum wheat semolina and spaghetti at different stages during manufacture

In order to define more precisely the changes that occur in semolina proteins during spaghetti manufacture, SDS-extracts of durum semolina and pasta were examined by gel-filtration chromatography, according to the method described in Appendix I.3.

Chromatograms of proteins extracted from semolina, and from spaghetti at the end of the main drying stages are presented in Figure 32. Comparison of the chromatograms obtained from material at different stages of processing indicated that the components most affected during pasta manufacture were the glutenins, especially those of very high molecular weight, which are excluded from the Sepharose CL-4B matrix. This was illustrated more clearly, when the areas beneath the regions of the chromatogram corresponding to glutenins that are excluded from the CL-4B matrix (region A),

included glutenins (region B), gliadins (region C) and albumins plus globulins (region D) were measured, and expressed as a percentage of the total area beneath the chromatogram. The data are summarised below.

Molecular size distribution of durum semolina proteins at different stages of commercial spaghetti manufacture

	%A	%B	%C	%D
Semolina	10.8	35.2	33.2	20.8
Dough	7.7	31.6	36.8	24.0
Freshly-extruded spaghetti	4.4	30.5	33.0	32.0
Ex-predrier	3.4	29.0	33.7	33.9
Ex-main drier	4.7	29.1	33.7	32.4

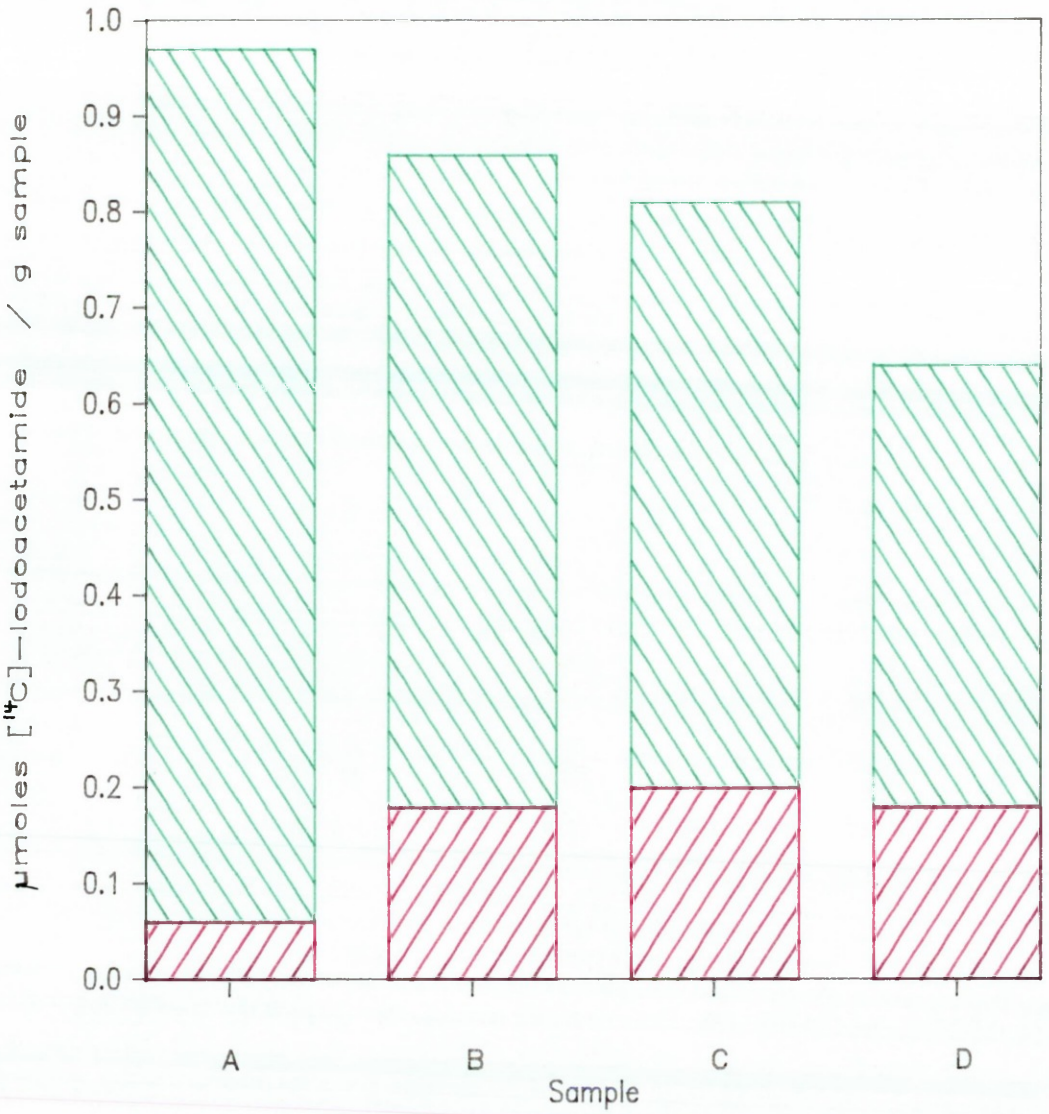
Values for each region of the chromatogram are expressed as a percentage of the total area beneath the chromatogram. The different regions are as indicated in Figure 32.

As semolina was processed, the proportion of material that was eluted in the column void volume (region A) fell from 10.8% to less than 5% of the total extractable protein, with the greatest change occurring during the dough mixing and dough extrusion stages. The proportion of lower molecular weight glutenin, i.e. that included in the column (region B), also decreased, the major change occurring at the dough mixing stage. The proportion of gliadin remained approximately constant.

There was an increase in the amount of material that was eluted in Region D of the chromatogram, i.e. an increase in the amount of albumin- and globulin-type protein in the SDS extract of pasta from later processing stages. This suggests that most of the decrease in protein extractability on pasta processing (i.e. from 94% - 74%) was due to reduced extractability of the gluten proteins, whilst the amount of low molecular weight material extractable in SDS probably remained approximately unchanged. Thus, as a percentage of the total SDS-extractable protein, albumins and globulins increased. In other solvents, the amounts of water-, salt- and acid-extractable proteins all decreased during pasta processing, but since SDS is a very effective dissociating agent this is probably not such a great contradiction as it appears.

Therefore, the decrease in the protein extractability during pasta processing appears to be due to the glutenins, particularly those of highest molecular weight, being rendered unextractable in SDS. In a study of changes in semolina proteins during pasta processing, Dexter and Matsuo (1977a) were unable to observe any changes in molecular size distribution by gel filtration on Sephadex G-150. Wasik (1978) was able to demonstrate some small, but significant changes in the low molecular weight protein components by gel-filtration on Sephadex G-200, which he suggested were the result of protein breakdown during pasta processing. However, the chromatography media used in these previous studies are not capable of resolving the very large glutenin aggregates, and therefore, it was not possible to examine changes in the high molecular weight fraction. The use, in the present study, of Sepharose CL-4B, has shown clearly, that changes occur in the very large glutenin

Figure 33: Distribution of SH groups between the SDS-extractable and -unextractable proteins of durum semolina and pasta at different stages of spaghetti processing.



Samples are A = durum semolina, B = freshly extruded pasta, C = pasta ex-predrier, D = pasta ex-maindrier

= µmoles [14C]-Iodoacetamide incorporated into SDS-extractable protein fraction.

= µmoles [14C]-Iodoacetamide incorporated into SDS-unextracable protein fraction.

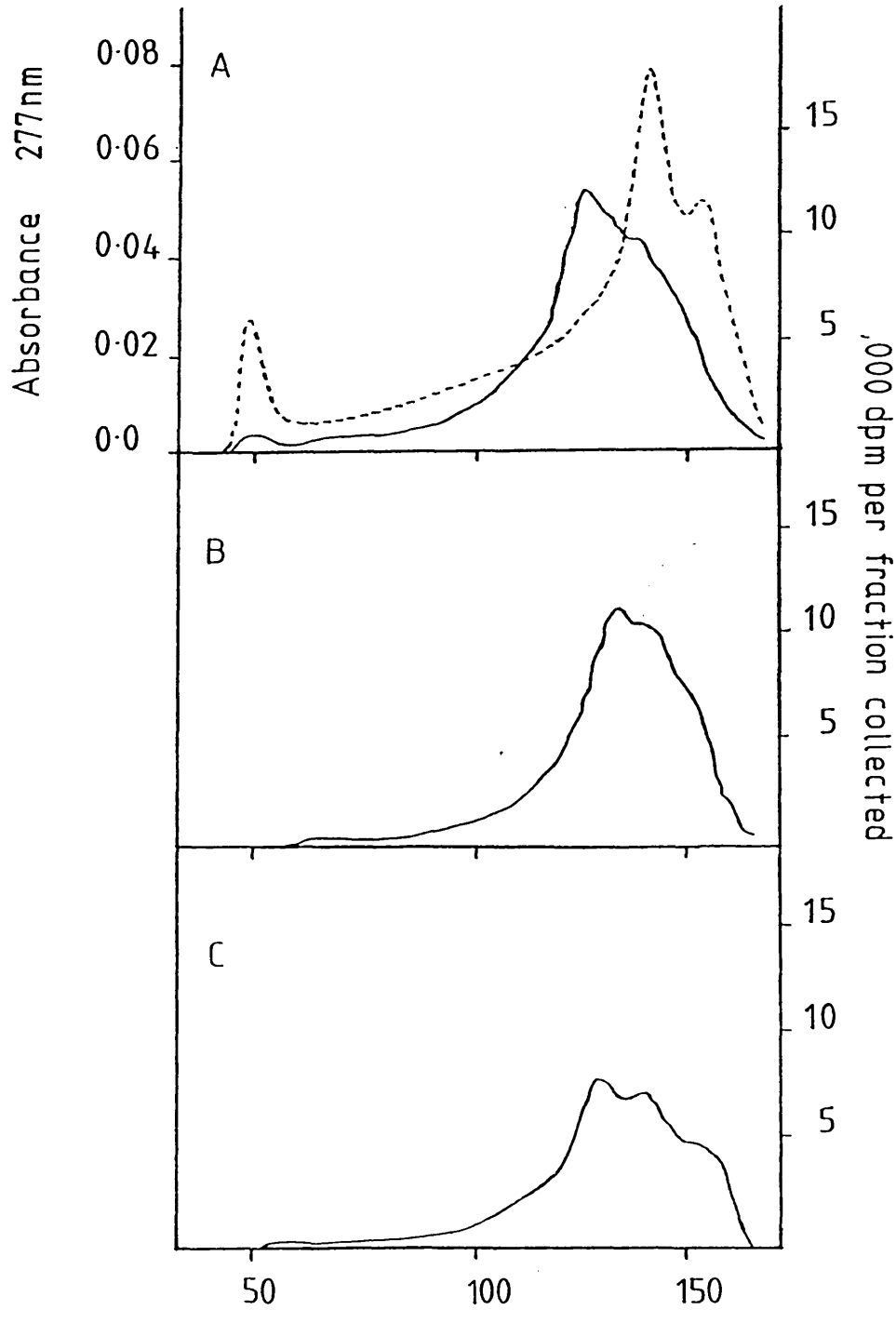
aggregates.

VI.3.3 Free sulphydryl groups of semolina and pasta at different stages of processing

In order to determine whether or not oxidation of sulphydryl groups or SS/SH interchange reactions played any part in the changes in solvent extractability and molecular weight distribution of durum wheat proteins, previously observed, free sulphydryl groups of semolina and pasta at different stages of spaghetti manufacture were determined, by carboxymethylation with [^{14}C]-iodoacetamide, according to the method described in Appendix I.13. Data for the distribution of free SH groups in durum protein fractions are presented courtesy of Dr. R.C. Bottomley.

Results presented in Figure 33, indicate that the total number of sulphydryl groups available for alkylation by this technique, fell during pasta processing. Dexter and Matsuo (1977a), using different techniques observed a similar decrease. Determination of the distribution of the label indicated a marked fall in free sulphydryl groups in the SDS-extractable fraction and a substantial increase in the SDS-unextractable fraction during the early stages of processing. These data suggest that there may be rearrangement of disulphide bonds by SS/SH interchange during the early stages of pasta processing, such as occurs during heating of gluten in the wet state (Schofield *et al.*, 1983) in addition to some loss of sulphydryl groups. During the later stages of processing there is a further decrease in radiolabel in the SDS-extractable fraction, but no further increase in the SDS-unextractable fraction, suggesting

Figure 34: Molecular size distribution of [^{14}C]-Iodoacet-amide labelled SDS-extractable proteins of durum semolina and pasta at different stages during processing.



Samples are A = durum semolina, B = freshly extruded pasta, C = dried spaghetti. The broken line shown in (A) is an optical density (277nm) profile to indicate the elution positions of the various protein classes.

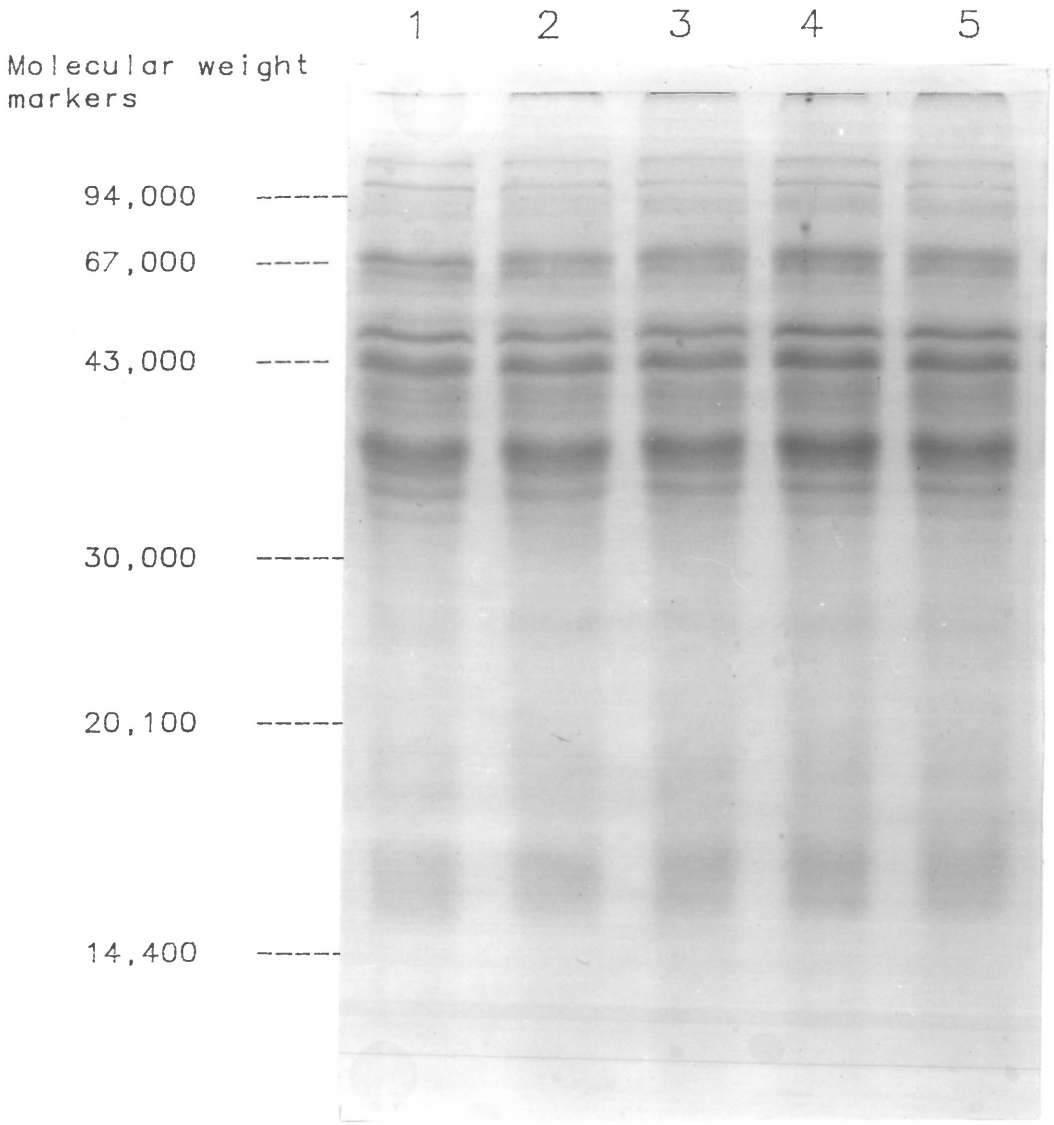
disulphide bond formation rather than SS/SH rearrangements.

To determine which protein classes were involved in these changes in free sulphydryl groups, the SDS-extractable, C-labelled proteins were chromatographed on Sepharose CL-4B and the distribution of label was determined. The results for semolina, freshly-extruded spaghetti and finished spaghetti are presented in Figure 34. The greatest concentration of free sulphydryl groups occurred in the region of the chromatogram preceding the gliadin peak (peak C), where the lower molecular weight disulphide-linked glutenin aggregates are eluted, although components in all regions of the chromatogram were also labelled. The most substantial loss of free sulphydryl groups on processing appeared to involve the lower molecular weight glutenin aggregates. These observations, together with findings that during the early stages of processing free sulphydryl groups are converted from an SDS-extractable to an SDS-unextractable form, suggest that aggregation of the semolina proteins occurs during the early stages of pasta processing involving sulphydryl-disulphide rearrangement, and that free sulphydryl groups in the low molecular weight glutenin components are particularly involved in these events. Feillet et al. (1977) have also concluded that both disulphide bond cleavage and new disulphide bond formation occurs during pasta dough sheeting.

VI.3.4 Protein subunit composition of durum semolina and pasta, by SDS-PAGE analysis

To investigate modifications to the semolina proteins during pasta processing, for example, by proteolysis or cross-linking other than

Figure 35: Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) of proteins of whole durum semolina and spaghetti at different stages during processing.



Samples are:
1. Durum semolina
2. Pasta dough
3. Freshly-extruded pasta
4. Spaghetti ex-predrier
5. Spaghetti ex-main drier

through disulphide bond formation, whole semolina and pasta samples were examined by SDS-PAGE according to the method described in Appendix I.4. SDS-extractable and -unextractable protein fractions were prepared by the method described in Appendix I.3, dialysed against distilled water to remove SDS, and then freeze-dried. The freeze-dried fractions were suspended in the electrophoresis sample buffer and applied to polyacrylamide slab gels as before.

