MECHANISM OF GAS CELL STABILITY IN BREADMAKING

by

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B.S., Punjab Agricultural University, India, 1999
M.S., Punjab Agricultural University, India, 2001

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
College of Agriculture

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Abstract

Expansion of dough and hence breadmaking performance is postulated to depend on a dual mechanism for stabilization of inflating gas bubbles. Two flours were used in this study, one from the wheat variety Jagger (Jagger) and the other from a composite of soft wheat varieties (soft). The primary stabilizing mechanism is due to the gluten-starch matrix surrounding the bubble. The secondary mechanism operates when gas bubbles come into close contact during later proofing and early baking. When discontinuities occur in the gluten-starch matrix surrounding gas bubbles, thin liquid lamellae stabilized by adsorbed surface active compounds, provide a secondary stabilization.

A key parameter in the primary stabilizing dough film is thought to be the property of strain hardening. Jagger flour gave higher test-bake loaf volume than soft wheat flour and higher strain hardening index for dough. Rheological properties of doughs were varied by addition of protein fractions prepared by pH fractionation. Fractions were characterized by SE-HPLC and MALLS. The molecular weight distribution (MWD) of fractions progressively shifted to higher values as the pH of fractionations decreased. Mixograph peak development time paralleled the MWD. However, the strain hardening index and the test-bake loaf volume increased with increasing MWD up to a point (optimum), after which they declined. At a given strain rate the behavior at the optimum appeared to result from slippage of the maximum number of statistical segments between entanglements, without disrupting the entangled network of polymeric proteins. Shift of MWD to MW higher than the optimum results in a stronger network with reduced slippage through entanglement nodes, whereas a shift to lower MWs will decrease the strength of the network due to less number of entanglements per chain.

In order to study the secondary stabilizing mechanism, different lipid fractions were added incrementally to the defatted flours. No effects were observed on the rheological properties of the dough. However, large effects on the loaf volume were measured. The additives used were the total flour lipid and its polar and non polar fractions and the fatty acids palmitic, linoleic and myristic. Polar lipids and palmitic acid had positive or little effect on loaf volume respectively. Non polar lipid, linoleic and myristic acids had negative effects on loaf volume.

The different effects of the lipid fractions are thought to be related to the type of monolayer that is formed. Polar lipid and palmitic acid form condensed monolayers at the air/water interface whereas non polar lipid, linoleic and myristic acids form expanded monolayers.

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Approved by:

Major Professor
Dr. Finlay MacRitchie

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Dedication

To my Mom and Dad

CHAPTER 1 - Introduction

Bread is the most widely consumed food product around the globe, making the bread industry worth billions of dollars. Technology of breadmaking is probably as old as the very first human civilizations. Since then it has improved by leaps and bounds. Science of breadmaking has been benefited by developments in engineering, chemistry, biochemistry, material science, polymer science, etc. The approaches of these fields relevant to breadmaking have helped understand properties and behavior of various ingredients used in breadmaking. The focus has always been to improve loaf volume and crumb grain. It is this cellular structure of wheat bread, which gives it a soft spongy texture – a reason for its great demand.

J.C. Baker in the 1940s did some groundbreaking research and discovered that the foundation for cellular structure is laid during mixing when air is incorporated in the form of small gas cells. During baking, it is these gas cells that are converted to foam and then to sponge, thus making air a vital bread ingredient. Since then, much work has been done by cereal scientists to improve cellular structure and ensure its stability during breadmaking. Various separate studies looked into rheology of wheat flour dough or the surface activity of wheat flour components such as proteins and lipids, as a requirement for gas cell stability. There has been no concerted effort to reveal the mechanism-in-total that stabilizes the gas cell during breadmaking. The array of divergent views and hypotheses therefore complicated the puzzle rather than resolving it. Nevertheless, some of these explanations, if looked at in conjunction with each other do seem plausible.

Based on one such hypothesis that proposes the presence of a dual film of gluten-starch matrix and liquid lamellae around expanding gas cells, the present study was designed with the following objectives:

1. To investigate and seek evidence for the presence of liquid lamellae and their ability to stabilize gas cells i.e. whether or not the dual film hypothesis holds true.
2. If the presence of liquid lamellae is confirmed as a support for expanding gas cells, to understand the possible mechanism by which the gas cells are stabilized.

3. To understand rheological properties of the gluten-starch matrix in relation to its molecular structure-function behavior required to stabilize expanding gas cells.

Understanding of the mechanism by which these building blocks i.e. gas cells are stabilized has numerous advantages for the food processing industry and agricultural research. Some more obvious ones are: maintaining high and consistent quality of leavened cereal products, by overcoming problems like environmental damage to wheat (e.g. heat stress, sulfur deficiencies, etc) and other batch to batch variations in raw material; utilization of underutilized cereals, e.g. sorghum that will also assist in achieving required agricultural diversification; new product development e.g. health breads for celiac patients; and help to focus on specific genetic manipulation to produce wheat and other cereals capable of producing high quality leavened end products.

CHAPTER 2 - Literature Review

2.1 Air: An Important Bread Ingredient

Bread is mainly relished due to its spongy texture. A recipe normally has no mention of one important ingredient i.e. air, which is incorporated from the atmosphere during the initial physical process of mixing to which flour, water and other ingredients are subjected. It is this air that provides sponginess to bread. Thus, only those flours and ingredients are used in manufacture of bread with desired texture and volume, which can incorporate and retain air/gas or assist in this during the entire process of breadmaking.

Among different cereal flours, wheat is capable of giving the best texture breads due to its ability to retain the incorporated air (He and Hoseney, 1991). It is during mixing that the air is occluded in the form of small nuclei/cells (Baker and Mize, 1946) in the liquid phase of the dough (MacRitchie, 1976a). The number of gas cells occluded during mixing is $10^2 – 10^5$ per mm^3 (Bloksma, 1990b). With relative volume (total volume of dough to volume of dough without air) of gas occluded being 1.07 after mixing, the diameter of these gas cells is quite small and it is appropriate to refer to them as gas nuclei at this stage of breadmaking. No further occlusion of gas in the form of cells occurs in succeeding stages (sheeting, molding, proofing, etc.) (Baker and Mize, 1946). These gas cells expand during proofing due to release of fermentation gases into them, and during baking due to expansion of gases as temperature increases. As the size of gas cells increases, the size distribution of gas cells skews towards larger ones (Gan et al., 1990; Li and Dobraszczyk, 2004). Subsequent sheeting or molding causes subdivision of already existing gas cells, thus improving their number and size distribution (MacRitchie, 1976a).

The proofing and baking stages of bread making are characterized by fast biaxial expansion of gas cells, expanding at strain rates of $10^{-3} – 10^{-4}$ s^{-1} and $10^{-2} – 10^{-3}$ s^{-1}, respectively (Dobraszczyk, 1997). The stability and extent of expansion of gas cells at these stages determines final volume and crumb structure of the bread. Ideal bread is one which has high volume and uniform gas cell size distribution i.e. thin walled, elongated, and small to medium sized gas cells.

2.2 Developing Visco-Elastic Dough and Gas Occlusion

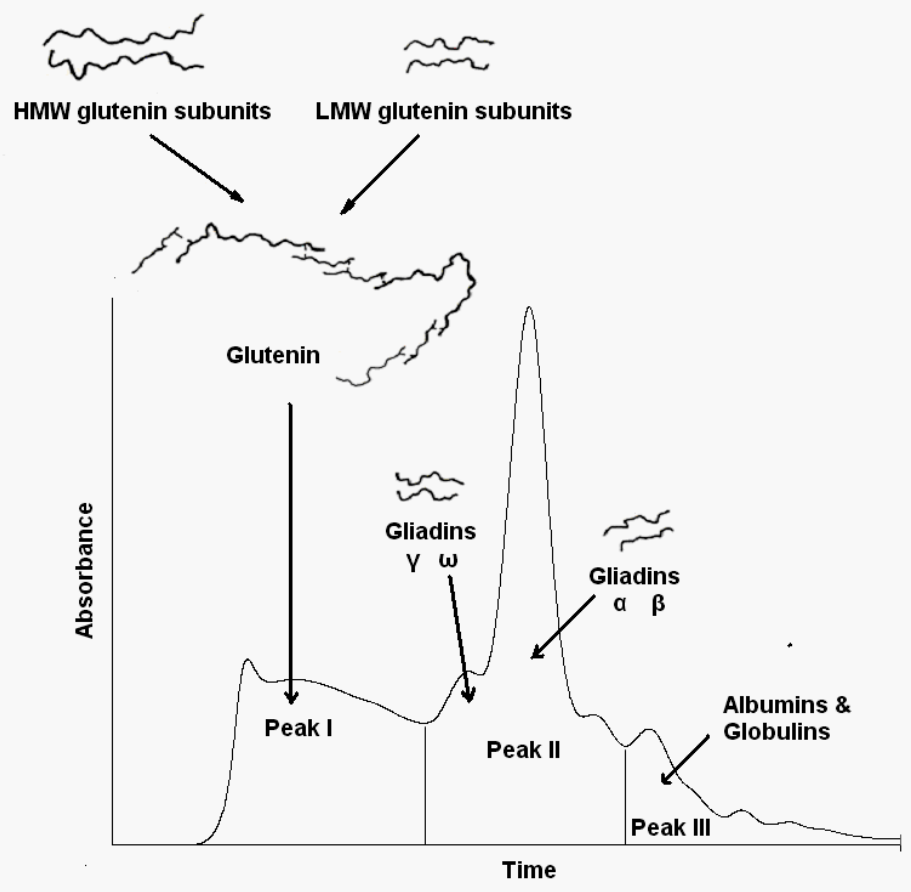
2.2.1 Molecular Weight Distribution (MWD) of Flour Proteins – Basis of Visco-Elasticity

Wheat flour is most widely used for bread production, as it is unique in giving visco-elastic dough. Based on polymer rheological concepts (Singh and MacRitchie, 2001), visco-elasticity of wheat flour dough is attributed to the components of gluten protein; the large MW proteins (MW in the range of 100,000 to 10,000,000) are also known as polymeric proteins or glutenins as glutenin is a major polymeric protein fraction, and the small MW fraction or monomeric proteins are called gliadins (MW range between 20,000 to 70,000) (MacRitchie, 1987; MacRitchie, 1992; Southan and MacRitchie, 1999). Glutenins are formed by polymerization of high-molecular weight subunits (HMW-GS) (80,000-120,000) and low-molecular weight subunits (LMW-GS) (30,000-55,000) and are eluted first during size-exclusion high-performance liquid chromatographic (SE-HPLC) analysis (Fig. 2.1) (MacRitchie and Lafiandra, 1997) Gliadins (α, β, γ and ω) do not polymerize, due to structural limitations (Wrigley, 2004), and are eluted later in the chromatogram (MacRitchie, 1992; MacRitchie, 1999; Southan and MacRitchie, 1999). The ratio of LMW-GS to HMW-GS is approximately 3:1 (MacRitchie, 1992) and that of gliadins to glutenins is about 60:40.

Some observations of high MW synthetic polymers can help us better understand how MW and MWD of wheat flour proteins affect dough rheological properties (Singh and MacRitchie, 2001). Fig. 2.2 (Kraus and Gruver, 1965) shows structure-function relationship of a linear polymer (polybutadiene) and how changes in MW affect its rheological properties (zero shear viscosity, μ_0). M_C is a critical MW beyond which linear chains are capable of forming entanglements, which are physical constraints that restrict relative motion of polymers. When MW increase above M_C there is rapid increase in μ_0, as is evident from the slope of the second part and at any given MW in this region $\mu_0=MW^{3.4}$. Bersted and Anderson derived a relationship between tensile strength and MW and MWD of polydispersed polymers (Bersted and Anderson, 1990). According to them, stable and effective entanglements are formed by those polymers, which have MW greater than a threshold MW (M_T) and it is this fraction i.e. with MW greater than M_T that is responsible for tensile strength of polymers. Stable entanglements are possible if

$$M_T > 2 \times M_C \qquad (2.1).$$

Fig 2.1 SE-HPLC elution profile of total wheat proteins extracted using SDS buffer, pH 6.9, sonicated at 6 W for 15s, and with 1% SDS/NaPhos, pH 7.0 as mobile phase. Peaks I, II, III, correspond to glutenins, gliadins, and albumins/globulins. Diagramatic representation illustrates how HMW and LMW subunits polymerize to give polymeric proteins (Adopted from; MacRitchie and Lafiandra, 1997).



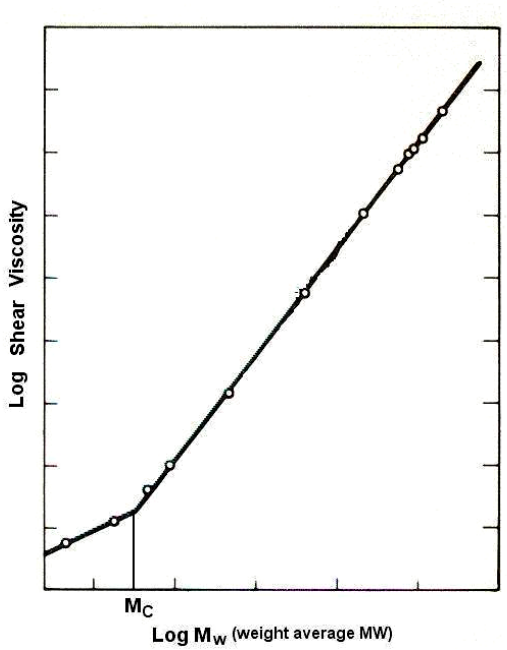
M_T for glutenins has been estimated to be 250,000 by SE-HPLC and Extensograph analysis (Bangur et al., 1997), assuming similar conformation for glutenin polymers and standard proteins that were used for calibration. The value of M_T will vary with concentration of polymer and type of solvent (Ferry, 1980). Equation 2.1 can be used to calculate the approximate value of M_C for glutenin proteins:

$$M_C \leq 125{,}000 \qquad (2.2).$$

The large glutenin polymers (MW > M_T) open up due to mixing (reviewed in the later part of this section) and entangle with each other, thus inhibiting relative motion of polymers and conferring elastic behavior. On the other hand, smaller gliadins act as plasticizers between

entangled glutenin networks allowing some relative motion, conferring viscous behavior. The underlying polymeric basis of each physical process will be reviewed under respective sections of this chapter.

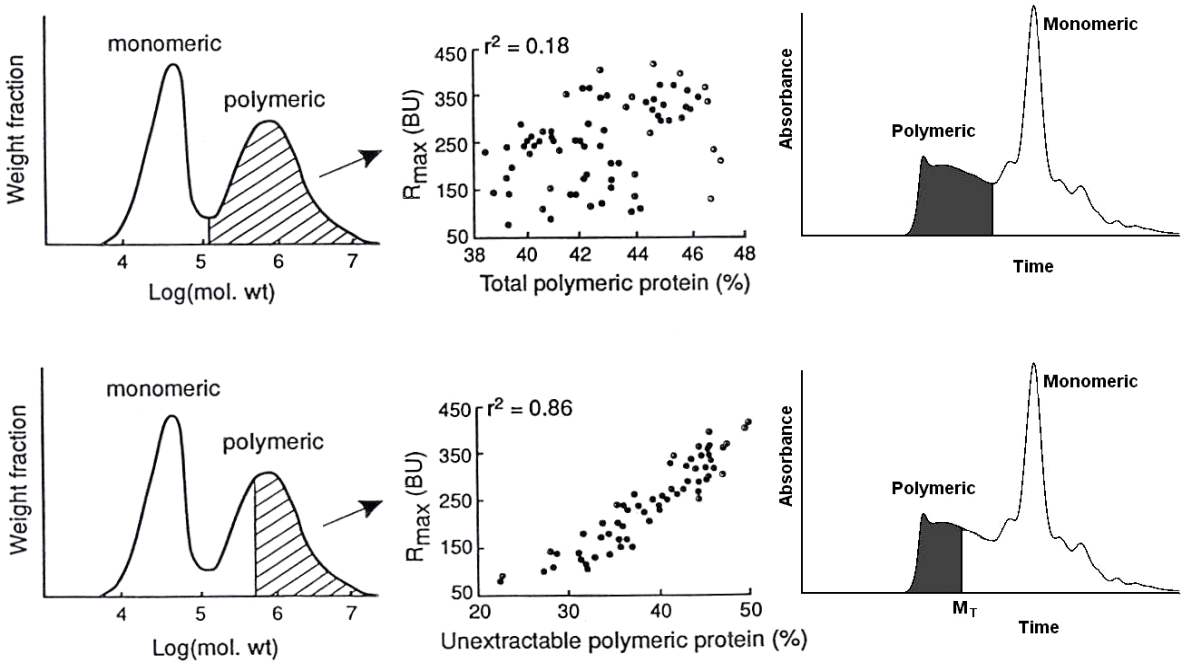
Fig 2.2 Effect of molecular weight (MW) on shear viscosity of linear polybutadiene polymers. MWs greater than the critical MW for entanglements (M_c) give a sharp rise in shear viscosity as $MW^{3.4}$ (Kraus and Gruver, 1965).



The MWD of total wheat proteins and that of its polymeric fraction is therefore a major deciding factor of a dough's rheological properties. A portion of polymeric fraction of total wheat proteins is insoluble in 0.5% SDS solution owing to its high MW and is referred to as unextractable polymeric protein (UPP) (Gupta et al., 1993). Based on the Osborne fractionation of gluten proteins, some studies have reported these as insoluble glutenin/polymeric proteins (IPP) i.e. the fraction that remains unextractable by 0.1N acetic acid after the extraction of albumins, globulins and gliadins (Khan and Bushuk, 1978). UPP and IPP are more or less the same fraction of total polymeric proteins with MW ≥ 158,000 as reported by (Gupta et al., 1993). Physical properties of wheat dough, like Mixograph development time, Extensograph maximum resistance (R_{MAX}), and loaf volume show strong positive correlation with percentage UPP and

not so significant correlation with percentage total polymeric protein (Fig. 2.3) (Khan and Bushuk, 1978; Gupta et al., 1990; Gupta et al., 1993; Park et al., 2006). The percentage UPP and MWD of this portion of the total polymeric fraction depends on the relative proportions of HMW and LMW subunits. As the ratio of LMW to HMW subunits decreases the percentage UPP increases and its MWD is skewed towards higher MW. Effect of subunit composition due to allelic variations on MWD of wheat proteins and physical properties of the dough have been reviewed in detail, elsewhere (MacRitchie and Lafiandra, 2001). We can now rephrase the first statement of this passage as; the rheological properties of wheat dough are determined by the relative proportion of UPP and the MWD of this fraction of total polymeric proteins.

Fig 2.3 Schematic representation showing that only part of the polymeric protein fraction with molecular weight greater than a threshold molecular weight (M_T) is highly correlated with R_{max}. M_T estimated to be ≥ 250,000 (Bangur et al., 1997), while UPP estimated to be ≥ 158,000 (Gupta et al., 1993), (Adopted from: MacRitchie and Lafiandra, 1997).



2.2.1.1 Determination of MWD

The ideal method for MWD determination of wheat proteins is one which is capable of resolving the highest MW fraction, since physical properties of wheat dough are highly

correlated with this fraction. A procedure using sonication and elution through a size-exclusion column of high-performance liquid chromatography (SE-HPLC) (Singh et al., 1990; Batey et al., 1991) is most widely used. It is capable of resolving total wheat endosperm proteins into three separate peaks (Fig. 2.1); (I) a polymeric (glutenins), (II) a monomeric (gliadins), and (III) a soluble (albumins and globulins) protein peak. Sonication treatment for a short time (15 to 30 seconds at 6 watts output) can solubilize very large sized glutenins without changing the chromatogram, as the sound energy is just sufficient to cause degradation of very large sized polymers only (Singh et al., 1990) and help achieve their solublization. The separation of proteins in SE-HPLC is based on their hydrodynamic radii, the largest being excluded first through a column packed with porous beads, while smaller ones get trapped in the pores and are eluted later (Autran, 1994). This separation is not a representation of weight-average MW (M_w) of proteins because, apart from mass of the protein, hydrodynamic radius also depends on conformation of individual protein molecules in solution. Nonetheless, the size-exclusion profile of wheat proteins is a good indication of their number-average MW. Despite the high resolving power (up to 10^6 - 10^7) of modern size-exclusion columns, some of the very high MW fraction, with MW greater than the upper limit of the column remains unresolved and is eluted in the first minute or so, known as the void volume (Autran, 1994; Carceller and Aussenac, 2001; Ueno et al., 2002). Some smaller polymeric, and/or monomeric proteins may also be eluted in the void volume in complexes with the largest ones (Ueno et al., 2002). By using calibration standards, we can approximate the MW (M_w) of protein molecules eluted at different times in SE-HPLC, but there may be some uncertainity.

Multiangle laser light scattering (MALLS) technique has been used in conjunction with SE-HPLC analysis to overcome the limitations of the size-exclusion chromatographic procedure (Bean and Lookhart, 2001; Carceller and Aussenac, 2001). The laser light scattered from individual wheat protein molecules is detected by an array of photodiodes in a flow cell and is related to the M_w of the proteins (Bean and Lookhart, 2001). The M_w profile as determined by MALLS can be overlaid on the SE-HLPC profile and provides a good description of MWD of wheat proteins (Bean and Lookhart, 2001). The importance of knowing M_w is that the rheological properties of the polydispersed polymers are more related to M_w of the polymeric fraction with MW greater than M_c.

Another more recent technique to determine MWD of wheat endosperm proteins is flow field-flow fractionation (Stevenson and Preston, 1996). The procedure also uses difference in hydrodynamic radii of the proteins for size based separation. As solvent with proteins flows down the channel, the largest MW proteins having lower diffusivities flow near the bottom of the channel and relatively smaller ones in a layer above them. This leads to formation of different layers according to MWs (Southan and MacRitchie, 1999). The smallest will lead at the front of parabolic flow and are eluted first, while solvent percolates through a membrane on the lower part of the channel. The procedure overcomes void volume limitations of SE-HPLC (Stevenson And Preston, 1996; Stevenson et al., 2003) and can be coupled with MALLS (Stevenson et al., 2003; Lemelin et al., 2005).

2.2.2 Mixing: A Key Bread Making Stage

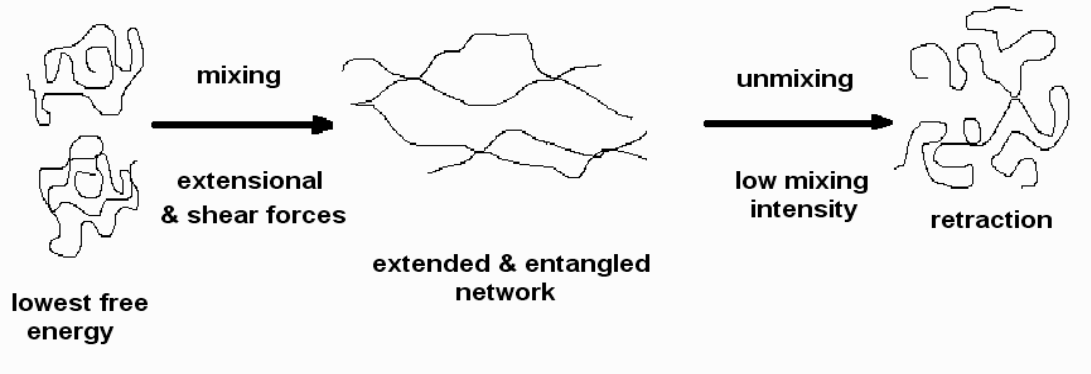
The first step in breadmaking is mixing of flour and other ingredients with water. Objectives of mixing are; to develop a nearly homogenous and visco-elastic dough and occlusion of gas cells in the form of small nuclei.

2.2.2.1 Developing a Visco-Elastic Dough

Mixing is characterized by decrease in intensity and scale of segregation (MacRitchie, 1986). At a molecular level, mixing is characterized by extension (stretching) of glutenin polymers and entangling of these stretched polymers (Fig. 2.4) (MacRitchie, 1986). This is achieved by work input and mixing intensity that must be above a certain minimum critical level (Kilborn and Tipples, 1972; Tipples and Kilborn, 1975). Initially in flour particles, glutenin polymers are present in the form of coiled structures at lowest free energy. When flour is hydrated with water and mixed at an intensity above the critical level, the randomly coiled structures, experiencing extensional and shear forces, begin to open up and are stretched out and oriented in the direction of shear in the form of long chains. Polydispersed glutenins have a range of MWs with the smallest ones probably opening up first and the largest ones at the end (MacRitchie, 1992). The onset of the development stage probably begins with extension of those glutenin polymers, which have MW greater than M_c, and due to continuous mixing action, these begin to entangle with each other, thus increasing the viscosity of the system. Due to the ability of elongated molecules to recoil to their lowest free energy state, elastic restoring forces are generated. Entanglement coupling prevents this retraction and elasticity is maintained even

during resting (Singh and MacRitchie, 2001). At a given work input and intensity of mixing, peak mixing time, referred to as Mixograph dough development time (MDDT) probably corresponds to the extension and entangling of the largest glutenin molecules (Singh and MacRitchie, 2001). This might be a reason why we are not able to form dough with gliadin and starch alone. The rheological properties of developed dough are these of a continuous gluten starch matrix in which starch acts as a filler.

Fig 2.4 Diagrammatic representation of changes in polymeric proteins of wheat flour in response to mixing intensity i.e. for optimum and lower intensity mixing (MacRitchie, 1986).



The work input and mixing intensity, of the flour is therefore function of its MWD. It has been observed that when the percentage of UPP increases and/or the MWD of the polymeric fraction is shifted to higher MWs, the MDDT increase, as requirements for work input and mixing intensity increase (Gupta et al., 1993; Park et al., 2006). MacRitchie and co-workers (MacRitchie, 1987; Lundh and MacRitchie, 1989; Gupta, 1990; MacRitchie et al., 1991) reported quite similar results when gluten fractions of increasing molecular weight, obtained by pH fractionation (MacRitchie, 1985) of wheat gluten, were added back to the base flour. The MDDT increased with a shift of MWD to higher MWs. However, in certain cases, i.e. upon addition of the latest fractions (MacRitchie, 1987; Lundh and MacRitchie, 1989), MDDT reached a maximum and then decreased. This has been attributed to the presence of large MW globulin-like proteins in these latest fractions (MacRitchie, 1987). Another explanation for the decrease in MDDT with addition of latest fractions of the largest MW glutenins is probably that the mixing action (mixing intensity/strain rate) is not sufficient to fully develop the dough

(Kilborn and Tipples , 1972; Tipples and Kilborn, 1975). The same work input and mixing intensity (in other words the same strain rate) was used in the study to determine mixing behavior of the flours. Presence of very high MW glutenins and decrease in polydispersity will significantly increase strain rate requirements to extend the largest glutenins (Singh and MacRitchie, 2001).

2.2.2.2 Air Occlusion

The development of visco-elasticity in wheat dough seems to be necessary for the accompanying phenomenon of air occlusion in the form of gas cells. The air occlusion takes place during the later stages of mixing (Baker and Mize, 1946) when dough has attained sufficient visco-elasticity and a continuous liquid phase is present (MacRitchie, 1976a). Air probably is beaten into the liquid phase of the dough, by virtue of the mixing action, and is retained in the form of gas cells. Some minimum visco-elasticity is required to retain occluded gas cells, a reason why other cereals like rice and corn lose the occluded air and are not able to produce breads with good loaf volume and crumb grain (He and Hoseney, 1991). The shearing action during mixing may cause further subdivision of occluded air into small gas nuclei.

At this stage, two things are very important for sufficient gas occlusion and stability of occluded gas cells during the entire baking process. Firstly, the ease with which gas is occluded and this determines the concentration of gas cells, and secondly, uniformity of the size of occluded gas cells (MacRitchie, 1986); uniformity of size is important for stability of gas cells (vanVliet, 1995).

Rheology or visco-elasticity of the dough will affect the ease of gas cell occlusion. Baker and Mize (1946) found that weaker flours occlude more air and at a more rapid rates compared to strong ones. In addition, bread baked from dough mixed to the time when rate of air occlusion just begins to rise steeply, i.e. before peak mixing time, gives finer crumb grain compared to one mixed to the peak when the rate of air occlusion is highest (Baker and Mize, 1946). Increase in visco-elasticity, as occurs near peak mixing time, may also reduce the shearing action and prevent subdivision of gas cells, giving uneven gas cell size distribution. However, good visco-elastic behavior of strong flour may help in retaining the gas cells at later stages of breadmaking (Baker and Mize, 1946; MacRitchie, 1976a; Dobraszczyk et al., 2003).

Apart from visco-elasticity of the dough, surface properties of the liquid phase play a vital role in entrainment, and uniformity of gas cell size distribution. Importance of the liquid phase can be judged from the fact that reasonable bread volume begins to appear only when liquid in mixed dough is sufficient to be present as a continuous phase (MacRitchie, 1976a). The liquid phase of dough is rich in surface active components such as lipids and proteins. It is these surface active components that adsorb at the gas/liquid interface of occluded gas cells affecting surface properties and stability of the gas cells (MacRitchie and Gras, 1973; MacRitchie, 1976a; MacRitchie, 1986). Various studies on surfactants (Junge and Hoseney, 1981; Junge et al., 1981) show that these added ingredients improve loaf volume and crumb grain by incorporating more and smaller gas cells during mixing.

After mixing, sheeting is another dough development stage, when dough is subjected to large extensional strains (10 s^{-1}) (Dobraszczyk, 1997; Dobraszczyk and Morgenstern, 2003). This not only enhances visco-elastic properties of the dough but also subdivides the gas cells, increasing their concentration and uniformity of size.

2.2.2.3 Estimation of Occluded Air and Gas Cell Size Distribution

Density measurement is a simple procedure to determine the extent to which dough can incorporate and retain the air in the form of gas cells. This procedure has shown that strong flours occlude less air than weak flours during mixing (Baker and Mize, 1946; Campbell et al., 2001). For a particular flour, density measurements can be effectively used to discriminate between various ingredients and processes based on their ability to occlude air (Junge et al., 1981; Campbell et al., 2001).

