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Review

Wheat flour constituents: how they impact bread quality, and how to impact their functionality

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The vast majority of bread is traditionally produced from wheat flour. Apart from its major constituent starch, wheat flour also contains many other types of substances of which the gluten, the non-starch polysaccharides as well as the lipids are the most important in terms of their impact on the processability of the raw material and in terms of the quality of the final products. We here provide the basics on the processability and quality determining wheat flour constituents and present common concepts on their fate during the breadmaking process as well as on approaches targeted to influence their functionality.

Introduction

For several thousand years, bread has been one of the major constituents of the human diet, making the baking of yeast-leavened and sourdough breads one of the oldest biotechnological processes. Wheat is by far the most important cereal in breadmaking, although in some parts of the world the use of rye is quite substantial. Other cereals are used to a lesser extent. In wheat breadmaking, flour, water, salt, yeast and/or other micro-organisms (often with the addition of non-essential ingredients, such as *e.g.* fat and sugar) are mixed into a visco-elastic dough, which is fermented and baked. Wheat flour is the major ingredient and consists mainly of starch (*ca.* 70–75%), water (*ca.* 14%) and proteins (*ca.* 10–12%). In addition, non-starch polysaccharides (*ca.* 2–3%), in particular arabinoxylans (AX), and lipids (*ca.* 2%) are important minor flour constituents relevant for bread production and quality.

During all steps of breadmaking, complex chemical, biochemical and physical transformations occur, which affect and are affected by the various flour constituents. In addition, many substances are nowadays used to influence the structural and physicochemical characteristics of the flour constituents in order to optimise their functionality in breadmaking. We here focus on these essential processing and quality determining flour constituents (*i.e.* starch, gluten proteins, AX and lipids). More in particular, we provide a basic overview of their structure and properties, their role in breadmaking and how their functionality can be affected.

Starch

Structural and physicochemical aspects

Starch, the most important reserve polysaccharide and the most abundant constituent of many plants, including cereals, occurs as semi-crystalline granules. It has some unique properties, which determine its functionality in many food applications, in particular breadmaking. Its structure and physicochemical properties have been the subject of many extensive reviews (*e.g.* Buléon, Colonna, Planchot, & Ball, 1998; Eliasson & Gudmundsson, 1996; Hizukuri, 1996; Parker & Ring, 2001) and will here be concisely dealt with.

Starch granule structure

The major components of starch are the glucose polymers amylose and amylopectin. *Amylose* is an essentially linear molecule, consisting of α-(1,4)-linked D-glucopyranosyl units with a degree of polymerisation (DP) in the range of 500–6000 glucose residues. It is now

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well recognised that a fraction of the amylose molecules is slightly branched by α-(1,6)-linkages (Hizukuri, Takeda, & Yasuda, 1981; Shibanuma, Takeda, Hizukuri, & Shibata, 1994) (Fig. 1e). In contrast, *amylopectin* is a very large, highly branched polysaccharide with a DP ranging from 3×10^5 to 3×10^6 glucose units. It is composed of chains of α-(1,4)-linked D-glucopyranosyl residues which are interlinked by α-(1,6)-bonds (Zobel, 1988). These chains can be classified as either the unbranched outer chains (A) or either the branched inner chains (B) (Peat, Whelan, & Thomas, 1956). The latter can be further divided in B1, B2, B3 and B4 chains (Hizukuri, 1986). In addition, there is a single C chain per molecule which contains the sole reducing residue (Peat *et al*., 1956). The cluster model of the amylopectin structure is nowadays widely accepted (French, 1984; Robin, Mercier, Charbonnière, & Guilbot, 1974) (Fig. 1e). In this model, the short (A and B1) chains form double helices, which are organised in discrete clusters, while the longer B2, B3 and B4 chains extend into 2, 3 or 4 clusters, respectively.

The amylose/amylopectin ratio differs between starches, but typical levels of amylose and amylopectin are 25–28% and 72–75%, respectively (Colonna & Buléon, 1992). However, the starches of some mutant genotypes of *e.g.* maize, barley and rice contain either an increased amylose content (*i.e.* high amylose or amylostarch with up to 70% amylose) or an increased amylopectin content (*i.e.* waxy starch with 99–100% amylopectin). In the past 10 years, several waxy wheat cultivars have been developed as well, as discussed by Graybosch (1998).

Starch is present as intracellular water-insoluble granules of different sizes and shapes, depending on the botanical source. In contrast to most plant starches, wheat, rye and barley starches show a bimodal size distribution. The small (B) granules are spherical with a diameter up to *ca.* 10 μm (mean diameter 5 μm), while the large (A) granules are lenticular with a mean diameter of *ca.* 20 μm (Karlsson, Olered, & Eliasson, 1983; Moon & Giddings, 1993).

When viewed in polarised light, the native starch granules are birefringent and a 'Malteser cross' can be observed. This indicates a degree of order in the starch granule and an orientation of the macromolecules perpendicular to the surface of the granule (Buléon *et al*., 1998; French, 1984). In addition, native starch is partially crystalline with a degree of crystallinity of 20–40% (Hizukuri, 1996), which is predominantly attributed to structural elements of amylopectin.

Several levels of granule organisation have been described. At the lowest structural level, the starch granule can be defined in terms of alternating amorphous and semi-crystalline growth rings or shells with a radial thickness of 120–400 nm (Buléon *et al*., 1998; French, 1984) (Fig. 1a). The amorphous shells are less dense and contain amylose and probably less ordered (not crystalline) amylopectin, while the semi-crystalline shells are composed of alternating amorphous and crystalline lamellae of about 9–10 nm (Jenkins, Cameron, & Donald, 1993). The latter are made up by amylopectin double helices packed in a parallel fashion, while the former consist of the amylopectin branching regions

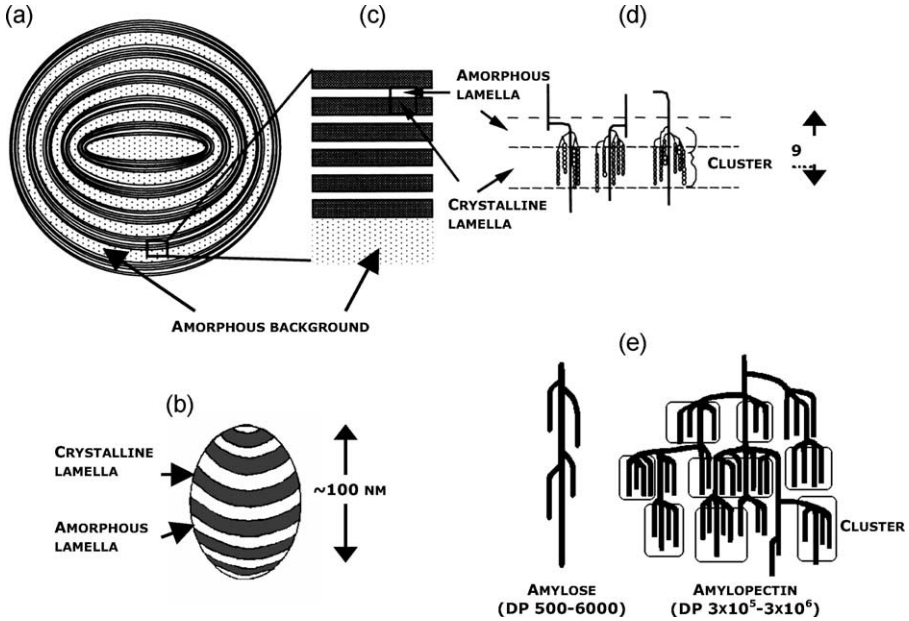

Fig. 1. Schematic representation of different structural levels of a starch granule (adapted from Donald et al. (1997) and Buléon et al. (1998)): (a) granule with alternating amorphous and semi-crystalline shells; (b) expanded view of a blocklet structure, the building blocks of the shells (Gallant et al., 1997); (c) expanded view of the semi-crystalline layer, consisting of alternating crystalline and amorphous lamellae; (d) the cluster structure of amylopectin within the semi-crystalline shell; (e) schematic representation of amylose and amylopectin.

(and possibly some amylose) (Fig. 1c and d). There are indications that these lamellae are organised into larger, somewhat spherical structures, named 'blocklets', which range in diameter from 20 to 500 nm (Gallant, Bouchet, & Baldwin, 1997) (Fig. 1b). Different packing of the amylopectin side-chain double helices gives rise to different crystal types. The A type is found in most cereal starches, while the B type is found in some tuber starches, high amylose cereal starches and retrograded starch. The B crystal type is a more highly hydrated and open structure (Buléon *et al*., 1998; Hizukuri, 1996).

A significant fraction of the starch granules (*ca.* 8%) is damaged during milling. This mechanical damage to the granule structure greatly affects starch properties. Damaged starch has lost its birefringence, has a higher water absorption and is more susceptible to (fungal) enzymic hydrolysis (Hoseney, 1994).