Electrophoresis of the fully reduced, whole semolina and pasta samples showed that the protein subunits present at different stages of processing were essentially unchanged. No new bands appeared and none were lost (Figure 35). There was evidence, however, of material that stained as protein but which streaked from the top of the gel instead of forming a discrete band. This material increased in intensity at each stage of processing, which may indicate that a proportion of the protein was becoming cross-linked, to form polydisperse covalently-linked aggregates of high molecular weight. These aggregates appear to be stabilised by bonds other than disulphide bonds since they were not cleaved by relatively high concentrations of the reducing agent dithiothreitol.

The electrophoretic characteristics of the protein components of the SDS-extractable and SDS-unextractable fractions at different stages of processing were also examined. The most marked change in the SDS-extractable fraction was a decrease in the relative proportion of glutenin subunits of molecular weights between 85,000 - 120,000 (Figure 36). Electropherograms of the reduced SDS-unextractable protein (Figure 37) show clearly that the protein-staining background, which increased during processing, was associated with

Figure 36: SDS-extractable proteins of durum semolina and pasta

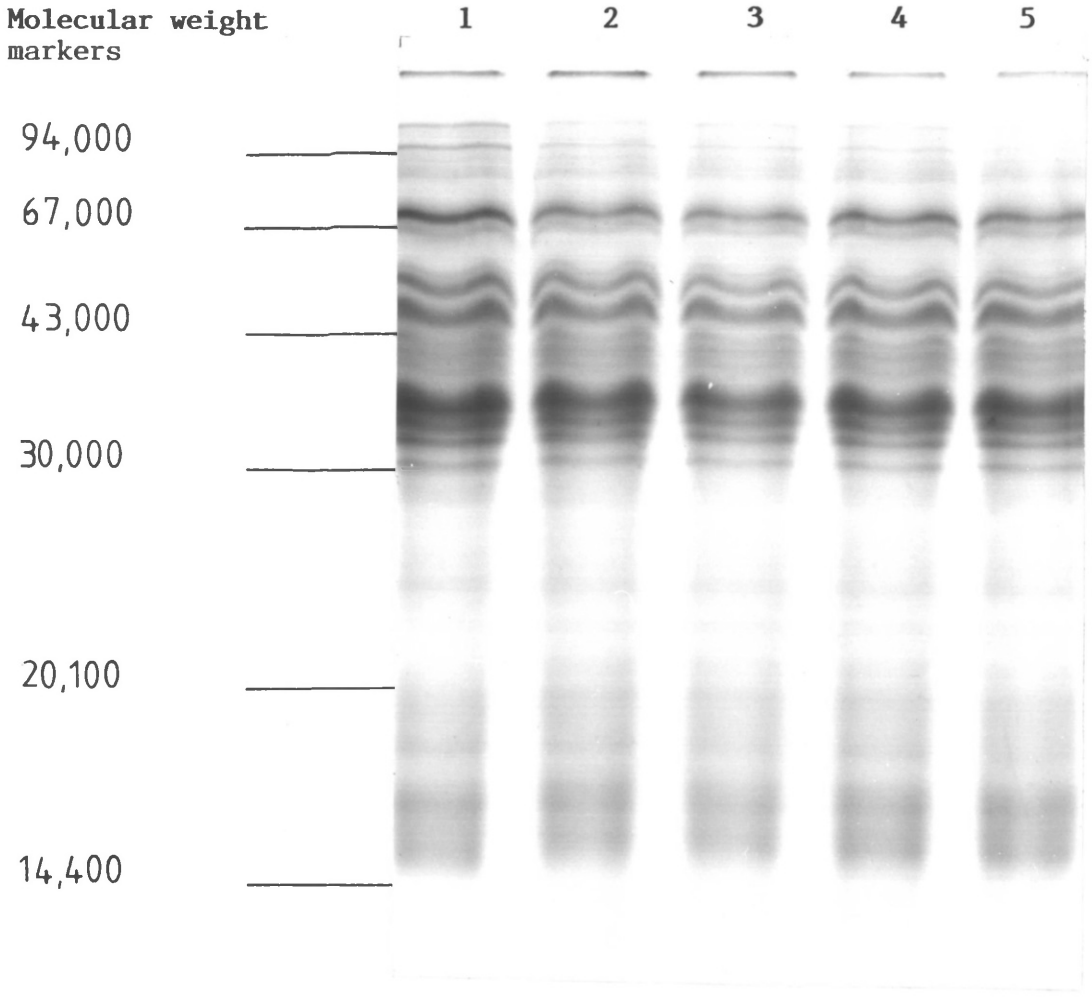
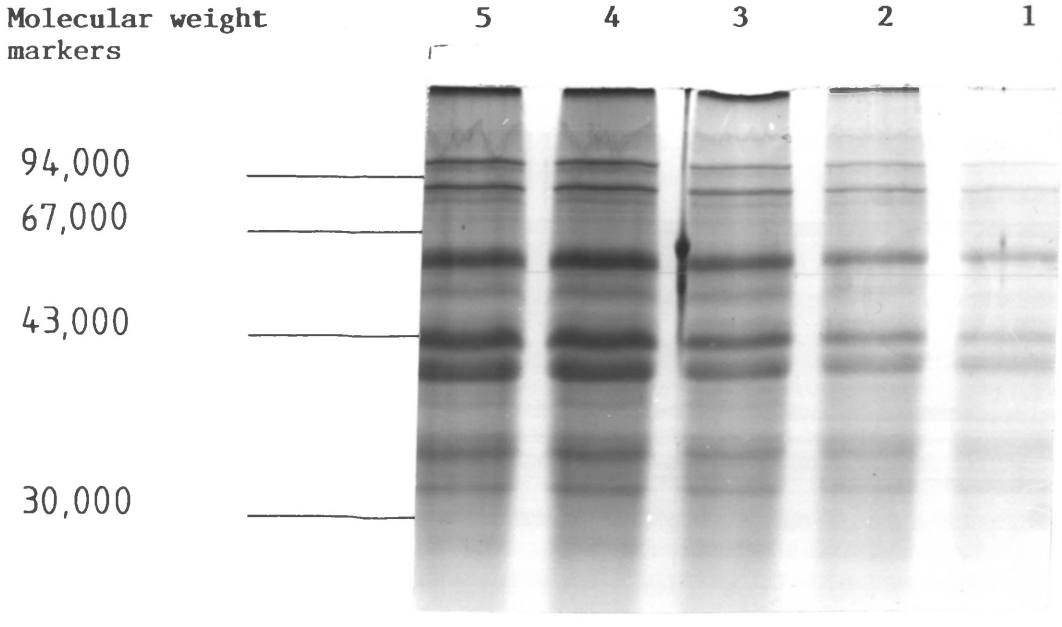
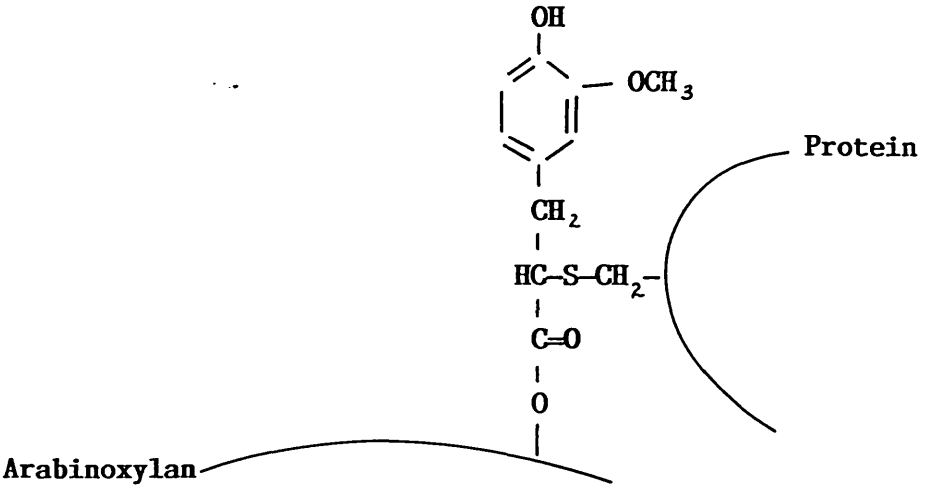


Figure 37: SDS-unextractable proteins of durum semolina and pasta



1. Durum semolina, 2. Pasta dough, 3. Freshly-extruded pasta, 4. Spaghetti ex-predrier, 5. Spaghetti ex-main drier.

components in the SDS-unextractable fraction. Dexter & Matsuo (1977a) have also observed a loss of free sulphydryl groups during processing, but they were unable to demonstrate any change in the level of disulphide bonds. This streaking of protein may be related to the disappearance of free sulphydryl groups. A possible explanation for these observations may be the postulated reaction between thiyl radicals derived from protein disulphide groups and the activated double bond of the ferulic acid moiety of pentosans (Hoseney & Faubion, 1981). Such a covalent grafting of polypeptide and polysaccharide chains, as represented below, would create a multiple cross-linked entity of high molecular weight. The postulated cross-linking would be reflected in changes in a dough's rheological properties. However, there is no direct evidence to support such a reaction at this date.



VI.4 Conclusions

The extractability of durum semolina proteins in a variety of solvents decreased during the early stages of spaghetti manufacture. There was relatively little change in protein characteristics during the long main drying stage. Gel-filtration chromatography data suggested that the proteins particularly affected by processing were the glutenins. Polymerisation of the glutenins through sulphydryl-disulphide interchange reactions may account in large part for the changes observed during the early stages of processing. The level of free sulphydryl groups declined continuously during processing, suggesting, also, the formation of new disulphide bonds. Electrophoretic analysis indicated, however, that some polymerisation of proteins occurred during processing other than through disulphide bonding.

Chapter VII: Changes in Wheat Flour Proteins during Preparation of Flour-Water Batters

VII.1 Introduction

The water:flour ratio used in baked goods depends upon the type of product being made and the associated process. The ratio may vary from 20 parts water:100 parts flour in a biscuit dough, to 150 parts water:100 parts flour in a batter system that is used for such products as French pancakes (crèpes). For a bread dough 57 - 61 parts of water are usual. The different water requirement also relates to the ease of handling of the product during manufacture; the biscuit dough is stiff to permit rolling and sheeting, while the liquid batter is a suspension that will flow freely over cooking surfaces.

Batters comprising flour, egg and milk are used in a wide variety of products, in which the structure is provided by gelatinisation of the starch and coagulation of the proteins of flour, egg and possibly also milk during baking.

Batters used for making crèpes and for coating foods, such as fruit and fish, must be well beaten to incorporate air, which, together with the steam generated during heating, provides the gas necessary to give the desired texture in the finished product. Whereas most of the air is lost from crèpes, in fritters the batter expands on contact with the hot fat, the outer surface then sets preventing escape of all the generated steam and the air, and surface blisters result.

An almost identical batter is used by commercial bakers to produce sugar wafers, which are baked between two hot plates, similar to a waffle iron, but with much smaller indentations. In the wafer, the gases are dispersed as a fine foam in a flour gel. Subsequent dehydration yields a thin brittle sheet of low moisture content containing a large number of very small air cells (Yamazaki & Lord, 1978).

Pancakes and waffles are made from a slightly modified batter recipe that includes shortening and sugar, in addition to eggs and milk. Pancake batters have a somewhat higher viscosity than those used for crèpes. This helps to entrap the gases generated during cooking and so produces a lighter product.

Some cakes, such as true sponge and chiffon cakes, are also produced from batters. Generally known as "foam cakes" these rely entirely on air as leavening agent. Angel food cakes, for example, contain sugar, egg whites and flour, with cream of tartar, (potassium hydrogen tartarate), added to increase foam stability. Cream of tartar reduces the pH of the medium thus decreasing the solubility of the egg protein and enhancing its whipping properties. The soft wheat flour used in cake manufacture is usually treated with chlorine gas. This causes some protein breakdown and increases starch viscosity. Flour chlorination improves cake quality, and efforts have been made to determine the mechanism of the action.

Early work (Sollars, 1958; Lamb & Bode, 1963) showed that most of the cake improvement resulting from flour chlorination was due to changes in the prime starch fraction. Wilson et al. (1964)

demonstrated that chlorination was a random reaction, the extent of reaction being dependent on surface area. Thus, the improvement in cake quality due to chlorination appeared to be a function of the starch surface exposed to the gas.

Youngquist et al. (1969) suggested that chlorine reacted with unsaturated lipids present on starch, and prevented formation of starch-lipid complexes. However, chlorinated starch has been shown to have a higher oil-binding capacity than its unchlorinated counterpart (Shuey et al., 1963; Seguchi, 1984).

Other workers have suggested that chlorine affects the baking behavoir of cake flours by modifying minor components, such as proteins or lipids, at the surface of the starch granule (Cauvain et al., 1977). The enhanced hydrophobicity of wheat starch after chlorination was shown to be reversed by treatments designed to remove protein from the surface of the stach granule, therefore, indicating that proteins were the surface components involved in the modification by chlorine. Recently, distinct proteins have been identified in wheat starch granules (Gough et al., 1985) and more specifically at their surface (Lowy et al., 1981).

Recently, Greenwell et al. (1985) suggested that starch surface proteins play a significant part in the manner in which enzymic degradation of the granule takes place: a sub-set of starch granule surface proteins appeared to channel or direct the amyloglucosidase (AMG) digestion of starch, resulting in pitting of the granule's surface. Chlorination altered the pattern of the AMG-catalysed degradation, apparently by altering this particular sub-set of

surface proteins. The significance of these observations in relation to the improving effect of chlorine on "high-ratio" cake flours remains, as yet, unknown.

Soft wheat flours seem to be uniquely suited to the manufacture of the wide range of products described above. The biochemical basis for this is unclear. However, the preference for soft wheats in these applications is based on numerous observations that their flours give products that are larger in size/greater in volume, more tender and of superior internal structure compared with the same derived from other types of wheat flour. The low protein content and water absorption capacity and fine granulation probably all contribute to the functional properties of soft wheat flours that are required for successful batter making.

It has been suggested that the degree of flour hydration may be the principle factor differentiating flours of various batter-making potential (Yamazaki, 1955). More syrup is formed in the dough of a "low hydration" flour from the added sugar and the available water. This high level of syrup makes the dough slacker at elevated temperatures and allows a greater degree of expansion and spread to take place in the oven before heat-setting occurs (Fuhr, 1962).

The hydration of the flour components is affected not only by the nature of the flour, e.g. the level of damaged starch, but also by the nature of other ingredients in the batter or dough system. In batters and cookie doughs soluble ingredients, such as sugar and salts, are at a high concentration in the aqueous phase. The high water-binding capacity of these solutes inhibits water absorption by

other flour components. Yamazaki (1969) found that the gelatinisation temperature of wheat starch was increased from 60°C in water to 85°C in 50% sugar solution. Gelatinisation of starch results when water enters the granule during heating and is trapped within the 3-dimensional lattice structure and/or bound to the exposed hydroxyl groups on the glucose units of the starch molecule. Substances that compete with starch for water, therefore, hinder swelling.

Baxter and Hester (1958) studied the effect of sucrose on gluten development in a soft wheat flour and showed that additions of 15% or 30% of sucrose (flour weight basis) increased dough development times in the Mixograph. Sucrose levels of 60% - 120% prevented dough formation, which they attributed to competition between the sucrose and the gluten proteins for the available water. Gluten development is not, anyhow, desirable in batter systems, since this would produce a tough product and reduce batter stability.

In commercial practice, wafer batters are often mixed at a central point and held in a tank from which the batter is distributed to the wafer ovens. When batter stability is low, gluten aggregates may be deposited in the distribution system. In extreme cases, the system may become completely occluded, leading to wastage of raw materials, variability in the final product and unacceptable production delays. Thus, stability of the protein component during processing is an important criterion determining flour quality for commercial wafer batter production.

A batter-mixing process must blend all of the ingredients and allow

time for hydration of the flour. Commercial mixing times range from 2.5 - 6.0 min (Pritchard & Wade, 1972). However, Pritchard and Stevens (1973) established that 4 min was ideal to achieve homogeneity. Generally, a high shear mixer is preferred since lower speed mixers may allow gluten strand formation to occur, producing strings in the final batter. Some uncertainty exists about the cause of gluten aggregation. Possible explanations include overmixing of the batter or the use of a flour with too high a protein content. Generally, a flour of medium strength, i.e. 9.5% protein is used for the production of wafer batters (Whiteley, 1971; Smith, 1972).

Pritchard et al. (1975) suggested that the flour particle size distribution was the single most important factor influencing the properties of wafer sheets, and that other flour properties, including protein content were relatively unimportant. Flour granulation is of undoubted importance in cookie baking, but the manner in which this is achieved is also relevant. Yamazaki (1959) showed that a fine flour fraction produced by sieving, increased cookie spread to a greater extent than a coarse fraction. On the other hand, a fine flour fraction produced by progressive milling decreased cookie spread (Brenneis, 1965). This was attributed to the increased hydration of the flour resulting from a higher level of damaged starch. The effect of flour particle size on cake volume has been reported for a small group of different wheat cultivars (Yamazaki & Donelson, 1972; Chaudhary et al., 1981). However, these included hard and soft varieties and so introduced differences due to wheat class in addition to variations in particle size. Gaines (1985) evaluated soft red and soft white wheat flours for

associations between various physicochemical properties (kernel hardness, milling quality, protein content) and indices of soft wheat quality (cake volume and cookie spread). Gaines concluded that larger cake volume and greater cookie spread were generally associated (among cultivars) with soft textured wheats of low protein content, which produced high break flour yield and had smaller flour particle size. Wheats of better milling quality were generally coarsely granulating and produced smaller cakes and cookies. If the particle size of any particular flour were reduced by more severe milling, cookie size decreased. Thus, soft wheat flour particle size analysis was proposed as a general indicator of cake and cookie flour quality if the milling procedure was standardised.