Limitations of density measurements can be overcome by use of a technique called digital image analysis as it provides more detailed information such as gas cell size distribution. Image analysis at different stages of breadmaking can help us learn about the stability pattern during the entire process. Digital image analysis is a simple photographic procedure used to determine gas cell size distribution by evaluating differences in the intensity of reflected light with the help of a computer program (Sapirstein, 1999; Zghal et al., 1999; Rouille et al., 2005; Whitworth et al., 2005). However, this procedure has lower size limitation and can not determine cells smaller than one pixel i.e. 0.094 cm. C-Cell is the most widely used digital image analyzing equipment in bread research (Whitworth et al., 2005). Fast X-ray computed tomography is

another image analyzing technique that provides a three dimensional view of the cellular structure of bread (Babin et al., 2006). It can be easily used on the finished product; however, real time imaging needs to overcome many hurdles.

2.3 Theory of Gas Cell Stability

Loaf volume is a measure of the extent to which gas cells can expand without failure (He and Hoseney, 1991; Gandikota and MacRitchie, 2005). Image analysis of gas cell size distribution in bread and biscuit dough showed vast variations in two flours and at different stages of baking (Li and Dobraszczyk, 2004). At all stages of breadmaking and in final bread, biscuit flour has a greater number of coarser gas cells. In both flours at a later stage of proofing and during baking, the number of larger gas cells increased and that of smaller decreased; in other words, the cell size distribution was skewed towards larger ones. In order to produce good breads, stability of gas cells and uniformity of their size distribution needs to be maintained during the entire breadmaking process. To understand the reason for deviations from normal, it is important to know the physical factors affecting gas cell stability.

2.3.1 Physics of the Gas Cell Stability

As gas cells are occluded in the liquid phase of dough, various physical instability processes come into action; these have been discussed in detail elsewhere (vanVliet, 1995). Two most important, from a breadmaking point of view, are Ostwald ripening (or disproportionation) and coalescence.

Disproportionation is the expansion of larger gas cells at the cost of smaller ones in the vicinity. It occurs due to Laplace pressure differences between the gas cells. Laplace pressure (P) inside a gas cell is

$$P = 2\gamma/R \qquad (2.3).$$

Where γ is the interfacial tension at the gas-liquid interface and R is the radius of the gas cell (vanVliet, 1995). The pressure P inside smaller gas cells will be higher compared to larger gas cells. This leads to diffusion of gas from smaller gas cells to larger ones in the vicinity through the liquid phase of the dough (Hoseney, 1992; vanVliet, 1995). The gas from smaller gas cells, which are separated by a continuous gluten-starch matrix from larger ones, may not diffuse because of this stronger barrier to diffusion. According to equation 2.3, the pressure P required to

create a new gas cell will be infinitely large since radius R will be zero. This explains why new gas cells can not be created during yeast fermentation, when carbon dioxide (CO_2) is produced (Hoseney, 1992). During fermentation of yeast, the CO_2 will diffuse into the liquid phase of dough and, upon its saturation, CO_2 will diffuse into the gas cells. There will be preferential diffusion into larger gas cells due to lower pressure in them (Bloksma, 1990b). This explains why uniformity in gas cell size distribution is important for good loaf volume and crumb structure. As pressure inside the gas cells increases, the gas cells expand, normalizing the pressure.

If we consider uniform gas cell size distribution, then according to Bloksma (1990b), they will remain spherical until the relative volume (total volume of dough to volume of dough without air) of 3.83 is achieved. Beyond this point, gas cells will be pushed against each other and the shape of cells will become polyhedral. Since white breads normally have relative volumes of 4-5 (Bloksma, 1990b), we can expect cells to be polyhedral. This will happen during the last stages of proofing and/or during initial baking; i.e. oven spring, when maximum increase in volume occurs. It is at this stage that the gluten-starch matrix (vanVliet et al., 1992; Dobraszczyk and Roberts, 1994; Dobraszczyk et al., 2003) and/or the monolayer at the gas-liquid interface (Gaines, 1966; MacRitchie, 1976b; MacRitchie, 1990) may fail to allow further expansion. This leads to rupture of membranes (cell walls) separating two cells. If the gluten-starch matrix is not set, which occurs only during baking (Bloksma and Nieman, 1975; Bloksma, 1990a), the two cells will fuse together to form one with lower free energy and surface to volume ratio. This phenomenon is called coalescence.

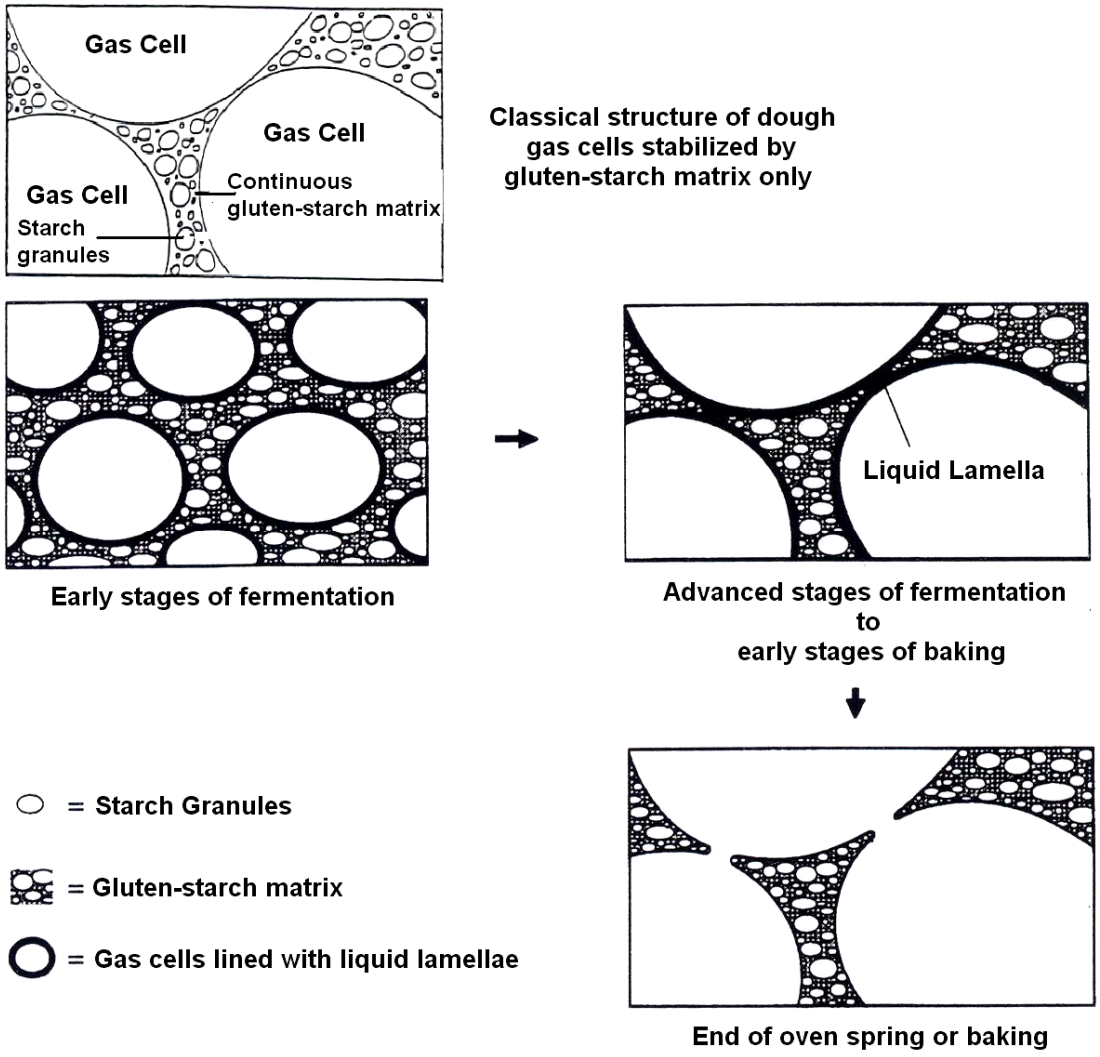
Thus to bake ideal bread with high loaf volume and good crumb grain, equal growth of gas cells needs to be ensured by preventing disproportionation and coalescence.

2.3.2 Hypothesis of Gas Cell Stability

Much work has been done to develop better understanding of gas cell stability in breadmaking (Baker and Mize, 1946; MacRitchie and Gras, 1973; DeStefanis and Ponte, 1976; MacRitchie, 1976a; MacRitchie, 1978; MacRitchie, 1980; Junge and Hoseney, 1981; Bloksma, 1990a; Bloksma, 1990b; Gan et al., 1990; He and Hoseney, 1991; vanVliet et al., 1992; Paternotte et al., 1993; Dobraszczyk and Roberts, 1994; Hayman et al., 1998). This led to emergence of mainly two schools of thought. One believes that it is entirely the gluten-starch

matrix that surrounds and stabilizes the gas cells (Bloksma, 1990b; Hoseney, 1992). According to this school, air is occluded in the form of gas cells into the gluten-starch matrix and not in the liquid phase of dough. In addition, during yeast fermentation, the CO_2 is saturated into the matrix from where it then diffuses into the gas cells. During baking, cross-linking of protein and gelatinization of starch increases viscosity and tensile stress in the matrix thus rupturing cell walls, transforming foam into sponge.

Fig 2.5 Dual film model by Gan and co-workers, showing role of gluten-starch matrix and liquid lamellae in stabilizing gas cells of bread dough. Liquid lamellae are secondary protection stabilizing gas cells when discontinuities in gluten-starch matrix appear. Figure on top shows classical structure of dough (Gan et al., 1990 and 1995).



Scanning electron micrographs of bread doughs (Gan et al., 1990) show the appearance of discontinuities near the end of the 50 min proofing in the Chorleywood bread process, with gas cells still being intact. Another study (Gandikota and MacRitchie, 2005) showed that dough can expand to its maximum capacity, which is an inherent property of the dough, and this expansion limit can be reached in the bake stage or during proofing or even under vacuum expansion. These results indicate the presence of a secondary stabilizing factor around expanding gas cells apart from the primary gluten-starch matrix. This is what the second school of thought believes. In a model proposed by Gan et al (1990), the expanding gas cells are stabilized against disproportionation and coalescence by the primary gluten-starch matrix with a secondary liquid lamella on its inner side, enveloping the gas cell (Fig. 2.5). The surface activity of compounds adsorbed at the gas-liquid interface of liquid lamellae, internal pressure and extensibility of the gluten-starch matrix determine integrity of the gas cells. The hypothesis seems plausible in view of evidence from various studies. MacRitchie (1976a) has shown that sufficient presence of a continuous liquid phase is required to get reasonable loaf volume. Further, in this study, analogous variations in foaming properties of dough liquor, and bread loaf volume and crumb structure were observed. Also, very low amounts (1 – 1.5% on flour weight basis) of natural lipid fractions produced significant effects on baking performance, with polar lipids improving loaf volume and crumb grain, whereas non-polar had detrimental effects (Ponte and DeStefanis, 1969; MacRitchie and Gras, 1973). All this confirms the presence of liquid lamellae and the surface action of compounds adsorbed at the gas-liquid interface of the lamellae.

2.4 Stability of Gluten-Starch Matrix

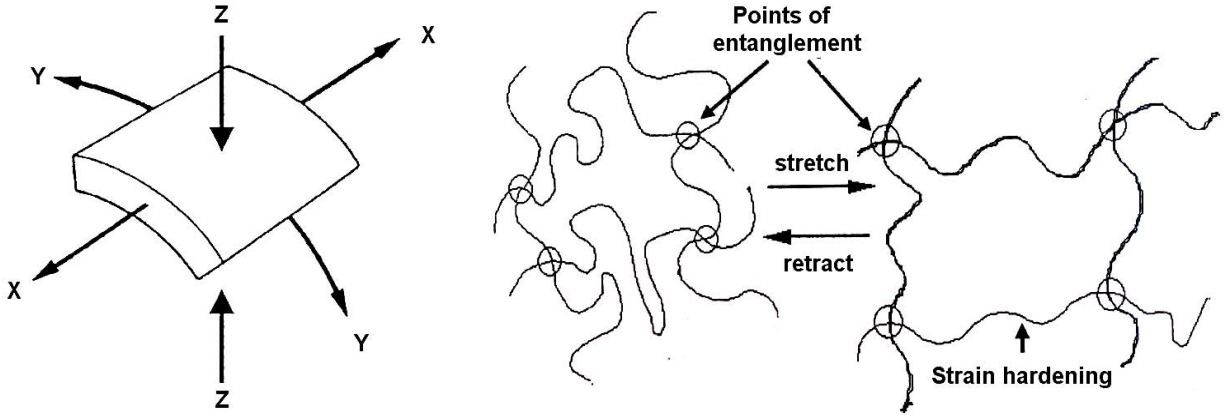
The gluten-starch matrix surrounding expanding gas cells in bread dough is a primary support and is vital to maintain equal growth of expanding gas cells. Stability of the gluten-starch matrix ensures uniform cell size distribution during proofing and baking, giving good crumb structure. This is achieved through the rheology of the gluten-starch matrix, which makes it extensible enough to allow desired expansion of the gas cells and strong enough to resist their collapse.

2.4.1 Rheology of Gluten-Starch Matrix

The gluten-starch matrix around a gas cell expands biaxially, due to excess pressure created in the gas cell by diffused CO_2 during proofing or due to thermal expansion of gases

during baking. The biaxial expansion means that the gluten-starch matrix is tangentially extended in two directions perpendicular to each other and radially compressed in a direction perpendicular to both tangential vectors (Fig. 2.6) (Dobraszczyk, 1999). Since the volume of the gluten-starch matrix remains constant, the strain in the radial direction is twice the strain in the planar direction. This causes thinning of gas cell walls. In case the gas cell wall continues to expand along this thin region, it may rupture. However, due to more than a proportional increase in stress compared to strain in the thin region, the thin region is stabilized against any further deformation or rupture and the gas cell continues to expand along the thicker parts of its wall. This phenomenon of localized increase of stress in response to strain, preventing failure of gas cell walls, is called strain hardening (vanVliet et al., 1992; Dobraszczyk and Roberts, 1994). Tendency to strain harden has been shown to be a good determinant of breadmaking potential of

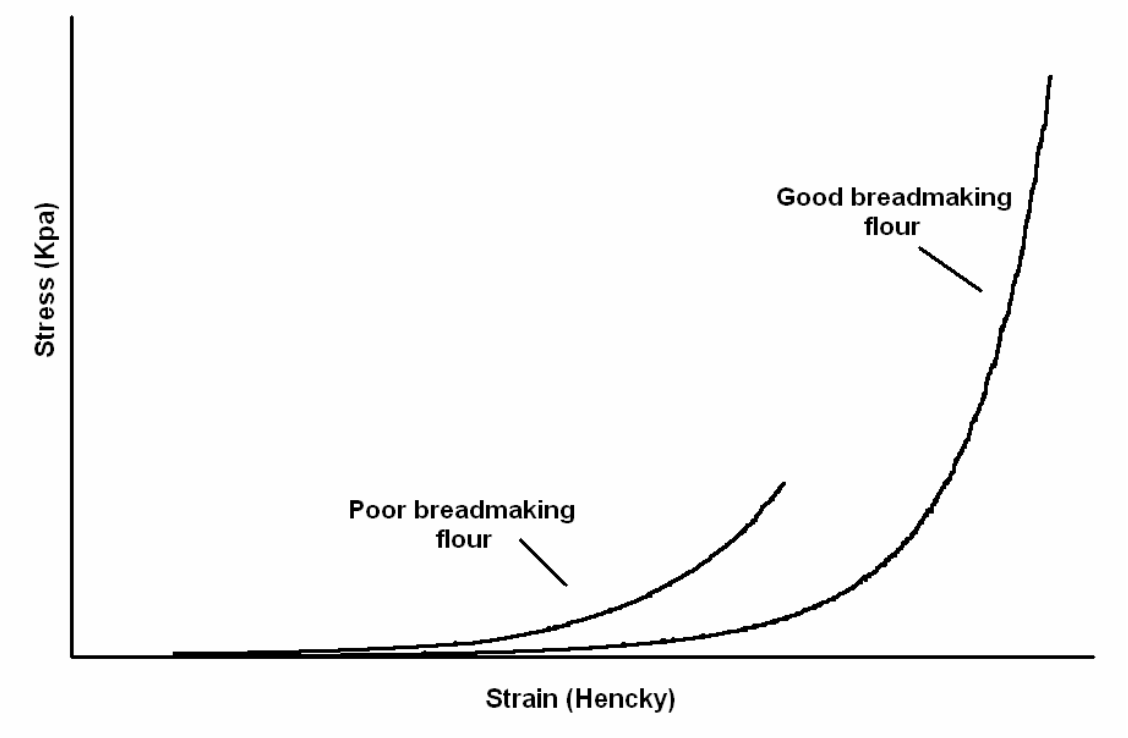
Fig 2.6 Diagrammatic representation of biaxial extension of the gluten-starch matrix surrounding expanding gas cells and corresponding changes in entangled polymer molecular network of wheat polymeric proteins (Adopted from: Dobraszczyk, 1997; Dobraszczyk and Morgenstern, 2003).



wheat flours (Dobraszczyk and Roberts, 1994; Kokelaar et al., 1996; Dobraszczyk, 1997). During baking, strain hardening has been observed to decrease with increase in temperature (Dobraszczyk et al., 2003). Nevertheless, good breadmaking doughs are able to retain this property at temperatures higher than those of poor breadmaking flours.

Apart from inherent dough properties, the tendency of the gluten starch matrix to strain harden depends on forces to which it is subjected and the rate of their application. The Considere

Fig 2.7 Stress vs. strain (Hencky) curves of poor and good bread making flours showing differences in their tendencies to strain harden. The poor breadmaking flour fails at relatively lower strains.



criterion for instability in extension of polymers has been used to predict strain hardening behavior of an expanding gluten-starch matrix (vanVliet et al., 1992; Dobraszczyk and Roberts, 1994). The criterion states that there is a critical value of strain for every dough at a given strain rate and if the gluten-starch matrix is stretched beyond this limiting strain it will continuously thin and rupture (Dobraszczyk and Roberts, 1994). The force at any point of thinning is

$$F = \sigma A \qquad (2.4).$$

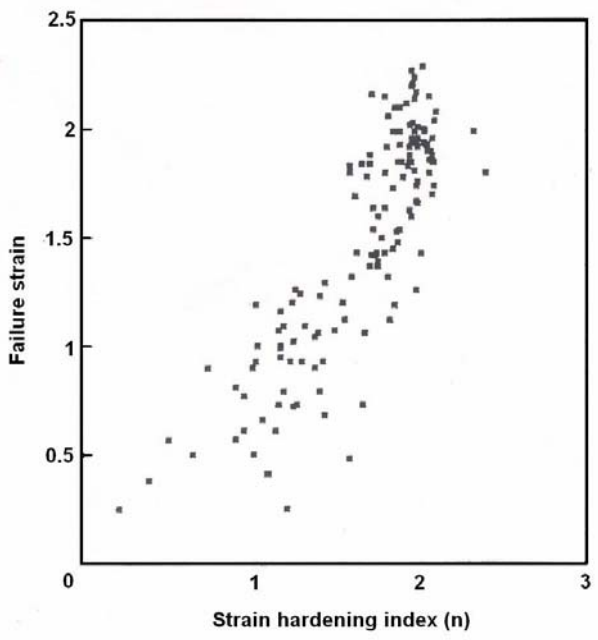
Where F is the applied force, σ is the true stress at the point of thinning and A is the area of cross section at the point. In equation 2.4, as cross-sectional area A in the thin region is reduced, the stress σ will increase, causing continuous thinning of the region. However, the thin region is protected against continuous thinning or failure as the force in this region increases more than the force in the thick region. This greater stress requirement of the thin region compared to the thick region prevents further expansion along the thin region. At any stage

during expansion, if the force in the thin region decreases, rupture of the gluten-starch matrix becomes inevitable (Dobraszczyk and Roberts, 1994).

A parabolic relation between stress and Hencky strain represents strain hardening (Fig. 2.7) (Dobraszczyk, 1999). The stress-strain curve of the gluten-starch matrix follows a power law

$$\sigma = K\,\varepsilon^{n} \qquad (2.5).$$

Fig 2.8 Failure strain vs. strain hardening index (n), obtained from bubble inflation data of different flours under a range of conditions, exhibiting a positive linear relation (Dobraszczyk and Roberts, 1994).



Where σ is the true stress, K is a constant, ε is strain and n is strain hardening index. Here, n must be greater than 1 in order to have some parabolic relation between stress and Hencky strain as shown in Fig. 2.7. In case the value of n is equal to 1, if not less than 1, power law equation 2.5 becomes the equation of a straight line with no strain hardening effect. Therefore, for gas cells to be stabilized, the strain hardening index must be greater than 1 (Dobraszczyk and Roberts, 1994; Dobraszczyk, 1999), allowing the gas cells to expand to larger volumes. It has been observed that the maximum strain also known as critical strain (ε_{crit}) at

which instability occurs is directly related to the strain hardening index n of the dough (Dobraszczyk and Roberts, 1994).

$$n = \varepsilon_{crit} \qquad (2.6).$$

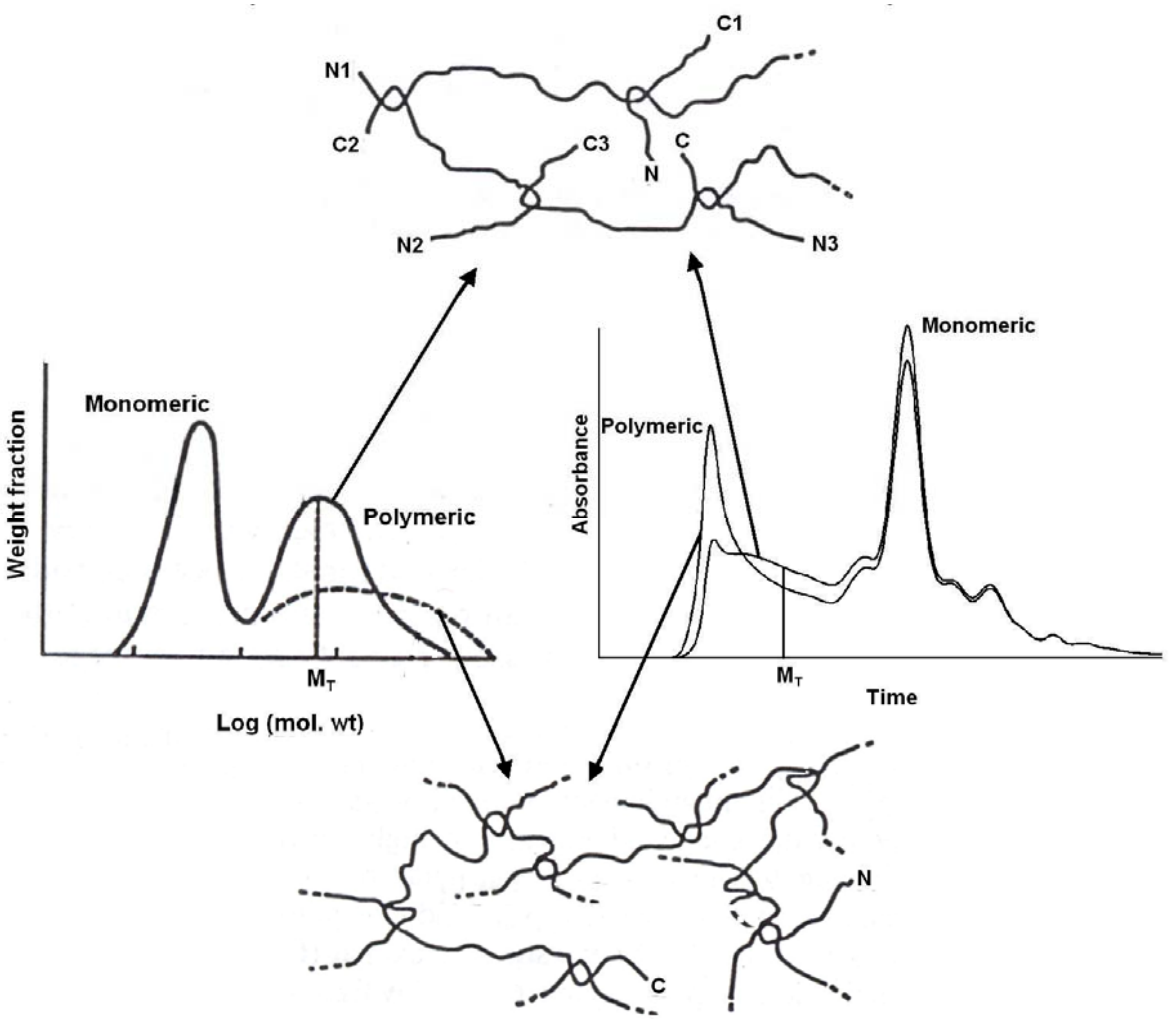
This shows that as the failure strain increases, the strain hardening index and thus the tendency to strain harden also increases. Dobraszczyk and Roberts (1994) found a linear relation between strain hardening index n and failure strain (Fig. 2.8) at different strain rates, temperatures and dough development conditions.

2.4.2 Polymer Molecular Basis of Strain Hardening

The concept of strain hardening explains the requirements for maximum inflatability of gas cells in breadmaking without failure or rupture to give good loaf volume and crumb grain. In a gluten-starch matrix, gluten proteins form a continuous network and the rheology of the matrix is that of a continuous gluten protein network. It is only the fraction of glutenins with MW greater than M_T (equation 2.1) that confers strength, whereas smaller ones act as diluents preventing additional physical constraints or entanglements and confer viscosity (Bersted and Anderson, 1990; MacRitchie, 1992; Singh and MacRitchie, 2001). As MWD shifts towards larger glutenins, the strength increases (Fig. 2.3) (Gupta et al., 1993). To get maximum inflatability of the gas cells, the gluten-starch matrix around them must stretch to its maximum extensibility without breaking. For this purpose, we must have sufficient concentration of large glutenins/ polymeric proteins ($MW > M_T$) and these must be stretched to their maximum length through entanglements. It has been well recognized that the underlying process determining rheology of large polymers is due to physical interaction of large polymer molecules (Ferry, 1980). Chemistry might be playing some role but the cumulative effect of physical interactions is much larger, such that chemical interactions can be ignored.

As the gluten-starch matrix surrounding expanding gas cells biaxially extends, the gluten protein network is also biaxially stretched (Fig. 2.6). This leads to breakage of non covalent interactions (mainly van der Waals interactions) and glutenin polymer chains between entanglements are stretched, leading to some elongation and necking or reduction of cell wall thickness (Termonia and Smith, 1987). Any further elongation of the polymers will happen only if they begin to slip through entanglements without disentangling the network. The activation energy for slippage through entanglements is higher than that required to break van der Waals

Fig 2.9 Effect of molecular weight distribution on number of entanglements per chain and molecular weight between two entanglements (Adopted from: MacRitchie and Lafiandra, 1997).



interactions (Termonia and Smith, 1987). This will cause increase in stress for any further extension of the matrix, inducing a strain hardening effect. When this desired activation energy for slippage through entanglements is reached, a significant extensibility of the polymer chains and the matrix occurs (Termonia and Smith, 1987). Slippage through entanglements and extensibility of the polymer chains depend on; number of entanglements per chain, MW between two entanglements or entanglement network density, elongation rate or rate at which polymers are stretched, and temperature (Termonia and Smith, 1987 & 1988; Termonia et al., 1988). If we consider M_T to be greater or equivalent to 250,000 (equation 2.1) (Bangur et al., 1997), that is the MW required to give stable entanglements (Bersted and Anderson, 1990), then maximum MW between two entanglements (M_e) is

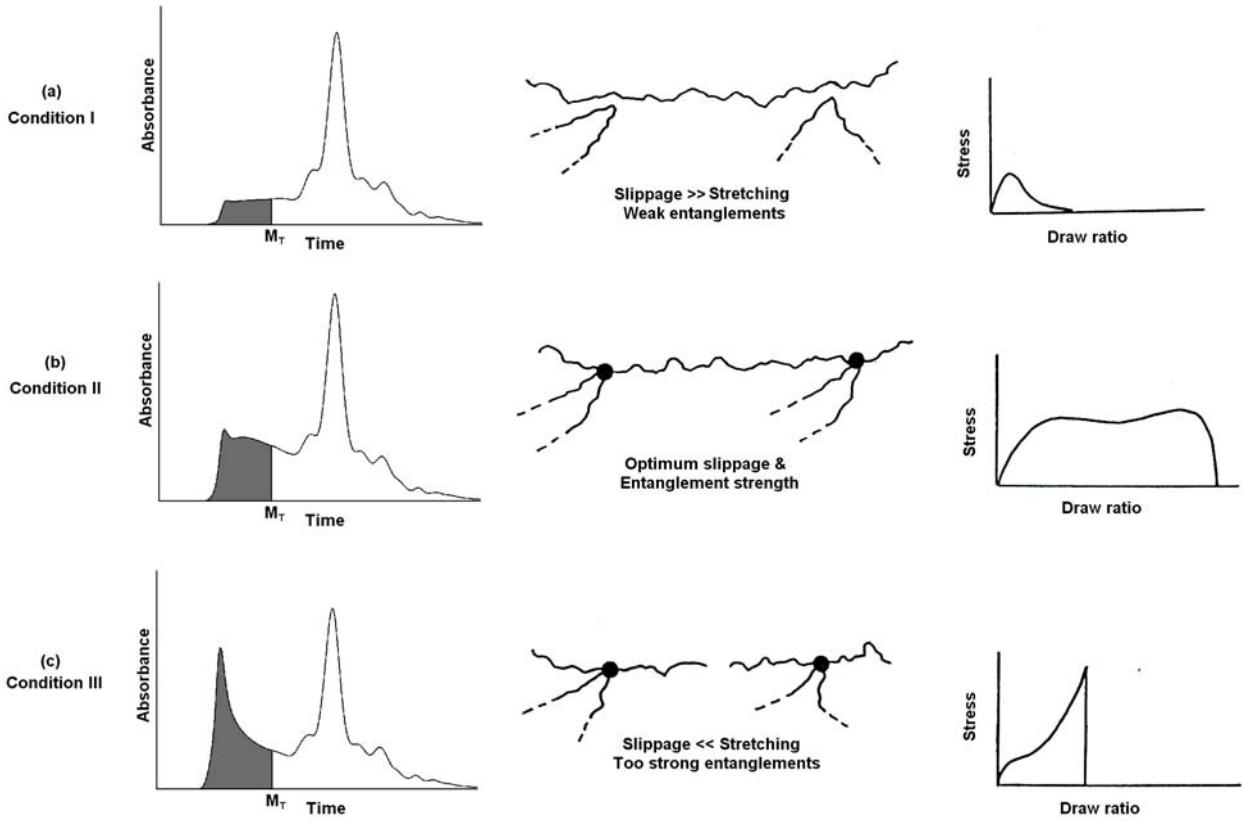
$$M_e \sim 125{,}000 \qquad (2.7).$$

Increase in MW will lead to increase in the number of entanglements per molecule provided the solvent and/or diluent concentration remains the same. Also shift in MWD towards larger MW and decrease in polydispersity, or in other words, if the concentration of largest polymeric molecules is increased, the M_e will be reduced, giving a greater number of entanglements for the same chain length; i.e. entanglement network density will be increased (Fig. 2.9) (Ferry, 1980; MacRitchie and Lafiandra, 1997). Termonia and Smith (1988) introduced a term called entanglement spacing factor (Φ)

$$\Phi \sim (M_e)^{melt} / (M_e)^{soln} \qquad (2.8).$$

Where $(M_e)^{melt}$ is MW between entanglements in pure polymer, and $(M_e)^{soln}$ is MW between entanglements in presence of solvent. On reduction of polydispersity, Φ will increase in the same way as entanglement network density. An entanglement model (Fig. 2.10) derived from the work of Termonia and Smith (Termonia and Smith, 1987 & 1988, Termonia et al., 1988) has been used earlier to explain tensile properties of dough (MacRitchie and Lafiandra, 1997; Singh and MacRitchie, 2001). The same is being used here to explain the molecular basis of strain hardening in the expanding gluten-starch matrix in accordance with theories presented by Termonia and co-workers (Termonia and Smith, 1987 & 1988, Termonia et al., 1988), Bersted and Anderson (1990) and MacRitchie and co-workers (MacRitchie and Lafiandra, 1997; Singh and MacRitchie, 2001).