Transformations of starch during heating and cooling in the presence of water

At room temperature and in sufficient water, starch granules absorb up to 50% of their dry weight of water. Thereby, they swell to a limited extent only (French, 1984). Below a characteristic temperature (the gelatinisation temperature), this process is reversible. When the starch suspension is heated above this temperature, it undergoes a series of changes which eventually lead to the irreversible destruction of the molecular order of the starch granule. This process is termed *gelatinisation* (Fig. 2) (Atwell, Hood, Lineback, Varriano-Marston, & Zobel, 1988). Heating and hydration of the non-crystalline regions facilitate molecular mobility in these regions and initiate an irreversible molecular transition. This includes the dissociation of the amylopectin double helices, and the melting of the crystallites (Tester & Debon, 2000; Waigh, Gidley, Komanshek, & Donald, 2000). These endothermic transitions, related to the loss of birefringence and crystallinity, can easily be monitored by differential scanning calorimetry (DSC). The onset temperature (T_o; *ca.* 45 °C), as determined by DSC, reflects the initiation of this process, which is then followed by peak (T_p; *ca.* 60 °C) and conclusion (T_c; about 75 °C) temperatures. However, at limited and intermediate water contents gelatinisation occurs more slowly and the DSC endotherm separates in two peaks (Eliasson & Gudmundsson, 1996).

Besides the loss of molecular order and crystallinity, the gelatinisation process is also associated with granule swelling and distortion (due to increased water absorption) and a limited starch solubilisation (mainly amylose leaching), which increases the viscosity of the starch suspension. Amylose leaching can be attributed to the phase separation of amylose and amylopectin because of mutual immiscibility (Kalichevsky & Ring, 1987).

During further heating and above T_c, swelling and leaching continue and a suspension of swollen, amorphous starch granules and solubilised macromolecules (mainly amylose) or *starch paste* is formed (Fig. 2). The granule structure remains until more extensive temperatures and/or shear have been applied (Eliasson & Gudmundsson, 1996; Tester & Debon, 2000).

When the amorphous starch paste is cooled, the starch polysaccharides reassociate to a more ordered or crystalline state. This process is defined as *retrogradation* (Atwell *et al*., 1988) (Fig. 2). At starch concentrations above 6%, a gel is formed. It consists of amylopectin-enriched gelatinised starch granules, also referred to as granule remnants, which are embedded in and reinforce, on crystallisation, a continuous amylose matrix. Initially, double helices are formed between the amylose molecules, which were solubilised during gelatinisation and

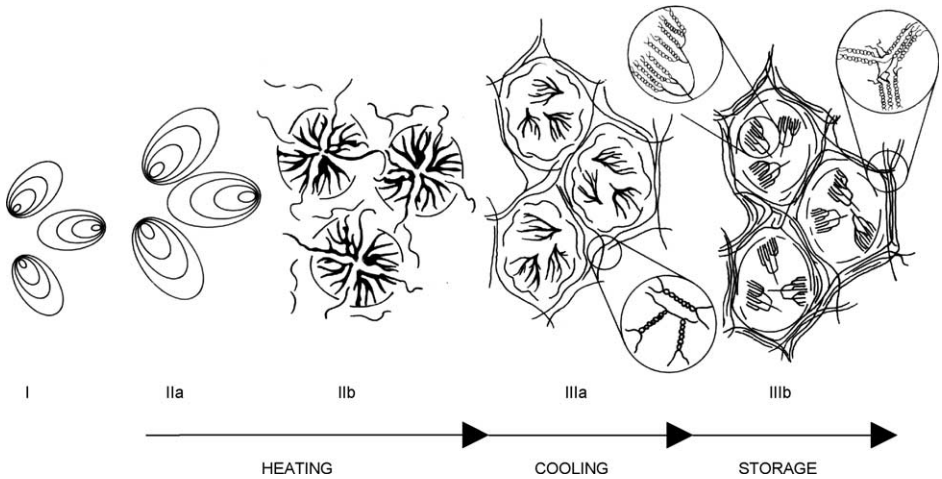

Fig. 2. Schematic representation of changes that occur in a starch–water mixture during heating, cooling and storage. (I) Native starch granules; (II) gelatinisation, associated with swelling [a] and amylose leaching and partial granule disruption [b], resulting in the formation of a starch paste; (III) retrogradation: formation of an amylose network (gelation/amylose retrogradation) during cooling of the starch paste [a] and formation of ordered or crystalline amylopectin molecules (amylopectin retrogradation) during storage [b].

pasting, and a continuous network develops (*gelation*) (Miles, Morris, Orford, & Ring, 1985). After some hours, these double helices form very stable crystalline structures. The recrystallisation of the short amylopectin side-chains is a much slower process (several days or weeks) and occurs in the gelatinised granules or granule remnants (Miles *et al*., 1985). Therefore, amylose retrogradation determines to a great extent the initial hardness of a starch gel, while amylopectin retrogradation determines the long-term development of gel structure and crystallinity in starch systems (Miles *et al*., 1985). The amylopectin crystallites melt at *ca.* 60 °C and with DSC a melting endotherm, the so-called 'staling endotherm', can be measured at this temperature. Therefore, this technique is often used to evaluate amylopectin retrogradation. Starch (amylopectin) retrogradation is influenced by a number of conditions and substances, including the pH and the presence of salts, sugars and lipids (see Eliasson & Gudmundsson, 1996 for an overview).

Amylose–lipid complexes

An important characteristic of amylose is its ability to form helical inclusion complexes with a number of substances, in particular polar lipids. Amylose forms a left-handed single helix and the hydrocarbon chain of the lipid is situated in the central cavity (French & Murphy, 1977). The inclusion complexes give rise to a V type X-ray diffraction pattern. The presence of polar lipids affects starch properties to a large extent, in particular its gelatinisation and retrogradation characteristics (Eliasson & Gudmundsson, 1996).

Role of starch in breadmaking

The dough stage

Starch is present in the dough in the native state where it appears as distinct semi-crystalline granules (Hug-Iten, Handschin, Conde-Petit, & Escher, 1999). During dough preparation, starch absorbs up to about 46% water. Its role in dough is still not very clear. Starch has been suggested to act as inert filler in the continuous protein matrix of the dough (Bloksma, 1990), while Eliasson and Larsson (1993) described dough as a bicontinuous network of starch and protein. Other studies reported that the rheological behaviour of wheat dough is influenced by the specific properties of the starch granule surface (Larsson & Eliasson, 1997) and by the presence of amylolytic enzymes (Martínez-Anaya & Jiménez, 1997a).

In the oven: the baking phase

Due to the combination of heat, moisture and time during baking, the starch granules gelatinise and swell. However, their granular identity is retained (Hug-Iten *et al*., 1999; Varriano-Marston, Ke, Huang, & Ponte, 1980). A small amount of starch (mainly amylose) is leached into the intergranular phase. Furthermore, due to phase separation, amylose and amylopectin are not homogeneously distributed in the granules: the centre of the large granules is enriched in amylose, while the outer granule layers are enriched in amylopectin (Hug-Iten *et al*., 1999). Part of the (solubilised) amylose forms inclusion complexes with both added (if any) and endogenous wheat polar lipids, as evidenced by the V crystal type of fresh bread crumb.

Fresh and aging bread

Upon cooling, the solubilised amylose forms a continuous network, in which swollen and deformed starch granules are embedded and interlinked. Because of its rapid retrogradation, amylose is an essential structural element of bread and is a determining factor for initial loaf firmness (Eliasson & Larsson, 1993). Indeed, flours containing no amylose [either reconstituted with waxy starches (Lorenz, 1995) or derived from waxy wheat (Morita *et al*., 2002)] were not suited for breadmaking as they yielded breads with very poor crumb characteristics.

During storage, bread gradually loses its freshness and stales. The staling process comprises several aspects: the crust toughens, the crumb becomes more firm and less elastic and moisture and flavour is lost (Hoseney, 1994). Bread staling is often evaluated by measuring crumb firmness. However, this property is also influenced by loaf volume and crumb structure. Bread staling is a complex phenomenon in which multiple constituents and mechanisms take part (Gray & BeMiller, 2003). In most views, water migration and transformations in the starch fraction are the most important factors in this process. Some researchers proposed that crumb firming can be attributed to some extent to gluten–starch interactions (*cf.* infra) (Every, Gerrard, Gilpin, Ross, & Newberry, 1998; Martin, Zelaznak, & Hoseney, 1991). However, in most staling models, the firming of the crumb during aging is mainly attributed to amylopectin retrogradation, in particular the formation of double helical structures and crystalline regions (Gray & BeMiller, 2003; Kulp & Ponte, 1981; Schoch & French, 1947; Zobel & Kulp, 1996). Because amylose is already almost completely retrograded in the bread after cooling, it is considered to have little, if any, contribution to crumb firming. The amylopectin side-chain reorganisation leads to an increased rigidity of the swollen granules. However, the formation of ordered amylose structures in the centre of the granules may also contribute to granular rigidity (Hug-Iten, Escher, & Conde-Petit; 2003; Hug-Iten *et al*., 1999). In addition, the starch is slowly transformed from an amorphous structure to a partially crystalline state and a B type X-ray diffraction pattern can be observed. However, starch crystallinity is often poorly correlated with crumb firmness. This may indicate that the formation of a structured network, as is the case when large starch molecules (amylopectin and/or amylose) pass through multiple crystalline and amorphous regions, can be a more important factor in gel or bread rigidity than the extent or quality of crystallinity (Zobel & Kulp, 1996). In this respect, Hug-Iten *et al*. (2003) proposed

that both molecular reorganisation of the amylopectin-rich and amylose-rich regions in the starch granules, resulting in an increased granular rigidity, and the formation of a structured network consisting of interlinked crystallites, contribute to crumb firming. Whatever be the case, stale bread can be temporarily refreshed by heating it to 50–60 °C, resulting in the melting of the amylopectin crystallites. In the case of sourdough bread formulas, bread firming is often delayed due to a combination of acidification by the lactic acid bacteria, which affects retrogradation, and microbial starch and protein hydrolysis (Corsetti *et al*., 1998).