The contribution of different flour components in the cookie test have been evaluated by fractionation and reconstitution techniques (Sollars, 1956). Yamazaki (1955) isolated a "purifed starch tailings" component that had a great capacity for imbibing water, and that reduced cookie spread when added to flour in small quantities. Sollars & Bowie (1966) fractionated tailings and demonstrated that a pentosan-rich fraction and a damaged starch component reduced cookie diameter more than the original tailings.

Pentosans are polysaccharides largely composed of the pentose sugars, D-xylose and L-arabinose. They are mainly found in plant-cell walls, where they are associated with cellulose and help to make up the framework of the cell wall. The majority of the pentosan material is, therefore, found in the bran portion of the wheat grain, which is almost entirely cell-wall material, but

pentosans are also present in the thin-walled endosperm cells that are packed with starch grains and proteins. Variation in the pentosan content of soft wheat flours appears to be a varietal characteristic (Yamazaki, 1955).

Most of the pentosans in the bran are insoluble in water, but dissolve in dilute alkali. Some of the water-insoluble pentosans seem to become highly hydrated and swollen with water without passing into solution. These are intimately associated with the proteins and glucosans (small starch grains and damaged starch) and are found in the starch tailings. Upton & Hester (1966) separated starch tailings into:

i) a supernatant liquid comprising 52% protein and 5% pentosans,
ii) a mucilaginous material comprising 16% protein and 18% pentosan,
iii) a polysaccharide-protein complex comprising 5% protein and 95% pentosans.

This last named fraction showed some resistance to hydrolysis and was, apparently, derived from the cell-wall material.

Straight grade flours from hard wheats contained more starch tailings than soft wheats, suggesting that varietal differences in cookie baking potential may be attributable to quantitative differences in tailings. Low-grade flours that are rich in starch tailings give poor cookies.

About 20 - 25% of the wheat flour pentosans are soluble in water

and dissolve to produce highly viscous solutions. Interest in the role of the water-soluble pentosans in bread-making originated with Baker et al. (1943) who found that solutions of water-soluble pentosans formed gels after addition of minute amounts of oxidising agents. Neukom et al. (1967) proposed the glycoprotein nature of the water soluble pentosans, and Pomeranz (1968) suggested that the large size and complexity of molecular structure of pentosans may contribute to their water-binding role and interaction with other flour constituents.

Pentosans of different wheat flours have been compared by several workers. According to Medcalf et al. (1968) durum pentosans contained a higher proportion of arabinose than those from hard red spring (HRS) and soft white wheats, indicating a more highly branched structure. Lineback et al. (1977) also found differences in the carbohydrate composition of pentosan fractions isolated from HRS, hard red winter (HRW), and soft red winter (SRW) wheat flours. Ciacco & D'Appolonia (1982) examined the relationship between composition and gelling capacity of the water-soluble pentosans of different wheat classes, i.e. HRW, HRS, SRW, durum and western white wheats. They found that the most abundant polysaccharide was an arabinoxylan, and that whereas durum arabinoxylan had the highest degree of branching, that from SRW had the lowest. Arabinoxylans had higher intrinsic viscosities than any other polysaccharide fraction, but the lowest intrinsic viscosity values were obtained for arabinoxylans from SRW.

Most of the research on soft wheat flour to date has been concerned with the study of its behavoir in the baking of specific products,

e.g. cookies. Underlying these investigations have been efforts to understand the nature of the reactions and phase changes that take place during mixing and baking, and the role of the flour components in these activities. The presence of other ingredients in the batter and dough systems further complicates the analysis.

Little is known of the changes that occur in the gluten proteins during the preparation of a simple batter or of the physicochemical reactions that are responsible for the stability of the protein component. This work examines the changes in protein extractability and molecular size distribution of four flours, covering a range of batter-making potential, during batter preparation. A theory for gluten protein interactions is advanced to explain the observed phenomenon, and proposals for modification of the batter recipe to improve the performance of a poor quality flour are tested.

VII.2 Experimental, results and discussion

VII.2.1 Flour characteristics and performance in a batter-mixing test procedure

Four flours were used in the study: a flour used in the commercial manufacture of batters (CBF), a flour prepared by laboratory milling of a grist of mixed varieties of U.S. soft red winter (SRW) wheats and flours derived from pure samples of the varieties Bounty and Kinsman. Flours were analysed for protein (P) and moisture (M) content, starch damage (SD) and water absorption capacity (WA).

Batters were prepared from the four flours by the procedure

described in Appendix I.14. and summarised below. Samples of batter were withdrawn at different stages during preparation, as indicated, frozen immediately in liquid N_2, and later freeze-dried and milled.

Batter-mixing test and sampling stages:

1. 120 parts water : 100 parts flour were mixed for 120s.
   Samples were taken after 60s and 120s of mixing.
   Batter viscosity was determined after 120s mixing.

2. The batter was transferred to a high shear blender and mixed until gluten development occurred.
   Samples were taken after 20s (= 140s total mix time) and at the point of gluten development, which is the measure of protein stability (s).

3. Further high shear mixing re-dispersed gluten aggregates.
   The final sample was taken.

The batter mixing test is known to predict the suitability of a flour for commercial batter manufacture. The method measures the batter viscosity, using a Ford cup, and protein stability on mixing at high shear rates in a domestic blender. A flour with good processing characteristics will typically have a Ford cup flow time of 80s - 100s, and will demonstrate no tendency for gluten aggregation and separation up to 220s of mixing. The analytical data and batter-mixing characteristics of the four flours are shown in Table 8.

Table 8: Flour Characteristics and Performance in a Test Batter-Mixing Procedure

Flour	M (%)	P (%)	SD (%)	WA (%)	Viscosity (s)	Stability (s)
Kinsman	14.2	9.3	22	54.4	>1000	120
Bounty	14.8	8.1	31	56.6	250	154
SRW	14.0	9.1	N/A	N/A	135	251
CBF	14.5	9.8	<10	50-54	75	184

The flours examined represented a wide range of qualities with respect to batter making potential. None of the flours met the ideal specification on both viscosity and protein stability criteria. However, the commercial flour, and the flour from the SRW wheat, would be acceptable for use on a commercial plant on the basis of these data. Neither of the monovarietal flours examined, (Bounty and Kinsman) would be acceptable for batter manufacture.

VII.2.2 Extractability of flours and batters taken at different stages of processing, into buffers containing sodium dodecyl sulphate

Protein extractability in buffers containing 2% SDS was determined, according to the method described in Appendix I.2., for the four flours and the freeze-dried samples of batter taken at various stages during preparation.

The proportion of total wheat protein extractable in SDS increased on batter mixing for Bounty, Kinsman and the commercial flours, although the observed changes were small, (Table 9). For the commercial flour, the greatest increase in extractability occurred after extended mixing times, i.e. >120s. For the SRW flour, there was an initial decrease, followed by an increase in protein extractability on batter-mixing, which may be related to changes in protein molecular size.

Table 9: SDS Extractability of Protein from Flours and Batters at Different Stages of Mixing

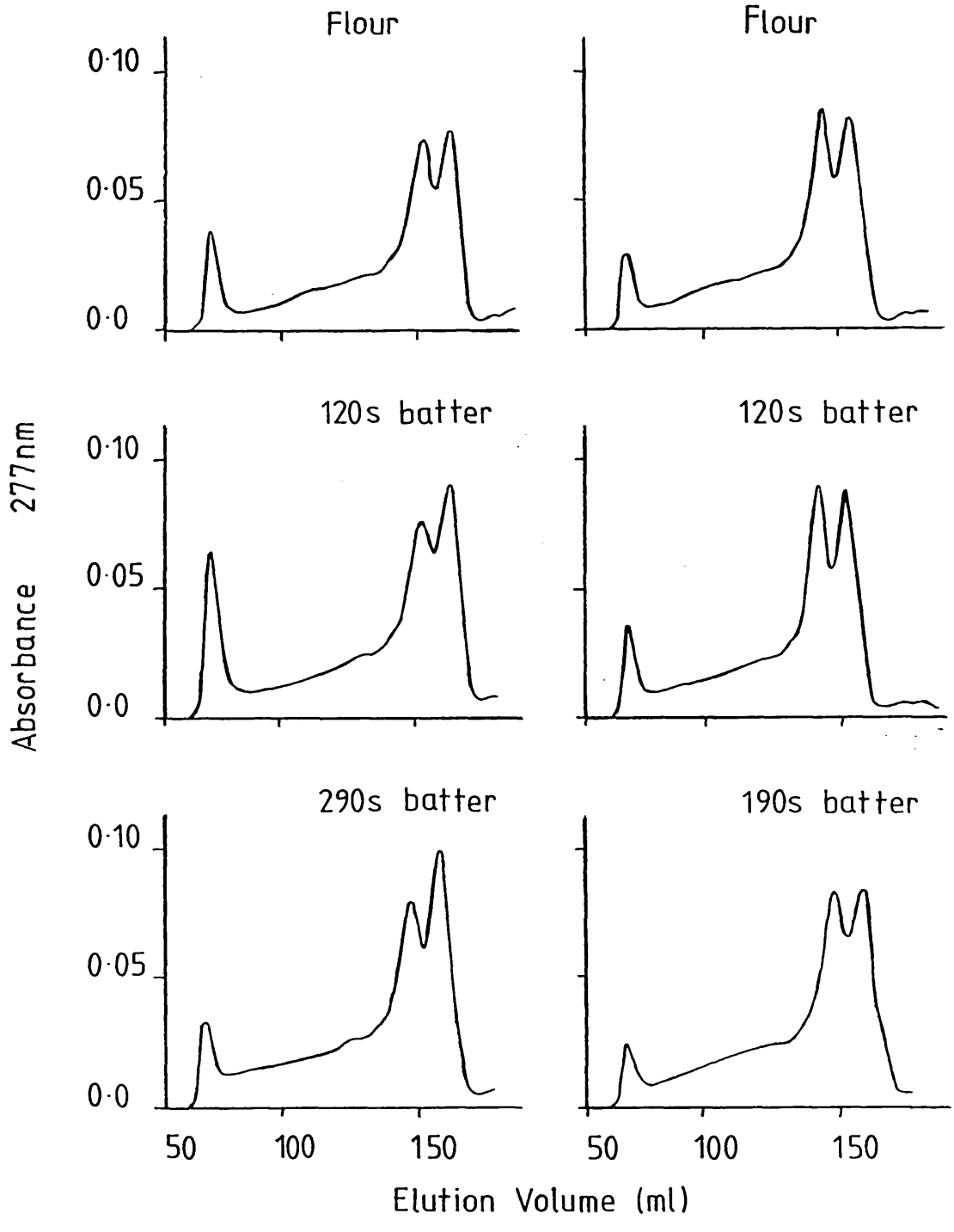
		Extractability *			
Sample	Mix Time (s)	Kinsman	Bounty	SRW	CBF
Flour	0	90.3	95.3	93.4	90.3
Batter	120	90.3	96.8	90.3	90.8
Gluten	190	91.5	-	-	-
Redispersal	290	-	96.4	91.5	93.3

* Extractability is expressed as the percentage of protein extracted of the total recovered protein.

VII.2.3 Molecular size distributions of SDS-extractable proteins of flours and batter samples

In order to define more precisely the changes that occur in flour proteins during batter preparation, SDS extracts of the four flours

Figure 38: Molecular size distribution of SDS-extractable proteins of wheat flours differing in batter-making potential, at different stages during a batter-mixing test.



SRW = good quality batter-making flour

Kinsman = poor quality batter-making flour

and batters taken at different stages during mixing were examined by gel-filtration chromatography according to the method described in Appendix I.3.

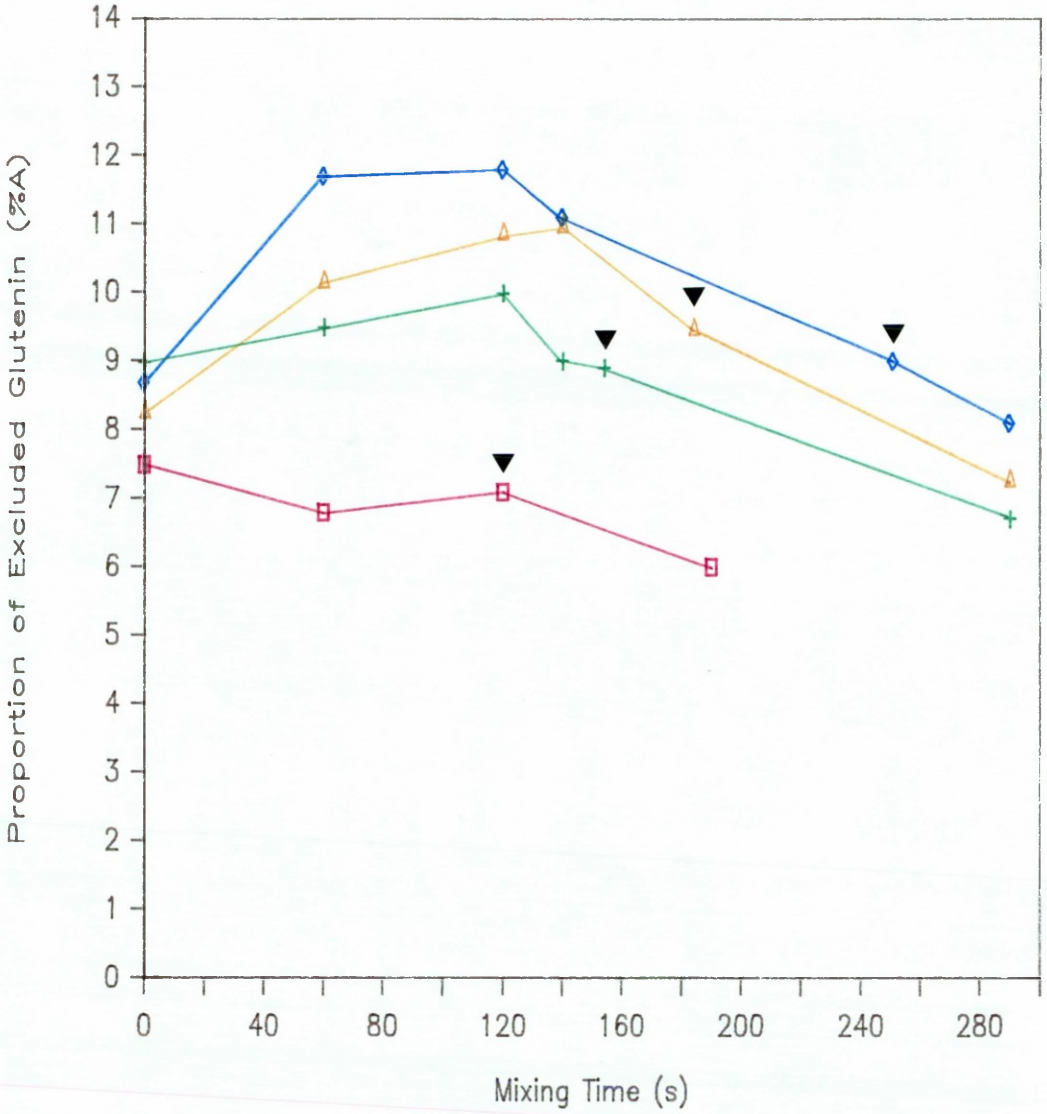
Typical chromatograms of the SRW and Kinsman batters are presented in Figure 38. Comparison of the chromatograms of each flour and its batters indicated that the glutenin components were most affected during batter-mixing, particularly those of very high molecular weight that were excluded from the Sepharose matrix.

This is illustrated more clearly if the area beneath the chromatogram corresponding to the excluded glutenins (region A) is expressed as a percentage of the total area beneath the chromatogram. Figure 39 shows the change in the proportion of high molecular weight glutenin in SDS extracts at different stages of batter mixing.

The proportion of the total extractable protein that was eluted at the column void volume was similar in each of the raw materials, although marginally lower in the Kinsman flour.

During the initial stage of batter mixing, (i.e. 0-120 s), the proportion of high molecular weight protein in the SDS extracts increased slightly in the case of Bounty and increased more markedly in the batters of the commercial and SRW flours. In contrast, the proportion of the total protein in chromatogram region A, decreased, or remained approximately constant, in protein extracts of Kinsman batters. Protein extractability in SDS was unchanged (Bounty and CBF) or decreased slightly (SRW) during this initial mixing phase.

Figure 39: Changes in the proportion of high molecular weight glutenin aggregates in SDS-extractable proteins of flours and batters at different stages of mixing.



SDS-extractable proteins were chromatographed on columns of Sepharose CL-4B. The UV asorbance of column effluents was monitored continuously. Excluded glutenin (%A) is the area of the A277 profile corresponding to the column void volume as a percentage of the total area of the curve.

◇ = SRW △ = Commercial + = Bounty □ = Kinsman

▼ = indicates the point of gluten separation

Thus, the increase in the amount of protein in chromatogram region A, presumably, did not result from depolymerisation of insoluble (extremely high molecular weight) glutenin that rendered it soluble.

On further mixing in the high shear blender, protein disaggregation occurred in all samples, although the relative differences between them were retained. For batters of SRW, commercial and Bounty flours, gluten separation occurred when the proportion of high molecular weight glutenin had fallen to similar levels (i.e. 8.9% - 9.5%). For Kinsman, gluten separation occurred at the onset of high energy mixing (120s total mix time). The proportion of high molecular weight protein in SDS extracts did not rise above 7.5%.

VII.2.4. Determination of flour sulphydryl groups and their distribution between SDS-extractable and unextractable protein fractions

In order to determine whether or not differences existed in the level or distribution of free sulphydryl groups in high- and low-quality batter-making flours, the SH groups of SRW and Kinsman flours accessible in SDS solution were determined by carboxymethylation with [^{14}C]-iodoacetamide (method as described in Appendix I.13). For each flour, the amount of radiolabel incorporated into the SDS-extractable and SDS-unextractable flour protein was determined, and hence the free sulphydryl content of each fraction (Table 10). Results are presented courtesy of Dr. R.C. Bottomley.