Fig 2.10 Effect of change in molecular weight distribution and relative proportion of polymeric and monomeric fractions on rheology (strain hardening) of gluten-starch matrix, and diagrammatic representation of corresponding behavior of polymeric protein network (Adopted from: MacRitchie and Lafiandra, 1997).



Condition I (Fig. 2.10 a); The MWD of gluten proteins skews towards lower MW i.e. towards the lower side of M_T and the relative proportion of monomeric fraction is quite high. This will lead to too few stable entanglements per chain and Φ will be quite low. The activation energy for chain slippage will be low in this case and application of small stress will lead to relatively higher elongation in comparison to slippage through entanglements. This is because of low MW that the polymer chains offer too few points of entangled resistance. The load is not transferred to the network and no strain hardening in the necking region is observed. The deformation in this region continues to propagate, causing rupture of the matrix. Also due to low MW of polymer chains, extensibility/strain is expected to be low.

Condition II (Fig. 2.10 b); The MWD of gluten proteins and the relative proportion of polymeric and monomeric fractions is optimum. Here optimum is being defined as one with best

bread making potential. This gives a sufficient number of stable entanglements per chain and optimum Φ or entanglement network density. When thinning at any part of the expanding gas cell wall occurs, the load is transferred to its neighboring region. More non covalent interactions in the region neighboring the necked area are broken. This not only prevents any damage to the entangled network of proteins in the region of necking but also causes further expansion of the gluten-starch matrix. In this manner, the phenomenon propagates to the entire network around the gas cell. The stress begins to increase and reaches the activation energy for slippage through entanglements. The activation energy for slippage is just optimum, that under regular baking conditions the rate of slippage is sufficient to reach the largest number of statistical segments between entanglements through slippage before disentanglement occurs (Termonia et al., 1988). This leads to maximum extensibility of the network without breakage of covalent bonds or disruption of the entangled network. Sufficient increase in stress and failure strain is achieved, exhibiting strain hardening (Termonia and Smith, 1987 & 1988).

Condition III (Fig. 2.10 c); Shows shift from optimum towards higher MW in terms of MWD and relative proportions. The value of Φ will increase and tend to approach 1 as MW increases and polydispersity decreases (Termonia and Smith, 1988), with very high entanglement network density. As in condition II the thin region will propagate in this case causing some stretching. However, the activation energy for slippage through entanglements will be sufficiently high because of chains being held by a significantly larger number of entanglements. This will lead to much higher stretching of the network without slippage of the polymer chains through entanglements, causing sharp increase in stress sufficient to break covalent bonds. Thus, under such circumstances, the extensibility of the gluten protein network is low and a sharp increase in stress exhibiting strain hardening will provide activation energy to disrupt the entangled network and/or break covalent interactions, leading to failure of gas cell walls.

The rate at which polymers are subjected to biaxial extension, at a given temperature, is another factor that determines the extent to which the gluten-starch matrix expands (Termonia et al., 1988, Dobraszczyk and Roberts, 1994). A decrease in rate of elongation or strain rate will lead to increase in extensibility or strain. This is possibly due to slow increase in activation energy, such that slippage is in equilibrium with elongation for sufficiently long time allowing more segments to slip through entanglements. Maximum values for strain are reached at a critical strain rate beyond which extensibility again decreases (Termonia et al., 1988) and the

most likely cause is that the desired activation level is not being reached to maintain extensibility at its maximum. Under such conditions the gas cells may not rupture, but will fail to expand any further, a condition analogous to lower loaf volumes due to yeast inactivity. The maximum in extensibility exists over a small range of strain rates.

The polymer molecular structure-function interpretations of strain hardening behavior of the gluten-starch matrix agree well with observations of some previous studies. MacRitchie and co-workers (MacRitchie, 1987; Lundh and MacRitchie, 1989; Gupta et al., 1990; MacRitchie et al., 1991) separated gluten proteins into fractions with increasing MWs, and MWD being shifted towards higher MW, obtained as supernatants at each step during step wise reduction of pH (MacRitchie, 1979; MacRitchie, 1985). It is based on the principle that, at the iso-electric pH (pH 5.5 – 7.0 for gluten proteins), solubility of proteins is lowest as they have an equal number of positive and negative charges generating sufficient intermolecular attractions (Scopes, 1994). As the pH is increased or decreased the net charge on proteins will be either negative or positive, respectively, increasing solubility of the proteins, though depending on ionizable groups present. As the pH of solution is reduced, the solubility of gluten proteins begins to increase, with the smallest (mainly gliadins) being extracted at the highest pH and, as pH is reduced stepwise, larger proteins become soluble, with largest MW glutenins being extracted as residue of the solution with lowest pH (Lundh and MacRitchie, 1989; Gupta et al., 1990, MacRitchie et al., 1991).

In these studies (MacRitchie, 1987; Lundh and MacRitchie, 1989; Gupta et al., 1990; MacRitchie et al., 1991), the fractions were added back to the base flour at a constant level of protein on a flour weight basis. The addition of fractions caused a shift in MWD and relative proportions of polymeric and monomeric proteins, the earliest extracted gliadin rich fraction causing a shift towards lower MWs and latest extracted glutenin rich fractions towards higher MWs (MacRitchie, 1987; Lundh and MacRitchie, 1989; Gupta et al., 1990). Mixograph analysis of the reconstituted flours showed that addition of fractions with successively increasing MW increased MDDT (Mixograph dough development time), since the requirement for mixing intensity increased. However, upon addition of the latest fractions with highest MW glutenins, the MDDT decreased. Though this has been attributed to the presence of high MW globulin type proteins, nevertheless, it is quite possible that these doughs have not been mixed to their optimum mixing intensities (Kilborn and Tipples , 1972; Tipples and Kilborn, 1975), which will

increase significantly with MW. Baking results were in accordance with MDDT (MacRitchie, 1987; Lundh and MacRitchie, 1989; MacRitchie et al., 1991). However, in most cases of addition of the latest fractions in different flours, it has been observed that the highest loaf volume for any particular flour corresponds to the fraction just preceding the one with highest MDDT. Since highest MDDTs have been achieved, it can be assumed that these flours with added fractions have been mixed to the optimum intensity. Decrease in loaf volume for the fraction with highest MDDT can be related to condition III described earlier in this section. Maximum loaf volume corresponds to the optimum (condition II). In contrast, decrease in loaf volume for the very latest fractions, which may not have been mixed to the optimum intensity as indicated by the decrease in MDDT, can be due to a shift from condition II to condition I.

Extensograph results (Gupta et al., 1990) confirm that with shift in MWD towards higher MW, the Extensograph maximum resistance (R_{max}) increases and extensibility decreases. Similar results have been reported by Gupta et al. (1993) (Fig. 2.3) and Uthayakumaran et al (2000). This suggests that an optimum of strength and extensibility is required for best loaf volume. These results are in accordance with those of Termonia and co-workers (Termonia and Smith, 1987 & 1988, Termonia et al., 1988) on tensile deformation of synthetic polymers, which have been used here to explain strain hardening of the gluten-starch matrix at a molecular level. Dobraszczyk and Roberts (1994) observed that strain hardening index (n), which is positively correlated with loaf volume, is also positively related to failure strain (equation 2.6 and Fig. 2.8).

2.4.3 Measuring Strain Hardening of Gluten-Starch Matrix

Strain hardening of dough is a good indicator of breadmaking potential of any flour. Commonly used methods to determine strain hardening tendency of a gluten-starch matrix around expanding gas cells either apply compression on the dough piece between lubricated flat plates (Janssen et al., 1996) or inflate a dough piece of particular thickness into a bubble (Dobraszczyk, 1997; Dobraszczyk and Morgenstern, 2003). The expanding gas cell walls during proofing and baking are subjected to biaxial extensional deformations of high strains (>> 100%) at strain rates of $10^{-3} – 10^{-4}$ s^{-1} and $10^{-2} – 10^{-3}$ s^{-1}, respectively (Dobraszczyk, 1997). Therefore, in order to understand its rheological behavior under actual baking conditions, the dough must be subjected to similar large deformational forces and rates. Various studies have compared use of

small and large deformation measurements to characterize dough on the basis of its rheology (Amemiya and Menjivar, 1992; Dobraszczyk and Morgenstern, 2003).

Inflation of a dough piece into a bubble is the most widely used rheological procedure since development of the Alveograph. However due to the empirical nature of the Alveograph testing, the results obtained are not in fundamental rheological units of stress, strain and time. Also strain rates are non-uniform and uncontrolled (Dobraszczyk and Morgenstern, 2003). This limits the comparison of tests performed in different labs and on different flours. Dobraszczyk (1997) developed a dough inflation system mounted on a TA-XT 2 Plus texture analyzer that tends to mimic biaxial extension of the gluten-starch matrix surrounding an expanding gas cell. The system tends to overcome limitations of empirical testing performed using the Alveograph by providing results in fundamental rheological units and subjecting the dough piece to a controlled strain rate. Various studies (Dobraszczyk and Roberts, 1994; Dobraszczyk et al., 2003; Chin and Campbell, 2005) report use of this technique to understand rheology of dough.

Biaxial extension of a dough piece by compression between two lubricated plates has also been used to study strain hardening behavior of flours of different bread making potential (Janssen et al, 1996). The procedure has some limitations as complete absence of friction between test piece and plates is important to give purely extensional flow (Dobraszczyk and Morgenstern, 2003), as any frictional forces may produce shearing action. Dough inflation systems overcome this, as the test piece is not in contact with any other material when blown.

2.5 Stability of Liquid Lamellae

The composition of the liquid phase of dough determines the ease with which the air is occluded in the form of small gas cells and the stability of these gas cells during the entire breadmaking process, due to the ability of individual compounds to modify surface chemistry at the gas-liquid interface. The chemical structures of these compounds decide the manner in which they will influence surface chemistry and hence gas cell stability.

2.5.1 Surface Active Compounds of Wheat Flour

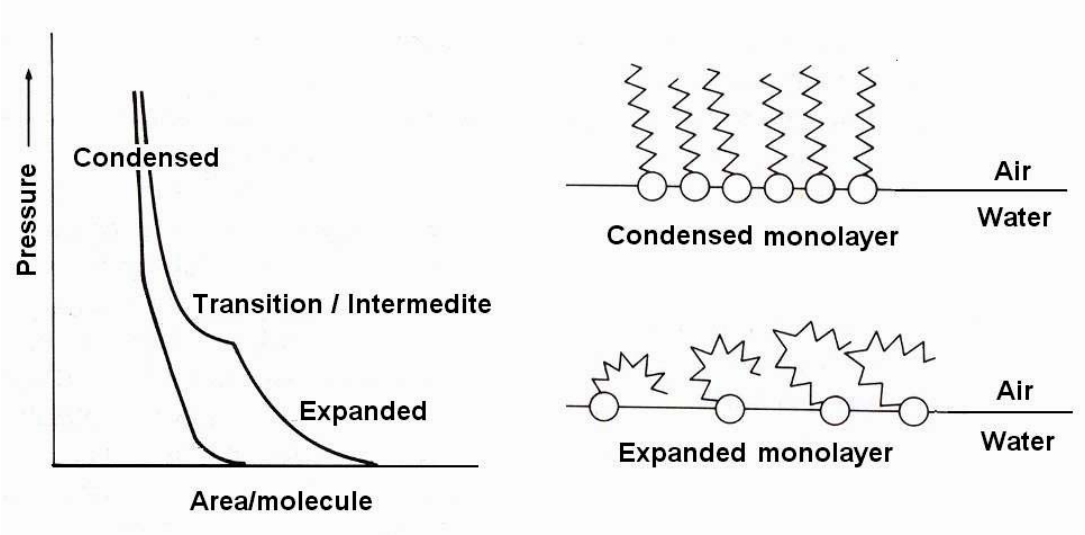
The liquid phase of dough is rich in various surface active compounds. This has been verified by different studies in which the liquid phase was separated by ultracentrifugation and analyzed (Baker et al., 1946; MacRitchie, 1976a; Gan et al., 1999; Salt et al., 2006). The

prominent surface active compounds of the liquid phase are various lipids and proteins of wheat flour, if we do not consider any added ingredients like synthetic surfactants. The lipid content of wheat flour varies between 1.5 – 2.0% and most of it is contributed by endosperm (Pomeranz, 1988; Day, 2004). Twenty five percent of total flour lipids are associated with starch and do not affect baking performance of the flour (Pomeranz, 1988; DeStefanis and Ponte, 1976). Chloroform is one of the best solvents to extract nearly all of the non starch lipids of wheat flour (MacRitchie, 1985; Finney et al., 1976). Non starch lipids include both non-polar and polar lipids in the ratio of 60 to 40, respectively (Ponte and DeStefanis, 1969). Non-polar solvents like petroleum ether can extract only up to 70% of non starch lipids from wheat flour, because more polar non starch lipids have relatively lower free energies in flour than in petroleum ether and are not extracted (MacRitchie, 1985). Solvents with alcohol groups, like water saturated butanol, may affect functional properties of wheat flour proteins. Suitable solvents can be used to fractionate non starch lipids into their polar and non-polar components (Ponte and DeStefanis, 1969; DeStefanis and Ponte, 1976). Extracted non starch lipids can be analyzed qualitatively and quantitatively using different techniques such as thin-layer chromatography (TLC) and densitometry (McCormack et al., 1991), High-performance liquid chromatography (HPLC) (Ohm and Chung, 1999) and gas chromatography (GC).

Based on polarity (i.e. relative size of hydrocarbon chain and polar head group), non starch lipids of wheat flour are classified as polar and non-polar lipids. Major non starch polar lipids with strong polar head groups are phospholipids (phosphatidyethanolamine and phosphatidylcholine) and glycolipids (digalactosyl diglyceride or DGDG, and monogalactosyl diglyceride or MGDG) (Ross and MacRitchie, 1995; Day, 2004). Phosphatidylethanolamine and DGDG are the most abundant polar lipids. The non-polar fraction is composed of free fatty acids (FFA), steryl esters, triglycerides, diglycerides and monoglycerides (DeStefanis and Ponte, 1976). The major portion of non-polar lipids (~58%) is triglycerides, which are the most non-polar because of the relatively large hydrocarbon part and lack of a strong polar head group. Mono and diglycerides have weak polar head groups, their polarity also depends on the size of fatty acid chains present. FFAs which compose 20% of non starch non-polar lipids are the least non-polar in the non-polar fraction. Linoleic acid (~60%) is the major unsaturated FFA and palmitic acid (~24%) is the major saturated FFA (Day, 2004).

Proteins constitute 2-3% of the liquid dough phase (Baker et al., 1946; MacRitchie, 1976a,). Salt et al. (2006) reported recovery of dough liquor composed of 0.5 – 0.75% of soluble protein, on a flour weight basis. Puroindolines (PINa and PINb) which are highly surface active (Dubreil et al., 1998; Gan et al., 1999), and the soluble fraction of wheat proteins i.e. albumins and globulins are expected to be the major protein fraction in the liquid dough phase (Gan et al., 1995). These and some lower MW gliadins might be responsible for surface action. The surface activity of proteins not only depends on their net hydrophobicity but also on their lowest free energy conformation in solution i.e. the liquid phase of dough (MacRitchie, 1990; MacRitchie, 1992).

Fig 2.11 Pressure-Area relationships of expanded and condensed phases of monolayers and diagrammatic representation of corresponding molecular conformation in two monolayer states. Polar head groups are depicted as circular and hydrocarbon chains as zig-zag lines (MacRitchie, 1990).



2.5.2 Interface Chemistry of Liquid Lamellae Stability

The surface active compounds of the liquid phase of dough are adsorbed at the gas-liquid interface and form a monomolecular layer (a monolayer), with polar head groups aligned towards water and hydrocarbon chains towards air (Fig. 2.11) (MacRitchie, 1990). Their affinity to get adsorbed at the interface to form a monolayer is determined by their relative solubility in

the liquid phase and a balance between their non-polar hydrocarbon chain and polar head group. The physical properties of monolayers formed by these surface active compounds determine stability of the liquid lamellae (DeStefanis and Ponte, 1976; MacRitchie, 1976a; MacRitchie, 1992).

If we consider gas cells of bread as foam, then two steps are important; firstly the ease with which this foam is formed and secondly its stability. The ease of foam formation is important for both good loaf volume and crumb grain. When the air is beaten into the liquid phase, surface active compounds immediately diffuse to adsorb at the freshly created gas-liquid interface. Initial adsorption depends on diffusion coefficients of these compounds (MacRitchie, 1990). The surface active compounds which have been adsorbed initially at the interface to form a monolayer will either stabilize or destabilize freshly occluded gas cells, thus affecting ease of foam formation (Larsson et al., 2006). Undoubtedly, the ease of foam formation also depends on dough rheology (Baker and Mize, 1946).

Once the foam is formed, it needs to be stabilized against coalescence and disproportionation. The monolayers formed may protect expanding gas cells against these instability factors depending upon their nature. The surface active compounds will either form a condensed or expanded monolayer at the gas-liquid interface (Fig. 2.11). Condensed monolayers are characterized by close packing of surface active molecules due to their saturated hydrocarbon chains. These monolayers have low compressibility (Fig. 2.11) because of close molecular packing that also leads to greater attraction between hydrocarbon chains or vice-versa, making the monolayer more stable and not easily desorbed (Gaines, 1966; MacRitchie, 1990; Ross and MacRitchie, 1995). The surface viscosity of such monolayers is high and with any change in interfacial area, large elastic restoring forces are generated, preventing coalescence and disproportionation. On the other hand, unsaturated hydrocarbons or other foam destabilizers will form expanded monolayers (Fig. 2.11). Molecular packing of expanded monolayers is loose, leading to greater compressibility (Gaines, 1966; MacRitchie, 1990). These are not so elastic in response to changes in the interfacial area and may get desorbed in the process. The desorption at the point of contact of two gas cells will lead to coalescence (MacRitchie, 1976b).

Surface active compounds of wheat flour such as phospholipids, DGDG and saturated FFAs (stearic acid, palmitic acid) are known to give condensed monolayers. Unsaturated FFAs

like linoleic acid give expanded monolayers. Any modification in the structure of a hydrocarbon chain such as inclusion of a double bond or decrease in the chain length will lead to transformation of condensed monolayers to expanded ones. This change can also be seen with increase in temperature (Gaines, 1966; MacRitchie, 1990; Ross and MacRitchie, 1995). With presence of a variety of surface active compounds in the liquid phase of the dough, it is quite possible that some of these molecules may possess a certain degree of mutual solubility. The monolayers of such surface active compounds will be of intermediate type, depending whether individual compounds give expanded or condensed monolayers (Paternotte et al., 1993; Larsson et al., 2006).

Various studies confirm the role of monolayers in gas cell stability. A very specific trend of loaf volume change is observed on addition of original flour lipids back into defatted flour. As the lipid level is increased, the loaf volume begins to decrease, and, after reaching a minimum, it again increases (MacRitchie and Gras, 1973; MacRitchie, 1976a). This is attributed to transformation from pure protein stabilized lamellae to mixed protein-lipid stabilized lamellae (Larsson et al., 2006; Salt et al., 2006). Studies have indicated that these differences are independent of differences due to gluten quality. Flours with better breadmaking properties will give relatively higher loaf volumes at analogous lipid treatments (MacRitchie, 1978; MacRitchie, 1980; McCormack et al., 1991). In addition, decrease in polar to non-polar ratio in original lipids will shift the minima to higher levels of lipid addition (MacRitchie, 1980).

The polar fraction of total flour lipids, which is rich in phospholipids and DGDG have positive effects on loaf volume (Ponte and DeStefanis, 1969; MacRitchie, 1978). On the other hand, in a study by DeStefanis and Ponte (1976), negative effects of non-polar fractions is attributed to the presence of the unsaturated FFA linoleic acid. In this study it was observed that other non-polar lipids such as triglycerides and saturated FFA palmitic acid do not affect loaf volume. Similar observations were reported by MacRitchie (1977), when incremental additions of different polar and non-polar rich fractions were made to defatted flour. Presence of some FFAs in polar fractions extracted from wheat flour caused some decrease in loaf volumes at lower levels of addition of these fractions back into defatted base flour. Analogous effects of these lipid types and fractions on foam stability (MacRitchie, 1976a; MacRitchie, 1977) further confirm the role of liquid lamellae in gas cell stability. Here a comparison between foaming behavior and baking properties indicated a possible positive effect of the molding step. The

inclusion of a molding step leads to improvement in loaf volume probably due to similarity of action with compression expansion cycles of monolayer studies (MacRitchie, 1990). The compression experienced by the gas-liquid interface may clear the lamella surface of any impurities that are relatively weakly surface active and are deleterious to the gas cell stability. Molding also subdivides gas cells, making the cell size distribution uniform.

Attempts have been made to understand the possible mechanism of action of shortenings and certain surfactants by which they improve loaf volume and crumb grain (Junge and Hoseney, 1981; Junge et al., 1981). Junge et al. (1981) observed no differences for air occluded on addition of different surfactants. Some surfactants like ethoxylated monoglycerides gave coarse crumb grain, others which produced fine crumb grain had no positive effect on loaf volume. In a separate study Junge and Hoseney (1981) found that addition of shortening to defatted flour caused decrease in loaf volume and that to whole flour caused increase in loaf volume. It is quite possible that shortening and other surfactants that are commercially used to improve loaf volume and crumb grain possibly act as sequestrants for film destabilizing compounds (the unsaturated free fatty acids) (MacRitchie, 1976a; MacRitchie, 1977).

2.2 References

Amemiya, J.I., and Menjivar, J.A. 1992. Comparison of small and large deformation measurements to characterize the rheology of wheat flour doughs. Journal of Food Engineering. 16: 91-108.

Autran, J-C. 1994. Size-exclusion high-performance liquid chromatography for rapid examination of size differences of cereal proteins. In: Kruger, J.E., Bietz, J.A. (Eds.), High-Performance Liquid Chromatography of Cereal and Legume Proteins, American Association of Cereal Chemists, St. Paul, MN, USA, pp326-372.

Babin, P., Della Valle, G., Chiron, H., Cloetens, P., Hoszowska, J., Pernot, P., Reguerre, A.L., Salvo, L., Dendievel, R. 2006. Fast X-ray tomography analysis of bubble growth and foam setting during breadmaking. Journal of Cereal Science 43, 393-397.

Baker, J.C., Mize, M.D. 1946. Gas occlusion during dough mixing. Cereal Chemistry 23, 39-51.

Baker, J.C., Parker, H.K., Mize, M.D. 1946. Supercentrifugates from dough. Cereal Chemistry 23, 539-544.

Bangur, R., Batey, I.L., McKenzie, E., MacRitchie, F. 1997. Dependence of extensograph parameters on wheat protein composition measured by SE-HPLC. Journal of Cereal Science 25, 237-241.

Batey, I.L., Gupta, R.B., MacRitchie, F. 1991. Use of size-exclusion high-performance liquid chromatography in the study of wheat flour proteins: an improved chromatographic procedure. Cereal Chemistry 68, 207-209.

Bean, S.R., Lookhart, G.L. 2001. Factors influencing the characterization of gluten proteins by size-exclusion chromatography and multiangle laser light scattering (SEC-MALLS). Cereal Chemistry 78, 608-618.

Bersted, B.H., Anderson, T.G. 1990. Influence of molecular weight and molecular weight distribution on the tensile properties of amorphous polymers. Journal of Applied Polymer Science 39, 499-514.

Bloksma, A.H. 1990a. Rheology of the breadmaking process. Cereal Foods World 35, 228-236.

Bloksma, A.H. 1990b. Dough structure, rheology, and baking quality. Cereal Foods World 35, 237-244.

Bloksma, A.H., Nieman, W. 1975. The effect of temperature on some rheological properties of wheat flour doughs. Journal of Texture Studies 6, 343-361.

Campbell, G.M., Herrero-Sanchez, R., Payo-Rodriguez, R., Merchan, M.L. 2001. Measurement of dynamic dough density and effect of surfactants and flour type on aeration during mixing and gas retention during proofing. Cereal Chemistry 78, 272-277.

Carceller, J.L., Aussenac, T. 2001. Size characterization of glutenin polymers by HPSEC-MALLS. Journal of Cereal Science 33, 131-142.

Chin, N.L., Campbell, G.M. 2005. Dough aeration and rheology. II. Effects of flour type, mixing speed and total work input on aeration and rheology of bread dough. Journal of the Science of Food and Agriculture 85, 2194-2202.

Day, L. 2004. Lipid chemistry. In: Wrigley, C., Walker, C., Corke, H. (Eds.), Encyclopedia of Grain Science 2, Elsevier Ltd., Oxford, UK, pp 157 – 165.

DeStefanis, V.A., Ponte, J.G.Jr. 1976. Studies on breadmaking properties of wheat-flour nonpolar lipids. Cereal Chemistry 53, 636-642.

Dobraszczyk, B.J. 1997. Development of a new dough inflation system to evaluate doughs. Cereal Foods World 42, 516-519.

Dobraszczyk, B.J. 1999. Measurement of biaxial extensional rheological properties using bubble inflation and the stability of bubble expansion in bread doughs. In: Campbell, G.M., Webb, C., Pandiella, S.S., Niranjan, K. (Eds.), Bubbles in Food, American Association of Cereal Chemists., St. Paul, Minnesota, USA, pp 173-182.

Dobraszczyk, B.J., Morgenstern, M.P. 2003. Rheology and the breadmaking process. Journal of Cereal Science 38, 229-245.

Dobraszczyk, B.J., Roberts, C.A. 1994. Strain hardening and dough gas cell-wall failure in biaxial extension. Journal of Cereal Science 20, 265-274.

Dobraszczyk, B.J., Smewing, J., Albertini, M., Maesmans, G., Schofield, J.D. 2003. Extensional rheology and stability of gas cell walls in bread doughs at elevated temperatures in relation to breadmaking performance. Cereal Chemistry 80, 218-224.

Dubreil, L., Meliande, S., Chiron, H., Compoint, J.-P., Quillien, L., Branlard, G., Marion, D. 1998. Effect of puroindolines on the breadmaking properties of wheat flour. Cereal Chemistry 75, 222-229.

Ferry, J.D. 1980. Viscoelastic Properties of Polymers, John Wiley and Sons, Inc., New York, USA.

Finney, K.F., Pomeranz, Y., Hoseney, R.C. 1976. Effects of solvent extraction on lipid composition, mixing time, and bread loaf volume. Cereal Chemistry 53, 383-388.

Gaines, G.L. 1966. Insoluble Monolayers at Liquid-Gas Interfaces. Prigogine, I. (Ed.), Interscience Monographs on Physical Chemistry, John Wiley and Sons, Inc., New York, USA.

Gan, Z., Angold, R.E., Williams, M.R., Ellis, P.R., Vaughan, J.G., Galliard, T. 1990. The microstructure of gas retention of bread dough. Journal of Cereal Science 12, 15-24.

Gan, Z., Ellis, P.R., Schofield, J.D. 1995. Gas cell stabilization and gas retention in wheat bread dough. Journal of Cereal Science 21, 215-230.

Gan, Z., van der Graaf, J., Leonard, S.A., Brooker, B.E., Parker, M.L., Schofield, J.D. 1999. The role of wheat proteins and polar lipids in the stabilization of the foam structure of dough. In: Campbell, G.M., Webb, C., Pandiella, S.S., Niranjan, K. (Eds.), Bubbles in Food, American Association of Cereal Chemists., St. Paul, Minnesota, USA, pp 89-93.