Ways to affect starch functionality in breadmaking

Amylases

Based on structural and amino acid sequence similarities, a variety of amylolytic enzymes capable of hydrolysing the α-(1,4)- and/or α-(1,6)-linkages in starch are grouped into glycoside hydrolase family 13 (the so-called α-amylase family) (Coutinho & Henrissat, 1999; MacGregor, Janeček, & Svensson, 2001; Svensson *et al*., 2002). α-Amylases (EC 3.2.1.1), which are typical endo-amylases, more or less randomly hydrolyse the α-(1,4)-linkages of starch, yielding low molecular weight α-dextrins (Bowles, 1996; Hoseney, 1994). Other family 13 amylolytic enzymes include maltogenic (EC 3.2.1.133) and other maltooligosaccharide-producing (*e.g.* EC 3.2.1.60, EC 3.2.1.98) amylases and debranching enzymes. The former are mainly exo-acting amylases which mainly release maltose or other maltooligosaccharides, like maltotetraose or maltohexaose, from starch. Debranching enzymes, like pullulanase (EC 3.2.1.41) and isoamylase (EC 3.2.1.68), hydrolyse the α-(1,6)-bonds, thus removing the side-chains. Typical inverting exo-amylases, such as β-amylase (EC 3.2.1.2; glycoside hydrolase family 14) and glucoamylase (EC 3.2.1.3; glycoside hydrolase family 15), hydrolyse the α-(1,4)-linkages at the non-reducing ends of the starch molecules. β-Amylase cannot hydrolyse α-(1,6)-linkages and its action stops at the branch points. It potentially degrades starch to β-maltose and β-limit dextrins (Bowles, 1996; Hoseney, 1994). Glucoamylase has a limited activity on α-(1,6)-linkages. Theoretically, this enzyme can completely convert starch to β-glucose (Bowles, 1996; Hoseney, 1994).

Amylases are routinely used in breadmaking. Their functionality depends mainly on their specificity, degradation products and thermostability. In general, fungal enzymes have a low thermostability and most of their activity is lost during starch gelatinisation (Bowles, 1996). In contrast, some bacterial α-amylases are very thermostable and may survive the baking process. They are hence difficult to control during baking and storage and can result in reduced crumb structure properties upon overdosing (Bowles, 1996). Addition of amylases mainly aims at optimising the amylase activity of the flour (*i.e.* flour standardisation) and at retarding bread staling.

Amylases for flour standardisation

Amylases are endogenously present in flour, but amylase activity can vary considerably. In general, unmalted flour has high levels of β-amylase, while its α-amylase activity is low, resulting in low bread volume and quality (Drapron & Godon, 1987). Therefore, flour is routinely supplemented to optimise the amylase activity. It is generally acknowledged that the primary goal of amylase addition is to increase the levels of fermentable and reducing sugars in flour (Bowles, 1996). By degrading the damaged starch particles during the dough stage and generating low molecular weight dextrins, supplemented α-amylase facilitates maltose production by the endogenous β-amylase (Kragh, 2003; Linko, Javanainen, & Linko, 1997). The maltose can then be used as fermentable sugar by the yeast or the sourdough microbial population. In addition, increased levels of reducing sugars promote the generation of Maillard reaction products, which intensify bread flavour and crust colour (Bowles, 1996; Drapron & Godon, 1987). In contrast, amylase supplementation may primarily affect dough viscosity during the initial stages of starch gelatinisation (Kragh, 2003). In this view, by delaying the viscosity increase due to amylose leaching during gelatinisation, amylases allow a prolonged oven spring and an increased loaf volume.

Flour standardisation is mostly performed at the mill or at the bakery by addition of a fungal α-amylase from *Aspergillus oryzae*, the so-called Taka-amylase, or malt. Malt amylases have a higher thermostability and generate longer maltodextrins, making them more susceptible to negative side effects upon overdosing than the fungal enzyme (Bowles, 1996).

Amylases for anti-staling

It has been known for a long time that certain amylases have an anti-firming effect and thus retard the staling of baked goods. The mechanism for the anti-staling effect of amylases is still much debated. Several authors suggested that dextrins of a particular size, formed by the amylases during starch degradation, exert an anti-staling effect by interfering with the reassociation and retrogradation of amylopectin (Defloor & Delcour, 1999; Léon, Durán, & de Barber, 1997; Min *et al*., 1998) and/or the formation of starch–protein cross-links (Martin & Hoseney, 1991) in the aging bread. This is somewhat in contradiction with the observation that amylase supplemented breads often have a higher degree of crystallinity (Hug-Iten *et al*., 2003; Zobel & Senti, 1959). Other researchers believe that the dextrins formed by amylase addition are an indication for the modification of the starch (Duedahl-Olesen, Zimmerman, & Delcour, 1999; Gerrard, Every, Sutton, & Gilpin, 1997). In this view, the anti-staling effect of amylases is due to the modified starch structure which has different retrogradation properties. Zobel and Senti (1959) suggested that (endo)amylases cleave the long starch chains, which can link different crystalline regions. This way, the network structure is weakened and less rigid,

resulting in softer bread. Alternatively, amylases, in particular exo-amylases, may act on the outer amylopectin branches, which protrude into the intergranular space and are the most accessible. Removal of these chains hinders double helices and crystal formation and/or cross-linking between amylose and amylopectin (Bowles, 1996; Zobel & Kulp, 1996).

Several amylase containing anti-staling products are commercially available. A maltogenic amylase from *Bacillus stearothermophilus* is one of the most effective anti-staling agents available to date. This enzyme, which shows sequence homology to cycloglycosyl transferases, has some unusual structural (Dauter *et al*., 1999) and starch degrading (Christopherson, Otzen, Norman, Christensen, & Schäfer, 1998) properties when compared to α-amylases. It produces mainly α-maltose from starch, and has some endo-activity as evidenced by its action on wheat amylose and β-limit dextrins (Christophersen *et al*., 1998; Outtrup & Norman, 1984). Hug-Iten, Conde-Petit, and Escher (2001) and Hug-Iten *et al*. (2003) related the efficiency of this maltogenic enzyme in retarding bread staling to its action on amylose and amylopectin. These authors suggested that the enzyme degrades the amylopectin side chains, thus hindering their reorganisation. Furthermore, the enzyme is believed to enhance the crystallisation of amylose, probably because amylose mobility is increased and its association facilitated due to a limited degradation via an endo-mechanism (Hug-Iten *et al*., 2001, 2003). This results in an increased initial bread firmness. The enhanced amylose aggregation possibly contributes to a reduced firming rate. It is conceivable that, due to the rapid aggregation of amylose, a weaker network is formed which rearranges less on aging or that cross-linking between starch compounds is hindered (Hug-Iten *et al*., 2001, 2003). In addition, the intermediate thermostability of the *B. stearothermophilus* maltogenic amylase contributes to its anti-staling properties (Hebeda, Bowles, & Teague, 1991). The enzyme is maximally active above the gelatinisation temperature and is inactivated during baking, making it less susceptible to overdosing than more thermostable enzymes (Hebeda *et al*., 1991; Outtrup & Norman, 1984).

Starch degrading and/or modifying enzymes as a means to retard staling are of much interest to the breadmaking industry and considerable research is still performed in this area (van der Maarel, van der Veen, Uitdehaag, Leemhuis, & Dijkhuizen, 2002). More in particular, several patents and publications deal with the use and properties of maltogenic and maltooligosaccharide-producing amylases (Ben Ammar *et al*., 2002; Cherry, Svendsen, Andersen, Beier, & Frandsen, 1999; Kragh, 2003; Kragh, Larsen, Rasmussen, Duedahl-Olesen, & Zimmermann, 1999; Nielsen & Schäeffer, 2000). In addition, several researchers aimed to modify the thermal stability of the enzymes either by mutagenesis (Cherry *et al*., 1999; Maeda, Hashimoto, Minoda, Tamagawa, & Morita, 2003) or by encapsulation (Horn, 2002).

Emulsifiers

Emulsifiers are substances possessing both hydrophobic (lipophilic) and hydrophilic properties. Emulsifiers are routinely used in breadmaking as dough strengtheners (*cf*. infra) and/or crumb softeners (Kulp & Ponte, 1981; Stampfli & Nersten, 1995). The latter enhance crumb softness and retard bread staling (Pisesookbunterng & D'Appolonia, 1983; Roach & Hoseney, 1995) and their use and properties have been comprehensively reviewed by Gray and BeMiller (2003), Knightly (1996), and Stampfli and Nersten (1995). Interactions between the emulsifiers and amylose, amylopectin and/or protein as well as their effect on starch swelling have been suggested to be responsible for their anti-staling properties (Gray & BeMiller, 2003).