Table 10: Distribution of Free Sulphydryl Groups in SDS-Extractable and -Unextractable Proteins of Flours for Batter-Making

Flour	Protein Content(%)	Protein Stability(s)	r	s	Total	r/s
Kinsman	9.3	120	180	787	967	0.23
SRW	9.1	251	424	813	1237	0.52

r = μmoles of [^{14}C]-iodoacetamide incorporated into the SDS-unextractable residue.

s = μmoles of [^{14}C]-iodoacetamide incorporated into the SDS-extractable flour protein.

Protein extractability in SDS-Tris buffer was 90.3% and 93.4% of the total flour protein for Kinsman and SRW respectively.

The total accessible, free sulphydryl content of Kinsman flour was 78% of that of the SRW flour. Only small differences in the sulphydryl content of the extractable protein fractions were observed between the flours. However, the residue protein fraction, which comprises mainly glutenin protein, contained a significant proportion of the total SH groups, particularly in the SRW flour. This flour also exhibited greater protein stability during batter mixing than the Kinsman flour.

Whilst it is likely that many factors influence protein stability, and, hence, the suitability of a flour for commercial batter manufacture, it appears from this investigation that protein quality

is an important factor. These data suggest that batter stability is related to the ability of the gluten proteins to aggregate. The very high molecular weight glutenin fraction, which contains a large proportion of the free SH groups in good "quality" flours, may play an important role in the aggregation process by participating in either sulphydryl/disulphide interchange or oxidation reactions. The aggregation resulting from such reactions appears to delay gluten development and separation from the batter system.

In "poor quality" flours, such as Kinsman (or Bounty), there are fewer accessible free sulphydryl groups, and these occur largely in the lower molecular weight SDS-extractable protein fraction. During the initial stages of mixing, little or no protein aggregation takes place, such that on the application of high shear rates, protein molecular size is reduced, presumably through rupture of disulphide bonds, and gluten development occurs.

VII.2.5 Effect of oxidising and reducing agents on batter viscosity and protein stability to mixing

If polymerisation of glutenins through disulphide bonding is related to the gluten development potential of a flour in the way suggested above, then mixing in the presence of oxidants should stabilise batters, whereas reducing agents should reduce batter stability. In order to test this, batters were prepared according to the test procedure (Appendix I.14) but incorporating either the oxidising agents azodicarbonamide (ADA) (45 ppm) or potassium bromate (50 ppm) or the reducing agent cysteine hydrochloride (150 ppm). The resultant batter viscosities and protein stability to mixing, were

determined for the samples of SRW and Bounty flour, used previously, and for a sample of Flanders flour prepared by laboratory milling. Results are presented below.

Effect of Oxidising and Reducing Agents on Batter Viscosity and Protein Stability to Mixing

Flour	(s)	Control	Cysteine (150ppm)	Bromate (50ppm)	ADA (45ppm)
SRW :	Viscosity	135	199	98	90
	Protein stability	251	129	318	>420
Bounty:	Viscosity	250	-	217	217
	Protein stability	154	-	187	187
Flanders:	Viscosity	342	>1000	-	158
	Protein stability	139	120	-	166

Additions of cysteine hydrochloride at a relatively high level (150 ppm) led to a significant decrease in protein stability, whilst additions of the oxidants, ADA or potassium bromate, increased protein stability and decreased batter viscosity. These data, therefore, reaffirm the central role that sulphydryl groups play in glutenin polymerisation and protein stability during batter mixing procedures.

VII.3 Conclusions

A batter system comprises free water and an aqueous phase in which the other ingredients are dispersed, and into which the leavening agents and yeast may release carbon dioxide and so produce a foam. During baking this foam rises, and is set in the later stages of heating (Handelman et al., 1961; Miller & Trimbo, 1965; Wilson & Donelson, 1963, 1965). In systems with a high water:flour ratio the importance of the gluten matrix is said to diminish (Pritchard et al., 1975). This has not been the conclusion of this investigation.

Two parameters were used as indices of flour quality for batter making, i.e. batter viscosity and protein stability during intensive mixing, both of which are important factors in commercial practice.

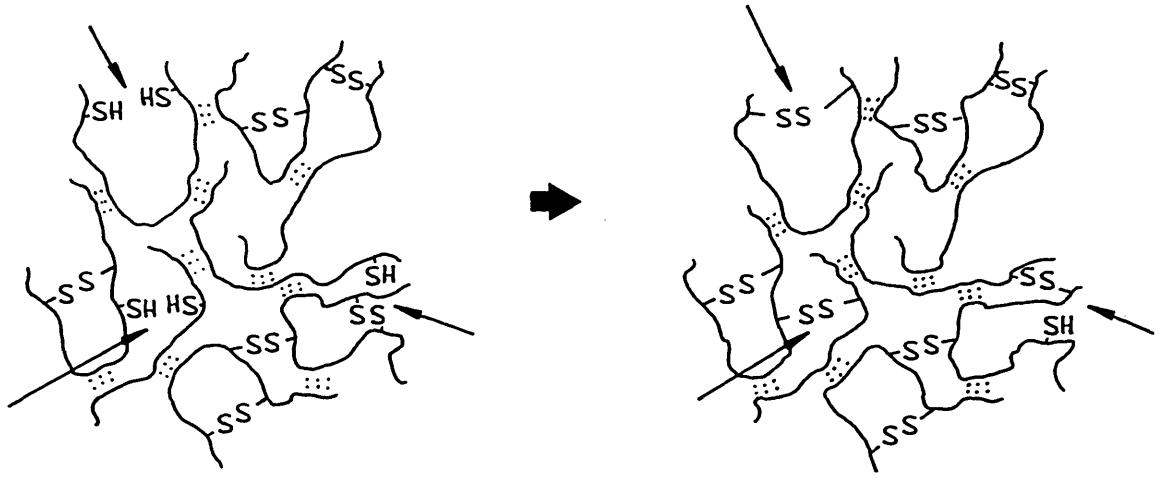
Many factors are likely to be of importance in determining the suitability of a flour for batter production, however, the ability of the glutenin proteins to aggregate and so delay gluten development in the batter system appears central to "flour quality".

The changes in protein extractability and molecular size distribution observed during batter preparation from "good-" and "poor-quality" flours, may be explained by the theory of gluten protein interactions advanced below, and illustrated in Figure 40.

1. In "good quality" flours, initial wetting and mixing at low shear results in some covalent aggregation through disulphide bonds.

Figure 40: Proposed reactions taking place in a "good quality" flour during batter mixing

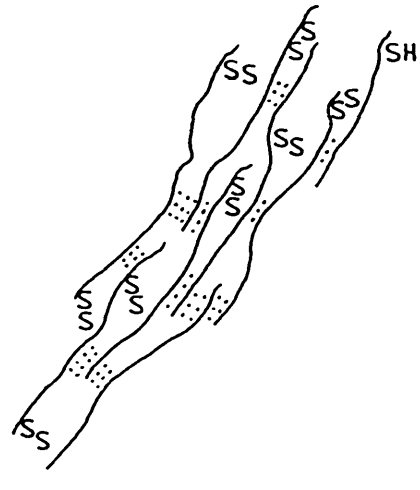
Initial wetting and mixing at low shear rates results in some covalent aggrega-tion through disulphide (SS) bond formation and and SS/SH interchange (⇀)



On application of higher shear rates (blending), protein disaggregation occurs through cleavage of SS and non-covalent (:::) bonds.

Glutenin polymers are re-aligned under the action of the shear forces. Non-covalent and covalent bonds reform resulting in gluten development.

Cleavage of SS bonds may also depend on the action of low molecular weight thiols compounds. Their involvement is suggested by the fact that BrO_3^- delays gluten development



2. On the application of higher rates of shear at the blending stage, protein disaggregation occurs through cleavage of both non-covalent and covalent (S-S) bonds.

3. This cleavage, presumably, allows alignment of the glutenin polymers and reformation of non-covalent interactions, such that gluten development takes place. Thus, for "good" flours, gluten development will be dependent to some extent on disaggregation involving cleavage of disulphide bonds, through the action of the shearing forces and probably through low molecular weight SH compounds also. The requirement for these reactions, presumably, introduces a delay in the occurrence of gluten development in a batter.

4. In "poor" flours aggregation through S-S bonds does not take place, and there may even be some disaggregation. Thus, in "poor-quality" flours the glutenin polymers may be more mobile allowing realignment to occur readily, even on initial wetting and low shear mixing.

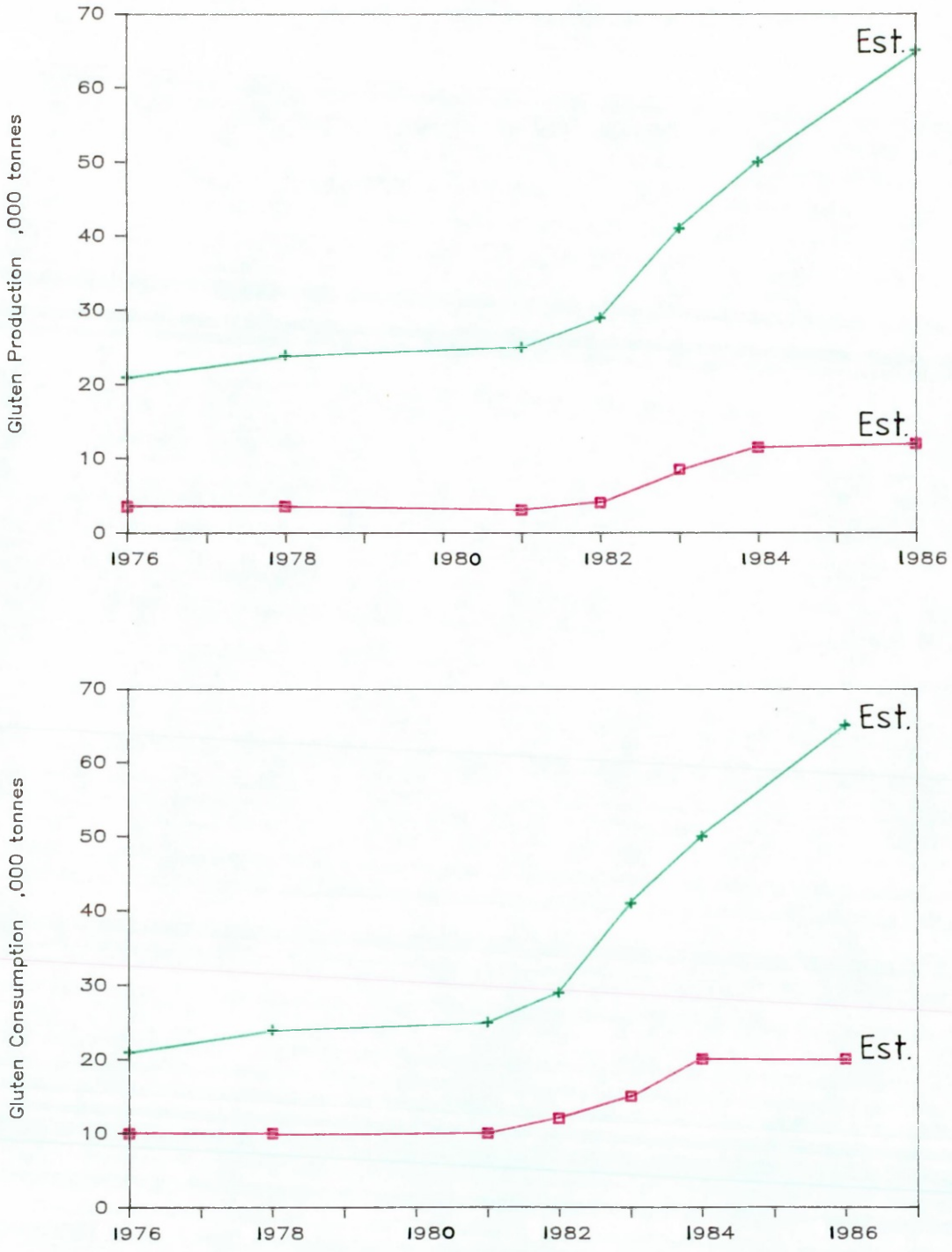
5. The involvement of low molecular weight thiol peptides is suggested by the fact that BrO_3^- prolongs development times, (BrO_3^- is envisaged as oxidising low molecular weight SH compounds to S-S peptides [Mair & Grosch, 1979]) and cysteine shortens development times.

The level of free sulphydryl groups in a flour, and their distribution between high and low molecular weight protein fractions may also be crucial in determining batter making potential of a

flour. "Good quality" flours had a higher total level of free sulphydryl groups than "poor quality" flours, and a greater proportion of the SH groups were found in the unextractable high molecular weight glutenin fraction.

Thus, the results presented here, demonstrate the essential protein characteristics required in a flour for successful batter production, and also indicate the means by which these may be achieved in "poor quality" flours by addition of oxidants.

Figure 41: Annual production and consumption of wheat gluten in the EEC and UK since 1976



Chapter VIII: Effect of moisture uptake on some functional and biochemical properties of commercial wheat gluten

VIII.1 Introduction

Many commercial plants have been established throughout the world, for separation of gluten from wheat flour. Figure 41 shows the growth in gluten production since 1976, which has been necessary to satisfy the demand for gluten (and the co-products derived from starch) for use in a variety of traditional and novel applications. Recent estimates of the actual UK and EEC production of vital wheat gluten are 12,000 and 55,000 tonnes respectively.

The market for vital wheat gluten has grown dramatically over the last 5 years and projections indicate that this trend is likely to continue in Europe. Figures presented below indicate that almost three quarters of the gluten used in the EEC is in flour fortification for bakery and confectionery applications.

Uses of Gluten in the EEC and the UK (% total consumption)

Application	EEC	UK
Flour fortification for baked goods	60 - 70	70
Bread improvers and flour mixes	15	<5
Pet foods	10	20
Breakfast cereals	5	5
Meat/Pasta/Miscellaneous products	5	5

The Flour Milling and Baking Research Association (FMBRA) have described the various bakery uses of commercial dried wheat gluten (Bennett et al., 1963).

1. As a bread improver, mixed with flour at the rate of addition of 1% - 2% of gluten (flour weight basis),
2. For the production of gluten bread, at an addition rate of 5% - 7%,
3. For high protein bread, containing 20% by weight of added gluten,
4. For gluten rolls containing equal weights of flour and gluten.

In recent years, gluten has also been a frequent component of breads made from high extraction flours or those supplemented with fibre. In the U.K., sales of brown bread doubled between 1971-1981, and now represent some 21.5% of total bread sales, (1984/5). Incorporation of germ, bran or other sources of dietary fibre, may have a deleterious effect on loaf volume and crumb structure, and flours with protein contents higher than those used for white bread manufacture are usually required to "carry" the load of these "inert" materials. Protein contents can be raised by the inclusion of gluten in the recipe for high fibre bread. This sustains loaf volume and improves the crumb texture of the bread, which would otherwise be dense and of low resilience.

Recently, considerable interest has been shown in the use of gluten to partly or wholly replace the protein in bread flour previously derived from North American and other third country (i.e. non-EEC) hard wheats.

Canadian wheat of approximately 14.5% protein content is available at a price of £220 per tonne (March 1985), including import levies imposed by the EEC. This is equivalent to a price of ~£1517 per tonne of protein. In contrast, dried wheat gluten of 72% protein is available at £970 per tonne, equivalent to £1347 per tonne of protein. Thus, measurable savings may be made if it is functionally acceptable to substitute Canadian wheat by EEC wheat fortified with gluten.

Substitution of third country wheats increases demand for home-grown wheats, both for milling base flours to which gluten may be added, and as the raw material for gluten isolation.

In the bakery, gluten is widely used to increase the "tolerance" of a dough during processing. "Tolerance" is difficult to define or demonstrate, but, generally, tolerant flours/doughs will consistently produce high quality products despite minor variations in plant operating conditions, such as mixing time, energy input, delays in dough handling, particularly during dividing and moulding, proof time etc. Flours of higher protein content are usually less sensitive to changes in these variables, and gluten additions can be made by the baker who experiences problems with batches of flour.

Gluten also finds application in breakfast cereals, in breading and batter mixes, in pasta made from low quality durum wheat, in meat analogues, and in processed meats and pet foods, where it may be employed for its nutritional properties as a source of protein and for its binding, adhesive and heat gelling properties (Schofield & Booth, 1983).

Thus, it is clear, that vital wheat gluten has an important role to play in the food industry in the traditional area of baked goods, and in the convenience and fast food markets, and also in pet food manufacture.

Most types of bread wheat (T. aestivum) can be used as raw material for the commercial separation of gluten. Isolation processes exist, using either wheat or flour as starting material, but most operations use flour for preference. The techniques employed commercially, are all basically similar wet separation systems; flour and water are mixed in the required proportions to form either a dough or a batter. The flour constituents are allowed to hydrate and are then separated by further washing and screening or centrifugation.

The quality of dried gluten can only be defined in terms of its performance in a particular application, e.g. its performance in some form of breadmaking assessment. There is no universally acceptable baking test, but, generally, the methods are based upon addition of gluten to a standard flour followed by the breadmaking procedure relevant to the interests of the investigators. However, the laborious and time-consuming nature of these procedures has prompted the search for a simple, rapid method of assessment which gives a reliable indication of the gluten baking quality.

Booth & Timms (1978) used a micro-mixing technique and measured the ratio of the time to gluten development (i.e. maximum dough viscosity in a mixer producing a recording of mixer torque) at two levels of water addition. Good correlations were obtained with the

baking performance of a synthetic flour that contained the test gluten sample as the sole source of protein, reconstituted with starch and water soluble material prepared from a standard CWRS flour.

Stenvert et al. (1981) measured differences in peak Farinograph development times at two levels of water addition to a starch/gluten blend containing 15.5% protein (dry basis). The Farinograph method of quality assessment correlated well with the baking behaviour of a base flour fortified with the test glutens (final protein level of 16.5%, dry solids basis), particularly with slow speed mixing, where the maximum differentiation of gluten quality was observed. Differences in baking quality, although less marked were still evident with medium intensity mixing, but were minimal under intensive mixing.

Objective assessment of gluten physical properties may also be made using conventional dough testing apparatus. Measurements with an extensometer (Kaminsky & Halton, 1964; Kretovich & Vakar, 1964), Extensograph (Tkachuk & Hlynka, 1968) or the Farinograph (Bushuk, 1963; Doguchi & Hlynka, 1967) have provided considerable information about the visco-elastic properties of hydrated masses of gluten, or gluten-fortified flours (Aitken & Geddes, 1938, 1939).