Gandikota, S., MacRitchie, F. 2005. Expansion capacity of doughs: methodology and applications. Journal of Cereal Science 42, 157-163.

Gupta, R.B., Bekes, F., Gras, P.W., MacRitchie, F. 1990. Functionality of glutenin, gliadin and secalin fractions as measured by extensograph and mixograph. In: Gluten proteins. Association of Cereal Research, Detmold. pp 550-559.

Gupta, R. B., Khan, K., MacRitchie, M. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quality and size distribution of polymeric protein. Journal of Cereal Science 18, 23-41.

Hayman, D., Hoseney, R.C., Faubion, J.M. 1998. Bread crumb grain development during baking. Cereal Chemistry 75, 577-580.

He, H., Hoseney, R.C. 1991. Gas retention of different cereal flours. Cereal Chemistry 68, 334-336.

Hoseney, R.C. 1992. Physical chemistry of bread dough. In: Schwartzberg, H.G., Hartel. R.W. (Eds.), Physical Chemistry of Foods, Marcel Dekker, Inc., New York, USA, pp 443-457.

Janssen, A.M., vanVliet, T., Vereijken, J.M. 1996. Rheological behavior of wheat glutens at small and large deformations. Effect of gluten composition. Journal of Cereal Science 23, 33-42.

Junge, R.C., Hoseney, R.C. 1981. A mechanism by which shortening and certain surfactants improve loaf volume in bread. Cereal Chemistry 58, 408-412.

Junge, R.C., Hoseney, R.C., Varriano-Marston, E. 1981. Effect of surfactants on air incorporation in dough and the crumb grain of bread. Cereal Chemistry 58, 338-342.

Khan, K., Bushuk, W. 1978. Glutenin: structure and functionality in breadmaking. Bakers' Digest 52, 14-20**.**

Kilborn, R.H., Tipples, K.H. 1972. Factors affecting mechanical dough development. I. Effect of mixing intensity and work input. Cereal Chemistry 49, 34-47.

Kokelaar, J.J., vanVliet, T., Prins, A. 1996. Strain hardening properties and extensibility of flour and gluten doughs in relation to breadmaking performance. Journal of Cereal Science 24, 199-214.

Kraus, G., Gruver, J.R. 1965. Rheological properties of multichain polybutadienes. Journal of Polymer Science 3, 105-122.

Larsson, K., Quinn, P., Sato, K., Tiberg, F. 2006. Lipids: Structure, Physical Properties and Functionality, The Oily Press, Bridgwater, England.

Lemelin, E., Branlard, G., Salvo, L., Lein, V., Aussenac, T., Dayde, J. 2005. Breadmaking stability of wheat flours: relation between mixing properties and molecular weight distribution of polymeric glutenins. Journal of Cereal Science 42, 317-326.

Li, W., Dobraszczyk, B.J. 2004. The dynamic development and distribution of gas cells in bread-making dough during proving and baking. In: Lafiandra, D., Masci, S., D'Ovidio, R. (Eds.), The Gluten Proteins, The Royal Society of Chemistry, Cambridge, UK, pp 255-258.

Lundh, G., MacRitchie, F. 1989. Size exclusion HPLC characterization of gluten protein fractions varying in bradmaking potential. Journal of Cereal Science 10, 247-253.

MacRitchie, F. 1976a. The liquid phase of dough and its role in baking. Cereal Chemistry 53, 318-326.

MacRitchie, F. 1976b. Monolayer compression barrier in emulsion and foam stability. Journal of Colloid and Interface Science 56, 53-56.

MacRitchie, F. 1977. Flour lipids and their effects in baking. Journal of The Science of Food and Agriculture 28, 53-58.

MacRitchie, F. 1978. Differences in baking quality between wheat flours. Journal of Food Technology 13, 187-194.

MacRitchie, F. 1979. A relation between gluten protein amide content and baking performance of wheat flours. Journal of Food Technology 14, 595-601.

MacRitchie, F. 1980. Protein-lipid interaction in baking quality of wheat flours. Bakers Digest 54, 10-13.

MacRitchie, F. 1983. Role of lipids in baking. In: Barnes, P.J. (Ed.), Lipids in Cereal Technology, Academic Press, Inc., New York, USA, pp 165-188.

MacRitchie, F. 1985. Studies of the methodology for fractionation and reconstitution of wheat flours. Journal of Cereal Science 3, 221-230.

MacRitchie, F. 1986. Physicochemical processes in mixing. In: Blanshard, J.M.V., Frazier, P.J., Galliard, T. (Eds.), Chemistry and Physic of Baking, Royal Society of Chemistry, London, UK, pp 132-146.

MacRitchie, F. 1987. Evaluation of contributions from wheat protein fractions to dough mixing and breadmaking. Journal of Cereal Science 6, 259-268.

MacRitchie, F. 1990. Chemistry at Interfaces. Academic Press, Inc., San Diego, CA, USA.

MacRitchie, F. 1992. Physicochemical properties of wheat proteins in relation to functionality. In: Kinsella, J.E. (Ed.), Advances in Food and Nutrition Research Vol. 36, Academic Press, Inc., New York, USA, pp 1- 87.

MacRitchie, F. 1999. Wheat proteins: characterization and role in flour functionality. Cereal Foods World 44, 188-193.

MacRitchie, F., Gras, P.W. 1973. The role of flour lipids in baking. Cereal Chemistry 50, 292-302.

MacRitchie, F., Kasarda, D.D., Kuzmicky, D.D. 1991. Characterization of wheat protein fractions differing in contributions to breadmaking quality. Cereal Chemistry 68, 122-130.

MacRitchie, F., Lafiandra, D. 1997. Structure-functionality relationships of wheat proteins. In: Damodaran, S., Paraf, A. (Eds.), Food Proteins and their Applications, Marcel Dekker, Inc., New York, USA, pp 293-324.

MacRitchie, F., Lafiandra, D. 2001. Use of near-isogenic lines to determine protein composition-functionality relationships. Cereal Chemistry 78, 501-506.

McCormack, G., Panozzo, J., MacRitchie, F. 1991. Contributions to breadmaking of inherent variations in lipid content and composition of wheat cultivars.II. Fractionation and reconstitution studies. Journal of Cereal Science 13, 263-274.

Ohm, J.B., Chung, O.K. 1999. Estimation of free glycolipids in wheat flour by HPLC. Cereal Chemistry 76, 873-876.

Park, S.H., Bean, S.R., Chung, O.K., Seib, P.A. 2006. Levels of protein and protein composition in hard winter wheat flours and the relationship to breadmaking. Cereal Chemistry 83, 418-423.

Paternotte, T.A., Orsel, R., Hamer, R.J. 1993. Interactions between flour proteins and flour lipids at the liquid/air interface. In: Gluten Proteins, Association of Cereal Research, Detmold, Germany, pp 207-217.

Pomeranz, Y. 1988. Wheat Chemistry and Tehnology II, Third Edition. American Association of Cereal Chemists, St. Paul, MN, USA, pp 285-328.

Ponte, J.G.Jr., DeStefanis, V.A. 1969. Note on separation and baking properties of polar and nonpolar wheat flour lipids. Cereal Chemistry 46, 325-329.

Ross, A., MacRitchie, F. 1995. Interactions of wheat proteins, carbohydrates and lipids. In: Gaonkar, A.G. (Ed.), Ingredient Interactions Effects on Food Quality, Marcel Deker, New York, USA, pp 321-356.

Rouille, J., Della Valle, G., Devaux, M.F., Marion, D., Dubreil, L. 2005. French bread loaf volume variations and digital image analysis of crumb grain changes induced by the minor components of wheat flour. Cereal Chemistry 82, 20-27.

Salt, L.J., Wilde, P.J., Georget, D., Wellner, N., Skeggs, P.K., Mills, E.N.C. 2006. Composition and surface properties of dough liquor. Journal of Cereal Science 43, 284-292.

Sapirstein, H.D. 1999. The imaging and measurement of bubbles in bread. In: Campbell, G.M., Webb, C., Pandiella, S.S., Niranjan, K. (Eds.), Bubbles in Food, American Association of Cereal Chemists., St. Paul, Minnesota, USA, pp 233-243.

Scopes, R.K. 1994. Protein Purification Principles and Practice, Third Edition. Springer-Verlag, Inc., New York, USA.

Singh, N.K., Donovan, G.R., Batey, I.L., MacRitchie, F. 1990. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. I. Dissolution of total proteins in the absence of reducing agents. Cereal Chemistry 67, 150-161.

Singh, H., MacRitchie, F. 2001. Application of polymer science to properties of gluten. Journal of Cereal Science 33, 231-243.

Southan, M., MacRitchie, F. 1999. Molecular weight distribution of wheat proteins. Cereal Chemistry 76, 827-836.

Stevenson, S.G., Preston, K.R. 1996. Flow field-flow fractionation of wheat proteins. Journal of Cereal Science 23, 113-119.

Stevenson, S.G., You, S., Izydorczyk, M.S., Preston, K.R. 2003. Characterization of polymeric wheat proteins by flow field-flow fractionation/MALLS. Journal of Liquid Chromatography and Related Technologies 26, 2771-2781.

Termonia, Y., Allen, S.R., Smith, P. 1988. Kinetic model for tensile deformation of polymers. 3. Effects of deformation rate and temperature. Macromolecules 21, 3485-3489.

Termonia, Y., Smith, P. 1987. Kinetic model for tensile deformation of polymers. 1. Effect of Molecular Weight. Macromolecules 20, 835-838.

Termonia, Y., Smith, P. 1988. Kinetic model for tensile deformation of polymers. 2. effect of entanglement spacing. Macromolecules 21, 2184-2189.

Tipples, K.H., Kilborn, R.H. 1975. Unmixing – the disorientation of developed bread doughs by slow speed mixing. Cereal Chemistry 52, 248-262.

Ueno, T., Stevenson, S.G., Preston, K.R., Nightingale, M.J., Marchylo, B.M. 2002. Simplified dilute acetic acid based extraction procedure for fractionation and analysis of wheat flour protein by size-exclusion HPLC and flow field-flow fractionation. Cereal Chemistry 79, 155-161.

Uthayakumaran, S., Newberry, M., Keentok, M., Stoddard, F.L., Bekes, F. 2000. Basic rheology of bread dough with modified protein content and glutenin –to-gliadin ratios. Cereal Chemistry 77, 744-749.

vanVliet, T. 1995. Physical factors determining gas cell stability in a dough during bread making. In: Schofield, J.D. (Ed.), Wheat Structure, Biochemistry and Functionality, The Royal Society of Chemistry, Cambridge, UK. pp 309-315.

van Vliet, T., Janssen, A.M., Bloksma, A.H., Walstra, P. 1992. Strain hardening of dough as a requirement for gas retention. Journal of Texture Studies 23, 439-460.

Whitworth, M.B., Cauvin, S.P., Cliffe, D. 2005. Measurement of bread cell structure by image analysis, using cereal science and technology for benefit of consumers. In: Cauvin, S.P., Salmon, S., Young, L.S. (Eds.), Proceedings of 12th International ICC Cereal and Bread Congress, Woodhead Publishing Ltd., Cambridge, UK, pp 193-198.

Wrigley, C. 2004. Structural features needed in a glutenin protein. Cereal Foods World 49, 36-38.

Zghal, M.C., Scanlon, M.G., Sapirstein, H.D. 1999. Prediction of bread crumb density by digital image analysis. Cereal Chemistry 76, 734-742.

CHAPTER 3 - General Mechanism of Gas Cell Stability

3.1 Introduction

During mixing, gas is occluded and concentrated in the liquid phase of dough in the form of small nuclei. Gas nuclei expand during proofing due to release of fermentation gases, and during baking due to expansion of these gases as temperature increases. No further occlusion of gas occurs in succeeding stages (sheeting, molding, etc.) of the bread making process (Baker and Mize, 1946). However, these subsequent stages (punching, sheeting and molding) cause subdivision of already existing gas cells, thus improving their number and size distribution. The proofing and baking stages of bread making are characterized by fast biaxial expansion of gas cells, expanding at strain rates of 0.001 - 0.0001s^{-1} and 0.01 - 0.001s^{-1}, respectively, generating large strains (>> 100%) (Dobraszczyk, 1997). The extent of expansion and stability of gas cells at these stages determine final volume and crumb structure of the bread.

Wheat flour dough due to its unique visco-elastic properties is capable of stabilizing the expanding gas cells. The classical view has emphasized only the gluten-starch matrix as the cell membrane that stabilizes the expanding gas cells (Bloksma, 1990b; Hoseney, 1992). However, scanning electron micrographs of dough at the end of the proofing stage show the existence of intact gas cells even when there are discontinuities in the gluten starch matrix (Gan et al., 1990). In the light of previous work, which shows a continuous liquid phase in dough (MacRitchie, 1976a), the presence of a secondary stabilizing feature was concluded (Gan et al. 1990). In a hypothesis proposed by Gan and co-workers based on electron micrographs of dough, (Gan et al., 1990 and 1995) the expanding gas cells are stabilized against coalescence and disproportionation by a primary gluten-starch matrix with a secondary liquid lamella on its inner side, enveloping the gas cell (Fig. 2.5). The hypothesis seems plausible in view of evidence from other studies.

The extent, to which the primary gluten-starch matrix around a gas cell can biaxially expand without rupturing, is a major factor determining gas cell stability. A biaxially expanding gluten-starch matrix is stabilized against rupture by virtue of its tendency to strain harden (Van Vliet et al., 1992; Dobraszczyk and Roberts, 1994; Dobraszczyk et al., 2003), making it a necessary rheological property for obtaining good bread volume. Doughs with a strain hardening

index of 1 or higher have the potential to retain gas cells and allow them to expand without undergoing disproportionation and coalescence and thus are good for bread making; the higher this value the better it is (Dobraszczyk and Roberts, 1994). MacRitchie and Gras (1973) found that Alveograms are not affected by variations in the original lipid fraction of the flour. However, small amounts of these lipids (1-1.5%) have significant effects on baking performance in terms of volume and crumb structure. It is probably due to their surface activity that these compounds (lipids and proteins) get adsorbed at the gas-liquid interface of the liquid lamellae and affect stability of the gas cells. Analogous variations in foaming properties of the dough liquor, and bread loaf volume and crumb structure (MacRitchie, 1976a) provide plausible evidence of their surface action at the interface.

The objective of this part of the study was to investigate and seek evidence for the presence of liquid lamellae and their ability to stabilize gas cells i.e. whether or not the dual film hypothesis holds true.

3.2 Materials and Methods

3.2.1 Flours

Jagger flour and soft wheat flour (a blend of soft wheat varieties) were two untreated and unbleached flours used in this study. The flours were milled in Buhler mill (73% milling extraction rate) in the department of Grain Science and Industry, Kansas State University, Manhattan, KS. The flours were evaluated for certain quality characteristics given in Table 3.1. These flours were stored at -20^{0}C until use. For the sake of brevity, Jagger flour and soft wheat flour will be referred to as Jagger and soft, respectively.

3.2.2 Reagents

Chloroform used was of HPLC grade, all other chemicals used in this were of ACS grade. These were purchased from Sigma-Aldrich, USA. Distilled deionized water, sterilized in an autoclave at a pressure of 120 psi for 20 min, was used in all stages of the experiments.

3.2.3 Analytical Procedures

- Moisture content was determined as per AACC method 44 – 15 A.
- Protein content was determined by the nitrogen combustion method using the LECO FP-2000 Nitrogen/protein analyzer with a factor of 5.7 to convert N to protein.
- Lipid content was determined by cold extraction with chloroform (HPLC grade) using a procedure by MacRitchie and Gras (1973).

3.2.4 Dough Mixing Properties

Mixing properties were evaluated using a 10 g National Mixograph (National Manufacturing Co., Lincoln, NE). Mixing parameters (peak development time, and weakening angle) were used for comparison. The procedure used was similar to AACC method 55-40 except that sodium chloride (1.5% w/w on flour weight basis) was added.

3.2.5 Size-Exclusion High-Performance Liquid Chromatography (SE-HPLC)

Size characterization of gluten proteins was done using SE-HPLC (Hewlett-Packard 1100. Proteins were fractionated with a Biosep SEC-4000 column (Phenomenex, Torrance, CA) using; 50 mM disodium orthophosphate (Na_2HPO_4) buffer (NaPhos), pH 7.0, containing 1%

sodium dodecyl sulfate (SDS), as mobile phase at a flow rate of 0.5 ml/min. Proteins were detected at 214 nm in SE-HPLC. Injection volume of samples for total and extractable protein analysis was 80 µl and that for unextractable protein analysis was 40 µl. Therefore, results obtained from chromatograms of unextractable proteins were multiplied by a factor of 2. SEC-HPLC data was analyzed using software program ChemStation (Agilent Technologies, USA).

3.2.6 Sample preparation for SE-HPLC

Basic procedure for SE-HPLC is described elsewhere (Batey et al., 1991). Samples were prepared to analyze total, extractable and unextractable polymeric protein (Gupta et al., 1993). Samples for total and extractable protein analysis were weighed in microfuge tubes. Sample size was determined based on protein content of each sample such that protein content in all samples was kept constant, using Jagger flour as standard and quantity for Jagger being 10.0 mg ± 0.1 mg. Weighed samples were suspended in 50mM NaPhos, pH 6.9, + 0.5% SDS and solubilized by vortexing for 10 min. For total protein analysis, to achieve solubilization of the largest molecular size fraction, samples were sonicated (Singh et al., 1990) at room temperature at an output of 6 watts for 15 s. No sonication was done for extractable protein analysis. The sonicator probe was placed at 1/3 distance from the bottom of the microfuge tube. Microfuge tubes with protein suspensions were then centrifuged at 12000 x g for 20 min. The supernatant was decanted in HPLC vials and sealed. To ensure stability of prepared samples, the vials with supernatant were heat treated in a water bath at 85^0C for 5 min to inhibit any intrinsic proteolytic activity. After heat treatment, vials were cooled with crushed ice and analyzed by SE-HPLC.

Residue from extractable protein was used for unextractable protein analysis. The same procedure was followed, except that suspensions were sonicated for 25s at an output of 6 watts.

3.2.7 Lipid Extraction from Flour

Original flour lipids were extracted using three batch extractions with chloroform in a glass beaker, followed by Buchner filtration through Whatman No. 1 filter paper (MacRitchie and Gras, 1973). 200 g of flour and 400 ml of chloroform were used for each extraction. The defatted flour was spread out on a flat glass tray in a fume hood for 12 hours to allow evaporation of solvent.

3.2.8 Addition of Lipids to Defatted Flour

For incorporation of intact original flour lipids into defatted flour, the parent flour was mixed with defatted flour in different proportions, for both the flours, to give different flour lipid levels.

3.2.9 Test Baking

Test loaves (35 g flour) were baked using a modified rapid bake test (MacRitchie and Gras, 1973). A lean formulation was used with no added shortening; flour (100%), sugar (6%), sodium chloride (1.5%), instant yeast (2.7%), potassium bromate (30ppm), water and mix time (as optimized from Mixograph analysis). Loaf volumes were measured by rapeseed displacement after cooling for 20 min.

3.2.10 Image Analysis

Image analysis of crumb grain of baked loaves was done after 12 hours of baking with a C-Cell, an image analyzing software and equipment (Calibre Control International Ltd., UK). Loaves were sliced using a rotary disc blade (unserrated Graef® blade) cutter. Central slices of 15 mm thickness were obtained. After slicing, image analysis was performed as soon as possible to avoid any shrinkage of crumb grain. Image analysis parameters (number of cells and average cell elongation) were used for comparison between different treatments.

3.2.11 Biaxial Extensional Rheology

Biaxial extensional rheological properties of the doughs were measured with a Stable Micro Systems dough inflation system mounted on a texture analyzer (TAXT 2plus) by means of the procedure established by Dobraszczyk (1997). Doughs for rheological testing were mixed in the same mixer as used for bake tests, using the same water absorption, mixing times and sodium chloride addition. After mixing, dough pieces were squashed by hand on a sheeting board without putting too much stress on the dough, and then allowed to relax for 5 min. They were then sheeted, rolled out slowly with several passes and rotated by 90 degrees after each pass. Sheeting was done for 5 min with relaxation of 10 s between each pass. Sheeting in all directions prevents anisotropic effects during dough inflation, allowing dough pieces to expand uniformly into spherical shapes. After sheeting, dough pieces were relaxed for 20 min. They were then cut into circular discs using a 55 mm cookie cutter, squashed to a height of 2.67 mm for 20 s into

oiled dough inflation system pots. Sample dough pieces (in pots) were then proofed at 35^0C for 25 min. During sample preparation, dough pieces were protected against loss of moisture using a fine coating of mineral oil (Saybold viscosity 335/358) and covering with shrink wrap film. Mineral oil of lower viscosity seems to penetrate dough pieces and may affect rheological measurements.

Dough pieces were inflated at a flow rate of 500cm^3/min at a strain rate of 0.1/s . Rheological parameters (peak stress, failure strain and strain hardening index) were used to compare between different treatments. Strain hardening index was calculated by fitting an exponential curve to the stress-strain (Hencky) curve, after transferring data to Microsoft Excel worksheet.

3.2.12 Statistical Analysis

Results were analyzed using analysis of variance (ANOVA). ANOVA was performed using a general linear model procedure to determine significant differences and interactions for the various treatments. Means were compared by using Fishers LSD procedure ($\alpha = 0.05$). Statistical analysis was performed using proc GLM in SAS (version 9.1; SAS Institute Inc., Cary, NC) software. Duplicates were prepared for each treatment and the order of treatment was not significant.

3.3 Results and Discussion

To investigate the presence of liquid lamellae and their ability to stabilize gas cells in breadmaking, the classical baking procedure of MacRitchie and Gras (1973) was conducted. In addition to baking, the effect of flour lipid variation on biaxial extensional rheology was tested.

3.3.1 Physico-Chemical Analysis of Flours

Two wheat flours, Jagger and soft, used for this study were analyzed for their chemical composition and physical dough mixing properties. Moisture (wet weight basis), protein (14% moisture basis) and lipid (14% moisture basis) contents of the flours are given in Table 3.1. Jagger (10.4%) was nearly 1% higher in protein content than soft (9.2%). However, soft gave higher mixing time (9.25 min) compared to Jagger (7.21 min). Relatively stronger behavior of soft flour dough is visible in its Mixograph chart (Fig. 3.1), where it does not show as definite development and breakdown as that of Jagger flour dough. Higher percentage of unextractable polymeric protein (UPP) (Table 3.2) in soft flour explains the higher mixing requirements for this flour. This is not typical of soft wheat flours, which normally are considered weak. However, some soft varieties like Caldwell are relatively stronger than others. Soft flour was milled from composite of soft wheat varieties grown in Missouri during 2004-2005 and obtained from grain elevators at Kansas City, MO. Increase in mixing requirements in terms of Mixograph dough development time (MDDT) with increase in percent UPP has been reported previously by Gupta et al (1993). Data on polymeric proteins was obtained by SE-HPLC analysis of the flours. Lipid content of Jagger and soft was 0.89% and 0.93%, respectively.

3.3.2 Effect of Original Flour Lipid Level Variation on Baking Performance

3.3.2.1 Breadmaking

Significant differences ($P<0.0001$) in loaf volumes of the two flours were observed in response to varying levels of flour lipids (Table 3.3, Fig. 3.2). Incremental addition of flour lipids back into defatted parent flour caused bread volume to decrease, and after reaching a minimum, to increase. At all levels of lipid addition, relatively lower volumes were observed for soft flour compared to Jagger. For soft flour, minimum volume is reached at a relatively lower percentage of original flour lipids (~ 30%) in comparison to Jagger (~ 50%) (Fig. 3.2).

Fig 3.1 Mixographs of (A) Jagger and (B) Soft wheat flours, showing differences in the mixing behavior of the two flours.

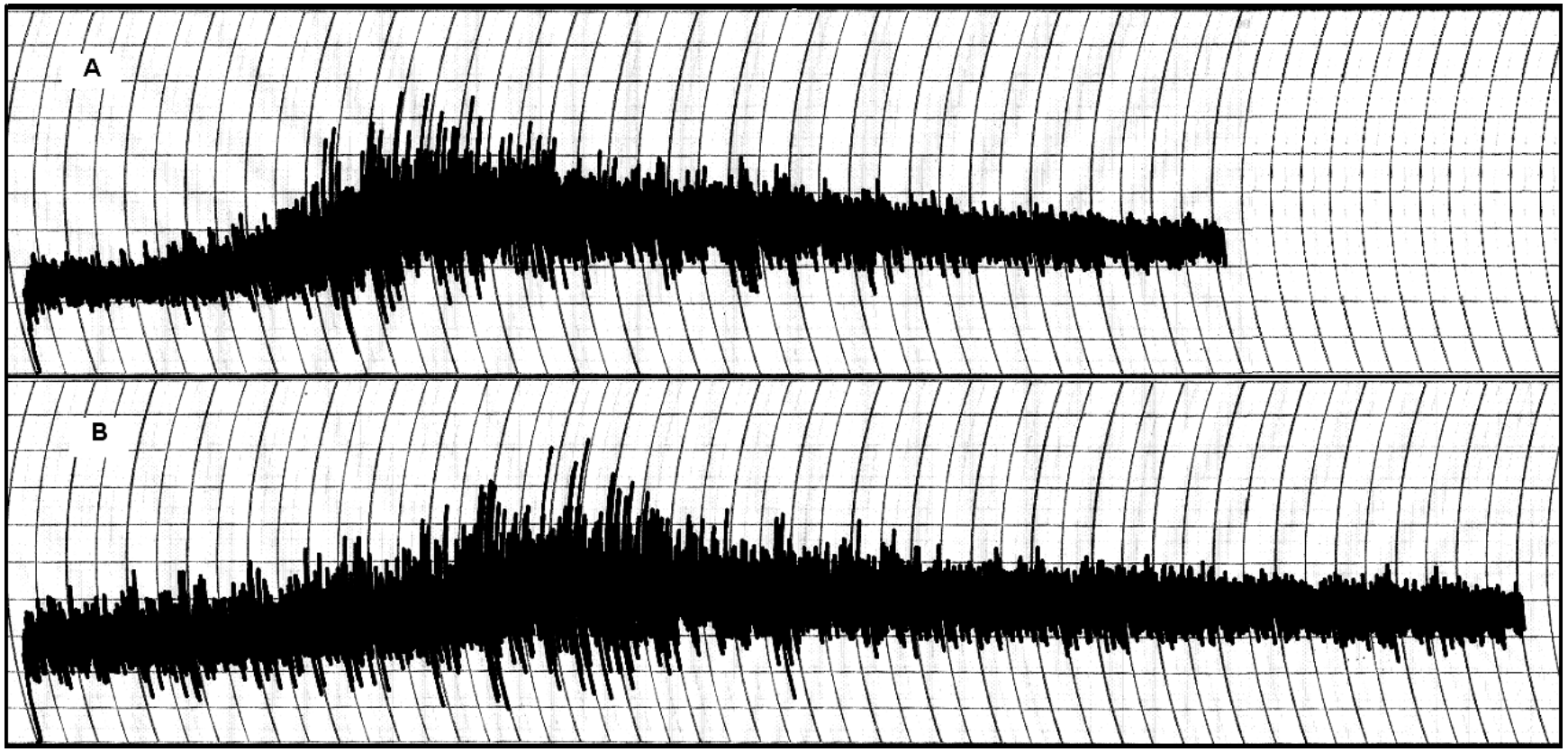


Table 3.1 Physico-Chemical analysis of Jagger and Soft wheat flours.

	Jagger Wheat Flour	Soft Wheat Flour
Moisture (%) (Wet Weight Basis)	12.5 ± 0.05	11.7 ± 0.01
Protein (%) (14% moisture basis)	10.4 ± 0.02	9.2 ± 0.03
Lipid Content (%) (14% moisture basis)	0.89 ± 0.03	0.93 ± 0.01
Mixograph Midline Peak Development Time (min)	7.21 ± 0.06	9.25 ± 0.00

Table 3.2 SE-HPLC relative composition of polymeric proteins in Jagger and Soft wheat flours.

Wheat Flours	Area (%) under Chromatogram Curve		
	TPP*	**EPP***	**UPP***
Jagger	36.4 ± 0.01	43.6 ± 0.02	56.4 ± 2.77
Soft	37.0 ± 0.05	39.0 ± 0.11	61.1 ± 3.40

* TPP - total polymeric protein; EPP - extractable polymeric protein; UPP – unextractable polymeric protein

The results of loaf volume variations in response to original flour lipid level variations agreed qualitatively with those of MacRitchie and co-workers (MacRitchie and Gras, 1973; MacRitchie, 1976a; McCormack et al, 1991). These studies also reported that the differences in loaf volume became visible only during oven spring; i.e. during the initial baking stage. However, in our case, these variations in loaf volumes were apparent during the advanced proofing stage for levels of lipid addition giving lower loaf volume, and during the early baking stage for levels giving higher loaf volume, for both the flours. This might be due to the differences in expansion capacity of the doughs created due to differences in the lipid levels, which became visible when the maximum expansion capacity was achieved. Gandikota and MacRitchie (2005), reported that maximum expansion capacity of dough can be achieved at any stage of baking by application of vacuum. The expansion capacity/loaf volume seems to be governed by the extent to which gas cells can expand without failure.

Scanning electron micrographs by Gan et al. (1990) showed the presence of intact gas cells with discontinuities in gluten-starch matrix at advanced stages of proofing. This means that the expansion capacity of the gas cells is not just controlled by the gluten-starch matrix and there is possibly a secondary factor contributing to it. This secondary factor as hypothesized by Gan et al (1990) is a liquid lamella present on the inner side of the gas cell (Fig 2.5). When liquid lamellae also fail, the presence of discontinuities in the gluten-starch matrix leads to coalescence of gas cells, thus decreasing volume significantly. It is quite possible that lipids due to their surface action might be affecting stability of liquid lamellae, thus causing variation in loaf volume. However, this needs to be investigated further to make sure that their (lipids) action is independent of the rheological properties of the gluten-starch matrix.