It is widely accepted that the anti-firming properties of these surfactants are related to their ability to form insoluble inclusion complexes with amylose. The complexed amylose does not participate in gel formation and recrystallisation. Therefore, emulsifier addition affects the amylose network structure after baking. Since different emulsifiers vary in complexation capacity (Knightly, 1996; Krog, 1971; Osman, Leith, & Fles, 1961), their efficiency to reduce the staling rate is different. Furthermore, several studies reported complex formation between certain emulsifiers (*e.g*. sodium stearoyl-2-lactylate (SSL) and several monoglycerides) and amylopectin, although to a lesser extent than their complexation with amylose (De Stefanis, Ponte, Chung, & Ruzza, 1977; Lagendijk & Pennings, 1970). Finally, addition of emulsifiers also reduces starch swelling and solubilisation during gelatinisation (Eliasson & Gudmundsson, 1996; Roach & Hoseney, 1995). This way, starch polymer mobility and amylose leaching is restricted, resulting in less crystallisation (Gray & BeMiller, 2003).

Proteins

Wheat protein classification

Classification based on solubility

Osborne (1924) introduced a solubility-based classification of plant proteins using sequential extraction in the following series of solvents: (1) water, (2) dilute salt solution, (3) aqueous alcohol and (4) dilute acid or alkali. Using this Osborne classification scheme, wheat proteins were classified in albumins, globulins, gliadins and glutenins, respectively (Table 1). However, a significant fraction of wheat proteins is excluded from the Osborne fractions because they are unextractable in all of the above-mentioned solvents. Furthermore, further research accompanied by significant improvements in tools for biochemical/genetical analysis gradually taught that the Osborne fractionation does not provide a clear separation of wheat proteins that differ biochemically/genetically or in functionality during breadmaking. Nowadays, the

Table 1. Overview of the different groups of wheat proteins

Osborne fraction	Solubility behaviour	Composition	Biological role	Functional role
Albumin	Extractable in water	Non-gluten proteins (mainly monomeric)	Metabolic and structural proteins	Variable
Globulin	Extractable in dilute salt	Non-gluten proteins (mainly monomeric)	Metabolic and structural proteins	Variable
Gliadin	Extractable in aqueous alcohols	Gluten proteins (mainly monomeric gliadins and low molecular weight glutenin polymers)	Prolamin-type seed storage proteins	Dough viscosity/ plasticity
Glutenin	Extractable in dilute acetic acid	Gluten proteins (mainly high molecular weight glutenin polymers)	Prolamin-type seed storage proteins	Dough elasticity/strength
Residue	Unextractable	Gluten proteins (high molecular weight polymers) and polymeric non-gluten proteins (triticins)	Prolamin-type (gluten) and globulin-type (triticin) seed storage proteins	Variable

names ‘gliadins’ and ‘glutenins’ are mostly used to indicate the functionally/biochemically related proteins instead of the exclusively soluble Osborne fractions. Nevertheless, the Osborne fractionation is still extensively used in studies relating protein composition to functionality in breadmaking. Furthermore, due to its relative simplicity, this fractionation method is often very useful as an initial separation step to obtain semi-pure protein fractions.

Classification based on functionality

From a functional point of view, two groups of wheat proteins should be distinguished: the non-gluten proteins, with either no or just a minor role in breadmaking, and the gluten proteins, with a major role in breadmaking.

The *non-gluten proteins* (between 15 and 20% of total wheat protein) mainly occur in the outer layers of the wheat kernel with lower concentrations in the endosperm. Most non-gluten proteins are extractable in dilute salt solutions and are, therefore, found in the Osborne albumin and globulin fractions. They are mostly monomeric physiologically active or structural proteins (Table 1). However, the non-gluten proteins also include a minor group of polymeric wheat storage proteins, called triticins, which belong to the globulin class of seed storage proteins. They are related to the major storage proteins in legumes and in the cereals oats and rice (Shewry & Halford, 2002; Shewry, Napier, & Tatham, 1995). These proteins are found in the residue after a classical Osborne fractionation. Their role in breadmaking remains unclear (Veraverbeke & Delcour, 2002).

The *gluten proteins* (between 80 and 85% of total wheat protein) are the major storage proteins of wheat. They belong to the prolamin class of seed storage proteins (Shewry & Halford, 2002; Shewry *et al*., 1995). Gluten proteins are found in the endosperm of the mature wheat grain where they form a continuous matrix around the starch granules. Gluten proteins are largely insoluble in water or dilute salt solutions. Two functionally distinct groups of gluten proteins can be distinguished: monomeric gliadins and polymeric (extractable and unextractable) glutenins. Gliadins and glutenins are usually found in more or less equal amounts in wheat. *Gliadins* represent a highly polymorphic group of monomeric gluten proteins with molecular weights varying between 30,000 and 80,000. Biochemically, three types (α, γ and ω) have been identified (Shewry, Tatham, Forde, Kreis, & Miflin, 1986; Veraverbeke & Delcour, 2002). They are all readily soluble in aqueous alcohols and are therefore the main components in the Osborne gliadin fraction (Table 1). *Glutenins* are a heterogeneous mixture of polymers with molecular weights varying from about 80,000 to several millions. Glutenins contain among the largest proteins found in Nature (Wrigley, 1996): the actual sizes of the largest glutenin polymers have not yet been accurately determined because of their enormous sizes. Another reason for the difficulties in the size determination of glutenins is their poor solubility in conventional buffers. In contrast with gliadins, only a very small part, corresponding to the smallest polymers, is soluble in aqueous alcohols. A larger part is soluble under dilute acid conditions (Table 1). However, a significant part cannot be solubilised without changing its structure. This large insolubility of glutenin explains why, despite significant efforts for already more than a century, relatively little details are available on the structure of glutenin. What is known, however, is that glutenin is built of glutenin subunits (GS) that are linked via disulphide bonds. These GS can be liberated by reduction of disulphide bonds with reducing agents such as β-mercaptoethanol or dithiothreitol. GS are biochemically related to the gliadins and are soluble in aqueous alcohols. Four different groups of GS can be distinguished: high molecular weight glutenin subunits (HMW-GS) with molecular weights between 65,000 and 90,000 and B-, C- and D-type low molecular weight glutenin subunits (LMW-GS) with molecular weights between 30,000 and 60,000. The structure of these GS and different hypotheses on the structure of the glutenin polymer are reviewed in more detail elsewhere (Shewry, Halford, & Tatham, 1992; Veraverbeke & Delcour, 2002).

Gluten protein functionality in breadmaking

Breadmaking quality of wheat flour is largely determined by its proteins. That both quantity and composition (quality) of proteins are important for breadmaking is evident from the observation that breadmaking performance of wheat flour is linearly related with its protein content and that different linear relationships exist for different wheat varieties (Finney & Barmore, 1948). Notwithstanding minor roles of different non-gluten proteins (*e.g.* certain enzymes, enzyme inhibitors, lipid-binding proteins and possibly also triticins) in breadmaking (Eliasson & Larsson, 1993; Veraverbeke & Delcour, 2002), the main quality determinants of breadmaking are the gluten proteins. Indeed, the unusual properties of the gluten proteins allow wheat flour to be transformed into a dough with suitable properties for breadmaking. These properties are unique and cannot even be found in cereals closely related to wheat such as barley and rye. Gluten proteins undergo various changes during the different steps of the breadmaking process, although the nature of these changes, like the native gluten protein structure itself, is poorly understood.

The dough stage

During *dough mixing*, wheat flour is hydrated and as a result of the mechanical energy input discrete masses of gluten protein are disrupted. The gluten proteins are transformed into a continuous cohesive visco-elastic gluten protein network (Singh & MacRitchie, 2001), as was visualised by microstructural studies (Amend, Belitz, Moss, & Resmini, 1991). This process is accompanied by a dramatic increase in the extractability of the gluten proteins (Graveland, Bosveld, Lichtendonk, & Moonen, 1980; Tanaka & Bushuk, 1973; Veraverbeke, Courtin, Verbruggen, & Delcour, 1999). The development of a gluten protein network during dough mixing can be monitored with recording dough mixers such as the Farinograph and the Mixograph (Walker & Hazelton, 1996). During mixing, the resistance of dough mixing first increases, then reaches an optimum and finally decreases during what is called 'over-mixing'. During *dough fermentation*, further changes in the gluten protein network are evident from the observation that gluten proteins again become less extractable (Graveland *et al.*, 1980; Veraverbeke *et al.*, 1999). The gluten protein network in the fermenting dough plays a major role in retaining the carbon dioxide produced during fermentation and during the initial stages of baking.

The quantity and quality of gluten proteins largely determine dough mixing requirement and sensitivity to over-mixing. Furthermore, they determine the rheological properties of the optimally mixed dough (Fig. 3) and as such contribute to the gas retention properties of the fermenting dough (Gan, Ellis, & Schofield, 1995). Gas retention properties in turn determine loaf volume and crumb structure of the resulting bread. Two important factors are believed to largely determine gluten protein quality in breadmaking (Fig. 3).