Some general criteria for assessing gluten quality have been proposed (Chamberlain, 1982; McDermott, 1984). The range of values encountered in such parameters measuring "quality" are shown in Table 11.

Table 11: Typical Ranges of some Criteria used to Estimate Commercial Gluten Quality

Moisture Content	4 - 10 %
Protein Content	75 - 85 % d.b.
Water Absorption	150 - 200 %
Rehydration Time	5 - 600 s
Ash Content	0.6 - 1.2 %
Free Fat	0.5 - 1.5 %
Acetic acid solubility	80 % minimum
Colour	Creamy
Particle size	<20 % below 100μ
Lactic acid sedimentation volume	<30 % reduction in turbidity in 3 min.

Since, however, the biggest single outlet for gluten is in baking, the ultimate test of gluten quality for this application is some form of breadmaking assessment. Early work by Aitken and Geddes (1938) reported the separation of gluten from a range of experimentally-milled flours, and fortification of the parent flour to a protein level of 13.7% with the dried gluten. The fortified flours were compared with a control prepared from No. 1. Manitoba wheat of the same protein content. The loaf volumes of all the fortified flours improved substantially, and the range of volumes was smaller than that of the unfortified flours. However, none of the gluten-enriched breads equalled the control in volume, and the added gluten had a deleterious effect on the colour of the bread crumb.

The same authors (Aitken & Geddes, 1939) and Harris (1940) demonstrated that the improving effect of dried gluten was proportional to the amount used. However, Aitken & Anderson (1943) found that the protein response on addition of dried gluten to low protein base flour, was only half that of native protein, for a range of graded Canadian wheats.

Later studies by Finney (1943) and Pence and coworkers (1951, 1959) were largely concerned with experimental reconstitution of flour components, as a means of evaluating their relative importance. A similar baking test was developed by Booth & Melvin (1979). However, instead of reconstituting all the components of the flour, they combined starch and water soluble material isolated from a standard flour with the gluten under examination. Baking then followed a traditional 3h bulk fermentation process (reconstitution to a "flour" protein content of 14%, at 14% moisture), and the bread produced was assessed for volume and texture. These techniques are very sensitive to quality differences in the test glutens, which provide the only effective protein, unlike most fortification procedures, which will also contain native, unfractionated flour protein.

Whether or not it is relevant to demonstrate differences between glutens that are manifested only at levels greatly in excess of the intended application, may be questioned. However, if the intention is to reveal potentially important differences between glutens then relatively high levels must be used. Glutens that had obvious differences in other characteristics, only revealed differences in their bulk-fermentation or CBP baking performance at the 5% level of

addition to flour (Bennett et al., 1963).

Recent work carried out at the FMBRA (Chamberlain, 1982) examined the comparative effectiveness of enhancing English flour of 10.1% protein content with Canadian flour or dried commercial gluten, increasing final protein level by 0.5%, 1.0% and 2.0%. The results showed that gluten additions at higher levels improved bread quality in all baking procedures examined, and that the loaf volume response to gluten protein was half that of Canadian flour protein in the CBP. The work also suggested that storage of the flour-gluten blends for 7 days at 21°C caused the beneficial effect of gluten on CBP loaf volume to be lost. Inability to duplicate this result prompted a second study of the effect of flour-gluten equilibration on the performance of a fortified flour. On this occasion, no loss of volume improvement due to storage of flour-gluten blends was observed compared with blends prepared at dough mixing.

Flour fortification with gluten frequently takes place at the flour mill. The length of time that elapses between gluten addition to a flour and its use in the bakery may vary considerably, but will usually exceed 7 days, given the necessity for movement of the flour from the mill to the bakery, and the probable rotation of flour stocks at both sites. If storage of fortified flours for periods of more than 7 days eliminates the beneficial effect of gluten, as suggested by the initial FMBRA investigation, then the practice of fortification at the mill should be replaced by gluten addition at the mixer in the bakery.

Recently, Wootton et al. (1982) showed that gluten hydration times

(i.e. the time taken for a specified amount of gluten to form a cohesive mass on mixing with excess water) increased when the glutens were stored at high relative humidites (RH), and that the deterioration in gluten rehydration properties was more rapid as the RH of storage increased. The rapid rise in rehydration time occurred when the RH of storage was sufficient to allow gluten moisture content to exceed 12%. These workers assumed that a rapid rate of hydration was a prerequisite for good baking performance. It would, indeed, seem logical to assume that in bakery applications, the rate and extent of hydration is crucial, in order that the gluten may be "instantly" functional on wetting and able to interact with flour proteins during mixing. Incomplete gluten rehydration during mixing could result in unsatisfactory performance of a dough during subsequent operations, such as dough sheeting or moulding.

Wootton and coworkers (1982) showed that glutenin protein was broken down under excessively moist conditions, and suggested that this would reduce the strength and stability of the gluten in dough, and so diminish gluten functionality. On the basis of these data, Wootton suggested that gluten should be stored at RH levels below 70% in order to maintain gluten moisture content below 10%.

The moisture content of commercial wheat flour is approximately 14%, and that of gluten about 8%. Thus, moisture transfer from flour to gluten may occur in mixtures of these two materials, such as in gluten-fortified flours. According to Wootton et al. (1982) this moisture uptake would lead to increased gluten rehydration times and loss of gluten functionality.

The loss of gluten functionality, reported by the FMBRA, on storage of fortified flours for periods of 7 days or longer, may therefore, be the result of moisture transfer from flour to gluten.

The following investigation was, therefore, undertaken, to determine the effect of moisture uptake by a commercial gluten on its rehydration characteristics and baking performance, and the relationship between these functional properties and the protein aggregation states.

VIII.2 Experimental, results and discussion

VIII.2.1 Moisture sorption isotherms of commercial wheat gluten and white bread flour

Moisture sorption isotherms of a commercial wheat gluten obtained from Tenstar Products Ltd., Ashford, Kent, and a commercial white bread flour were determined, in order to establish whether or not moisture transfer would take place in mixtures of these two materials.

Isotherms were determined by placing the samples in open petri-dishes (40 mm x 10 mm) and drawing a knife edge across the surface of the dish to remove excess flour or gluten and to produce layers of uniform thickness. Samples were then stored for 1-3 weeks in air-tight containers, each held at a different relative humidity using saturated salt solutions as detailed in Table 12. Storage temperature was that of the laboratory and varied between 20 - 25°C.

Figure 42: Water sorption isotherms of a commercial bread flour and sample of vital wheat gluten

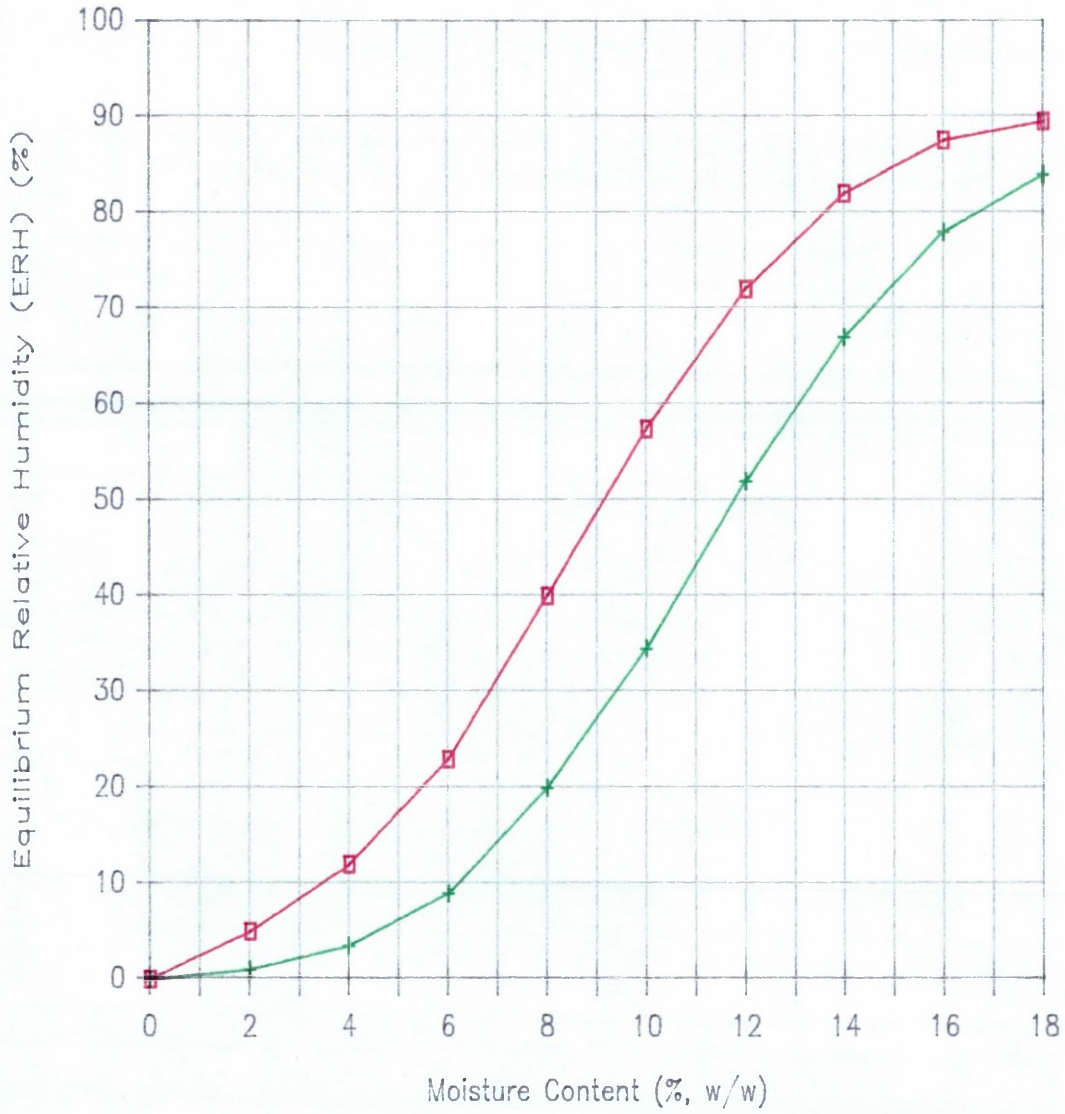


Table 12: Preparation of Controlled Humidity Cabinets Using Saturated Solutions of Salts

Salt	ERH (%)	Vapour Pressure (mm Hg)
Lithium chloride	11.1	2.31
Potassium chloride	22.9	4.76
Magnesium chloride	32.9	6.83
Potassium carbonate	43.9	9.12
Sodium dichromate	54.1	11.24
Sodium nitrate	64.8	13.47
Sodium chloride	75.5	15.69
Potassium chromate	86.5	17.96

ERH = Equilibrium relative humidity

At the end of the storage period, the moisture content of the conditioned samples was determined by drying at 105 $\pm$ 1° C to constant weight. Percent Equilibrium Relative Humidity (ERH) was determined using a Nova-Sina water activity meter : model en ZFB-4TEL. All moisture and ERH determinations were performed by Mr. C.D. James, Food Technology Dept.

The data presented in Figure 42, shows that the white bread flour had an ERH of 65% at its normal moisture content of 13.7%, whereas gluten had an ERH of 36% at its normal moisture content of between 7-8%. Thus, moisture would be transferred from flour to gluten in blends of the two materials. From Figure 42, the anticipated final

moisture of gluten, in blends where its addition rate was low (e.g. 1-2%) would be approximately 11%. Wootton et al. (1982) proposed that an increase in gluten moisture content to levels >10% would decrease the rate of gluten rehydration, and, as a result, decrease gluten functionality in breadmaking. Thus, in gluten-fortified flours, moisture transfer from flour to gluten, may be a cause of lost gluten functionality.

VIII.2.2 Moisture transfer in flour-gluten mixtures

In order to determine whether or not moisture transfer actually takes place in flour-gluten mixtures, initial ERH values were measured for the sample of commercial wheat gluten and the sample of commercial white bread flour.

The gluten sample was then passed through an "Endecott" 180μm mesh sieve to prepare a fraction having a particle size >180μm. The sample of white flour was then passed through an "Endecott" 125μm mesh sieve to prepare a fraction having a particle size <125μm.

The ERH of the fractions obtained by sieving, were determined. Equal weights of the flour and gluten fractions, were simultaneously "poured" onto a 125μm mesh "Endecott" sieve. The materials were then thoroughly stirred using a small brush, which also brushed the flour through the sieve mesh, and so effected a re-separation of the gluten and flour fractions. The final ERH values of the blended and separated fractions were determined. The time taken to separate the blended samples was 15s. The experiment was repeated, allowing a

longer period of contact between the two samples to elapse. Total time for mixing and sieving on this occasion was 90s. The recovery of material that passed through the sieve, and that was retained on the sieve was determined as a proportion of the initial weight of each fraction used.

The gluten and flour fractions were individually "poured" onto the sieve and brushed on/through the mesh for a period of 15s. The initial and final ERH values were determined for each fraction. All experiments were performed in a controlled environment room: 40% Relative Humidity, 21°C. Work is presented here courtsey of Mr. C.D. James, Food Technology Dept.

The results presented in Table 13 demonstrate that when a coarse fraction of gluten (>180μm) was mixed with a fine fraction of flour (<125μm), moisture transfer from the flour to the gluten took place rapidly, with equilibration probably being achieved within a few minutes. The physical treatment of brushing the sample over the mesh of the 125μm sieve, was not responsible for changes in the ERH of the two fractions.

Recovery determinations of the fractions after blending and sieving revealed that approximately 25% of the gluten passed through the sieve along with the flour. The action of brushing, obviously, disrupted some of the gluten particles, reducing their size. The transfer of gluten theough the sieve could account for some (~50%) of the observed fall in the flour ERH, but could not account for the rise in the ERH of the gluten retained on the sieve.

Table 13: Transfer of Moisture between Sieved Fractions of Commercial Flour and Commercial Wheat Gluten

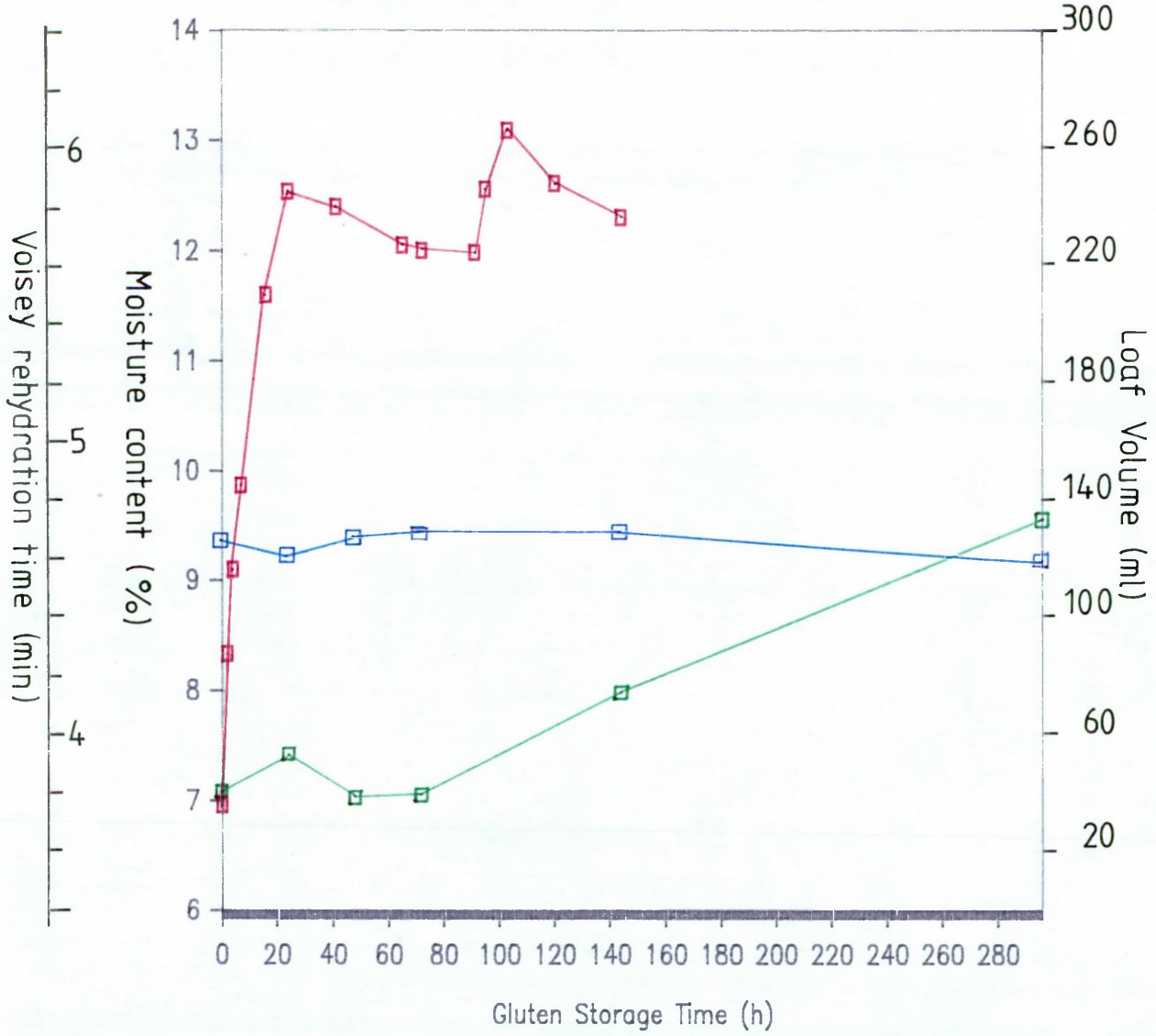
	<----------%ERH---------->			
	Flour	125μm Fraction	Gluten	180μm Fraction
Starting Material	55.0		32.0	
Sieved Fraction		53.0		33.0
...after 15s blending		45.1		40.6
...after 90s blending		45.1		42.4
Sieved, unblended fractions		55.0		33.0

The experiment described here was carried out in an environment of 40% ERH, 21°C. Thus, the samples would have tended to equilibrate not only with each other during mixing, but also with the surrounding atmosphere. However, the final ERH of the 180μm gluten fraction after 90s contact with flour was 42.4% i.e. an ERH higher than that of the environment, and it has been demonstrated in this laboratory, that flour exposed to ambient air takes over 5 min. to dry to 45% ERH. Therefore, it must be concluded that moisture transfer between flour and gluten takes place rapidly in initimate blends of the two materials.