Relatively lower loaf volumes at all levels of lipid addition in soft flour, could be attributed to inherent differences in gluten quality of the two flours, as previously suggested by MacRitchie (1978).

3.3.2.2 Crumb Structure

Image analysis of bread crumb (Table 3.4, Fig 3.5, Fig 3.6) showed that addition of different levels of flour lipids resulted in insignificant variations in number of gas cells ($P = 0.02$) (Table 3.4, Fig 3.3) and average cell elongation ($P = 0.94$ for Jagger and $P = 0.12$ for soft) (Table 3.4, Fig 3.4). These not so significant differences in number of gas cells further make it

Table 3.3 Loaf volume responses of Jagger and Soft wheat flours to original flour lipid levels. [A, B]

% of Original Flour Lipids	Loaf Volume (cc)	
	Jagger Wheat Flour	Soft Wheat Flour
0%	299.5 ± 0.71 a	214.0 ± 8.49 a
20%	246.0 ± 2.83 c,d	189.0 ± 1.41 c
40%	206.0 ± 1.41 d,e	148.0 ± 2.83 i,h
50%	194.5 ± 0.71 e, f,g	155.0 ± 0.00 g,h
60%	198.0 ± 2.83 g	161.0 ± 1.41 f,g
80%	213.5 ± 2.12 f,g	173.5 ± 2.12 d,e
100%	237.5 ± 3.54 b,c	180.0 ± 0.00 d

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Fig 3.2 Loaf volume vs. intact original flour lipids, added to defatted Jagger and Soft wheat flours as percentage of original flour lipids.

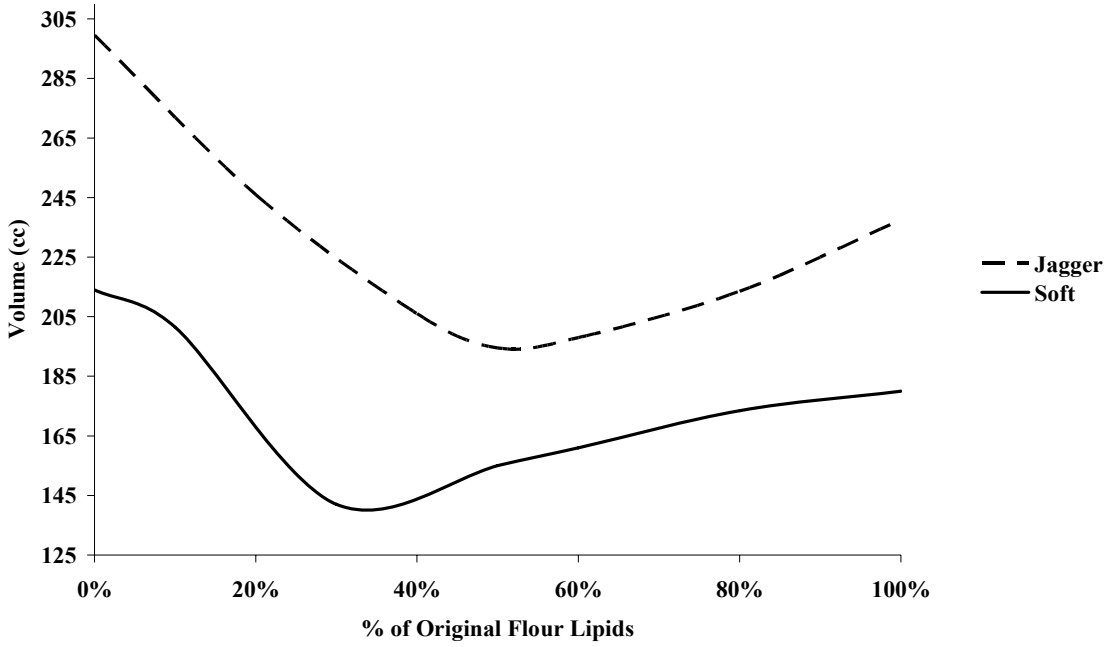


Table 3.4 Crumb structure responses of Jagger and Soft wheat flour breads to original flour lipid levels. [A, B]

% of Original Flour Lipids	Jagger Flour		Soft Flour	
	Number of Cells	Average Cell Elongation (C-Cell Score)	Number of Cells	Average Cell Elongation (C-Cell Score)
0%	2000.0 ± 11.3 a	1.58 ± 0.01 b	2544.5 ± 187.4 a,b	1.69 ± 0.01 a,b
20%	1935.0 ± 222.03 a,b	1.59 ± 0.05 b	2521.0 ± 110.3 a,b,c	1.68 ± 0.01 a,b,c
40%	1880.5 ± 78.5 a,b	1.64 ± 0.06 a,b	2210.5 ± 115.3 d	1.65 ± 0.03 b,c
50%	1898.0 ± 59.4 a,b	1.68 ± 0.04 a	2209.0 ± 79.2 d	1.65 ± 0.02 c
60%	1788.5 ± 81.3 a,b	1.67 ± 0.05 a,b	2347.5 ± 2.1 b,c,d	1.70 ± 0.02 a
80%	1907.5 ± 2.1 a,b	1.62 ± 0.01 a,b	2196.0 ± 86.3 d	1.66 ± 0.00 a,b,c
100%	1749.0 ± 41.0 b	1.63 ± 0.01 a,b	2229.0 ± 114.6 d	1.69 ± 0.03 a,b

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Fig 3.3 Number of cells vs. intact original flour lipids, added to defatted Jagger and Soft wheat flours as percentage of original flour lipids.

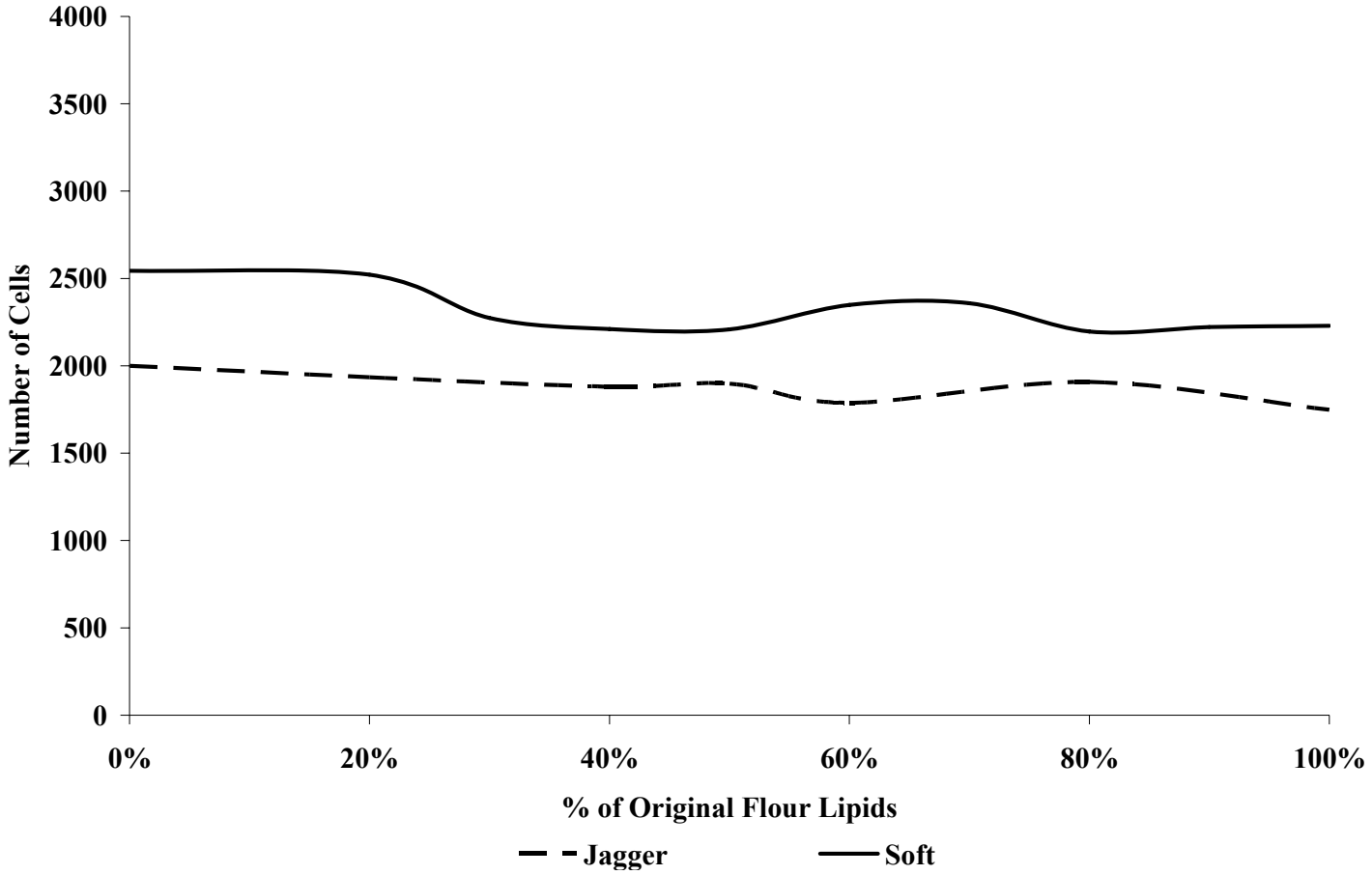


Fig 3.4 Average cell elongation vs. intact original flour lipids, added to defatted Jagger and Soft wheat flours as percentage of original flour lipids.

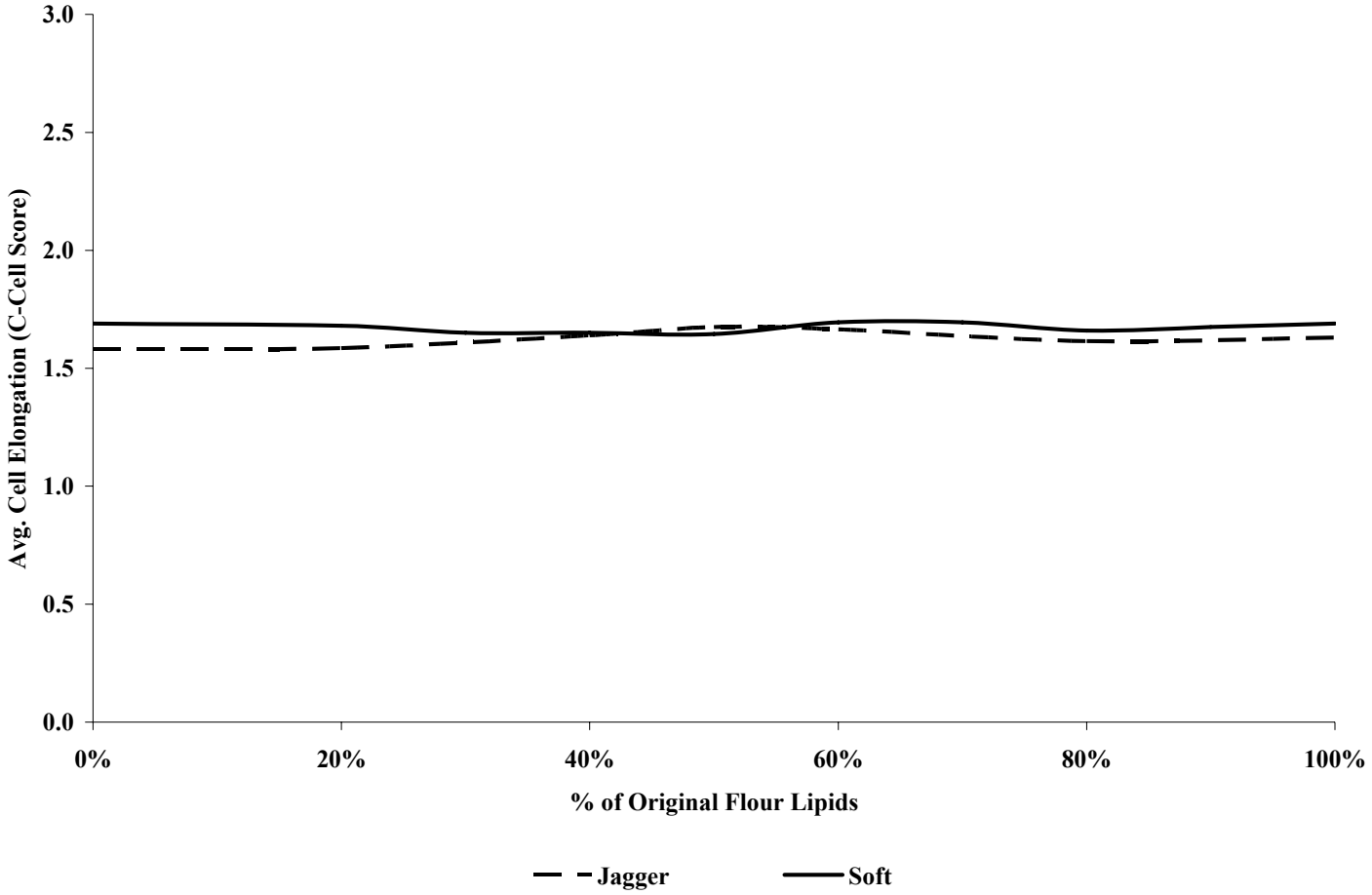


Fig 3.5 C-Cell images of Jagger wheat flour bread slices showing differences in gas cell expansion at different original flour lipid levels (represented as percentages) added to defatted flour.

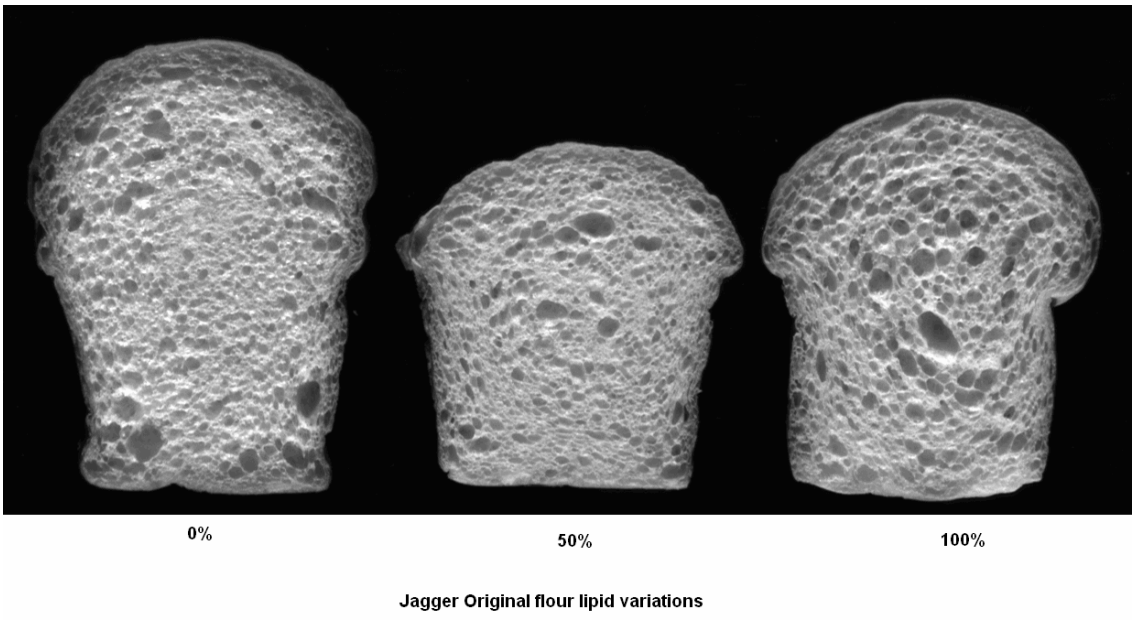
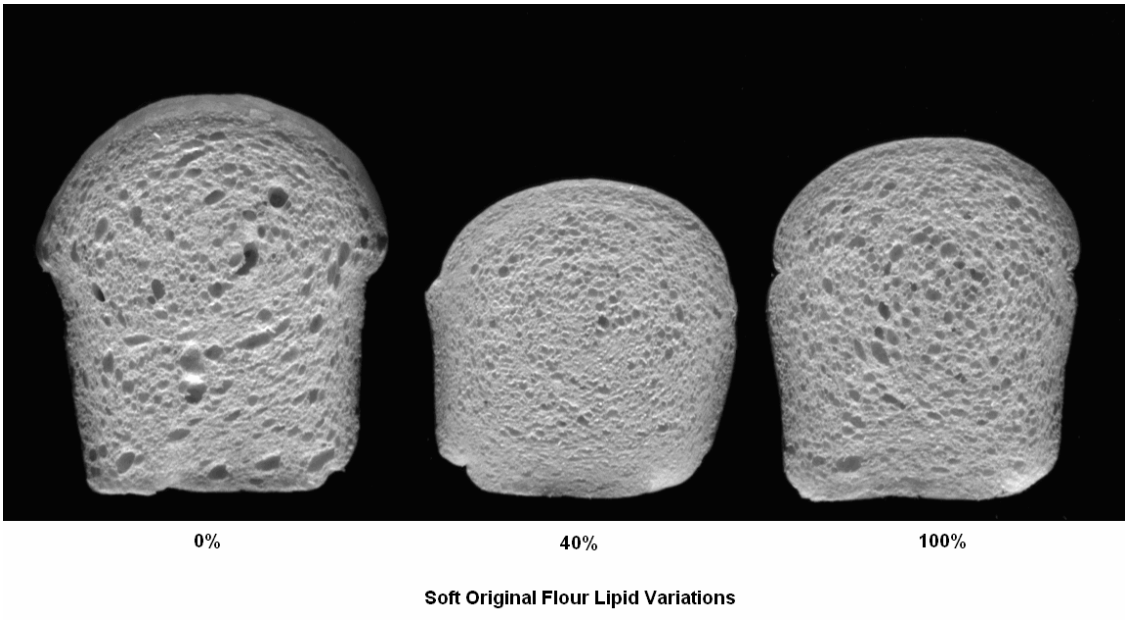


Fig 3.6 C-Cell images of Soft wheat flour bread slices showing differences in gas cell expansion at different original flour lipid levels (represented as percentages) added to defatted flour.



evident that differences in loaf volumes are due to differences in expansion capacity of the gas cells (Fig 3.5, Fig 3.6), and factors like gas cell concentration are not involved in this case. Negligible variations in average cell elongation indicated that lipids might not be causing any variations in rheology of the gluten-starch matrix, since cell elongation is thought to be associated with dough rheology (Gandikota and MacRitchie, 2005).

3.3.3 Effect of Original Flour Lipid Level Variation on Biaxial Extensional Rheology of Gluten-Starch Matrix.

In order to investigate possible independent action of lipids causing loaf volume variations, to indicate presence of liquid lamellae, biaxial extensional rheology tests were conducted on defatted Jagger and soft flour doughs, and at flour lipid levels where maximum and minimum loaf volumes were observed.

Biaxial extensional rheological parameters (maximum stress, failure strain and strain hardening index) were higher for Jagger doughs (Table 3.5) in comparison to soft flour doughs. Different treatments did not influence these parameters within doughs prepared from each flour type (Table 3.5, Fig 3.7, Fig 3.8). Though very minor differences were observed for strain hardening indices and failure strain of the doughs at some lipid levels, no specific trend was observed, thus attributing the variation to slight experimental scatter.

Higher values of biaxial extensional rheological parameters for Jagger, verify that rheologically Jagger is a better breadmaking flour. Higher strain hardening index and failure strains ensure that gas cells in Jagger doughs expand to a greater extent than soft flour doughs. Similar results differentiating between breadmaking potential of flours have been reported by Dobraszczyk and co-workers (Dobraszczyk and Roberts, 1994; Dobraszczyk et al, 2003). However, no effect of lipid treatments on dough rheology, besides the fact that these treatments produced major difference in loaf volumes, provides clear evidence for the role of lipids, independent of dough rheology as surface active compounds stabilizing liquid lamellae.

Table 3.5 Mean bubble inflation rheological response of Jagger and Soft wheat flour doughs to original flour lipid levels. [A, B]

% of Original Flour Lipid	Max. Stress (Kpa)	Failure Strain (Hencky)	Strain Hardening Index
Jagger Wheat Flour			
0%	571.62 ± 305.45 a	2.58 ± 0.18 a	2.21 ± 0.12 b,c
60%	491.68 ± 237.21 a	2.60 ± 0.12 a	2.38 ± 0.064 a,b
100%	598.62 ± 112.05 a	2.66 ± 0.04 a	2.44 ± 0.06 a
Soft Wheat Flour			
0%	120.80 ± 12.76 a	1.79 ± 0.00 a	1.76 ± 0.01 a
40%	125.42 ± 12.14 a	1.85 ± 0.02 a	1.74 ± 0.01 a
100%	102.40 ± 5.45 a	1.66 ± 0.05 b	1.64 ± 0.04 c

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Fig 3.7 Stress vs. strain (Hencky) curves of Jagger flour doughs with different original flour lipid levels measured in biaxial extension.

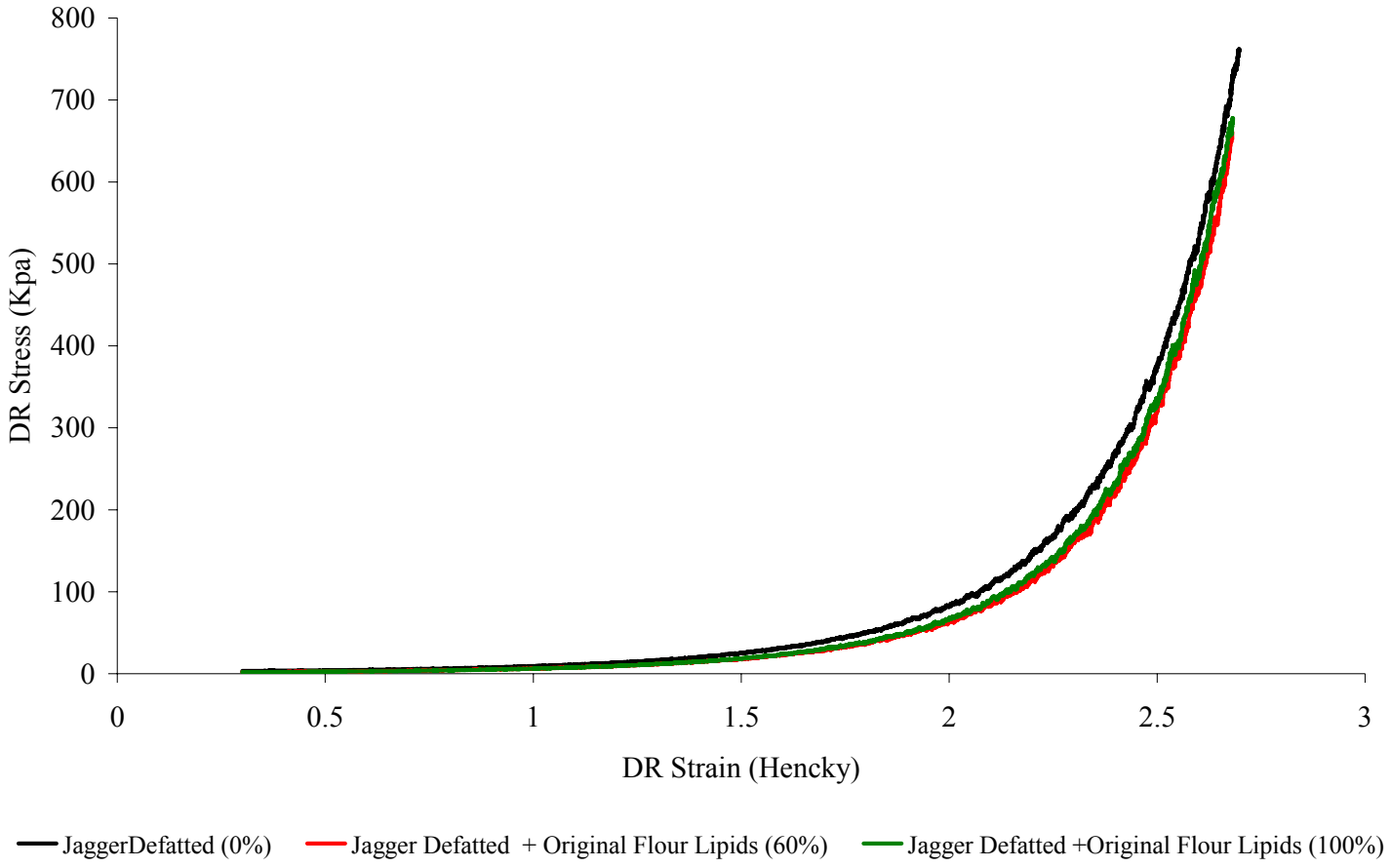
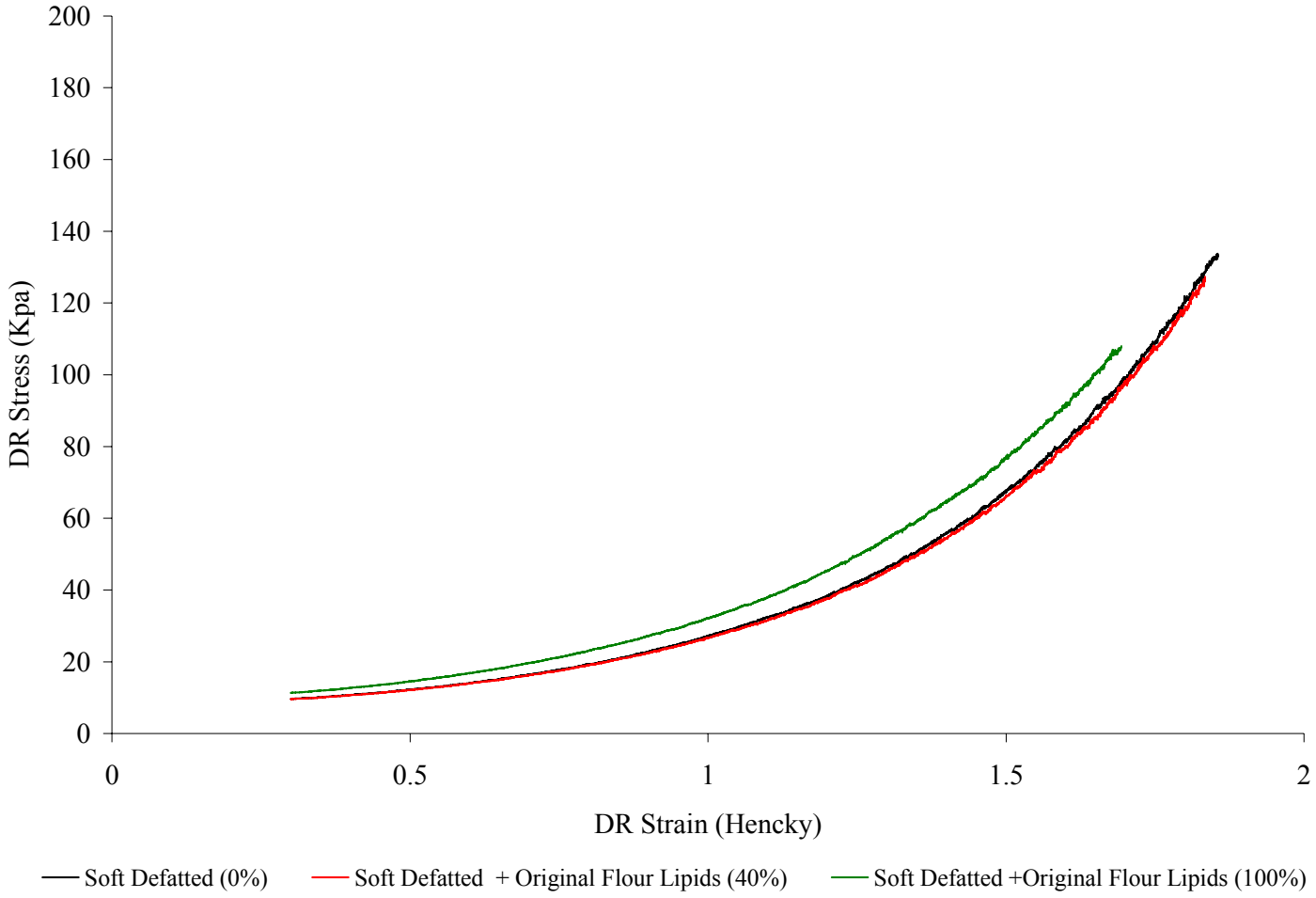


Fig 3.8 Stress vs. strain (Hencky) curves of Soft flour doughs with different original flour lipid levels measured in biaxial extension.



3.4 Conclusion

Results of the study provide evidence in support of the dual film hypothesis. It can be concluded that, during breadmaking, a primary gluten-starch matrix and secondary liquid lamellae enveloping them stabilize gas cells. The liquid lamellae are on the inner side of the gluten-starch matrix (Fig 2.5). The original flour lipids do not influence rheological properties of the gluten-starch matrix surrounding the gas cells, mainly due to their presence in very small quantities. However, rheology of the gluten-starch matrix plays an important role in gas cell stability and inherent differences in gluten quality, as indicated by biaxial extensional rheology of Jagger and soft doughs and can significantly affect the breadmaking potential. Lower loaf volumes of soft flour in comparison to Jagger at parallel lipid levels (Table 3.3, Fig 3.2) show the primary importance of the gluten-starch matrix in gas cell stability. On the other hand, wheat flour lipids are surface active compounds, and they influence gas cell stability by altering surface properties of liquid lamellae. Small amounts in which these lipids are present are sufficient to influence surface properties.