The first factor is the *gliadin/glutenin* ratio of the gluten proteins. This is a direct consequence of the fact that, within the visco-elastic gluten protein network of wheat flour doughs, gliadins and glutenins fulfil a different role. Due to their large size, glutenin polymers form a continuous network that provides strength (resistance to deformation) and elasticity to the dough (Belton, 1999; Ewart, 1972). On the other hand, the monomeric gliadins are believed to act as plasticisers of the glutenin polymeric system. In this way, they provide plasticity/viscosity to wheat flour doughs (Cornec, Popineau, & Lefebvre, 1994; Khatkar, Bell, & Schofield, 1995). For quality breadmaking, an appropriate balance between dough viscosity and elasticity/strength is required. Up to a certain limit, higher dough strength

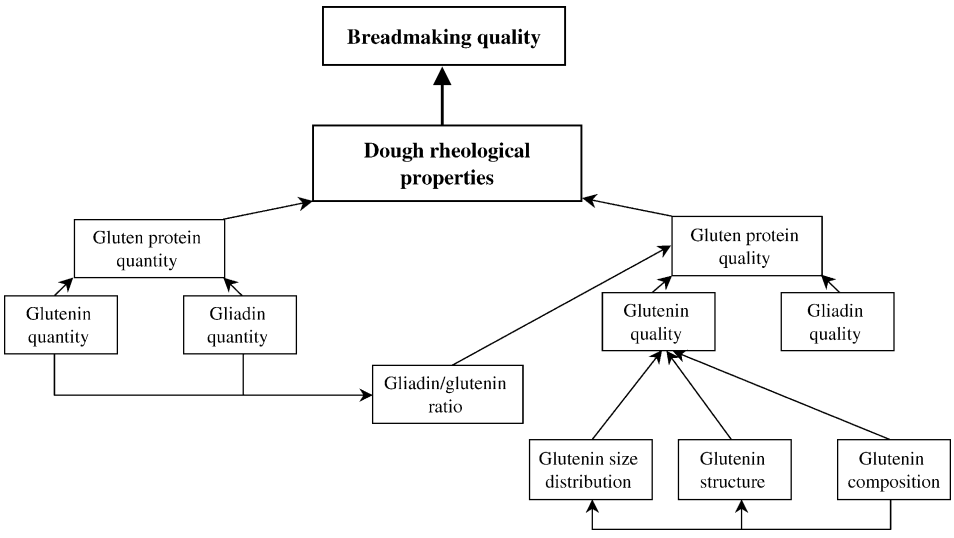

Fig. 3. Factors governing breadmaking quality and wheat dough rheological properties (adapted from Veraverbeke & Delcour, 2002).

increases loaf volume. However, dough rise is hindered with doughs that are too strong.

The second factor in gluten protein quality is the *quality* of its (extractable as well as unextractable) *glutenin fraction*. While differences in gliadin properties might certainly also have an effect, it is now generally believed that differences in glutenin properties are more important in explaining gluten protein quality differences in breadmaking. Although a lot of questions remain because of the lack of detailed knowledge of the molecular structure of glutenin and its contribution to elasticity, it can be assumed that differences in glutenin functionality in breadmaking result from differences in (i) composition, (ii) structure and/or (iii) size distribution of the glutenin polymers (Fig. 3). Firstly, differences in glutenin composition may result in differences in the non-covalent interactions that determine the elasticity of glutenin. Each wheat variety contains between 3 and 5 different HMW-GS (Shewry *et al*., 1992) and an estimate between 7 and 16 different LMW-GS (Gupta & Shepherd, 1990). Knowing that more than 20 different HMW-GS and more that 40 different LMW-GS (Gupta & Shepherd, 1990) have been detected so far in different wheat varieties, this explains an enormous variation in glutenin composition between different wheat varieties. Secondly, although it is hard to hypothesise on this matter because of the poor knowledge of the structure of glutenin, it can be assumed that (even subtle) differences in the structure of glutenin largely affect glutenin functionality in breadmaking. To a certain extent, differences in structure of glutenin may also result from differences in glutenin composition. For example, if the glutenin structure is indeed branched, as suggested from its rheological behaviour (Dobraszczyk & Morgenstern, 2003), GS composition may determine the degree of branching since some GS would allow for branching while others would not (Veraverbeke & Delcour, 2002). Thirdly, based on polymer theories, only the polymers above a certain size would contribute to the elasticity of the glutenin polymer network (MacRitchie, 1992; Singh & MacRitchie, 2001). This corresponds well with several reports in the literature on positive correlations between dough strength/breadmaking performance and levels of the unextractable/least extractable glutenin fractions and/or the largest glutenin polymers (Singh & MacRitchie, 2001; Veraverbeke & Delcour, 2002). As with the glutenin structure, differences in the glutenin size distribution may also (at least partly) be attributed to differences in GS composition. Size differences of GS, resulting in variations in *e.g.* HMW-GS/LMW-GS ratio, and/or different numbers of cysteine residues available in GS for cross-linking, influencing *e.g.* the ratio of 'chain terminator' GS (only one cysteine residue available for cross-linking) to 'chain extender' GS (two or more cysteine residues available for cross-linking), may significantly affect glutenin size distribution.

The baking phase

During baking, dramatic changes occur in the gluten proteins that are probably a combination of changes in protein surface hydrophobicity, sulphydryl/disulphide interchanges and formation of new disulphide cross-links (Jeanjean, Damidaux, & Feillet, 1980; Lavelli, Guerrieri, & Cerletti, 1996; Schofield, Bottomley, Timms, & Booth, 1983; Weegels, de Groot, Verhoek, & Hamer, 1994). As a result of these heat-induced changes as well as those of the starch, the typical foam structure of baked bread is formed.

The aging bread

In contrast to the widely accepted view that starch retrogradation plays a major part in bread staling, the role of gluten proteins in this process is still not clear. It is generally believed that starch–gluten interactions are somehow involved in bread firming (Gray & BeMiller, 2003). They may be qualitatively more important (Martin *et al*., 1991) or of equal importance (Every *et al*., 1998) than starch–starch interactions. This way, the formation of hydrogen bonds between gelatinised starch granules and the gluten network in bread ties together the continuous protein network and the discontinuous granule remnants (Martin *et al*., 1991). However, Maleki, Hoseney, and Mattern (1980) postulated that the flour component primarily responsible for differences in staling rate of bread is gluten and that its role in staling is something other than dilution of starch. In contrast, several researchers found no significant correlation between protein concentration and quality and crumb firmness (Gray & BeMiller, 2003). In this respect, the staling rate of a gluten-free bread was similar to a normal bread (Morgan, Gerrard, Every, Ross, & Gilpin, 1997).

Ways to affect gluten protein functionality during breadmaking

Oxidising and reducing agents

As stated above, the functionality of gluten in wheat flour breadmaking is strongly determined by the molecular weight of glutenin, the occurrence of covalent and non-covalent bonds between glutenin molecules and interactions between glutenin and other flour constituents. The most prominent linkages are disulphide bonds as they hold together the glutenin subunits. Hence, oxidising and reducing agents which have a strong impact on the dough thiol-disulphide system can affect the polymerisation of glutenin subunits and thereby change the mechanical and rheological properties of the dough (Fitchett & Frazier, 1986).

Already very low concentrations of endogenous glutathione drastically weaken dough and increase extensibility through thiol/disulphide interchange. Chemical oxidising agents, such as potassium iodate and potassium bromate, are able to reform some of the intermolecular disulphide bonds of gluten proteins restoring dough strength. Especially bromate, the slowest acting of these two oxidants, has been one of the most common bread

improvers. Whereas iodate mainly acts during dough kneading, the effect of bromate occurs during baking (Fitchett & Frazier, 1986). Nowadays, the use of bromate has been overshadowed by toxicological studies implicating that it could be carcinogenic and has largely been banned from the baking industry (Dupuis, 1997). Azodicarbonamide, acetone peroxide and L-threo-ascorbic acid (vitamin C) are often used as replacement oxidant and source of the replacement oxidant dehydroascorbic acid, respectively. The action of azodicarbonamide in dough is to contribute to the formation of a cohesive dry dough that can tolerate high water absorption while resulting in a good texture and volume of the loaf. During dough preparation, it is rapidly and completely converted to the metabolically inert, non-toxic and non-carcinogenic biurea which is stable in bread (Fitchett & Frazier, 1986). Acetone peroxide is regarded as an alternative to azodicarbonamide. However, in contrast to azodicarbonamide, acetone peroxide is active in dry flour.

L-threo-Ascorbic acid is the stereoisomer of ascorbic acid that most strongly enhances strength, handling and baking properties of dough. This stereospecificity suggests that at least one enzyme contributes to the effect of L-threo-ascorbic acid. In addition to chemical oxidation mediated by iron and copper ions, L-threo-ascorbic acid is oxidised enzymically by an endogenous ascorbic acid oxidase which uses molecular oxygen as an electron acceptor (Grosch, 1986). This results in L-threo-dehydroascorbic acid and hydrogen peroxide. The oxidised ascorbic acid acts as an electron acceptor in the oxidation of endogenous glutathione by wheat flour glutathione dehydrogenase drastically reducing the level of reduced glutathione which can weaken the dough (Fitchett & Frazier, 1986; Grosch, 1986).