VIII.2.3 Effect of moisture uptake by commercial wheat gluten on rehydration rate and baking performance

In order to determine whether or not moisture uptake by a commercial

Figure 43: Effect of moisture uptake on rehydration time (Voisey) and reconstitution baking performance of a commercial wheat gluten



□ = Gluten moisture content (%)

□ = Gluten rehydration time (min)

□ = Loaf volume (ml). Test gluten samples were reconstituted with starch and water -soluble material from a standard CWRS flour. Values in parentheses are loaf crumb texture scores.

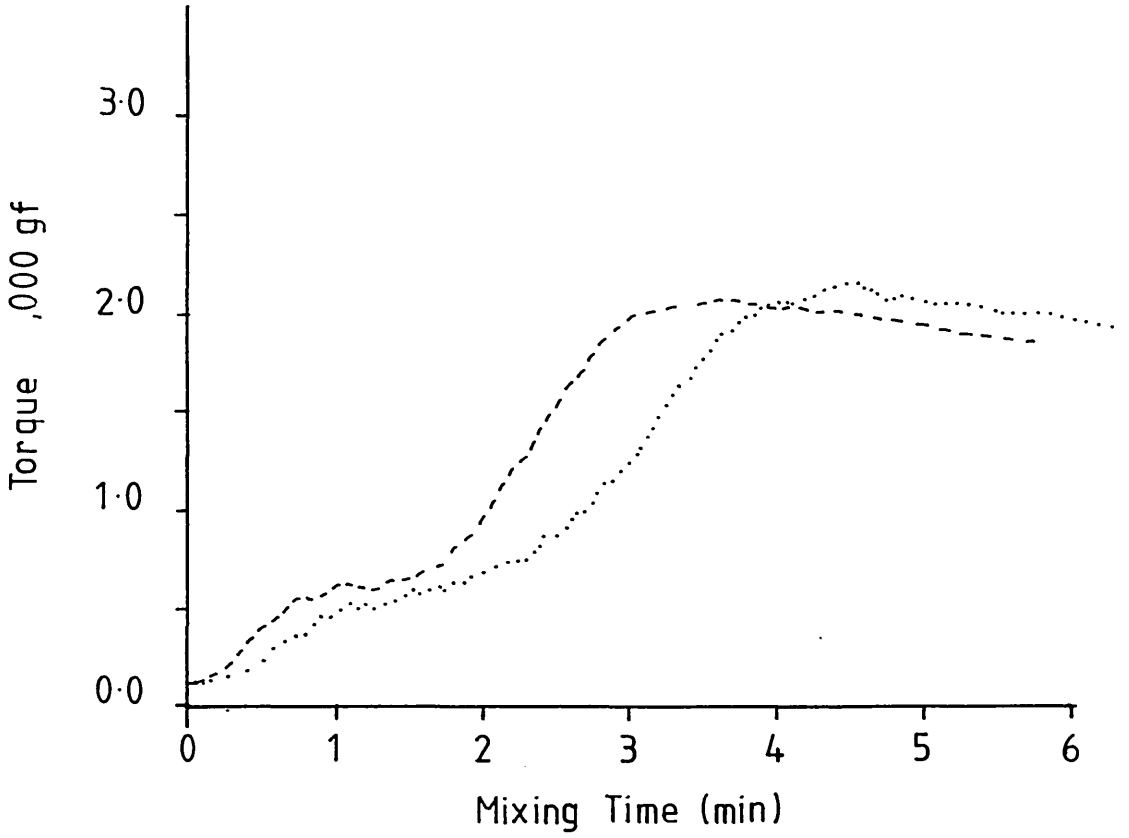
wheat gluten affects the gluten rehydration and development time and/or baking performance, gluten was placed in open petri-dishes, and a knife-edge was drawn across the surface to remove excess gluten and produce layers of uniform thickness (8mm). The gluten was stored at 75% RH/25°C for periods of up to 296h. Samples were withdrawn at intervals and their moisture contents determined by heating at 105 C ± 1°C to constant weight. The gluten rehydration and development times of the samples were determined by mixing in a Voisey micro-pin mixer (Voisey et al., 1966) by the method described in Appendix I.15.

The gluten samples were also assessed for baking performance, by reconstitution with starch and water-soluble material derived from CWRS flour, and baking according to the 3h bulk fermentation procedure described in Appendix I.7. The protein content of the reconstituted flours was 14% at 14% moisture content. Gluten, starch and water-soluble material were blended immediately prior to breadmaking and water addition was adjusted to make allowance for variations in the gluten moisture content.

Rate of Moisture Uptake

The time course for moisture uptake by gluten stored at 75% RH/25°C is shown in Figure 43. Under the conditions described, equilibrium moisture content of 12.5% was achieved in approximately 24h for an 8mm thick layer of gluten powder. However, it has been shown previously that moisture transfer between intimate mixtures of flour and gluten (as in a gluten-fortified flour) occurs very rapidly and that equilibrium is achieved within a few minutes.

Figure 44: Effect of moisture uptake on rehydration and development time of a commercial wheat gluten.



----- = Control gluten
..... = Gluten stored at 75% RH/25°C for 296 h. Dry solids content equal to control.

Effect of Moisture Content on Rehydration and Development Time

The Voisey micro-pin mixer (i.e. a small-scale planetary action mixer) records the torque transmitted by the rehydrating gluten mass in the mixing bowl from the rotating pins to the static pins. The time taken to achieve maximum torque value, therefore, gives an objective determination of the rate of gluten development. Typical traces for the control gluten (i.e. zero hours storage) and the same gluten stored for 296h at 75% RH/25°C are shown in Figure 44.

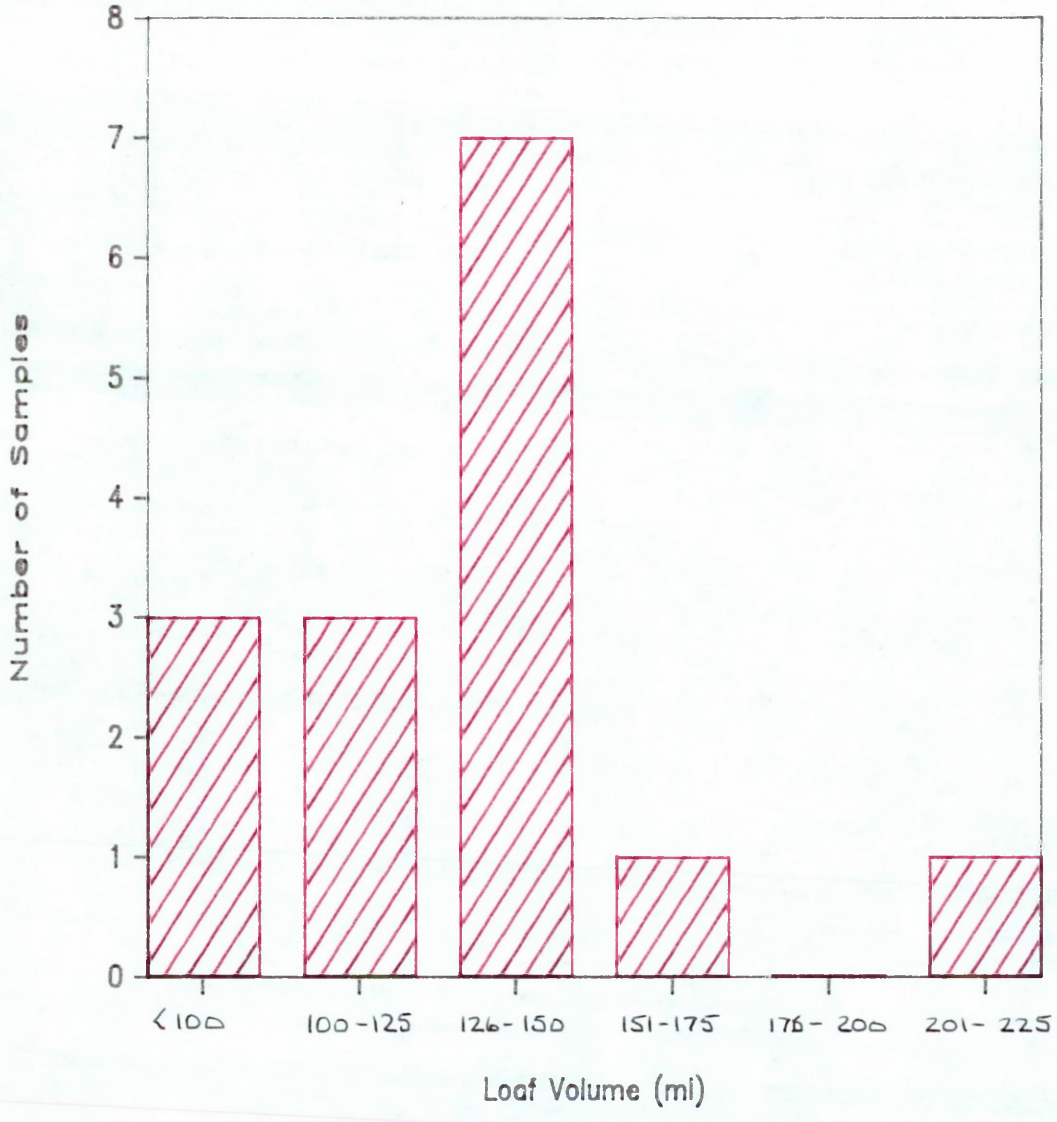
The gluten rehydration and development test involves mixing a constant weight of gluten dry solids with sufficient water to produce a final wet gluten of 65% moisture content. The dependence of the micropin development time on storage time at 75% RH/25°C is shown in Figure 43. It can be seen that whereas moisture transfer to the gluten occurred within 24h, changes in the development time were not apparent until at least 72h of storage. There was then a gradual increase in development time on storage up to 296h, which was the maximum period examined.

Effect of Gluten Moisture Content on Baking Quality

The results presented in Figure 43, show that baking performance of the commercial glutens was essentially unchanged over the whole period (up to 296h) of storage at 75% RH/25°C.

Reconstituted loaf volumes of the test glutens were in the range 119-129 ml. Loaf volumes of less than 100ml are sometimes observed in commercial glutens, when assessed by this baking procedure.

Figure 45: Comparison of gluten baking qualities (loaf volumes) for several commercially prepared samples. (Reproduced from Booth et al, 1980)



Glutens were compared in a bulk fermentation baking test using flours reconstituted at 14% protein content. (14% moisture basis)

Figure 45, illustrates the differences in functionality between a series of commercial gluten samples (Schofield & Booth, 1983). It is clear that although all may be termed "vital", quality varies widely when measured as breadmaking potential. There was, therefore, potential to observe a decrease in baking performance due to moisture uptake, even from the relatively low level seen in this experiment.

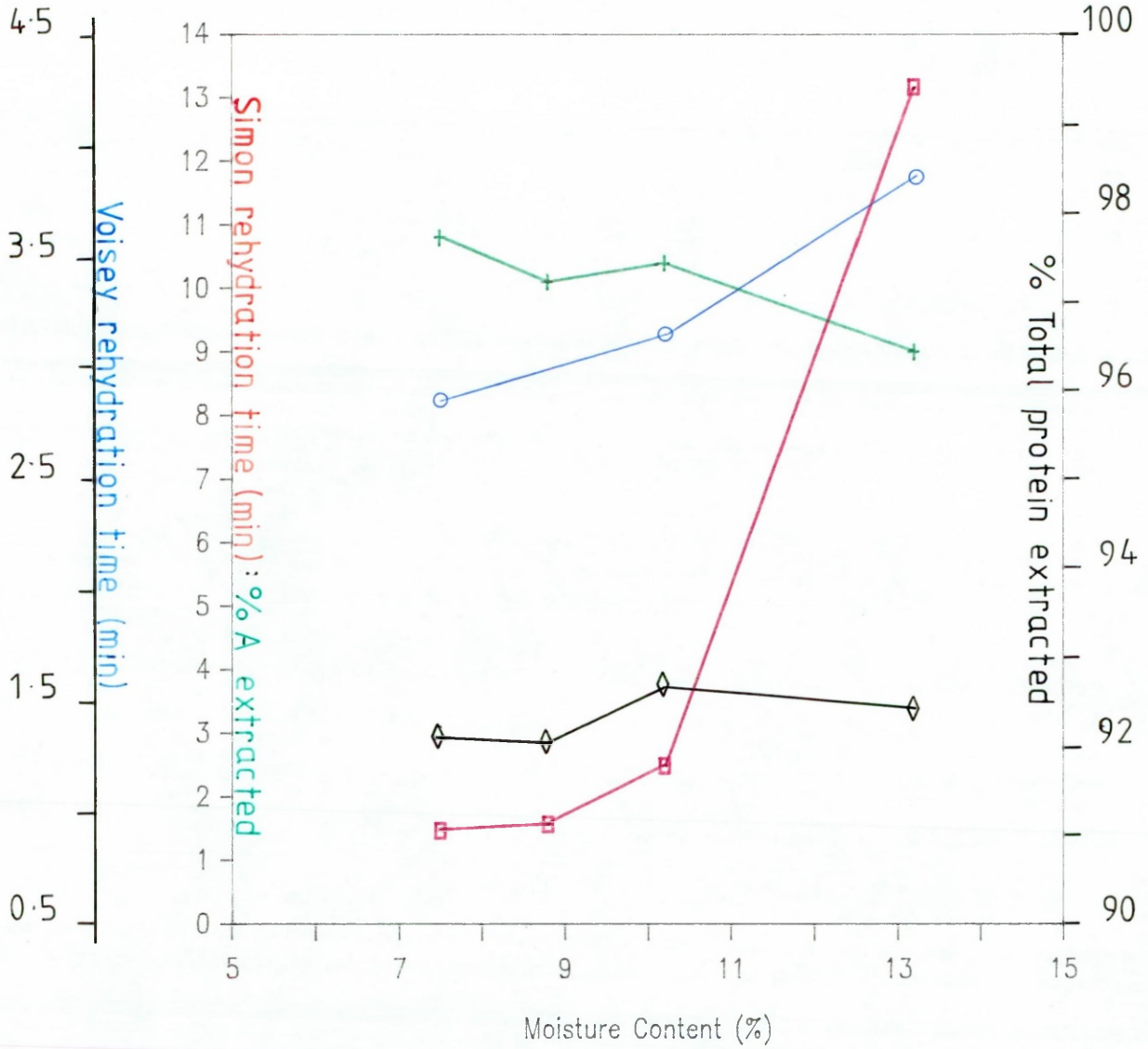
Thus, although storage at 75% RH resulted in moisture uptake and gave rise to increases in gluten rehydration and development times, the baking performance as measured by a sensitive small scale bulk fermentation baking test was not affected.

VIII.2.4 Effect of moisture uptake by commercial wheat gluten on rehydration rate and protein aggregation states

It has been shown that gluten storage at high RH (75% RH/25°C) increases rehydration and development time but does not affect gluten baking performance. The effect of storage at RH levels between 47% - 75% on gluten rehydration rate and protein aggregation states was determined.

Samples of commercial gluten were stored in air-tight containers, held at 47%, 65% and 75% RH using saturated salt solutions as previously. At the end of the storage period (3 weeks), moisture content of the conditioned samples was determined by drying at 105°C ± 1°C to constant weight. Gluten rehydration and development times were determined using the Voisey micropin mixer (Appendix I.15) and a Simon Minorpin mixer (Appendix I.16).

Figure 46: Effect of increased gluten moisture content on rehydration and development times and glutenin molecular size distributions.



○ = Voisey micropin mixer rehydration time (min)
□ = Simon minorpin mixer rehydration time (min)
◊ = Proportion of gluten protein extractable in SDS-containing buffers (% of total)
\+ = Proportion of high molecular weight glutenin in SDS-extracts of gluten (%)

Gluten samples were also assessed for changes in their protein extractability into SDS-solutions (Appendix I.2) and changes in the molecular weight distribution of SDS-extractable proteins (Appendix I.3) that occurred as a result of moisture uptake.

It has been shown (Figure 43) that the period of storage at 75% RH affected gluten rehydration time. The present glutens were all humidified for fixed periods of 3 weeks, therefore, differences in the rehydration rate and/or protein aggregation states resulted from the amount of moisture taken up by the gluten and not differences in the length of the storage period.

Results presented in Figure 46 show that rehydration time increased with the moisture content of the gluten sample, and that the relationship was essentially linear when rehydration time was determined in the Voisey micropin mixer. In contrast, changes in the gluten rehydration time were small at gluten moistures below 10%, when measured in a Simon Minorpin mixer. At gluten moisture contents >10%, rehydration time increased markedly.

The somewhat different relationship between gluten moisture content and rehydration rate when measured by the two mixers may be due to the more intensive mixing action of the Voisey micropin. However, the results agree with those of Wootton *et al.* (1982) who correlated a high gluten moisture content (>10%) with a decreased rate of rehydration.

Moisture uptake by gluten samples did not affect protein extractability into SDS; however, there was a small decrease in the

amount of high molecular weight glutenin in the SDS-extractable proteins, (Figure 46), i.e. a decrease in the proportion of extracted proteins that were eluted at the void volume of a Sepharose CL-4B column. This may indicate depolymerisation of glutenin, that was dependent on high gluten moisture content. However, the observed changes were small and probably not significant.

VIII.3 Conclusions

In intimate mixtures of two fine, particulate materials of different moisture content, such as are found in gluten-fortified flours, moisture transfer will take place between them. The rate of moisture uptake is rapid, and an equilibrium situation may be attained within a few minutes of blending. Subsequent to moisture uptake by gluten, but not necessarily the result of increased moisture content *per se*, certain gluten properties may be modified.