Comments on the exact mechanism by which these lipids and other surface active components (proteins) stabilize liquid lamellae is discussed in chapter 2. Nevertheless, a hypothesis can be suggested here for the mechanism of stabilization. It seems that liquid lamellae are stabilized by adsorption of these surface active components at the gas-liquid interface to form monolayers (Fig 2.11) (Ross and MacRitchie, 1995). The surface active components, depending on their molecular size or conformation, form either condensed or expanded monolayers (Gaines, 1966; MacRitchie, 1990). Condensed monolayers are more stable due to their lower compressibility and resistance to desorption when the interfacial area changes (MacRitchie, 1976b). The stability of liquid lamellae thus depend on stability of the monolayer. In defatted flours, proteins are the only surface active components forming monolayers. This results in a higher loaf volume and a more uniform crumb structure as proteins do not desorb easily and give stable monolayers. Addition of original flour lipids may lead to the formation of mixed monolayers as suggested by Ross and MacRitchie (1995). The occurrence of minima in loaf volume on addition of original flour lipids is analogous to the decrease in stability of mixed films reported by Paternotte et al (1993) on passage from a pure protein film to a pure lipid (monogalactosyl monoglyceride) film. Variations in percentage of original flour lipid at which

the minimum volume is reached could be attributed to differences in protein composition of the two flours.

3.5 References

Baker, J.C., Mize, M.D. 1946. Gas occlusion during dough mixing. Cereal Chemistry 23, 39-51.

Batey, I.L., Gupta, R.B., MacRitchie, F. 1991. Use of size-exclusion high-performance liquid chromatography in the study of wheat flour proteins: an improved chromatographic procedure. Cereal Chemistry 68, 207-209.

Bloksma, A.H. 1990b. Dough structure, rheology, and baking quality. Cereal Foods World 35, 237-244.

Dobraszczyk, B.J. 1997. Development of a new dough inflation system to evaluate doughs. Cereal Foods World 42, 516-519.

Dobraszczyk, B.J., Roberts, C.A. 1994. Strain hardening and dough gas cell-wall failure in biaxial extension. Journal of Cereal Science 20, 265-274.

Dobraszczyk, B.J., Smewing, J., Albertini, M., Maesmans, G., Schofield, J.D. 2003. Extensional rheology and stability of gas cell walls in bread doughs at elevated temperatures in relation to breadmaking performance. Cereal Chemistry 80, 218-224.

Gaines, G.L. 1966. Insoluble Monolayers at Liquid-Gas Interfaces. Prigogine, I. (Ed.), Interscience Monographs on Physical Chemistry, John Wiley and Sons, Inc., New York, USA.

Gan, Z., Angold, R.E., Williams, M.R., Ellis, P.R., Vaughan, J.G., Galliard, T. 1990. The microstructure of gas retention of bread dough. Journal of Cereal Science 12, 15-24.

Gan, Z., Ellis, P.R., Schofield, J.D. 1995. Gas cell stabilization and gas retention in wheat bread dough. Journal of Cereal Science 21, 215-230.

Gandikota, S., MacRitchie, F. 2005. Expansion capacity of doughs: methodology and applications. Journal of Cereal Science 42, 157-163.

Gupta, R. B., Khan, K., MacRitchie, M. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quality and size distribution of polymeric protein. Journal of Cereal Science 18, 23-41.

Hoseney, R.C. 1992. Physical chemistry of bread dough. In: Schwartzberg, H.G., Hartel. R.W. (Eds.), Physical Chemistry of Foods, Marcel Dekker, Inc., New York, USA, pp 443-457.

MacRitchie, F. 1976a. The liquid phase of dough and its role in baking. Cereal Chemistry 53, 318-326.

MacRitchie, F. 1976b. Monolayer compression barrier in emulsion and foam stability. Journal of Colloid and Interface Science 56, 53-56.

MacRitchie, F. 1978. Differences in baking quality between wheat flours. Journal of Food Technology 13, 187-194.

MacRitchie, F. 1990. Chemistry at Interfaces. Academic Press, Inc., San Diego, CA, USA.

MacRitchie, F., Gras, P.W. 1973. The role of flour lipids in baking. Cereal Chemistry 50, 292-302.

McCormack, G., Panozzo, J., MacRitchie, F. 1991. Contributions to breadmaking of inherent variations in lipid content and composition of wheat cultivars.II. Fractionation and reconstitution studies. Journal of Cereal Science 13, 263-274.

Paternotte, T.A., Orsel, R., Hamer, R.J. 1993. Interactions between flour proteins and flour lipids at the liquid/air interface. In: Gluten Proteins, Association of Cereal Research, Detmold, Germany, pp 207-217.

Ross, A., MacRitchie, F. 1995. Interactions of wheat proteins, carbohydrates and lipids. In: Gaonkar, A.G. (Ed.), Ingredient Interactions Effects on Food Quality, Marcel Deker, New York, USA, pp 321-356.

Singh, N.K., Donovan, G.R., Batey, I.L., MacRitchie, F. 1990. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. I. Dissolution of total proteins in the absence of reducing agents. Cereal Chemistry 67, 150-161.

van Vliet, T., Janssen, A.M., Bloksma, A.H., Walstra, P. 1992. Strain hardening of dough as a requirement for gas retention. Journal of Texture Studies 23, 439-460.

CHAPTER 4 - Mechanism of Stability of Liquid Lamellae

4.1 Introduction

High initial concentration of gas cells and their stability during breadmaking is essential for good loaf volume and crumb grain. It has been verified that the liquid lamellae surrounding expanding gas cells act as secondary protection together with the primary gluten-starch matrix, thus preventing their coalescence and disproportionation (Chapter 3). Stability of the liquid lamellae involves surface active compounds (proteins and lipids), present in the continuous liquid phase of dough (Baker et al, 1946; MacRitchie, 1976a), that are adsorbed at the gas-liquid interface. Analogous variations in foaming properties of the dough liquor, and bread loaf volume and crumb structure (MacRitchie, 1976a) provide plausible evidence of surface action of these compounds at the gas-liquid interface.

Contributions of liquid film stability to dough expansion can be assessed from different studies. Studies have shown that flour lipids, though present in very low amounts (1-1.5%), have significant effects on baking performance in terms of volume and crumb structure (MacRitchie and Gras, 1973). Polar lipids improve bread loaf volume and crumb grain, whereas non-polar lipids have the opposite effects (Ponte and DeStefanis, 1969; MacRitchie and Gras, 1973). DeStefanis and Ponte (1976) observed that an unsaturated free fatty acid (linoleic acid) has detrimental effects on loaf volume. The most probable mechanism by which these surface active components control stability of gas cells is through formation of monolayers at the gas-liquid interface (Ross and MacRitchie, 1995). Saturated free fatty acids like palmitic and stearic form condensed monolayers, which are highly incompressible and these molecules do not desorb easily from the monolayers. These condensed monolayers generate substantial elastic restoring forces (Fig. 2.11), which resist destabilization of liquid lamellae when changes in interfacial area occur (MacRitchie, 1976b). Molecules like digalactosyl diglyceride (DGDG) with strong polar head groups are expected to orient themselves strongly at the interface as condensed monolayers. Unsaturated free fatty acids like linoleic acid give expanded monolayers, which are relatively compressible and soluble in the sub phase, leading to instability of the liquid lamellae (MacRitchie, 1976b). It seems that the type of monolayer formed will either stabilize or destabilize the freshly occluded gas cells, and any changes in monolayer state will influence the gas cell stability at all stages of breadmaking.

Therefore, the stability of liquid lamellae determines the ease with which gas cells are concentrated during mixing and which ensure their permanence during the entire breadmaking process. The major objective of this part of the study is to understand the possible mechanism by which liquid lamellae are stabilized.

4.2 Materials and Methods

4.2.1 Flours

Jagger flour and soft wheat flour (a blend of soft wheat varieties) were two untreated and unbleached flours used in this study. The flours were milled in Buhler mill (73% milling extraction rate) in the department of Grain Science and Industry, Kansas State University, Manhattan, KS. The flours were evaluated for certain quality characteristics given in Table 3.1. These flours were stored at -20^{0}C until use. For the sake of brevity, Jagger flour and soft wheat flour will be referred to as Jagger and soft, respectively.

4.2.2 Free Fatty Acids

Palmitic acid (99%), linoleic acid (60%), and myristic acid (99%) were purchased from Sigma-Aldrich, USA. Values in brackets are of minimum purity by gas chromatography as per certificate of analysis by manufacturer. Linolenic acid was main impurity in linoleic acid.

4.2.3 Reagents

Chloroform used was of HPLC grade, all other chemicals used in this were of ACS grade. These were purchased from Sigma-Aldrich, USA. Distilled deionized water, sterilized in an autoclave at a pressure of 120 psi for 20 min, was used in all stages of the experiments.

4.2.4 Lipid Extraction from Flour

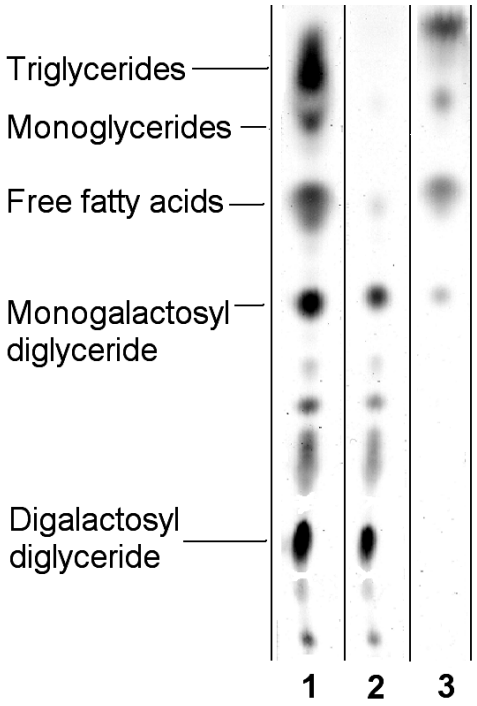
Original flour lipids were extracted using three batch extractions with chloroform in a glass beaker, followed by Buchner filtration through Whatman No. 1 filter paper (MacRitchie and Gras, 1973). 200 g of flour and 400 ml of chloroform were used for each extraction. The defatted flour was spread out on a flat glass tray in a fume hood for 12 hours to allow evaporation of solvent.

4.2.5 Lipid Fractionation

Original lipids of both flours in chloroform were collected together and concentrated under vacuum using a rotary evaporator at a temperature < 30^{0}C. The combined original flour lipids were then separated into polar and non-polar fractions by the method of Ponte and DeStefanis (1969). High purity grade silica gel without binder (Sigma-Aldrich, USA) of average particle size 5-25 μm was used in the procedure. The amount of original flour lipid extracted

with chloroform was 0.91% (flour weight basis). The amount of polar and non-polar fractions separated out were 0.36% and 0.55% (flour weight basis), respectively. These fractions i.e. original flour lipids, polar fraction and non-polar fraction, were analyzed for their composition, qualitatively, using the thin-layer chromatography (TLC) method of McCormack et al (1991). TLC patterns (Fig 4.1) show efficient separation of original flour lipids into polar and non-polar fractions. After extraction and analysis, the fractions were solubilized in chloroform and nitrogen was bubbled through the liquids to remove any solubilized oxygen. The liquids (fraction + solvent) were then refrigerated under nitrogen atmosphere until their use. For use in baking and rheology measurements, these were again concentrated under vacuum using a rotary evaporator.

Fig 4.1 Thin-layer chromatogram of (1) original flour lipid and its (2) polar and (3) non-polar fractions.



4.2.6 Addition of Lipids to Defatted Flour

Different lipid types (polar, non-polar) and free fatty acids (linoleic acid, palmitic acid and myristic acid) were added to the defatted flour in different amounts. For incorporation of intact original flour lipids into defatted flour, the parent flour was mixed with defatted flour in different proportions, for both flours, to give different flour lipid levels. All other lipid additions

were made as a percentage of total flour lipids; for example, addition of 60% non-polar fraction means 60% of 0.91g was added to 100 g of defatted flour.

4.2.7 Test Baking

Test loaves (35 g flour) were baked using a modified rapid bake test (MacRitchie and Gras, 1973). A lean formulation was used with no added shortening; flour (100%), sugar (6%), sodium chloride (1.5%), instant yeast (2.7%), potassium bromate (30ppm), water and mix time (as optimized from Mixograph analysis, Table 3.1). Loaf volumes were measured by rapeseed displacement after cooling for 20 min.

4.2.8 Image Analysis

Image analysis of crumb grain of baked loaves was done after 12 hours of baking with a C-Cell, an image analyzing software and equipment (Calibre Control International Ltd., UK). Loaves were sliced using a rotary disc blade (unserrated Graef® blade) cutter. Central slices of 15 mm thickness were obtained. After slicing, image analyses were performed as soon as possible to avoid any shrinkage of crumb grain. Image analysis parameters (number of cells and average cell elongation) were used to compare different treatments.

4.2.9 Biaxial Extensional Rheology

Biaxial extensional rheological properties of the doughs were measured with a Stable Micro Systems dough inflation system mounted on a texture analyzer (TAXT Plus) by means of the procedure established by Dobraszczyk (1997). Doughs for rheological testing were mixed in the same mixer as used for bake tests, using the same water absorption, mixing times and sodium chloride addition. After mixing, dough pieces were squashed by hand on a sheeting board without putting too much stress on the dough, and then allowed to relax for 5 min. They were then sheeted, rolled out slowly by several passes and rotated by 90 degrees after each pass. Sheeting was done for 5 min with relaxation of 10 s between each pass. Sheeting in all directions prevents anisotropic effects during dough inflation, allowing dough pieces to expand uniformly into spherical shapes. After sheeting, dough pieces were relaxed for 20 min. They were then cut into circular discs using a 55 mm cookie cutter, squashed to a height of 2.67 mm for 20 s into oiled dough inflation system pots. Sample dough pieces (in pots) were then proofed at 35^0C for 25 min. During sample preparation, dough pieces were protected against loss of moisture using a

fine coating of mineral oil (Saybold viscosity 335/358) and covering with shrink wrap film. Mineral oil of lower viscosity seems to penetrate dough pieces and may affect rheological measurements.

Dough pieces were inflated at a flow rate of 500cm^3/min at a strain rate of 0.1/s . Rheological parameters (peak stress, failure strain and strain hardening index) were used to compare different treatments. Strain hardening index was calculated by fitting an exponential curve to the stress-strain (Hencky) curve, after transferring data to Microsoft Excel worksheet.

4.2.10 Statistical Analysis

Results were analyzed using analysis of variance (ANOVA). ANOVA was performed using a general linear model procedure to determine significant differences and interactions for the various treatments. Means were compared by using Fishers LSD procedure ($\alpha = 0.05$). Statistical analysis was performed using proc GLM in SAS (version 9.1; SAS Institute Inc., Cary, NC) software. Duplicates were prepared for each treatment and the order of treatment was not significant.

4.3 Results and Discussion

To gain understanding of the mechanism by which surface active components (lipids and proteins) of wheat flour dough affect stability of the liquid lamellae surrounding expanding gas cells, baking trials were conducted by adding different lipid types (original flour lipid and its polar and non polar factions) and free fatty acids (linoleic, palmitic and myristic acids) to defatted flours at different levels of addition (on original flour weight basis). Effect of lipids was also tested on biaxial extensional rheology of the doughs.

4.3.1 Effect of Variations in Lipid Types and Free Fatty Acids, and their Levels on Baking Performance

4.3.1.1 Breadmaking

Loaf volume trends observed on incremental addition of different lipid types and free fatty acids were mainly parallel for both Jagger and soft wheat flours, except for some variations in certain cases (Table 4.1, Fig 4.2, Fig 4.3). Within each lipid type and free fatty acid, significant differences were observed in response to varying levels, except palmitic acid (Fig 4.2, Fig 4.3). Incremental addition of original flour lipids back into parent flours caused loaf volume to decrease, and, after reaching a minimum, to increase. Incremental addition of the polar lipids also displayed initial depression for small additions followed by increase in loaf volume. This increase in volume was appreciably larger than what was observed for the same levels of original flour lipids. Addition of increasing levels of the non-polar lipids and linoleic acid caused a continuous decrease in loaf volume for both flours. Incremental addition of palmitic acid produced no change in loaf volume of Jagger flour and slight depression in soft wheat flour. Incremental addition of myristic acid caused depression in loaf volume at initial levels, however, at considerably higher levels of addition, some increase in loaf volume was observed. This increase, i.e. in the case of myristic acid, leveled off at the volumes equivalent to those of intact flours (i.e. 100% of original flour lipids). Maximum addition of myristic acid in Jagger was 400% of original flour lipids. This was a very high level of addition in comparison to other lipid types and free fatty acids used in the study; this was done to have a clear picture of the loaf volume trend in this case.

Table 4.1 Loaf volume responses of Jagger and Soft wheat flours to different lipid types and levels, added to defatted flours as percentage of original flour lipids. [A, B]

% of Original Flour Lipids	Loaf Volume (cc)	
	Jagger Wheat Flour	Soft Wheat Flour
Original Flour Lipids		
0%	299.5 ± 0.71 a	214.0 ± 8.49 a
20%	246.0 ± 2.83 c,d	189.0 ± 1.41 c
40%	206.0 ± 1.41 d,e	148.0 ± 2.83 i,h
50%	194.5 ± 0.71 e,f,g	155.0 ± 0.00 g,h
60%	198.0 ± 2.83 g	161.0 ± 1.41 f,g
80%	213.5 ± 2.12 f,g	173.5 ± 2.12 d,e
100%	237.5 ± 3.54 b,c	180.0 ± 0.00 d
Flour Polar Lipids		
0%	300.5 ± 0.71 c	214.0 ± 5.66 d
20%	285.5 ± 0.71 d	183.0 ± 1.41 f
40%	218.5 ± 3.54 g	263.5 ± 2.12 c
66%	229.0 ± 1.41 f	266.5 ± 2.12 c
132%	376.0 ± 1.41 b	288.5 ± 2.12 b
200%	397.0 ± 1.41 a	306.0 ± 1.41 a
Flour Non Polar Lipids		
0%	301.5 ± 2.12 a	220.0 ± 2.83 a
15%	286.0 ± 1.41 b	175.5 ± 13.44 b
30%	263.0 ± 0.00 c	153.0 ± 2.83 c
60%	223.5 ± 9.19 d	147.0 ± 4.24 c
132%	176.0 ± 1.41 e	147.0 ± 2.83 c
200%	150.5 ± 0.71 f	—
Linoleic Acid		
0%	300.5 ± 0.71 a	221.0 ± 1.41 b
66%	208.0 ± 11.31 b	231.0 ± 5.66 a
132%	138.0 ± 11.31 c	148.5 ± 0.71 c
200%	132.5 ± 6.36 c	124.5 ± 0.71 d
250%	128.0 ± 0.00 c	123.0 ± 1.41 d
Palmitic Acid		
0%	300.5 ± 0.71 a	221.0 ± 1.41 b
66%	293.5 ± 12.02 a	210.0 ± 14.14 a
132%	296.0 ± 4.24 a	186.5 ± 3.54 b
200%	295.5 ± 0.71 a	180.0 ± 1.41 b
250%	293.0 ± 0.00 a	169.0 ± 4.24 b
Myristic Acid		
0%	296.5 ± 2.12 a	201.5 ± 2.12 a
66%	238.5 ± 2.12 b	135.0 ± 0.00 d
132%	211.5 ± 0.71 c,d	151.5 ± 0.71 c
200%	201.0 ± 1.41 d	180.5 ± 0.71 b
250%	192.5 ± 0.71 d	179.5 ± 0.71 b
330%	205.5 ± 0.71 c,d	—
400%	229.0 ± 1.41 b	—

[A] Values represent mean ± standard deviation for duplicate determinations
[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Fig 4.2 Loaf volume vs. different lipid types, added to defatted Jagger flour as percentage of original flour lipids.

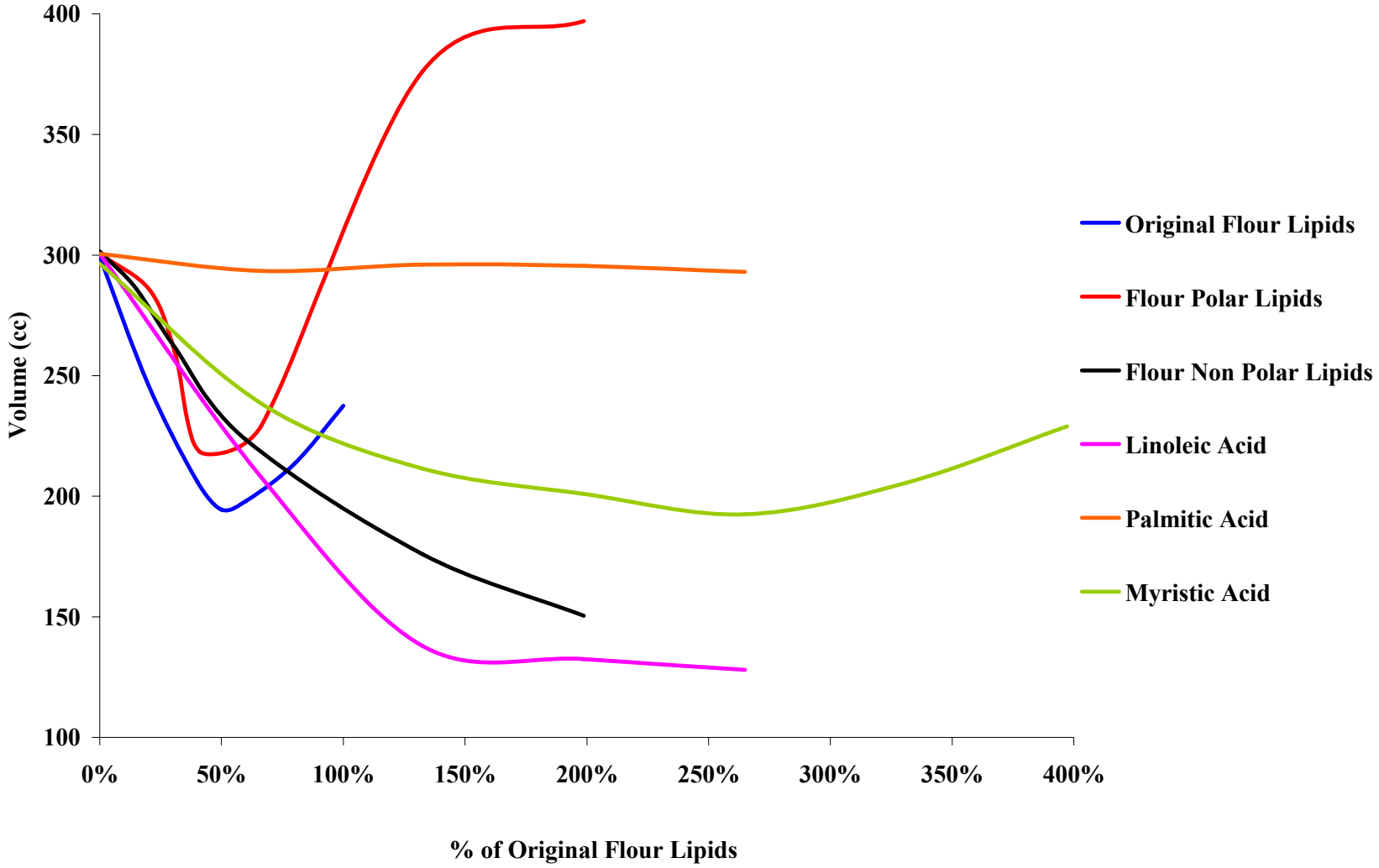
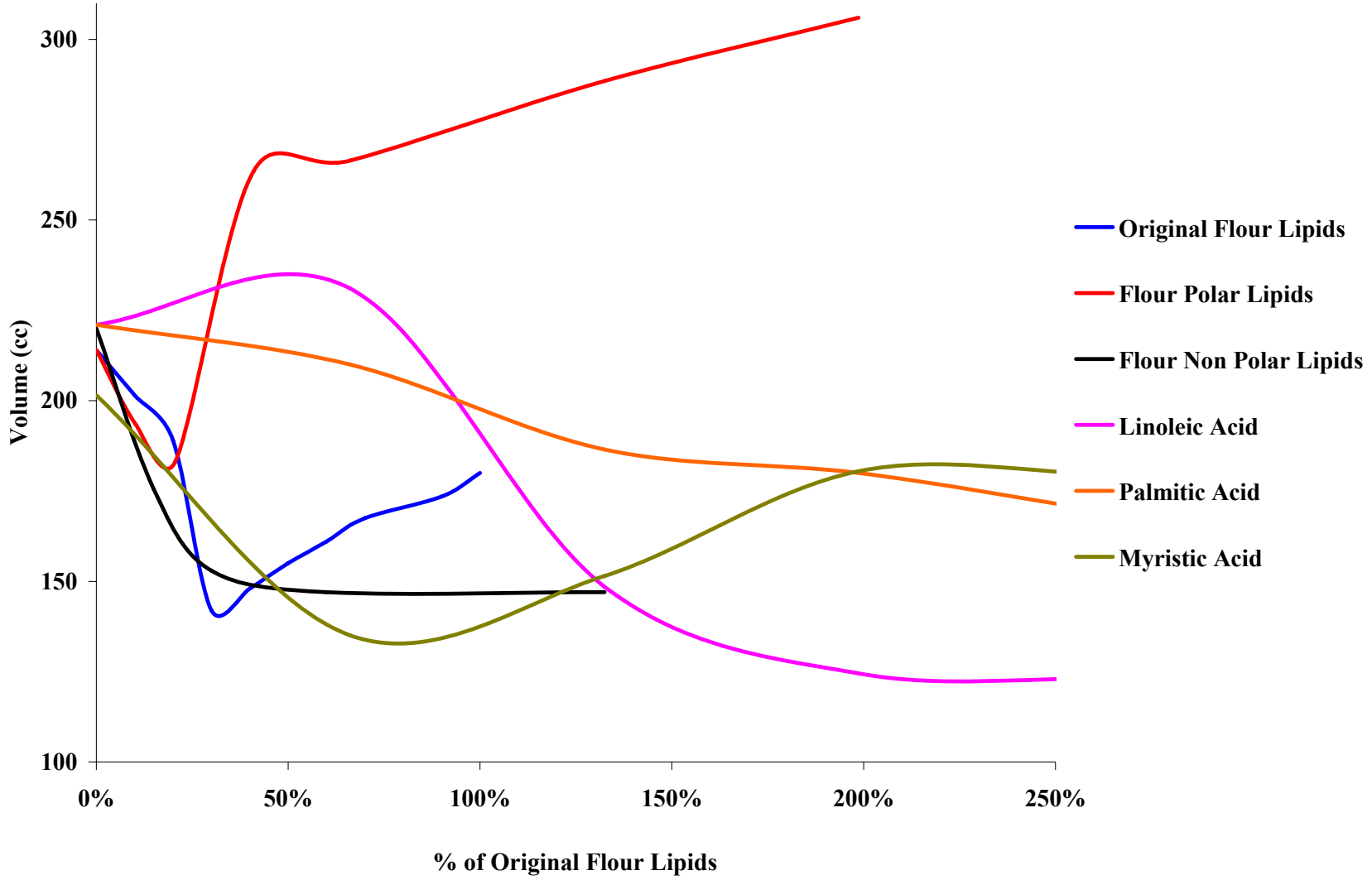


Fig 4.3 Loaf volume vs. different lipid types, added to defatted Soft wheat flour as percentage of original flour lipids.



The results for original flour lipids and their polar and non-polar fractions agreed qualitatively with those of MacRitchie and co-workers (MacRitchie and Gras, 1973; MacRitchie, 1977; MacRitchie, 1978; McCormack et al, 1991), and those of unsaturated (linoleic) and saturated (palmitic) free fatty acids concurred with results of DeStefanis and Ponte (1976). Effects of different lipid types on loaf volume and crumb structure were in agreement with results from film balance studies reported by various workers (Gaines, 1966; MacRitchie, 1990). This substantiated our earlier results (chapter 3) providing evidence for the presence of liquid lamellae around expanding gas cells. The results also indicated that the most probable mechanism by which different lipid types influenced the gas cell stability was through their adsorption as monolayers at the gas-liquid interface.

The monolayers formed are either condensed or expanded (Fig 2.11) (Gaines, 1966, MacRitchie, 1990). Condensed monolayers are characterized by close packing of surface active molecules, with polar head groups oriented towards water and non-polar hydrocarbon chains towards air. Expanded monolayers are formed due to loose packing of surface active molecules, occupying a much higher area per molecule. Condensed monolayers are incompressible and elastic compared to expanded ones and are less easily desorbed than expanded monolayers (MacRitchie, 1976b). On expansion of gas cells, condensed monolayers, due to their elastic nature, resist collapse of liquid lamellae with change of interfacial area (MacRitchie, 1976b).

In defatted flours, proteins are the only surface active components forming monolayers. This results in a higher loaf volume and a more uniform crumb structure as proteins provide quite stable monolayers and do not desorb easily from the gas-liquid interface. This has been attributed to suitable configurations adopted by protein molecules at the interface and to their large sizes (Larsson et al, 2006, MacRitchie, 1990). Addition of different lipid types may lead to the formation of mixed monolayers as suggested by Ross and MacRitchie (1995). The occurrence of minima in loaf volume on addition of original flour lipid and its polar fraction is analogous to the decrease in stability of mixed films reported by Paternotte et al (1993) on passage from a pure protein film to a lipid (monogalactosyl monoglyceride)-protein stabilized film. Variations in percentage of original flour lipids at which the minimum volume is reached could be attributed to differences in protein composition of the two flours. A recent study by Salt et al (2006) also indicated that mixed protein-lipid interfaces are relatively unstable compared to pure protein ones. The positive effect on loaf volume, due to incorporation of palmitic acid, a

saturated free fatty acid, and polar lipids having digalactosyl diglyceride (DGDG), were probably due to their tendency to form condensed monolayers (Gaines, 1966; MacRitchie, 1990). On the other hand, the probable formation of an expanded monolayer by linoleic acid resulted in detrimental effects on loaf volume. Myristic acid is a saturated free fatty acid but, due to its shorter chain length, forms expanded monolayers (MacRitchie, 1990), decreasing loaf volume. At higher levels of myristic acid addition, the reverse effect is observed (Table 4.1, Fig 4.2, Fig 4.3). This could be attributed to the presence of impurities in myristic acid, which is ~ 99% pure by gas chromatographic analysis. However, such purity analysis does not indicate surface chemical purity of surface active compounds like myristic acid (MacRitchie, 1990). The presence of impurities, even in minor amounts, may increase above the critical level to be adsorbed at the surface and influence surface properties. This might have been the case at higher levels of addition of myristic acid in bake trials. Therefore analysis of surface chemical purity (MacRitchie, 1990) of added surfactants, though not performed in this study, will be of great advantage in future studies.