In addition to the above discussed wheat flour endogenous oxido-reduction system, also exogenous oxidising enzymes such as tyrosinase, laccase, glucose oxidase, soybean lipoxygenase, glutathione oxidase and sulphydryl oxidase can impact dough and bread characteristics because of their impact on gluten (Reinikainen, Lantto, Niku-Paavola, & Buchert, 2003). Tyrosinase is able to oxidise accessible tyrosine residues in proteins to *o*-quinones which are able to condense with each other or with amino and sulphydryl groups of proteins in non-enzymic reactions. It is able to radicalise different aromatic components which can then react with each other non-enzymically (Reinikainen *et al*., 2003). During the oxidation of β-D-glucose to D-glucuronic acid, glucose oxidase produces hydrogen peroxide which can affect gluten as discussed above. The positive effect of soybean lipoxygenase (type II) on dough mixing tolerance, dough rheology and loaf volume can be ascribed to the formation of peroxyradicals. However, how these radicals react with gluten is far from understood. Glutathione oxidase and sulphydryl oxidase catalyse the formation of disulfide bonds. While the former oxidase is specific for glutathione and releases hydrogen peroxide, the latter may create highly cross-linked gluten fractions (Reinikainen *et al*., 2003).

Reducing agents such as L-cysteine and sodium metabisulfite may be added to weaken the dough structure. By reducing dough resistance to deformation, they help in moulding and shape forming without structural changes.

L-Cysteine can also be used in combination with oxidants. During kneading, cysteine cleaves disulphide bonds, which facilitates the gluten protein distribution in the dough and improves dough extensibility. Once a good gluten distribution is achieved the oxidant restores dough strength (Fitchett & Frazier, 1986).

Proteases

Another way to impact covalent interactions in the gluten network is the degradation of proteins with proteases. This reaction is not reversible: the peptide bonds and the original rheological condition cannot be restored, which is in contrast with the impact of chemical reducing agents. Proteases can be added to shorten mixing time, to reduce dough consistency, to regulate gluten strength in bread, to assure dough uniformity, to control bread texture, to solve baking problems in special flours (biscuits, wafers) and to improve flavour (Mathewson, 2000). When the reduction of mixing time is the objective of the application, an endo-acting enzyme is preferred since cleavage of internally located peptide bonds has a more dramatic rheological effect. On the other hand, liberation of free amino acids by exo-acting enzymes will have a great influence on colour of the product because the free amino acids readily undergo Maillard-type reactions with reducing sugar to form pigments. Exoproteases may also contribute to the flavour and aroma of the product since many flavours undoubtedly result from Maillard-type reactions with sugars and various fermentation intermediates.

Transglutaminases

Unlike proteases, transglutaminases (EC 2.3.2.13) build up new inter- and intramolecular bonds, which enable them to better tolerate the negative effects that can occur with overdoses of traditional baking enzymes. The enzyme catalyses acyl-transfer reactions, introducing covalent cross-links between the L-lysine and L-glutamine amino acid residues. Transglutaminase is a relatively new enzyme used in the manufacture of baked goods (Gottmann & Sprössler, 1994, 1995; Reinikainen *et al*., 2003).

Emulsifiers and shortening

Emulsifiers are fat-based ingredients that function both as dough stabilisers when the emulsifier interacts with the gluten protein in the dough and as crumb softeners when the emulsifier complexes with the gelatinising starch during baking (*cf*. supra). The emulsifiers with the best dough stabilising effect, such as diacetyl tartaric esters of monodiglycerides (DATEM) and ethoxylated

monoglycerides (EMG), are usually the worst crumb softeners, while the emulsifiers with the best crumb softening effect (monoacylglycerols) are usually inferior dough strengtheners (Stampfli & Nersten, 1995). The dough strengthening effect is not completely understood. It has been suggested that these emulsifiers are able to form liquid, lamellar films between the gluten and the starch, thus improving the film forming properties of the gluten (Stampfli & Nersten, 1995).

It has been known for some time that the addition of small amounts of *shortening* to bread dough leads to improved loaf volume and finer, more uniform crumb structure with thin cell walls. None of the numerous hypotheses discussed in literature provides an acceptable explanation for the mechanism.

Non-starch polysaccharides

In cereal science, non-starch polysaccharides (NSP) is a generic term for arabinoxylans (AX), β-glucan, cellulose and arabinogalactan-peptides, *i.e.* polysaccharides that differ from amylose and amylopectin either by the nature of their composing monosaccharides and/or by the nature of their linkages (Henry, 1985). Health promoting effects have been ascribed to some of these dietary fibre components (Lanza, Jone, Block, & Kessler, 1987). Up to 75% of dry matter weight of wheat endosperm cell walls consists of NSP of which AX are by far the most prominent group (85%) (Mares & Stone, 1973a,b). In contrast to what might be expected for a structural component of the cell wall, one-fourth to one-third of the 1.5–2.5% AX found in wheat flour endosperm is water-extractable (Meuser & Suckow, 1986). Their structure and aspect result in unique physicochemical properties which strongly determine their functionality in breadmaking. The latter and thus their impact on the breadmaking process can be modulated by means of xylanolytic enzymes.

Arabinoxylans and their structural and physicochemical properties

Although wheat endosperm AX can be divided in two polydisperse groups of water-extractable (WE-AX) and water-unextractable AX (WU-AX), one general structure applies. AX are made up of β-1,4-linked D-xylopyranosyl residues, substituted at the C(O)-3 and/or the C(O)-2 position with monomeric α-L-arabinofuranoside (Perlin, 1951a,b). Ferulic acid can be coupled to the C(O)-5 of arabinose through an ester linkage (Fausch, Kündig, & Neukom, 1963) (Fig. 4). An important parameter of AX is the arabinose to xylose (A/X) ratio, with a typical average value of 0.5–0.6 for the general wheat WE-AX population (Cleemput, Roels, Van Oort, Grobet, & Delcour, 1993), but extreme values of 0.31–1.06 for WE-AX subfractions (Dervilly, Saulnier, Roger, & Thibault, 2000). WE-AX are considered to consist of alternating strongly branched and open regions of which different proportions explain the

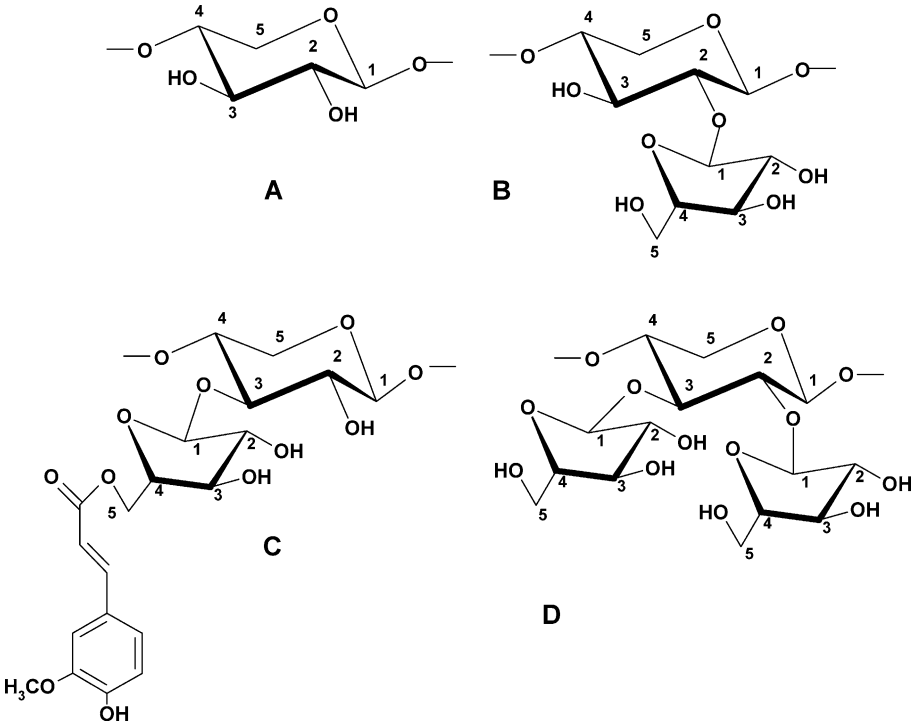

Fig. 4. Structural elements of AX: (A) non-substituted D-xylopyranosyl residue; (B) D-xylopyranosyl residue substituted on C(O)-2 with a L-arabinofuranosyl residue; (C) D-xylopyranosyl residue substituted on C(O)-3 with a L-arabinofuranosyl residue; (D) D-xylopyranosylresidue substituted on C(O)-2 and C(O)-3 with L-arabinofuranosyl residues. Structure C shows the link of ferulic acid to C(O)-5 of a L-arabinofuranosyl residue.

above-mentioned variation in A/X ratio (Goldschmid & Perlin, 1963; Gruppen, Komelink, & Voragen, 1993). The structure of WU-AX after alkaline extraction (AS-AX) largely corresponds to that of WE-AX (Gruppen, Hamer, & Voragen, 1992; Gruppen *et al*., 1993; Meuser & Suckow, 1986). Only small differences in molecular weight (Meuser & Suckow, 1986) and A/X ratios (Gruppen *et al*., 1993) were reported.