Data presented here indicates that gluten rehydration and development time increases following moisture uptake. The delayed nature of the response suggests that reactions may be taking place in the gluten that are facilitated by the higher moisture content. Analysis of the gluten protein components revealed a small decrease in the proportion of high molecular weight glutenin in SDS-extracts, although there was negligible change in total protein extractability. These data may be indicative of glutenin depolymerisation at high gluten moisture content.

These findings confirm those of others (Wootton *et al.*, 1982) who

demonstrated that gluten "quality", as measured by rehydration rate, deteriorated when samples were held at >10% moisture content. Wootton also found that the proportion of high molecular weight gluten protein (defined by extraction with AUC and chromatography on Sephadex G-150) was reduced under excessively moist conditions.

The mechanism by which hydration quality is lost is likely to be complex, and may involve such factors as microbial activity, activity of endogenous proteases, physical rearrangement of protein conformation and chemical changes, e.g. Maillard reactions.

However, the ultimate test of gluten suitability for use in bakery applications, is assessment of functionality in some form of baking test. The results presented here demonstrate that increased gluten moisture and subsequent increases in rehydration and development time do not affect the gluten baking performance as measured in a sensitive reconstitution baking test. Investigations at the FMBRA (Chamberlain, 1982) suggested that storage of flour-gluten blends for 7 days at 21°C caused the beneficial effects of gluten on CBP loaf volumes to be lost. However, these findings were subsequently amended, when a second study failed to show a loss of gluten functionality due to storage.

Therefore, the most recent results from the FMBRA and the findings of the present study suggest that the commercial practice of dry blending gluten with flour and storage prior to baking, will not reduce gluten functionality nor will it decrease the beneficial effect of gluten addition to bakery flours.

Chapter IX: Summary and Conclusions

It is generally recognised that the proteins of flour are primarily responsible for the unique cohesive-elastic properties that enable wheat to be used in the manufacture of a wide variety of foodstuffs. The protein fraction of greatest technological significance is that known as gluten, i.e. the proteinaceous material produced when a flour/water dough is washed with excess water to remove starch and other minor components. The major components of gluten are the glutenins and the gliadins. In the present work, gliadins have been taken to be those monomolecular proteins that comprise the bulk of the protein extractable in aqueous alcohol solutions, whereas glutenins are regarded as those protein complexes, linked by disulphide bonds, that are largely, but not wholly unextractable in aqueous alcohol, and extractable in dilute acid or alkali.

Many studies have sought to define the molecular basis of wheat "quality" for use in a given application, and correlations have been drawn between various wheat parameters and functionality. However, a complete definition of the gluten components in precise chemical terms is, so far, lacking and, therefore, our understanding of the nature of the interactions that take place during processing is similarly incomplete.

Investigations of the relationship between particular protein characteristics and processing performance have been considerably confused by the use of techniques that give incomplete extraction of the storage proteins and result in cross-contamination of the different fractions. For satisfactory investigations of the

chemical properties of proteins it is important that an efficient and convenient solvent system is chosen.

The author has demonstrated that buffered SDS solutions extract maximum amounts of protein from cereal flours, wheat doughs, batters and pasta etc., and that these solvents lend themselves readily to subsequent electrophoretic and chromatographic studies on the extracted proteins.

The reasons why SDS-containing buffers have been less-favoured than AUC as extractant and chromatography solvents for wheat proteins are not clear. The SDS-Tris buffer used here extracted substantially more protein from flour than either AUC or a related urea-CTAB solution buffered to pH 5.5 with sodium citrate. The high solvent efficiency of SDS may be due to the detergent's ability to penetrate the complex flour system and dissociate and extract proteins that are organised into macromolecules through disulphide bonds.

Hydrophobic interactions are thought to play an important part in the stabilisation of native conformation and the aggregation and binding properties of proteins generally, and wheat flour proteins are no exception. An hypothesis for glutenin structure has been put forward, in which all the disulphide bonds are present as internal links within the protein. In this model, the intramolecular disulphide bonds stabilise the three dimensional structure of the proteins in a form that enables aggregation to take place. The aggregates are then stabilised solely by hydrophobic interactions. It has been proposed that proteins of flours with good breadmaking properties are more hydrophobic than proteins of flours with poor

functionality. It might be expected, therefore, that higher levels of SDS would be required to saturate all the possible sites for interaction between gluten proteins from a good quality flour. The present study has shown, however, that flours of different breadmaking potential cannot be differentiated on the basis of their hydrophobicity, as measured by protein extractability into solutions containing different concentrations of SDS.

The desirable qualities of good bread are largely associated with the quantity and quality of the gluten proteins. The protein content of wheat is influenced mainly by environmental factors, particularly the N nutrition of the plant. Other workers have shown that S nutrition, especially when considered in relation to the N supply is also important in determining breadmaking properties. For grain produced under broadly similar nutritional conditions with a correct N:S balance, the author has demonstrated that protein content is a major factor accounting for variation in loaf volume and that the relationship is essentially linear.

The regression of loaf volume on protein content is different for different wheat varieties. These loaf volume:protein content regression lines for variety represent differences in protein quality. In the present study, insufficient data was available to determine the individual regression lines for different varieties.

Gliadins and glutenins differ in their physical properties, but most notably in their viscoelasticity: gliadin is cohesive, but with low elasticity, whereas glutenin is both cohesive and elastic. Thus, it might be expected that differences in the relative amounts of these

components in flours would affect functionality. Furthermore, the model advanced by Ewart to explain glutenin structure (the "linear glutenin hypothesis") predicts that dough strength, and hence baking performance, is related positively to the lengths of the disulphide-linked polymers in flours. Extraction and gel-filtration chromatography of wheat proteins has shown that approximately 30% of the variation in flour loaf specific volume can be accounted for by variations in the amount of high molecular weight glutenin in SDS-extracts of flours. This high molecular weight material may correspond to the unextracted protein residue, reported by other workers, that remains after extraction of flours with less efficient solvents. Thus, these data generally agree with the previous findings and provide some evidence in support of the Ewart theory.

In recent years correlations have been identified between the presence of particular polypeptides and indices of breadmaking quality. The high molecular weight glutenins appear to be of particular interest in this respect, and have been ranked in order of their influence on baking quality. Further work might, therefore, be undertaken to determine whether or not certain polypeptides/combinations of polypeptides are present in the high molecular weight complexes of glutenin of good breadmaking flours that are absent from poor quality flours. Thus, the observation that flours containing similar amounts of aggregated glutenin may produce loaves that differ markedly in loaf specific volume and texture, may in part, be explained.

Cereals differ markedly in their suitability for baking, and bread

made from rye, barley and oats is inferior in quality to that derived from wheat. The unique position of wheat among the cereals is due to the ability of wheat flour dough to retain carbon dioxide produced during yeast fermentation. A fine vesicular structure with expanding gas cells is built up and maintained during fermentation and proving until the texture is fixed in bread by protein denaturation and starch gelatinisation in the oven. There is still no satisfactory explanation why wheat-flour doughs retain their gas better than do the doughs from other cereals. However, this variation in processing capability has stimulated many workers to examine similarities and differences between the cereals. In this investigation, quantitative differences were demonstrated, both in the total protein content and also in the amounts of different solubility fractions present in four of the cereals.

A comparison of the aggregation states of the SDS-extractable proteins of wheat, barley, rye and oats also revealed differences with respect to the relative proportions of the different molecular size fractions. Rye and oats, in particular, had higher levels of the low molecular weight protein components than did wheat. However, it was observed that the endosperm proteins of barley, rye and oats occurred in polymeric form like those of wheat, in which subunits are linked by disulphide bonds, and in the case of barley, for example, the molecular size profile of SDS-extractable proteins was quite similar to that of wheat. Thus, as previously concluded, the presence of high molecular weight protein in cereal flours cannot be the only factor that confers breadmaking quality on wheat.

In breadmaking, mixing is generally considered a critical step that

influences the over-all bread quality. Dough mixing in the Chorleywood Bread Process, resulted in depolymerisation of glutenin proteins in better quality breadmaking flours, such that a greater percentage of the total protein was extractable in SDS, and there was an increase in the proportion of low molecular weight glutenins in SDS extracts, as measured by gel-filtration chromatography. The greatest changes in molecular size distribution occurred during the initial stages of mixing, with only small decreases in the amount of aggregated glutenin being observed on further mixing, even to work levels considerably in excess of those observed in commercial practice. A weak flour did not show any significant change in the molecular size distribution of the gluten proteins on CBP mixing, which may be an important factor relating to its poor breadmaking ability. These findings contradict the results of other workers, who have reported the greatest increases in protein extractability on mixing weak flours. However, most of these reported findings relate to low energy mixing conditions and not to an energy intensive process such as the CBP.

Graveland and coworkers have described a high molecular weight, SDS-insoluble gel protein that is rendered extractable by dough mixing. Graveland demonstrated that this gel protein probably comprised a backbone of HMW glutenin polypeptides linked head-to-tail via disulphide bonds to which lower molecular weight glutenin polypeptides were also linked through disulphide bonds. On resting the dough, in conditions analogous to those used in fermentation and proving, the gel protein reappeared, through repolymerisation of glutenins. The techniques employed here (gel-filtration chromatography and 2D-electrophoresis of the acid soluble proteins)

were unable to detect any movement of glutenin polypeptides between different molecular size classes, as a result of dough mixing to different work levels. Future investigations might also examine changes in the polypeptide composition of glutenin aggregates of different molecular size at various stages during the baking process, i.e. flour, dough ex-mixer, dough post 1st proof and post 2nd proof.

The content and composition of wheat endosperm protein is of prime importance in determining flour suitability for commercial bread manufacture. However, wheat is used in the manufacture of a wide variety of foodstuffs, and is subjected to many different processing techniques. Considerable variation is observed in the processing conditions and recipes employed in the manufacture of these foods, and the requirements of the different wheat-utilising industries may also vary considerably. Conversely, it has been shown that dough mixing, for example, results in an increase in the solvent extractability of flour proteins as a result of protein disaggregation. It might be expected, however, that different processing conditions exert different effects on the wheat protein constituents.

During commercial spaghetti manufacture, water to give an absorption of about 30% is mixed with semolina and the dough, so formed, is extruded through a die to give the required shape of pasta. The product is then dried in a two-stage process that may take up to 36h depending on the type of drier and the product.

It was observed that protein extractability decreased during

spaghetti manufacture, the greatest changes occurring during the mixing, extrusion and pre-drying stages. A large amount of mechanical work is imparted to the pasta dough during mixing and extrusion which, it might be expected, would cause gluten breakdown with a resultant increase in protein extractability. Others have reported that mixing pasta doughs at higher moisture levels (i.e. similar to those used in bread doughs) leads to increased protein extractability in acid solution. Thus, it was concluded that the low moisture content of pasta dough limits gluten development during mixing.

This investigation has shown that the decrease in protein extractability during the early stages of pasta processing results from the aggregation of semolina proteins through sulphydryl-disulphide interchange reactions, and that the free SH groups in the low molecular weight glutenin fraction are particularly involved in these reactions. There is also evidence to suggest that polymerisation of proteins through SS bond formation and involving cross-linking reactions other than through disulphide bonds may take place throughout the different stages of pasta processing.

Whereas bread, cookie and pasta doughs have a relatively low water:flour ratio, i.e 20 - 60:100, batter systems are typically prepared from 150 parts water:100 parts flour, and the importance of the gluten component of the flour is said to be small. It has been demonstrated, however, that the ability of the glutenin components of flour to aggregate is central to flour quality for batter-making. It is proposed that covalent aggregation of glutenins, through disulphide bond formation, delays gluten development and allows the

production of a stable batter. Flours that do not aggregate in this way produce poor quality batters from which the gluten component may readily separate. Presumably, glutenins in poor quality flours are more mobile and more easily realigned during mixing. Thus, strong secondary bonds may be formed between the proteins allowing gluten development to take place.

The ability of glutenin proteins to aggregate during batter mixing appears to be related to the level of free SH groups in the flour, and to the distribution of SH between high- and low-molecular weight protein fractions; good quality flours have a higher total level of free SH than poor quality flours and a greater proportion of these are found in a high molecular weight SDS-unextractable glutenin fraction.

According to the "linear glutenin hypothesis" glutenin polypeptides are joined head-to-tail by an average of one interchain disulphide bond per subunit to form the linear polymers known as "concatenations". The size of the polymer is determined by the reactivity of the terminal SH groups on the subunits that comprise the backbone of the polymer. Graveland proposed that the backbone of a high molecular weight SDS-insoluble gel protein which he termed Glutenin I, was composed of the subunits 10 and 1 or 3 and 5.

The present investigation has demonstrated the central role that glutenin aggregation/disaggregation reactions play during the processing of wheat flour into a variety of different foodstuffs, and the consequences of glutenin's "inability to participate" in such reactions. Future work should identify and characterise the

polypeptides that are specifically involved in these reactions, and determine whether or not differences exist in the chemical and/or physical properties of the subunits that can account for differences in the aggregation behavoir of flours of different quality.

It is, thus, evident that different manufacturing processes may affect protein aggregation states in different ways, according to such factors as the intensity of mixing, the temperature conditions employed, the water content of the system etc.

Even at very low moisture contents, reactions may take place in gluten proteins that affect their chemical and functional properties. The present investigation showed that commercial dry gluten will take up moisture when exposed to an environment of high relative humidity, and that rehydration and development time increases following moisture uptake. The delayed nature of the response suggests that reactions may be taking place in gluten that are facilitated by the higher moisture content. These reactions may include depolymerisation of glutenins, although the observed changes were small and possibly not significant. The rehydration test is frequently used as an index of gluten quality for use in bakery applications. However, although increased moisture content had a deleterious effect on rehydration times, the gluten functionality in a sensitive breadmaking test was unimpaired. Thus, it may concluded that uptake of moisture by gluten before use is unlikely to reduce its breadmaking performance, despite changes in the protein components.

In this thesis, methods are reported for the extraction and

subsequent characterisation of the extracted cereal endosperm storage proteins. These procedures have facilitated an investigation of the relationship between cereal protein aggregation states and flour functionality, and have also allowed examination of the effect of commercial food manufacturing procedures on the stability of these aggregation states. The proteins of wheat flour are crucial in determining a flour's suitability for processing, and both quantitative and qualitative factors are, evidently, of importance. Much work still remains to be done to elucidate the precise chemical nature of the gluten component of wheat, and the basis for gluten's functionality. However, the present investigation indicates that each process should be considered as a separate entity since the factors that determine a flour's suitability for one process may not be indicative of its quality in a different procedure, and furthermore, the effects of the different processes on the protein components may differ markedly.

Appendix I

Methods used in the Analysis of the Biochemical and Functional Properties Of Cereal Flours and Wheat Gluten

Appendix I.1: Fractionation of cereal proteins using a sequential extraction procedure

Cereal proteins were fractionated according to the procedure of Osborne (1907) as modifed by Chen & Bushuk (1970).

Wholemeal flour samples (5g) were extracted with 0.5M NaCl (20ml) by end-over-end mixing in a centrifuge tube for 1h. Each suspension was centrifuged at 1860g for 30 min and the supernatant was decanted. The residue was re-extracted with 0.5M NaCl (20ml) for 1h, and then with distilled water (20ml) for 30 min to remove residual salt. The three supernatants were combined, dialysed against distilled water for 48h and then centrifuged to separate the precipitated salt-soluble proteins. Water-soluble proteins remained in the supernatant. The residue from the salt solution stage was then extracted similarly with 2 x 20ml volumes of 70% (v/v) ethanol, and the resultant residue was further extracted twice with 0.5M acetic acid (20ml). Ethanol and acetic acid were removed from the extracts by dialysis against distilled water for 48h. The four soluble fractions and the final residue were freeze-dried and stored at -20°C.

The protein contents of the extracts and residue were determined as Kjeldahl N x 5.7.

Appendix I.2: Extractability of endosperm storage proteins in buffers containing sodium dodecyl sulphate

Samples were suspended in a buffer containing 2% (w/v) sodium dodecyl sulphate, 0.1M Na_2HPO_4/NaH_2PO_4, pH 7.6, at a solvent to solid ratio of 20:1 for wholemeals, flours and freeze-dried doughs, and 100:1 for isolated wheat gluten. Extractions were carried out in polycarbonate centrifuge tubes, by gently rotating for 17h at room temperature. Suspensions were then centrifuged at 50,000g for 1h to separate SDS-extractable and unextractable material. The protein contents, of the extracts and the freeze-dried residues, were determined as N x 5.7 by a micro-Kjeldahl procedure.

Appendix I.3: Molecular weight distributions of SDS-extractable endosperm storage proteins

Determination of the molecular weight distributions of SDS-extractable endosperm storage proteins was carried out according to the method of Bottomley et al. (1982).

Samples were extracted with 2% SDS (w/v), 0.1M Tris-HCl, pH 8.0, at a solvent to solid ratio of 20:1 for wholemeals, flours and freeze-dried doughs, and 100:1 for isolated wheat gluten. Extractions were carried out in polycarbonate centrifuge tubes by end-over-end rotation for 17h at room temperature. Hexane (0.5ml/20ml extraction buffer) was then added to each sample prior to centrifugation at 50,000g for 1h. The use of a hexane overlay was found to be necessary to produce a clear lipid free supernatant. The extract was carefully separated from the residue and heated in a boiling water bath for 5 min. The boiled extract was dialysed for 5h against the chromatography buffer, which was 0.1% SDS, 0.1M Tris-HCl, pH 8.0. For preparative chromatography, the dialysed extract (13 ml) was applied to a gel-filtration column of Sepharose CL-4B (dimensions 35mm diameter x 850mm) equilibrated with the chromatography buffer and eluted at a flow rate of 10 ml/h. Column effluents were monitored continuously at 277nm using an LKB Uvicord "S" monitor, and fractions of 3.5ml were collected. Fractions corresponding to a narrow range of molecular weights (as indicated in the appropriate section of this thesis) were combined, dialysed exhaustively against distilled water and freeze-dried.

For analytical chromatography, the dialysed extract (3ml) was

applied to a gel-filtration column of Sepharose CL-4B (dimensions 16mm diameter x 850mm) and eluted as previously.