4.3.1.2 Crumb Structure

Image analysis results similar to those reported in chapter 3 were observed in this study. Image analysis of bread crumb showed that addition of different lipid types and variations in their levels resulted in negligible differences in number of gas cells and average cell elongation (Table 4.2, Fig 4.4 to Fig 4.17). These non significant differences in the number of gas cells for a particular flour when treated with different levels of the same lipid type and free fatty acid, suggested that higher loaf volumes were due to an increase in expansion capacity of gas cells and not due to their number. That is, the film stabilizers (polar lipids and palmitic acid) allowed gas cells to expand, increasing the volume (Fig 4.8 to 4.17). With improvement in expansion capacity, the size of gas cells increased and crumb structure changed from fine to more uniformly distributed larger gas cells. The inability of gas cells to expand also explains the earlier observations of relatively fine crumb appearance with addition of non-polar lipids reported by Ponte and DeStefanis (1969).

However, an exception was observed in the case of linoleic acid addition to Jagger flour. Incremental additions of linoleic acid to Jagger flour caused small but continuous decrease in number of gas cells (Table 4.2). Linoleic acid might also be detrimental to initial concentration

Table 4.2 Crumb structure responses of Jagger and Soft wheat flour breads to different lipid types and levels, added to defatted flours as percentage of original flour lipids. [A, B]

% of Original Flour Lipids	Jagger Flour		Soft Flour	
	Number of Cells	Average Cell Elongation (C-Cell Score)	Number of Cells	Average Cell Elongation (C-Cell Score)
Original Flour Lipids				
0%	2000.0 ± 11.3 a	1.58 ± 0.01 b	2544.5 ± 187.4 a,b	1.69 ± 0.01 a,b
20%	1935.0 ± 222.03 a,b	1.59 ± 0.05 b	2521.0 ± 110.3 a,b,c	1.68 ± 0.01 a,b,c
40%	1880.5 ± 78.5 a,b	1.64 ± 0.06 a,b	2210.5 ± 115.3 d	1.65 ± 0.03 b,c
50%	1898.0 ± 59.4 a,b	1.68 ± 0.04 a	2209.0 ± 79.2 d	1.65 ± 0.02 c
60%	1788.5 ± 81.3 a,b	1.67 ± 0.05 a,b	2347.5 ± 2.1 b,c,d	1.70 ± 0.02 a
80%	1907.5 ± 2.1 a,b	1.62 ± 0.01 a,b	2196.0 ± 86.3 d	1.66 ± 0.00 a,b,c
100%	1749.0 ± 41.0 b	1.63 ± 0.01 a,b	2229.0 ± 114.6 d	1.69 ± 0.03 a,b
Flour Polar Lipids				
0%	2361.5 ± 7.8 a	1.56 ± 0.03 c,d	2107.0 ± 26.9 b	1.60 ± 0.02 c
20%	2307.0 ± 157.0 a	1.57 ± 0.01 b,c,d	1876.5 ± 37.5 c	1.66 ± 0.02 a,b
40%	2001.0 ±24.0 b	1.60 ± 0.04 a,b,c	2389.0 ± 0.0 a	1.64 ± 0.00 b
66%	1985.0 ± 0.0 b	1.62 ± 0.00 a	2183.0 ± 0.0 b	1.67 ± 0.00 a,b
132%	2285.0 ± 0.0 a	1.54 ± 0.00 d	2180.5 ± 23.3 b	1.69 ± 0.03 a
200%	2385.0 ± 0.0 a	1.61 ± 0.00 a,b	2092.0 ± 216.4 b	1.67 ± 0.01 a,b
Flour Non Polar Lipids				
0%	2436.0 ± 113.1 a	1.57 ± 0.01 b	2110.0 ± 22.6 a	1.60 ± 0.02 a
15%	2306.5 ± 126.6 a	1.57 ± 0.00 b	2036.5 ± 95.5 a,b	1.59 ± 0.01 a
30%	1989.0 ± 69.3 b,c	1.60 ± 0.04 b	1832.0 ± 144.2 b	1.68 ± 0.05 a
60%	2202.5 ± 187.4 a,b	1.61 ± 0.01 a,b	1950.0 ± 104.7 a,b	1.66 ± 0.05 a
132%	1937.5 ± 47.4 b,c	1.65 ± 0.01 a	2053.0 ± 14.1a,b	1.64 ± 0.01 a
200%	1761.5 ± 0.0 c	1.61 ± 0.00 a,b	—	—
Linoleic Acid				
0%	2452.5 ± 26.2 a	1.63 ± 0.06 a	2060.5 ± 29.0 a,b	1.60 ± 0.00 c
66%	1803.0 ± 220.6 b	1.68 ± 0.02 a	2290.0 ± 158.4 a	1.68 ± 0.02 b
132%	1796.5 ± 67.2 b	1.72 ± 0.04 a	1926.0 ± 116.0 b	1.69 ± 0.02 a,b
200%	1473.5 ± 169.0 b	1.70 ± 0.04 a	2019.0 ± 144.2 a,b	1.73 ± 0.01 a
250%	1505.5 ± 13.4 b	1.72 ± 0.00 a	1994.0 ± 120.2 a,b	1.71 ± 0.02 a
Palmitic Acid				
0%	2477.5 ± 139.3 a	1.61 ± 0.04 a	2060.5 ± 29.0 a	1.60 ± 0.00 b
66%	2438.5 ± 103.9 a	1.64 ± 0.02 a	2043.0 ± 69.3 a	1.64 ± 0.01 a,b
132%	2522.5 ± 171.8 a	1.63 ± 0.06 a	1940.5 ± 68.6 a	1.66 ± 0.06 a,b
200%	2331.0 ± 14.1 a	1.58 ± 0.00 a	1880.5 ± 17.7 a	1.72 ± 0.04 a
250%	2285.5 ± 126.5 a	1.59 ± 0.01 a	1829.5 ± 204.4 a	1.65 ± 0.03 b
Myristic Acid				
0%	2328.0 ± 63.6 a	1.62 ± 0.01 a	2100.0 ± 135.8 a	1.68 ± 0.01 b
66%	2368.0 ± 190.9 a	1.62 ± 0.02 a	1954.0 ± 24.0 a	1.68 ± 0.01 b
132%	2404.5 ± 54.5 a	1.61 ± 0.01 a	2263.5 ± 54.5 a	1.68 ± 0.01 b
200%	2447.5 ± 150.6 a	1.64 ± 0.00 a	2270.5 ± 147.8 a	1.68 ± 0.01 b
250%	2244.5 ± 17.7 a	1.65 ± 0.00 a	2156.5 ± 188.8 a	1.68 ± 0.01 b
330%	2324.0 ± 84.9 a	1.66 ± 0.00 a	—	—
400%	2632.0 ± 35.4 a	1.65 ± 0.00 a	—	—

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Fig 4.4 Number of cells vs. different lipid types, added to defatted Jagger flour as percentage of original flour lipids.

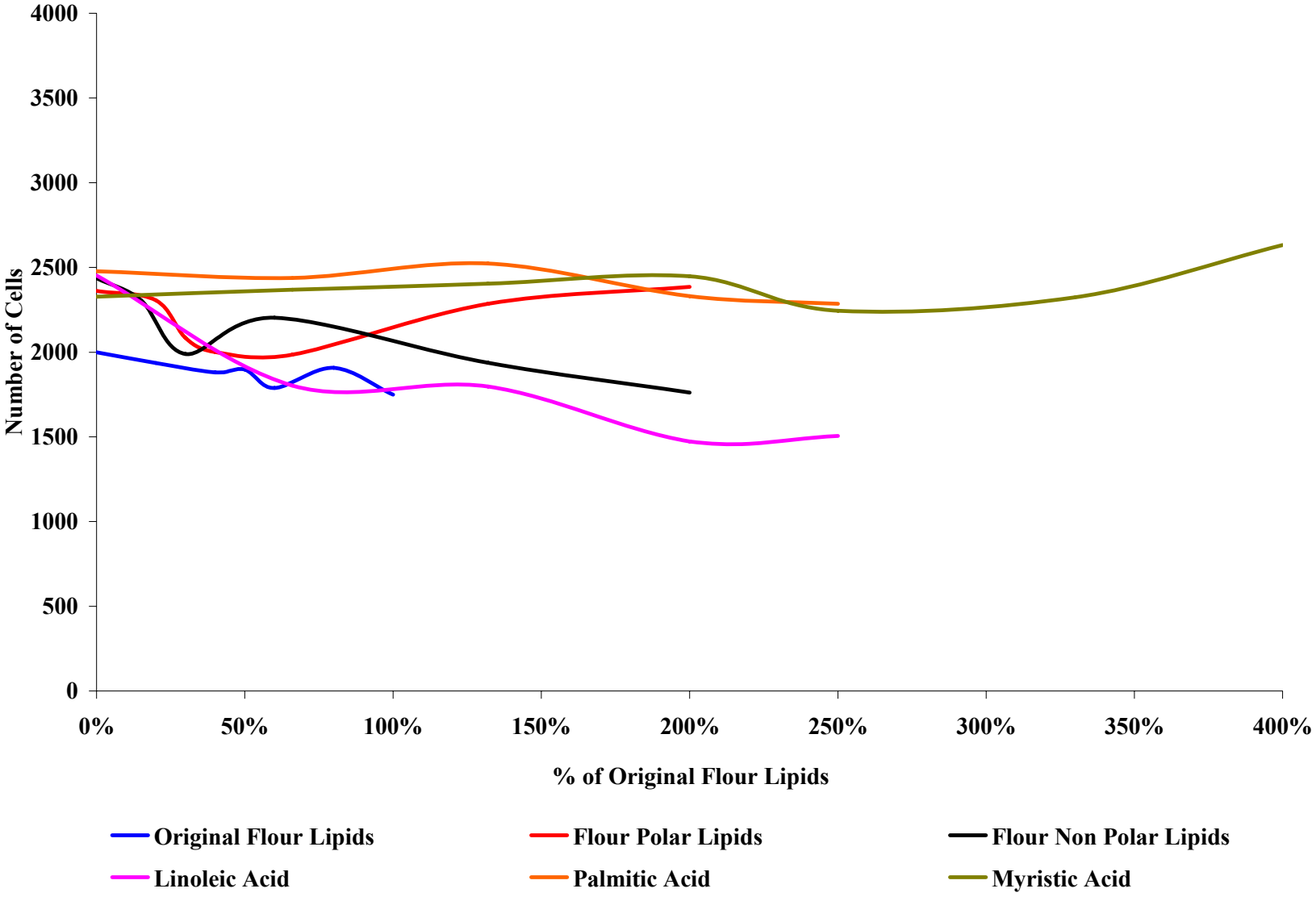


Fig 4.5 Average cell elongation vs. different lipid types, added to defatted Jagger flour as percentage of original flour lipids.

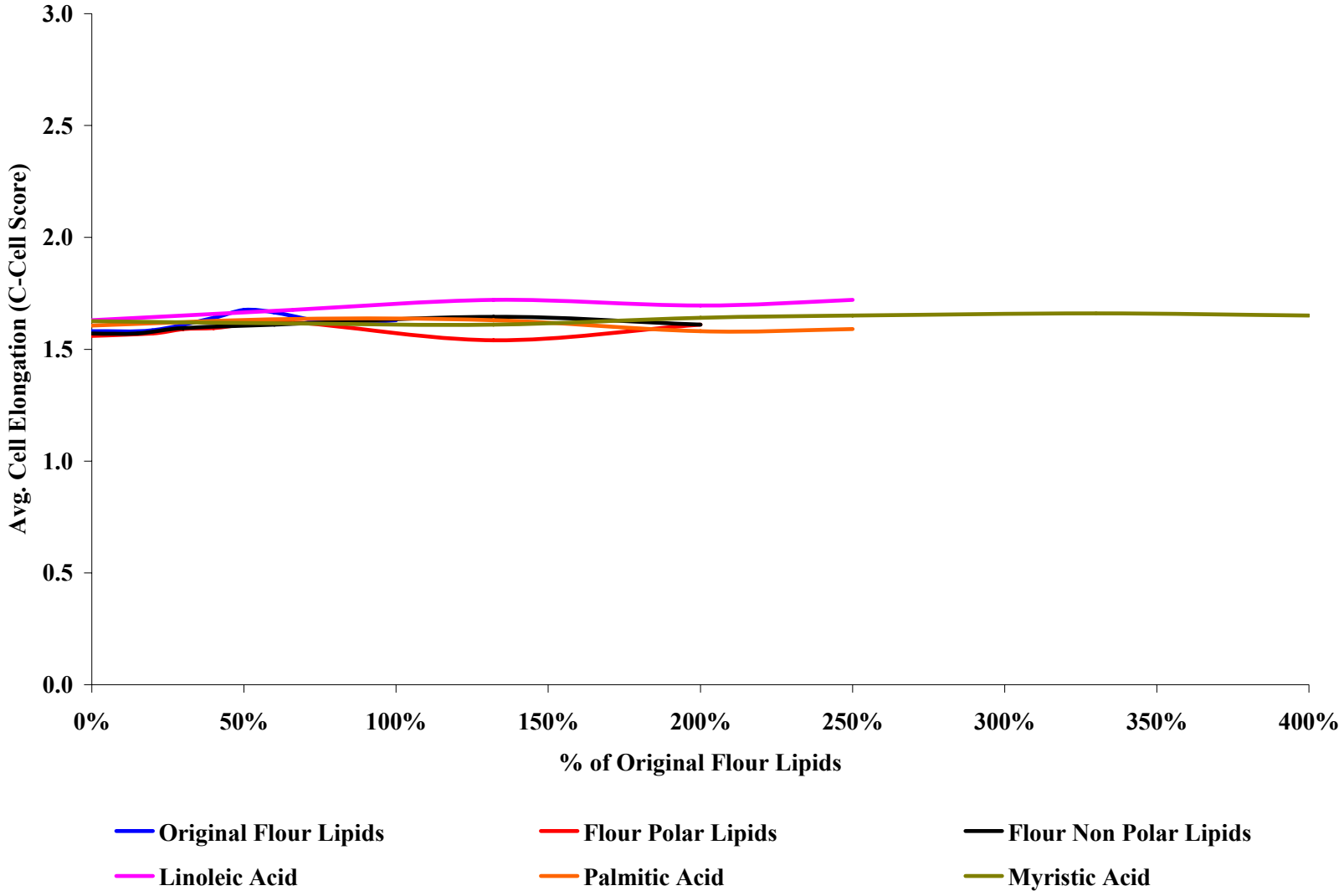


Fig 4.6 Number of cells vs. different lipid types, added to defatted Soft wheat flour as percentage of original flour lipids.

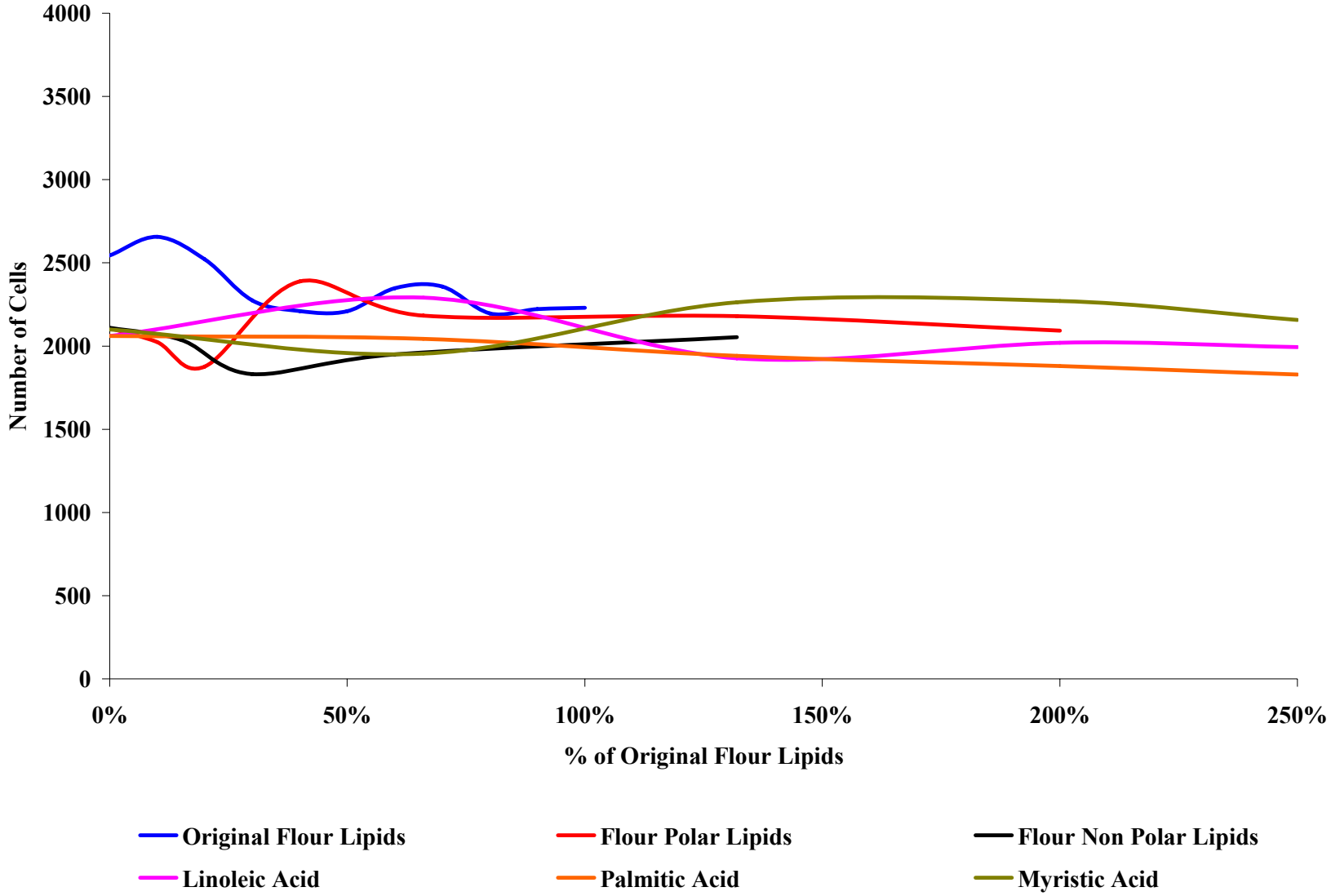


Fig 4.7 Average cell elongation vs. different lipid types, added to defatted Soft wheat flour as percentage of original flour lipids.

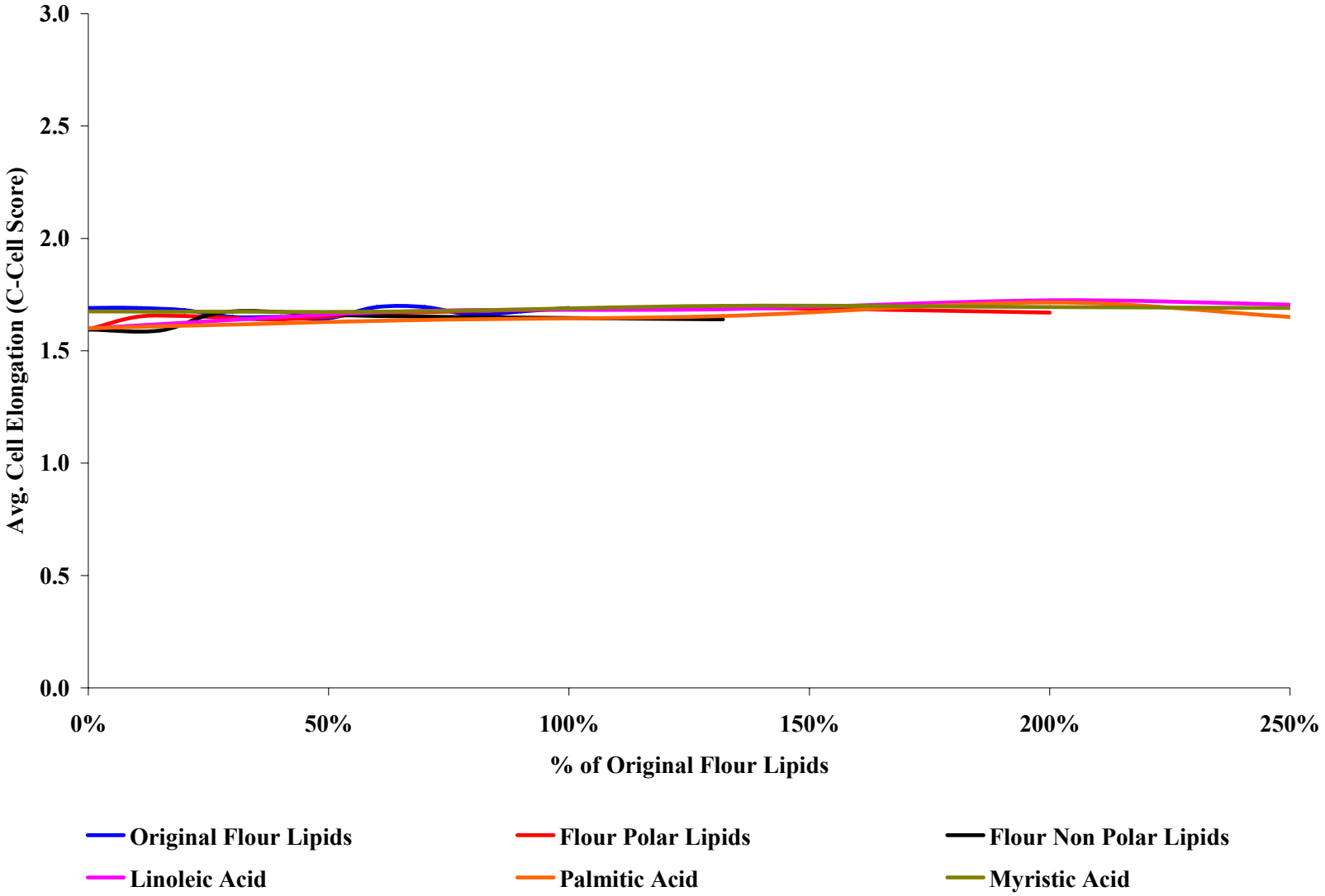


Fig 4.8 C-Cell images of Jagger wheat flour bread slices showing differences in gas cell expansion at different polar lipid levels (represented as percentages) added to defatted flour as percentage of original flour lipids.

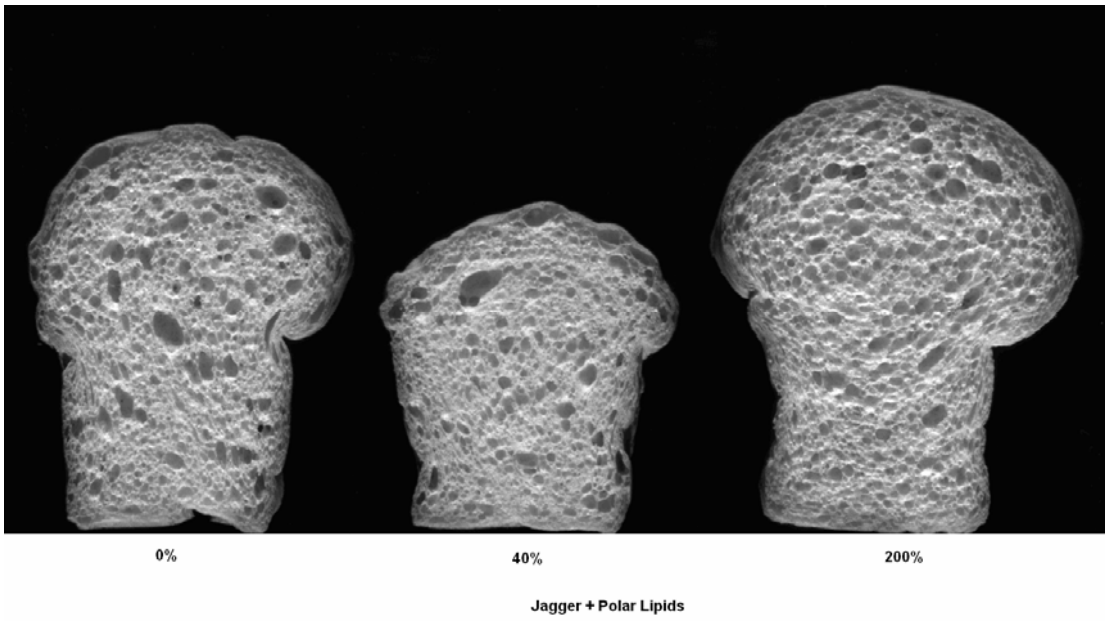


Fig 4.9 C-Cell images of Jagger wheat flour bread slices showing differences in gas cell expansion at different non polar lipid levels (represented as percentages) added to defatted flour as percentage of original flour lipids.

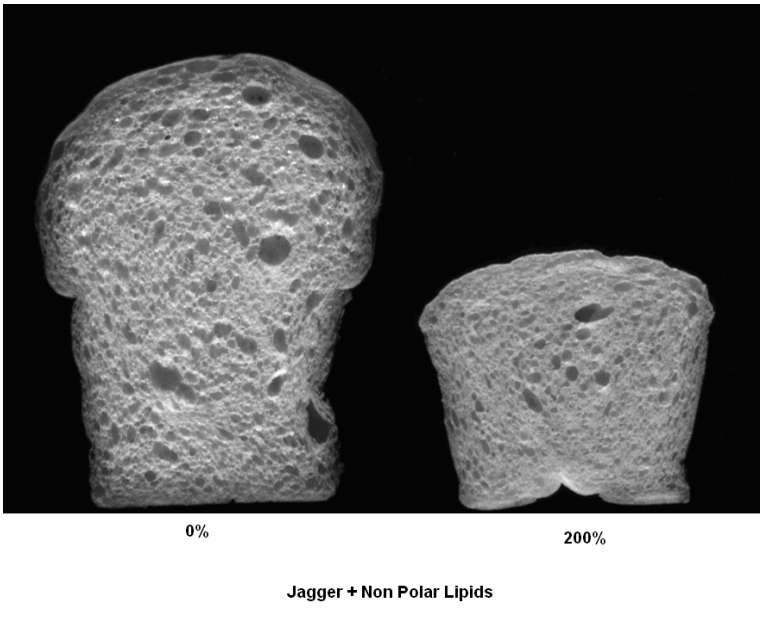


Fig 4.10 C-Cell images of Jagger wheat flour bread slices showing differences in gas cell expansion at different linoleic acid levels (represented as percentages) added to defatted flour as percentage of original flour lipids.

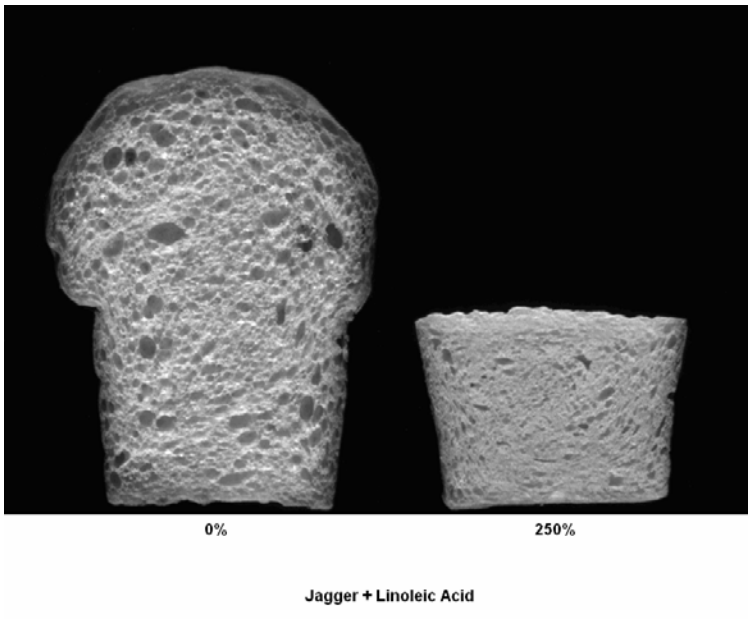


Fig 4.11 C-Cell images of Jagger wheat flour bread slices showing differences in gas cell expansion at different palmitic acid levels (represented as percentages) added to defatted flour as percentage of original flour lipids.

Fig 4.12 C-Cell images of Jagger wheat flour bread slices showing differences in gas cell expansion at different myristic acid levels (represented as percentages) added to defatted flour as percentage of original flour lipids.

Fig 4.13 C-Cell images of Soft wheat flour bread slices showing differences in gas cell expansion at different polar lipid levels (represented as percentages) added to defatted flour as percentage of original flour lipids.

Fig 4.14 C-Cell images of Soft wheat flour bread slices showing differences in gas cell expansion at different non polar lipid levels (represented as percentages) added to defatted flour as percentage of original flour lipids.

Fig 4.15 C-Cell images of Soft wheat flour bread slices showing differences in gas cell expansion at different linoleic acid levels (represented as percentages) added to defatted flour as percentage of original flour lipids.