AX has unique physicochemical properties. The solubility of WE-AX, AS-AX and enzymatically solubilised AX (ES-AX) in water or water–ethanol mixtures is favoured by high substitution degrees (Andrewartha, Phillips, & Stone, 1979; Neukom, Providoli, Gremli, & Hui, 1967), substitution patterns with high proportions of substituted regions (Andrewartha *et al*., 1979; Rybka, Sitarski, & Raczynska-Bojanowska, 1993) and low molecular weights (Courtin & Delcour, 1998). WE-AX, AS-AX and ES-AX can form highly viscous solutions, the viscosity of which obviously depends on AX chain length, substitution degree and substitution pattern (Izydorczyk & Biliaderis, 1995). Approximately two-third of the intrinsic viscosity of flour extracts is attributed to WE-AX (Udy, 1956). Under oxidising conditions, WE-AX can cross-link by covalent coupling of two ferulic acid residues (Figueroa-Espinoza, & Rouau, 1998; Vinkx, Van Nieuwenhove, & Delcour, 1991). This results in a strong viscosity increase of the AX solution or, at high AX concentrations, the formation of a gel (Izydorczyk, Biliaderis, & Bushuk, 1990). Furthermore, WE-AX stabilise protein films against thermal disruption but lower initial foam formation. Both phenomena can presumably be attributed to an increase in liquid viscosity (Izydorczyk, Biliaderis, & Bushuk, 1991; Sarker, Wilde, & Clark, 1998) and the mediation of interactions between proteins in the absorbed layer (Sarker *et al*., 1998). WE-AX are reported to possess surface active properties, possibly attributed to associated proteins (Eliasson & Larsson, 1993). WU-AX are cross-linked in the cell wall structure and therefore water-unextractable (Iiyama, Lam, & Stone, 1994; Lam, Iiyama, & Stone, 1992). As such, they have high water holding capacity. This property is equally attributed to WE-AX. It has been estimated that in freshly prepared dough, AX hold up to one quarter of the water (Atwell, 1998).

Arabinoxylan functionality in breadmaking

The dough stage

WE-AX added to *dough* increase dough consistency and stiffness and decrease mixing time (Jelaca & Hlynca, 1972). On a same dough consistency basis, WE-AX addition increases baking absorption (Biliaderis, Izydorczyk, & Rattan, 1995; Jelaca & Hlynca, 1971, 1972; Kulp, 1968), does not affect (Kulp, 1968) or increases mixing time (Biliaderis *et al*., 1995; Jelaca & Hlynca, 1971; Michniewicz, Biliaderis, & Bushuk, 1991), lowers the energy input to achieve optimal mixing (Jelaca & Hlynca, 1971) and enhances resistance to extension and decreases extensibility (Jelaca & Hlynca, 1972). WE-AX of high average molecular weight (M_r 201,000–555,000) exert greater effects on baking absorption and development time than their lower molecular weight counterparts (M_r 50,000–134,000) (Biliaderis *et al*., 1995; Courtin & Delcour, 1998). Addition of WU-AX has similar effects as that of WE-AX (Jelaca & Hlynca, 1971; Kulp, 1968; Michniewicz *et al*., 1991), but does not alter dough extensibility properties (Jelaca & Hlynca, 1971; Kulp & Bechtel, 1963). A positive correlation between flour WU-AX level and baking absorption was equally shown for endogenous WU-AX through fractionation-reconstitution breadmaking experiments (Courtin, Roelants, & Delcour, 1999). Using this approach, extensibility decreased and resistance to extension increased with increasing flour WU-AX content (Courtin *et al*., 1999). This would feed the hypothesis that the WU-AX rich cell wall fragments interfere with optimal gluten formation during dough mixing.

According to Hoseney (1984), WE-AX function somewhat as gluten during *fermentation* as they slow down the diffusion rate of carbon dioxide out of the dough, thus contributing to gas retention. However, they lack elastic properties. Presumably, WE-AX increases dough foam stability because they increase the viscosity of the dough aqueous phase which in its turn stabilises the gas cells liquid films (Gan *et al*., 1995). Others attributed the positive impact of WE-AX to the formation of a secondary, weaker network enforcing the gluten network (Jelaca & Hlynca, 1972). Upon addition of WU-AX, Kulp and Bechtel (1963) observed that gas retention and evolution of dough were similar to those of the control dough. This observation is in contrast to the postulated negative impact of WU-AX which suggests that they: (i) destabilise gas cells by forming physical barriers for gluten during dough development, (ii) absorb a large amount of water which consequently is not available for gluten development and film formation, and/or (iii) perforate the gas cells which causes them to coalesce (Courtin & Delcour, 2002; Courtin *et al*., 1999).

The baking phase

It is assumed that, during the initial phase of baking, AX affect breadmaking by mechanisms equal to those observed for fermentation. Stabilisation of gas cells by WE-AX will prolong the oven rise and improve bread characteristics (crumb firmness, structure and texture, loaf volume) (Gan *et al*., 1995), while WU-AX enhance gas cell coalescence and decrease gas retention, resulting in poorer bread quality (Courtin & Delcour, 2002). Indeed, fractionation-reconstitution experiments demonstrated that loaf volume was increased both when decreasing the WU-AX content and increasing the level of WE-AX of medium and high molecular weight in dough (Courtin *et al*., 1999).

The aging bread

As outlined above, during storage, bread stales and it is generally accepted that this is mainly due to starch retrogradation. According to some authors, the presence of AX may sterically interfere with the starch intermolecular associations and, hence, may lower retrogradation (Kim & D'Appolonia, 1977a,b). Others attribute the effect of AX on bread staling mainly to their strong effect on the water distribution in dough (Biliaderis *et al*., 1995; Eliasson & Larsson, 1993; Gudmundsson, Eliasson, Bengston, & Åman, 1991). After all, on the one hand, the rate of starch retrogradation depends on the amount of available water while, on the other hand, the available water may act as a plasticiser in the gluten–starch matrix (Levine & Slade, 1990).

Endoxylanases affect arabinoxylan functionality in breadmaking

Whatever be the functionality of different AX-fractions in breadmaking, on a commercial scale it is at present not possible to change dough properties by AX addition, due to the lack of industrially feasible AX isolation procedures and, therefore, the lack of commercial AX products. Optimisation of AX functionality in breadmaking, however, can be obtained through the use of selected microbial endoxylanases (EC 3.2.1.8), *i.e.* enzymes that are able to hydrolyse the AX xylan backbone internally. Endoxylanases are mainly classified in glycoside hydrolase families 10 and 11 (Coutinho & Henrissat, 1999; Henrissat, 1991), with different structures and catalytic properties (Biely, Vrsanskà, Tenkanen, & Kluepfel, 1997; Jeffries, 1996; Törrönen & Rouvinen, 1997). Although endoxylanases of both families have similar catalytic residues and mechanisms, family 10 endoxylanases are regarded as less specific and more catalytically versatile, releasing shorter fragments than family 11 endoxylanases. The latter enzymes are more easily impeded by the arabinose substituents of AX (Biely *et al*., 1997; Jeffries, 1996).

Specific microbial endoxylanases are routinely used in breadmaking to improve dough properties (softness, stability, elasticity and extensibility) (Courtin, Gelders, & Delcour, 2001; Rouau, El-Hayek, & Moreau, 1994), oven spring (Sprössler, 1997) and loaf volume (Courtin *et al*., 2001; Rouau *et al*., 1994; Sprössler, 1997). The impact of endoxylanases on bread staling remains a controversial issue (Courtin *et al*., 2001; Martínez-Anaya, & Jiménez, 1997b).

The impact of endoxylanases on AX functionality in breadmaking strongly depends on their selectivity towards WE-AX and WU-AX substrates (Courtin *et al*., 2001). In view of the above-mentioned functionalities of WE-AX and WU-AX in breadmaking, it can easily be understood that optimal use of endoxylanases that preferentially attack the WU-AX, generally causing a reduction in the level of WU-AX and an increase in the level of solubilised AX, has a positive impact on dough and bread properties (Courtin *et al*., 2001, 1999) (Fig. 5). More specifically, the use of a suitable endoxylanase in the first place increases dough stability. This means that during fermentation the dough retains its optimal volume for a longer period of time and is more resistant to mechanical stress. Furthermore, it implies that during the initial stage of baking the oven rise is significantly prolonged leading to a higher loaf volume and a finer, softer and more homogeneous bread crumb (Courtin *et al*., 2001, 1999). In contrast, endoxylanases that preferentially hydrolyse WE-AX or solubilised AX, thus reducing their molecular weight, yield little if any improvement (Courtin *et al*., 2001, 1999) (Fig. 5). In both cases, excessive levels of endoxylanase result in slack and sticky doughs and loaves with poor crumb structure and colour, gas cell distribution and crust colour. This is due to an extensive degradation of the overall AX population, and the