Appendix I.4: Polypeptide/protein composition of cereal endosperm storage proteins by polyacrylamide gel electro--phoresis in the presence of SDS, (SDS-PAGE)

Electrophoresis was carried out on polyacrylamide slab gels in the presence of SDS according to the method of Laemmli (1970) and employing the modifications of Holt et al. (1981).

The reservoir buffer was 0.188M glycine/0.003M SDS/0.025M Tris, pH 8.3. The separating gel (150mm x 150mm x 1.5mm) contained 175 g/l acrylamide monomer and 0.79 g/l NN'-methylene bisacrylamide, and the stacking gel contained 30 g/l acrylamide and 0.43 g/l NN'-methylene bisacrylamide.

Samples were prepared for electrophoresis by suspending them in 0.05M dithiothreitol/0.069M SDS/0.125M Tris-HCl, pH 6.8 and heating at 100°C for 5 min. After stacking the samples at 15 mA, they were electrophoresed at 10 mA overnight.

Protein bands were visualised using a two stage staining procedure. Gels were stained for 2-5 d in a solution containing 0.02% (w/v) Coomassie Blue R-250/5% (v/v) ethanol/6% (w/v) trichloroacetic acid, washed for 1-2d with distilled water and then immersed in Blakesley's stain, which consisted of 0.08% (w/v) Coomassie Blue G-250/2% (v/v) sulphuric acid/5% (w/v) potassium hydroxide/12% (w/v) trichloroacetic acid. The stained gels were photographed for permanent record.

Appendix I.5: Bulk fermentation baking text for Buhler-milled flours

Recipe

Buhler-milled flour (600g), salt (11.3g), yeast (10.0g), potassium bromate (16 ppm flour weight basis). Flours were supplemented with malt flour to raise the diastatic activity to a level of 16 Farrand Units (Farrand, 1964). Bakery water addition was determined by mixing 50g of the test flour with water in a Do-corder, to achieve a dough consistency of 600 B.U.* Water temperature was adjusted to give a final dough temperature of (26-27)°C.

Procedure

Doughs were mixed in a Simon Majorpin mixer for 3 min, and then left to ferment for 2h at 27°C in a sealed polythene tub. The dough was then "knocked-back" by mixing for 2 min in the Majorpin mixer. The dough was returned to the tub and left to ferment for a further 1h at 27°C. At the end of the fermentation period, the dough was scaled (2 x 453g dough pieces), moulded into a ball and rested for 5 min. It was then flattened four times using a rolling pin, folded and rested for a further 2 min. The dough piece was turned through 90°, and rolled again four times. The dough was turned through a further 90° and the top end of the dough then folded over by approximately 2.5cm, and pressed down firmly. The dough piece was moulded by hand to form a sausage-shape and the ends of the "sausage" and the open-flap were sealed by hand. The dough piece (approximately 16cm long) was placed in a greased baking tin, and

proved for 60 min at 37.8°C, 80%-85% humidity. Loaves were baked in a Simon oven at 225°C, for 30 min. Loaf volume was assessed by seed displacement. Texture score was assessed by a trained panel on a scale of 0 (poor) - 7 (good).

* The Do-corder method for determination of bakery water addition correlates with the "Farinograph Handbook" method, where water is added to 300g of flour in a Brabender Farinograph, to achieve a dough consistency of 500 B.U. (Farinograph Handbook, Editors L. Locken, S. Loska and W. Shuey. Publ. by the AACC as proceedings of the AACC Farinograph Workshop, American Institute of Baking, Chicago, Illinois. pp43, 1960)

Appendix I.6: Flour fractionation into gluten and starch plus water soluble material

Distilled water (20°C) was added to the flour (solvent:solid ratio of 1:1) in a Kenwood Major Mixer. The batter was mixed at minimum speed setting for 30s and then the sides of the bowl scraped down to ensure incorporation of all the flour into the batter. The batter was then mixed at maximum speed until the development of a gluten matrix had occurred. (i.e. when the batter turned from a viscous fluid to a cohesive, slightly elastic mass). An equal volume of distilled water was then added to the mixer-bowl, and the dough-water slurry was stirred slowly for 5 min. The gluten was separated from the starch using a coarse mesh sieve (1.0mm) and was rewashed twice before freeze-drying. The wash waters were combined and freeze-dried. All dried components were ground in a laboratory Retsch centrifugal mill (0.5mm screen) and stored in sealed bags at + 4°C. Gluten and starch plus water soluble material were analysed for protein and moisture content.

Appendix I.7: Reconstitution baking procedure for isolated wheat gluten

Test gluten samples were blended with starch and water soluble material from Canada Western Red Spring (CWRS) wheat flour, to produce reconstituted "flours" of 14% protein content. These "flours", were equilibrated in controlled atmosphere cabinets to bring their moisture content to 14%.

Reconstituted "flours" incorporating the test gluten were baked according to the procedure of Booth & Melvin (1979) which is based on previous methods of Finney & Barmore (1943, 1945) and McCluggage (1943).

Recipe

Flour (50g), sugar (83.0g), salt (0.75g), shortening (1.5g), yeast (1.0g), skimmed milk powder (2.0g) and potassium bromate (16 mg/kg flour). Composite flours were supplemented with malt flour to raise their α-amylase activities to 8 Farrand Units (Farrand, 1964). Bakery water addition was determined by the Brabender Farinograph.

Procedure

Doughs were mixed for 3 min in a Simon Minorpin mixer then left to ferment for 3h at 30°C in sealed polythene tubs (180 ml volume). After 105 min of the fermentation period the dough was rolled twice, folded into three layers and replaced in the tub. This was repeated after a further 50 min. At the end of the fermentation period, the

dough was scaled (56g dough pieces) moulded into a ball and rested for 5 min. It was then flattened twice using a rolling pin, folded into three layers and rested for a further 5min. The dough piece was then turned through 90°, rolled and folded, and this procedure was repeated before placing the dough into a small greased tin, and proving for 55 min at 38°C. The loaves were baked for 15 min in a Simon Rotatory oven at 225°C with steam addition for 7 min. Loaf volume was measured by seed displacement. Texture score was assessed by a trained panel on a scale of 0 (poor) - 7 (good).

Appendix I.8: Assessment of wheat flour baking performance by the Chorleywood Bread Process

Recipe

Flour (4500g), yeast (96.4g), salt (96.4g), fat (32.0g), *ascorbic acid (30 ppm), *potassium bromate (45 ppm). Bakery water addition was determined by Brabender Farinograph ("Farinograph Handbook" pp 43, 1960).

* Variations from the standard rate of oxidant addition are specified in the appropriate section of this thesis.

Procedure

All doughs were mixed in the Tweedy 10 mixer under a vacuum of 50.5kPa (15 in of Hg). Total work input was varied between 17.8 - 71.8 kJ/kg. Target dough temperature was 30 $\pm$ 1°C. Small pieces of dough were taken immediately after mixing, placed in labelled polythene bags and frozen in liquid N_2. A 480g dough piece was used for determination of ex-mixer dough consistency after 1 min mixing on the Farinograph. The remaining ex-mixer dough was divided into six 900g pieces, which were moulded, covered with a plastic sheet to prevent development of a "skin" on the dough surface, and then rested for 10 min at ambient room temperature. The dough pieces were remoulded and placed in greased loaf tins in a proving cabinet at 37.5°C, with humidity provided by a wet sponge. After 60 min final proof, the heights of three of the 900g dough pieces in the bread tins were determined. The six loaves from each mix were then

baked at 225°C for 20 min. Loaves were cooled overnight at 21°C, 70% RH.

Loaf volume was determined the following day by seed displacement with pearl barley. Crumb texture was assessed by an expert panel, on a scale 0 (poor) - 8 (good).

The dough samples taken immediately after mixing were freeze-dried and ground to a fine powder in a Moulinex coffee mill. Dough samples were stored at -20°C in sealed polythene bags.

Appendix I.9: Fractionation of acetic acid-extractable proteins from flours and freeze-dried doughs by gel-filtration chromatography

Flour and dough samples were extracted with 0.1M acetic acid, at a solvent to solid ratio of 20:1. Extractions were carried out in polycarbonate centrifuge tubes by end-over-end rotation for 1h at room temperature. The samples were then centrifuged at 50,000g for 1h to produce a clear supernatant. The extract was carefully separated from the starch residue and 10 ml were applied to a gel filtration column of Sephadex G-150 (dimensions 35mm diameter x 60mm) equilibrated previously in 0.1M acetic acid and eluted at a flow rate of 30 ml/h. Column effluents were monitored continuously using an LKB Uvicord "S" monitor, and fractions of 3.5ml were collected. Fractions corresponding to a narrow range of molecular sizes were combined, as indicated in the appropriate section of this thesis, dialysed exhaustively against distilled water and freeze-dried. Protein contents of the acetic acid extract and residue after extraction were determined as Kjeldahl N x 5.7.

Appendix I.10: Subunit compositions of acetic acid extractable proteins of flours and doughs, by two dimensional electrophoresis

Acetic acid extractable proteins of flour and freeze-dried doughs were fractionated according to the method described in Appendix I.9. The subunit composition of the different molecular size fractions was examined by two dimensional electrophoresis of the fully reduced samples.

1st dimension separation of polypeptides

Gels of 75mm length x 5mm diameter were prepared in glass tubes from a solution containing 861 g/l Aristar urea, 62.63 g/l acrylamide monomer, 32 g/l Nonidet P-40 and Ampholines pH 8-9.5, pH 7-9, pH 5-7 and pH 4-6 all at 17.0 ml/l, and Ampholine pH 3.5-10 at 15.66 ml/l. The gels were overlaid with distilled water and left to polymerise for 2h. At the end of this period, the rubber caps at the bottoms of the tubes are replaced with dialysis tubing, and the rods were set into the "Shandon" disc electrophoresis apparatus, which contained 0.01M, H_3PO_4 in the lower reservoir. Each gel was overlaid with enough 8M urea to fill half of the gap at the top of the tube. The remaining gap was then filled with 0.02M NaOH, which was also used to fill the upper reservoir. The electrodes were connected (+ ve at the top) and the gels prefocussed at 200V for 15 min, 300V for 30min and 400V for 30 min. The NaOH in the upper reservoir was replaced with fresh solution and the samples applied by underlayering. Electrofocussing was performed at 400V for 15h and then 800V for 1h.

Samples were prepared for isoelectrofocussing (IEF) by suspending them in a buffer containing 20 g/1 SDS, 0.1 g/1 Pyronin Y, 10% (v/v) glycerol and 5% (v/v) 2-mercaptoethanol for 2h at room temperature, with occasional mixing, followed by heating at 100°C for 2-5 min. Insoluble material was removed by centrifuging at 13,000 rpm for 30 min.

After electrofocussing, the rods were extruded from the glass tubes into an equilibration buffer of 9.8% (w/v) glycerol, 8.6 g/1 Tris, 23.0 g/1 SDS and 5% (v/v) 2-mercaptoethanol (pH 6.8), and placed in a water bath at 35°C for 2h prior to electrophoresis in the second dimension.

2nd dimension separation of polypeptides

Electrophoresis was carried out on polyacrylamide slab gels in the presence of SDS according to the method described in Appendix I.4. with the following modifications:-

The main separating gel was poured to a level of approximately 1.5cm below the top of the glass plate and overlayered with water. After polymerisation for 1h, the water was removed and the gel surface rinsed twice with water and then stacking gel solution. The stacking gel was then poured to within 2-3mm of the top of the plates, and overlayered with water. When the stacking gel had polymerised, the water overlay was removed and the remaining space between the top of the gel and the top of the plates was filled with a hot solution of 1% (w/v) Agarose in 0.06M Tris-HCl (pH 6.8) and containing enough bromophenol blue to colour. The first dimension

rods were then set into the hot agarose, (tops to the centre), excluding all air bubbles from the underside. A fully-reduced sample standard was dissolved in the same hot agarose and placed in the centre of the slab gel between the two rods.

After stacking the samples for 1h at 30mA, electrophoresis was continued at 10mA overnight and for a further 1h after the bromophenol blue had migrated off the bottom of the slab gel. Gels were stained by the method previously described for analytical polyacrylamide slab gels.

Appendix I.11: Determination of levels of salt- and water-extractable protein in durum semolina and pasta

Semolina or a ground spaghetti sample (5g) was suspended in 0.5M NaCl (20ml) in a 50ml centrifuge tube: the tube was shaken in a horizontal position at 4°C for 90 min. The suspension was then centrifuged at 2,000g for 30 min at 4°C. The supernatant was decanted and retained. The pellet was re-extracted with 0.5M NaCl (20ml), followed by distilled water (20ml). The combined supernatants were then dialysed against distilled water for 48h at 4° C (3 changes). Proteins that precipitated during dialysis were removed by centrifugation at 10,000g for 30 min. The protein contents of the pellet and supernatant were determined as Kjeldahl N x 5.7.

Appendix I.12: Acetic acid extractability of durum wheat semolina and pasta proteins

Durum semolina, or the ground pasta sample (1g), was suspended in 0.05M acetic acid (25ml) in a 50ml centrifuge tube, and the contents were shaken for 2h at room temperature. After centrifugation at 10,000g for 30 min at 4°C, the supernatant was decanted, and the protein content of both supernatant and residue fractions were determined as Kjeldahl nitrogen x 5.7.

Appendix I.13: Determination of free sulphydryl groups of SDS-extractable and SDS-unextractable proteins

Free sulphydryl groups in SDS-extractable and SDS-unextractable proteins were determined by carboxymethylation with [^{14}C]-iodoacetamide. Samples (300mg) were suspended in 2% (w/v) SDS/0.1M Tris-HCl (pH 8.0) extraction buffer (7ml), to which was added 0.1M [^{14}C]-iodoacetamide, (specific activity 618 dpm/nanomole), (0.1ml). After extraction by end-over-end mixing for 17h at room temperature, the sample was centrifuged at 50,000g for 1h, and the supernatant was then dialysed against 0.1% SDS, 0.1M Tris-HCl (pH 8.0). The radioactivity in an aliquot of the dialysed supernatant was determined by counting in a Beckman LS200B liquid scintillation counter using a commercially available scintillation solvent ("Liquid Scintillation Cocktail T", Hopkin and Williams Ltd., Chadwell Heath, U.K.).

Protein in the SDS-insoluble residue remaining after extraction and centrifugation, was solubilised by resuspending the pellet in 2% (w/v) SDS, 0.1M Tris-HCl (pH 8.0), 20mM dithiothreitol (5ml). The sample was centrifuged, the supernatant dialysed and an aliquot counted as before.

The radiolabelled protein extractable in SDS in the absence of reducing agent (i.e. that extracted initially) was also chromatographed on Sepharose CL-4B (according to the method described in Appendix I.3) to determine the distribution of free sulphydryl groups in the different protein molecular size groups. The column effluent was collected in fractions of approximately 2ml

volume and 0.4ml aliquots of each fraction were taken for determination of radioactivity.

Appendix I.14: Batter-mixing procedure

Batters were prepared from the test flours by addition of 120 parts water (on flour weight basis) at 43°C, to 100 parts flour in a Kenwood Major Mixer (Model A707A). The flour/water mixture was blended for 30s at the lowest speed setting, and the sides of the mixing bowl were scraped down to ensure incorporation of all the flour into the batter. The slurry was then mixed at maximum speed for 120s. Samples were withdrawn after 60s and 120s of rapid mixing and frozen in liquid N_2. The batter viscosity at the end of mixing was determined as the flow time (s) of 500g batter from a standard Ford Cup (BS. 1733, 1955), type B5.

The batter (250g) was then transferred to the high shear blender attachment of the Kenwood mixer and mixed at maximum speed until gluten development occurred. Mixing beyond this point caused the gluten aggregates to be redispersed. Samples were withdrawn and immediately frozen in liquid nitrogen after 20s of high shear mixing (i.e. 140s total mix time), at the point of gluten development and after re-dispersion. Protein stability was measured as the period of mixing to the time of gluten development.

All samples were freeze-dried and then milled in a Retsch centrifugal mill (0.5mm screen). Ground samples were stored at room temperature in a desiccator over NaOH pellets.

Appendix I.15: Determination of gluten rehydration and development time (Voisey Micropin Mixer Method)

Gluten rehydration and development times were determined according to the following procedure, based on that of Booth & Timms (1978).

The control dry gluten (4g) was placed in the temperature-controlled bowl of a Voisey (1966) micropin mixer. Distilled water was added to give a final gluten ball moisture content of 65%. The planetary motion mixing head was lowered into position and mixing commenced.

The torque transmitted by the rehydrating gluten mass, from the rotating pins to the static pins in the mixing bowl was recorded on a previously calibrated chart recorder, (i.e. mixing time to gluten development was followed by the increase in torque).

Gluten development time was then determined for the test sample. The weight of gluten added to the mixing bowl was adjusted to maintain the level of dry solids equivalent to the control. Water addition was adjusted to give a moisture content of 65% in the gluten ball.

Appendix I.16: Determination of gluten rehydration and development times (Minorpin Mixer Method)

The control dry gluten (10g) was placed in the bowl of a Simon Minorpin Mixer. The mixing head was lowered and distilled water was added, whilst the pins were rotating, to give a final gluten ball moisture content of 65%.

The rehydration and development time was determined as the mixing period required for the gluten/water slurry to form into a coherent mass.

Gluten development time was then determined for the test sample. The weight of gluten added to the mixing bowl was adjusted to maintain the level of dry solids equivalent to the control. The water addition was adjusted to give 65% moisture content in the final gluten ball.

Appendix I.17: Amino acid analysis of wheat flour and gluten proteins

Amino acid analyses were carried out according to the method of Bottomley et al. (1982).

Analyses were carried out using a Rank Hilger "Chromaspek" analyser, fitted with a fluorimetric detection system. Samples were hydrolysed at 108°C for 21 h in sealed, evacuated tubes using constant boiling HCl (Fisons "Primar"; 1.0 ml/mg for high protein samples, 0.25 ml/mg for low protein samples). Norleucine was added as an internal standard (0.2µmol/ml of acid). The hydrolysates were filtered through glass fibre discs and dried under reduced pressure at 45°C.

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