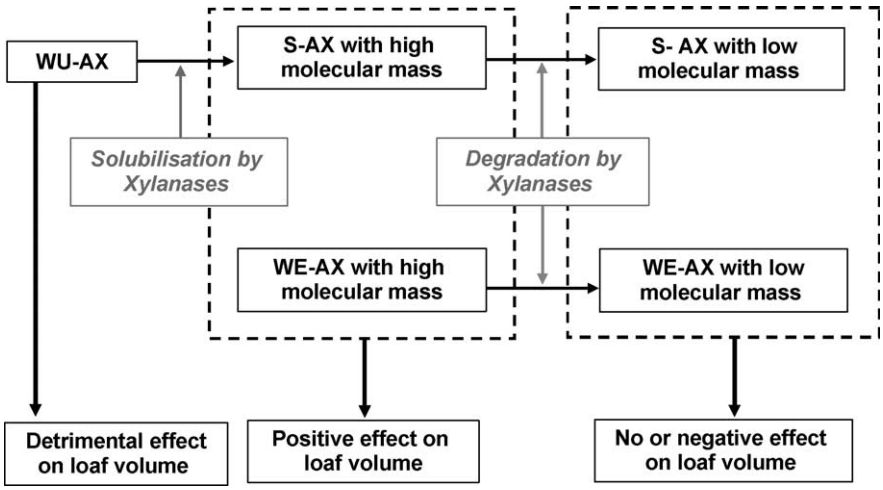

Fig. 5. Schematic representation of the enzymic degradation of AX by endoxylanases and the effect of the different AX fractions on bread loaf volume (based on Courtin et al., 1999).

accompanying loss in water holding capacity of the dough (Courtin *et al*., 2001, 1999; McCleary, Gibson, Allen, & Gams, 1986; Rouau *et al*., 1994).

A second factor which, besides endoxylanase substrate selectivity, is able to strongly influence endoxylanase functionality in breadmaking is their sensitivity towards endoxylanase inhibitors. Indeed, endoxylanases are, to variable extent, prone to inhibition by cereal endoxylanase inhibitors. These proteins have been recently reviewed (Gebruers *et al*., 2004; Goesaert *et al*., 2004; Juge, Payan, & Williamson, 2004). In wheat, two structurally different endoxylanase inhibitors have been identified, *i.e. Triticum aestivum* endoxylanase inhibitor (TAXI) (Debyser, Peumans, Van Damme, & Delcour, 1999; Gebruers *et al*., 2001) and endoxylanase inhibiting protein (XIP) (McLauchlan *et al*., 1999). While TAXI inhibits family 11 endoxylanases (Gebruers *et al*., in press; 2001), XIP has a bias for fungal family 10 and 11 endoxylanases (Flatman *et al*., 2002). Inhibitor concentrations in wheat flour are in the range of 15 mg/kg or higher (Gebruers, Brijs, *et al*., 2002) for both TAXI and XIP and can vary two- to three-folds depending on variety (Gebruers, Courtin, *et al*., 2002). These endogenous inhibitor levels will almost invariably exceed the level of endoxylanase added for bread-improving purposes, potentially resulting in significant reduction of the activity of the inhibition sensitive endoxylanases frequently used in breadmaking (Gebruers, Courtin, *et al*., 2002). In the dough phase, inhibition kinetics determine to a large extent the impact of the inhibitors. Some authors postulate that the inhibitors may affect the substrate selectivity of the endoxylanase by inhibiting enzymic degradation of WE- and WU-AX fractions to different degrees (Sibbesen & Sørensen, 2001). Recently, a mutant *B. subtilis* endoxylanase XynA was developed by molecular engineering which is insensitive towards wheat endoxylanase inhibitors and has improved breadmaking functionality (Sørensen, 2003).

Lipids

Classification

The lipids in wheat flour originate from membranes, organelles and spherosomes (membrane-bound emulsion droplets) and comprise different chemical structures. Based on solubility in selective extraction conditions, they are classified as starch lipids and free and bound non-starch lipids (Eliasson & Larsson, 1993; Hoseney, 1994). *Non-starch lipids* (NSL) comprise approximately 2/3–3/4 of the total wheat flour lipids and consist predominantly of triglycerides, as well as of other non-polar lipids, and digalactosyl diglycerides (DGDG). The non-polar lipids are mainly present in the free NSL fraction, while the glyco- and phospholipids are mainly associated with proteins and present in the bound NSL fraction (Eliasson & Larsson, 1993; Hoseney, 1994). Lysophospholipids, in particular lysophoshatidylcholine or lysolecithin, are the major constituents of the *starch lipids*. They are important minor constituents typical of cereal starches in levels positively correlated to amylose content (0.8–1.2% for wheat starch) (Morrison & Gadan, 1987). As a result, amylose–lipid complexes are formed during gelatinisation of cereal starches. In addition, Morrison, Law, and Snape (1993) indicated that these complexes occur in native starches as well.

The fatty acid pattern of the flour lipids is dominated by linoleic acid (C18:2) with lower amounts of palmitic (C16:0) and oleic acids (C18:1) (Eliasson & Larsson, 1993; Hoseney, 1994).

Lipid functionality in breadmaking

It is well known that flour lipids, in particular the NSL fraction, significantly affect the breadmaking quality. Starch lipids are too strongly bound in the starch granules and are essentially unavailable to affect dough processing before starch gelatinisation occurs. When non-polar wheat lipids are added back to defatted flour, bread loaf volume is reduced. This observation has been ascribed to free fatty acids. Polar lipids can have a similar detrimental effect, but at higher concentrations, they increase loaf volume (Eliasson & Larsson, 1993). In addition, the ratio of non-polar to polar lipids and the galactolipid content of the free NSL are strongly correlated with loaf volume (Gan *et al*., 1995; Matsoukas & Morrison, 1991). Presumably, lipid functionality is related to their effect on the stability of the gas cells. In this respect, the positive influence of the polar lipids is attributed to their ability to form lipid monolayers at the gas/liquid interphase of the gas cells, thus increasing the gas retention of the dough (Gan *et al*., 1995). Furthermore, polar flour lipids positively contribute to dough handling properties as well (Graybosch, Peterson, Moore, Stearns, & Grant, 1993).

In addition, during dough mixing two processes occur which affect the lipids and hence the breadmaking performance of the flour. First, most of the free NSL 'bind' to gluten or the starch granule surface and, as a consequence, their extractability is reduced (Addo & Pomeranz, 1991; Chung & Tsen, 1975). Secondly, polyunsaturated fatty acids are oxidised by wheat lipoxygenase, yielding hydroxyperoxides and free radicals. These compounds can oxidise other constituents, such as proteins and carotenoids, thus affecting dough rheological properties and crumb colour (Hoseney, 1994).

Lipases affect lipid functionality in breadmaking

The use of lipases (EC 3.1.1.3) in breadmaking is quite recent when compared to that of other enzymes. Lipases hydrolyse the ester bonds of (mainly) the triglycerides, yielding mono- and diglycerides and free fatty acids. In particular, 1,3-specific lipases, which preferentially remove fatty acids from the 1- and 3-positions, improve dough

rheological properties as well as the quality of the baked product (Olesen, Qi Si, & Donelyan, 1994; Qi Si, 1997) and may provide an alternative for the use of chemical dough strengthening emulsifiers. Lipases increase dough strength and stability, thus improving dough machinability. Furthermore, because they increase volume and result in an improved, more uniform crumb structure, crumb softness is improved (Olesen *et al*., 1994; Qi Si, 1997). In addition, lipases may have anti-staling properties (Johnson & Welch, 1968; Olesen *et al*., 1994).

Presently, the positive effects of lipases in breadmaking are still insufficiently understood. Part of the released polyunsaturated fatty acids are oxidised by the wheat lipoxygenase (Castello, Jollet, Potus, Baret, & Nicolas, 1998). Furthermore, it has been postulated that lipases modify the interaction between the flour lipids and gluten (Olesen *et al*., 1994). The increased level of monoglycerides, which can form amylose–lipid complexes, has been suggested to retard staling (Johnson & Welch, 1968). However, the production of monoglycerides *in situ* is probably insufficient to explain all reported effects (Qi Si, 1997).

A new lipase with a high activity towards the polar lipids has recently become commercially available. Its dough stabilising effect can be related to the selectivity and rate of hydrolysis (Christiansen, Vind, Borch, Heldt-Hansen, & Spendler, 2003). Other lipolytic enzymes may improve breadmaking as well. Indeed, phospholipase A (EC 3.1.1.4), which liberates one fatty acid from phospholipids, is claimed to improve dough handling properties, to suppress dough stickiness and to increase loaf volume (Inoue & Ota, 1986).

Conclusions

The transformation of flour to a loaf of bread, which then stales during storage, comprises a series of complex chemical, biochemical and physical processes, which are not completely understood. Flour and bread processing and bread quality are affected to a variable extent by the various flour constituents, which in their turn can be affected in many ways by a variety of additives. Nowadays, breadmaking and bread quality are mainly optimised by changing starch and NSP (AX) properties, which is mostly done by addition of selected enzymes, and/or by influencing the gluten functionality, which is usually performed by the use of oxidising and reducing agents. However, despite much research, the use of these additives (alone or in different combinations) is still considerably empirically driven. Therefore, a lot of research is still needed to gain more insight into the role of various wheat constituents in breadmaking and how to affect their functionality. Undoubtedly, this will be aided by the use of new, well-characterised enzymes and additives and more precise equipment.

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