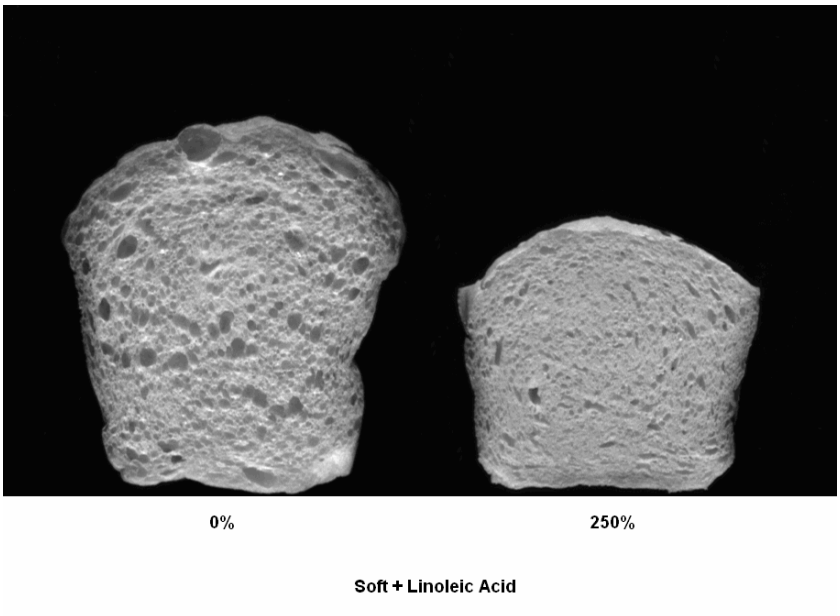


Fig 4.16 C-Cell images of Soft wheat flour bread slices showing differences in gas cell expansion at different palmitic acid levels (represented as percentages) added to defatted flour as percentage of original flour lipids.

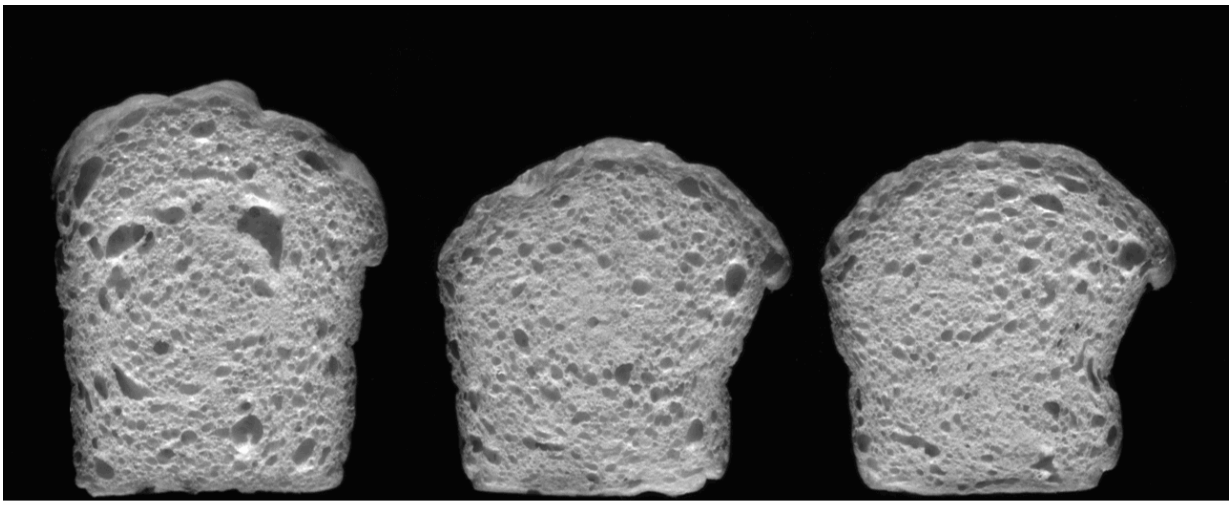


Fig 4.17 C-Cell images of Soft wheat flour bread slices showing differences in gas cell expansion at different myristic acid levels (represented as percentages) added to defatted flour as percentage of original flour lipids.

and stability of gas cells during mixing, thus hindering the ease with which gas cells are occluded into the liquid phase of dough. This initial adsorption depends upon the diffusion coefficient of surface active compounds (lipids and proteins) in the liquid phase of the dough (MacRitchie, 1990), and these will vary from flour to flour. The nature of surface active compounds which have been adsorbed initially at the interface to form a monolayer will stabilize or destabilize freshly occluded gas cells, thus affecting ease with which they are concentrated (Larsson et al, 2006).

Negligible variations in average cell elongation indicated that different lipid types and free fatty acids at the levels of addition used might not be influencing rheology of the gluten-starch matrix, since cell elongation is thought to be associated with dough rheological properties (Gandikota and MacRitchie, 2005).

4.3.2 Effect of Variations in Lipid Types and Free Fatty Acids, and their levels on Biaxial Extensional Rheology of Gluten-Starch Matrix.

Biaxial extensional rheological tests were performed on Jagger and soft flour doughs with different lipid types and their levels, in order to investigate possible independent action of lipids on baking performance (loaf volume and crumb structure). Different lipid types and free fatty acids at the levels used did not influence biaxial extensional rheological parameters (maximum stress, failure strain and strain hardening index) of the doughs (Table 4.3, Table 4.4, Fig 4.18, Fig 4.19). However, higher values were observed for Jagger than soft wheat flour. Though minor differences were observed for strain hardening indices within a particular flour at some treatments, no specific trend was observed, thus attributing the variation to slight experimental scatter.

As in the case of the earlier part of this study (chapter 3), higher values of biaxial extensional rheological parameters were observed in the case of Jagger flour, indicating its superior breadmaking potential (Dobraszczyk et al, 2003). Inability of different lipid types and free fatty acids to cause any effect on biaxial extensional rheology, demonstrates that their action is independent of rheology of the gluten-starch matrix and that they act purely as surface active compounds at these levels of addition.

Table 4.3 Mean bubble inflation rheological responses of Jagger flour doughs to different lipid types and levels, added to defatted flours as percentage of original flour lipids. [A, B]

% of Original Flour Lipid	Max. Stress (Kpa)	Failure Strain (Hencky)	Strain Hardening Index
Original Flour Lipids			
0%	571.62 ± 305.45 a	2.58 ± 0.18 a	2.21 ± 0.12 b,c
60%	491.68 ± 237.21 a	2.60 ± 0.12 a	2.38 ± 0.064 a,b
100%	598.62 ± 112.05 a	2.66 ± 0.04 a	2.44 ± 0.06 a
Flour Polar Lipids			
40%	480.85 ± 67.26 a	2.56 ± 0.04 a	2.18 ± 0.03 c
200%	660.22 ± 130.74 a	2.64 ± 0.05 a	2.22 ± 0.04 b,c
Flour Non Polar Lipids			
60%	936.93 ± 560.68 a	2.74 ± 0.16 a	2.44 ± 0.09 a
Linoleic Acid			
132%	650.21 ± 18.07 a	2.66 ± 0.04 a	2.36 ± 0.10 a,b,c
Palmitic Acid			
132%	598.55 ± 239.23 a	2.62 ± 0.12 a	2.23 ± 0.09 b,c
Myristic Acid			
250%	613.71 ± 90.70 a	2.65 ± 0.03 a	2.32 ± 0.10 b,c
Over all average	623.58 ±236.59	2.63 ± 0.10	2.31 ± 0.12

[A] Values represent mean ± standard deviation for duplicate determinations
[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Table 4.4 Mean bubble inflation rheological responses of Soft wheat flour doughs to different lipid types and levels, added to defatted flours as percentage of original flour lipids. [A, B]

% of Original Flour Lipid	Max. Stress (Kpa)	Failure Strain (Hencky)	Strain Hardening Index
Original Flour Lipids			
0%	120.80 ± 12.76 a	1.79 ± 0.00 a	1.76 ± 0.01 a
40%	125.42 ± 12.14 a	1.85 ± 0.02 a	1.74 ± 0.01 a
100%	102.40 ± 5.45 a	1.66 ± 0.05 b	1.64 ± 0.04 c
Flour Polar Lipids			
20%	105.49 ± 3.52 a	1.68 ± 0.02 b	1.66 ± 0.00 b,c
200%	130.17 ± 1.61 a	1.84 ± 0.02 a	1.72 ± 0.02 a,b
Flour Non Polar Lipids			
60%	108.71 ± 27.51 a	1.83 ± 0.02 a	1.73 ± 0.01 a,b
Linoleic Acid			
132%	104.35 ± 12.49 a	1.81 ± 0.06 a	1.75 ± 0.07 a
Palmitic Acid			
132%	116.21 ± 0.52 a	1.82 ± 0.01 a	1.79 ± 0.01 a
Myristic Acid			
250%	115.44 ± 23.14 a	1.78 ± 0.02 a	1.72 ± 0.03 a
Over all average	114.19 ± 13.69	1.79 ± 0.07	1.72 ± 0.05

[A] Values represent mean ± standard deviation for duplicate determinations
[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Fig 4.18 Stress vs. strain (Hencky) curves of Jagger flour doughs with different lipid types and levels measured in biaxial extension.

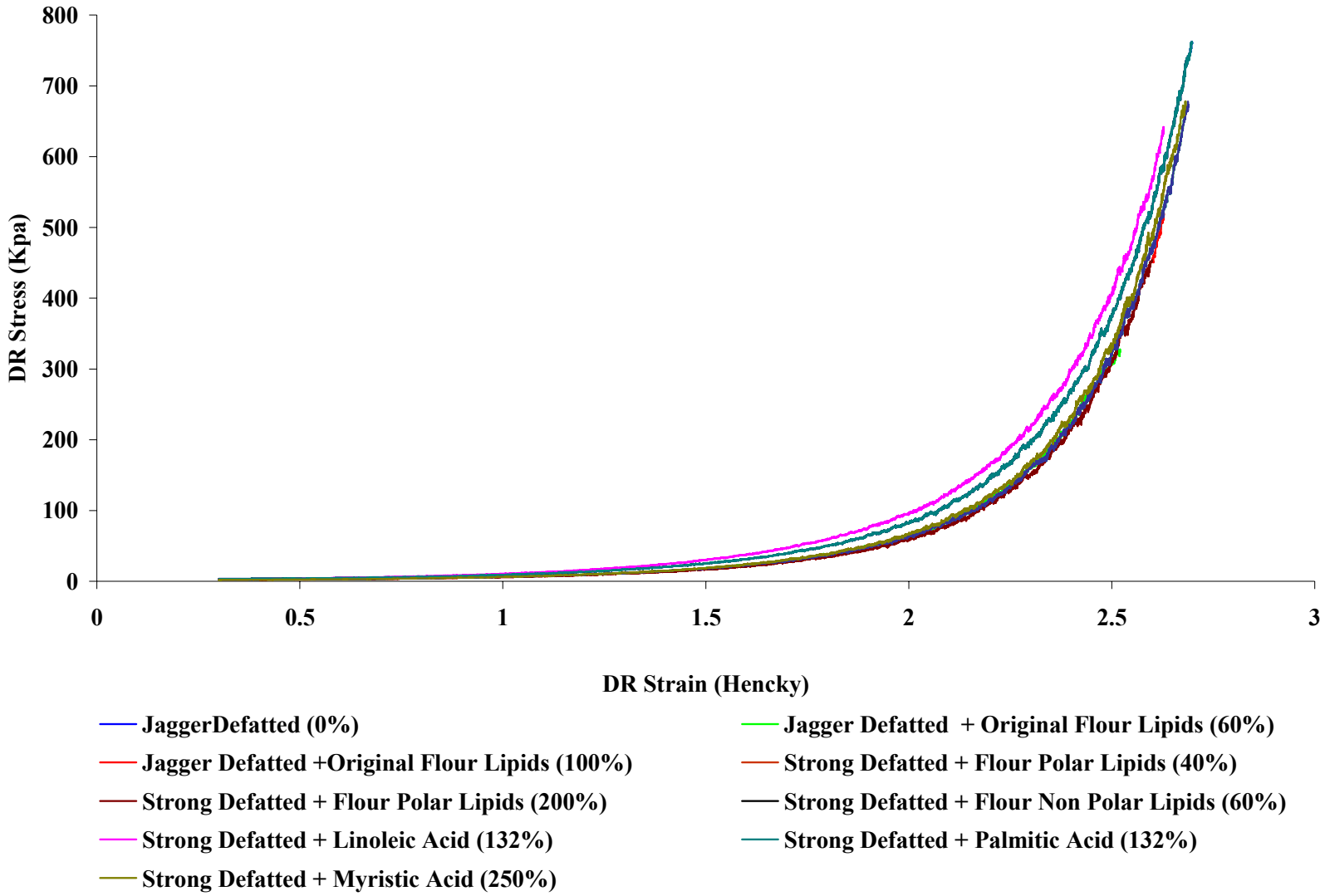
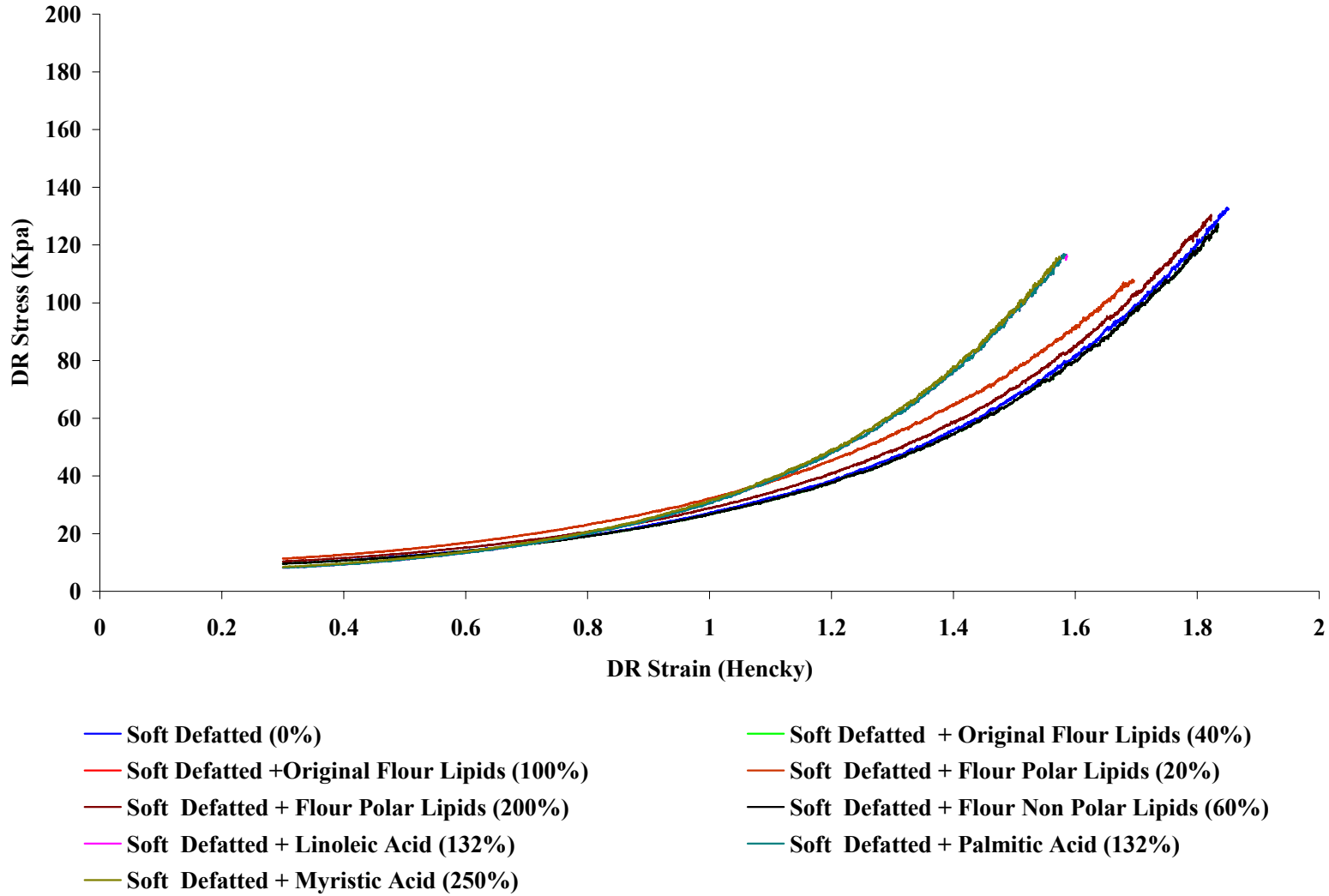


Fig 4.19 Stress vs. strain (Hencky) curves of Soft flour doughs with different lipid types and levels measured in biaxial extension.



4.4 Conclusion

Liquid lamellae, apart from the primary gluten-starch matrix, act as a secondary support to expanding gas cells and delay their failure during proofing and baking. The stability of liquid lamellae depends on surface active compounds (proteins and lipids) present at the gas-liquid interface and their ability to form monolayers. Surface active compounds like lipids, depending on their structure i.e. degree of saturation in the hydrocarbon chain, hydrocarbon chain length and size of polar head groups, will either form condensed or expanded monolayers (Fig 2.11). Condensed monolayers are characterized by close packing of surface active molecules due to their saturated hydrocarbon chains. These monolayers have low compressibility (Fig. 2.11) because of close molecular packing that also leads to greater attraction between hydrocarbon chains, making the monolayer more stable and not easily desorbed. The surface viscosity of such monolayers is high and with any change in interfacial area, large elastic restoring forces are generated preventing coalescence and disproportionation (MacRitchie, 1976b). On the other hand, unsaturated hydrocarbons or other foam destabilizers will form expanded monolayers (Fig. 2.11). Molecular packing of expanded monolayers is loose, leading to greater compressibility. These are not so elastic in response to changes in the interfacial area and may get desorbed in the process. The monolayer desorption at the point of contact of two gas cells will lead to coalescence.

Surface active compounds of wheat flour such as phospholipids, DGDG and saturated free fatty acids (stearic acid, palmitic acid) are known to give condensed monolayers. Unsaturated free fatty acids like linoleic acid give expanded monolayers. Any modification in the structure of a hydrocarbon chain such as inclusion of a double bond or decrease in the chain length will lead to transformation of condensed monolayers to expanded ones. With the presence of a variety of surface active compounds in the liquid phase of the dough, it is quite possible that some of these molecules may possess a certain degree of mutual solubility. The monolayers of such surface active compounds will be of intermediate type, depending whether individual compounds give expanded or condensed monolayers. The use of surface active components capable of forming condensed monolayers as bakery additives can help improve bread loaf volume.

4.5 References

AACC. 2001. Approved Methods of the AACC, Tenth Edition. American Association of Cereal Chemists, St. Paul, Minnesota, USA.

Baker, J.C., Parker, H.K., Mize, M.D. 1946. Supercentrifugates from dough. Cereal Chemistry 23, 539-544.

DeStefanis, V.A., Ponte, J.G.Jr. 1976. Studies on breadmaking properties of wheat-flour nonpolar lipids. Cereal Chemistry 53, 636-642.

Dobraszczyk, B.J. 1997. Development of a new dough inflation system to evaluate doughs. Cereal Foods World 42, 516-519.

Dobraszczyk, B.J., Smewing, J., Albertini, M., Maesmans, G., Schofield, J.D. 2003. Extensional rheology and stability of gas cell walls in bread doughs at elevated temperatures in relation to breadmaking performance. Cereal Chemistry 80, 218-224.

Gaines, G.L. 1966. Insoluble Monolayers at Liquid-Gas Interfaces. Prigogine, I. (Ed.), Interscience Monographs on Physical Chemistry, John Wiley and Sons, Inc., New York, USA.

Gandikota, S., MacRitchie, F. 2005. Expansion capacity of doughs: methodology and applications. Journal of Cereal Science 42, 157-163.

Larsson, K., Quinn, P., Sato, K., Tiberg, F. 2006. Lipids: Structure, Physical Properties and Functionality, The Oily Press, Bridgwater, England.

MacRitchie, F. 1976a. The liquid phase of dough and its role in baking. Cereal Chemistry 53, 318-326.

MacRitchie, F. 1976b. Monolayer compression barrier in emulsion and foam stability. Journal of Colloid and Interface Science 56, 53-56.

MacRitchie, F. 1977. Flour lipids and their effects in baking. Journal of The Science of Food and Agriculture 28, 53-58.

MacRitchie, F. 1978. Differences in baking quality between wheat flours. Journal of Food Technology 13, 187-194.

MacRitchie, F. 1990. Chemistry at Interfaces. Academic Press, Inc., San Diego, CA, USA.

MacRitchie, F., Gras, P.W. 1973. The role of flour lipids in baking. Cereal Chemistry 50, 292-302.

McCormack, G., Panozzo, J., MacRitchie, F. 1991. Contributions to breadmaking of inherent variations in lipid content and composition of wheat cultivars.II. Fractionation and reconstitution studies. Journal of Cereal Science 13, 263-274.

Paternotte, T.A., Orsel, R., Hamer, R.J. 1993. Interactions between flour proteins and flour lipids at the liquid/air interface. In: Gluten Proteins, Association of Cereal Research, Detmold, Germany, pp 207-217.

Ponte, J.G.Jr., DeStefanis, V.A. 1969. Note on separation and baking properties of polar and nonpolar wheat flour lipids. Cereal Chemistry 46, 325-329.

Ross, A., MacRitchie, F. 1995. Interactions of wheat proteins, carbohydrates and lipids. In: Gaonkar, A.G. (Ed.), Ingredient Interactions Effects on Food Quality, Marcel Deker, New York, USA, pp 321-356.

Salt, L.J., Wilde, P.J., Georget, D., Wellner, N., Skeggs, P.K., Mills, E.N.C. 2006. Composition and surface properties of dough liquor. Journal of Cereal Science 43, 284-292.

CHAPTER 5 - Mechanism of Stability of the Gluten-Starch Matrix

5.1 Introduction

Loaf volume can be defined as extent of dough expansion (Gandikota and MacRitchie, 2005), which depends upon how thin a cell wall can be stretched before reaching its expansion limit. Rheology of gluten films is important in the bread making process as it determines their extensibility and strength. The gluten-starch matrix around gas cells expands biaxially to large strains (>> 100%) due to excess pressure produced in the gas cells by diffusion of carbon dioxide during proofing, and by thermal expansion of gases during baking. This causes thinning of gas cell walls and, if a gas cell continues to expand along this thin region, it may rupture. However, if the stress in the thin region increases more than proportionally to strain, the thin region of cell wall or gluten-starch matrix will resist further deformation and the gas cell will continue to expand along thicker parts of the cell wall. This localized increase of stress in response to strain, preventing failure of the gas cell walls, is called strain hardening (vanVliet *et al*, 1992; Dobraszczyk and Roberts, 1994).

A parabolic relation between stress and Hencky strain (Fig. 2.7, equation 2.5) represents strain hardening. In equation 2.5, n is a strain hardening index, and it must be greater than 1 in order to have a parabolic relation between stress and Hencky strain. Strain hardening index is a reliable criterion for differentiating flours based on bread making potentials. At a molecular level, strain hardening is believed to be due to entanglement coupling of large glutenin molecules (Singh and MacRitchie, 2001), with molecular weight (MW) greater than a threshold MW M_T (equation 2.1) (Bersted and Anderson, 1990). For wheat flour doughs, the strain hardening index shows a positive linear relation with failure strain, i.e. with increase in strain hardening index, the ability of glutenin molecules to slip through entanglements without breaking the entangled network increases.

Dough strength in terms of R_{max} (Extensograph maximum resistance to extension) shows positive correlation with the glutenin fraction with MW greater than a certain critical MW. This fraction is roughly equivalent to the unextractable polymeric protein (UPP) (Fig 2.3) (Gupta et al., 1993). The shift in MW distribution (MWD) of this fraction towards higher MW or increase

in relative proportion of this fraction will increase the number of entanglements per chain and reduce the MW between entanglements (Termonia and Smith, 1988). This will lead to increase in strength and decrease in extensibility (Gupta et al., 1990). Successive addition of glutenin fractions with increasing molecular weight to a base flour at constant protein level causes loaf volume to increase, and, after reaching a maximum, to decrease (MacRitchie, 1987; Lundh and MacRitchie, 1989; MacRitchie et al., 1991). The optimum here possibly indicates a balance between strength and extensibility, beyond which decrease in extensibility might result in lower strain hardening indices.

The objective of this part of the study is to understand rheological properties of the gluten-starch matrix in relation to its molecular structure-function behavior required to stabilize expanding gas cells.

5.2 Materials and Methods

5.2.1 Flours

Jagger flour and soft wheat flour (a blend of soft wheat varieties) were two untreated and unbleached flours used in this study. The flours were milled in Buhler mill (73% milling extraction rate) in the department of Grain Science and Industry, Kansas State University, Manhattan, KS. The flours were evaluated for certain quality characteristics given in Table 3.1. These flours were stored at -20^{0}C until use. For the sake of brevity, Jagger flour and soft wheat flour will be referred to as Jagger and soft, respectively.

5.2.2 Reagents

Chloroform used was of HPLC grade, all other chemicals used were of ACS grade. These were purchased from Sigma-Aldrich, USA. Distilled deionized water, sterilized in an autoclave at a pressure of 120 psi for 20 min, was used in all stages of the experiments.

5.2.3 Analytical Procedures

- Moisture content was determined as per AACC method 44 – 15 A.
- Protein content was determined by the nitrogen combustion method using the LECO FP-2000 Nitrogen/protein analyzer using a factor of 5.7 to convert N to protein.

5.2.4 Dough Mixing Properties

Mixing properties were evaluated using a 10 g National Mixograph (National Manufacturing Co., Lincoln, NE). Mixing parameters (peak development time, and weakening angle) were used for comparison. The procedure used was similar to AACC method 55-40 except that sodium chloride (1.5% w/w on flour weight basis) was included in the formulation.

5.2.5 Size-Exclusion High-Performance Liquid Chromatography (SE-HPLC) and Multiangle Laser Light Scattering (MALLS)

Size characterization of gluten proteins was done using SE-HPLC (Hewlett-Packard 1100 system) in conjunction with MALLS (Multiangle light scattering detector DAWN EOS of Wyatt Technology Corp., Santa Barbara, CA). Proteins were fractionated with a Biosep SEC-4000 column (Phenomenex, Torrance, CA) using; 50 mM disodium orthophosphate (Na_2HPO_4) buffer

(NaPhos), pH 7.0, containing 1% sodium dodecyl sulfate (SDS), as mobile phase at a flow rate of 0.5 ml/min. Proteins were detected at 214 nm in SE-HPLC. Injection volume of samples for total and extractable protein analysis was 80 µl and that for unextractable protein analysis was 40 µl. Therefore results obtained from chromatograms of unextractable proteins were multiplied by a factor of 2. Reduced injection volume for unextractable protein analysis was used to avoid problems in MALLS analysis due to high concentration of very large glutenin protein.

SEC-HPLC data was analyzed using software program ChemStation (Agilent Technologies, USA). MALLS data was analyzed with software program ASTRA 4.50 (Wyatt Technology Corp.) using 0.31 as dn/dc value, as per procedure by Bean and Lookhart (2001).

5.2.6 Sample preparation for SE-HPLC and MALLS

Basic procedure for SE-HPLC is described elsewhere (Batey et al., 1991). Samples were prepared to analyze total, extractable and unextractable polymeric protein (Gupta et al., 1993). Samples for total and extractable protein analysis were weighed in microfuge tubes. Sample size was determined based on protein content of each sample such that protein content in all samples was kept constant, using Jagger as standard and quantity for Jagger being 10.0 mg ± 0.1 mg flour. Weighed samples were suspended in 50mM NaPhos, pH 6.9, + 0.5% SDS and solubilized by vortexing for 10 min. For total protein analysis, to achieve solubilization of the largest molecular size fraction, samples were sonicated (Singh et al., 1990) at room temperature at an output of 6 watts for 15 s. No sonication was done for extractable protein analysis. The sonicator probe was placed at 1/3 distance from the bottom of the microfuge tube. Microfuge tubes with protein suspensions were then centrifuged at 12000 x g for 20 min. The supernatant was decanted in HPLC vials and sealed. To ensure stability of prepared samples, the vials with supernatant were heat treated in a water bath at 85^0C for 5 min to inhibit any intrinsic proteolytic activity. After heat treatment, vials were cooled with crushed ice and analyzed by SE-HPLC.

Residue from extractable protein was used for unextractable protein analysis. The same procedure was followed, except that suspensions were sonicated for 25s at an output of 6 watts.

5.2.7 Extraction of Gluten Proteins

Gluten proteins were extracted from defatted Jagger flour following the procedure developed by MacRitchie (1985). The wet gluten mass obtained was freeze dried and ground to a

particle size less than 150 microns. Powdered gluten was stored at a temperature of -20^{0}C until its use.

5.2.8 pH Fractionation of Gluten Protein

Powdered gluten extracted from Jagger was subjected to pH fractionation, using the procedure established by Gupta et al (1990). Gluten was stirred in water at pH 5.3 (35 g / 1000 ml) for 10 min and centrifuged at 2300x g for 10 min. The residue, after collection of supernatant, was subjected to two more extractions at the same pH. The total supernatant collected together was named as pH 5.3 gluten fraction. In a similar manner, gluten was fractionated into 6 fractions by sequential lowering of pH. Fractions were obtained at pH 5.3, 4.9, 4.1, 3.5 and 3.1, getting two fractions at pH 3.1 i.e. supernatant of pH 3.1 and residue of pH 3.1. These were then frozen and freeze dried. Freeze dried fractions were ground to particle sizes less than 150 microns. The fractions were stored in poly bags in containers with desiccant at temperature -20^{0}C until their use.

Fractions were analyzed for percentage protein and moisture. Protein size characterization of the fractions was done using SE-HPLC and MALLS. Fractions retained their functional properties (dough mixing properties) when reconstituted in proportions relative to their yield, with starch (Midsol-50, MGP Ingredients, Inc., Hutchison, KS) to form a flour with protein content equal to that of the original flour (Jagger).

5.2.9 Addition of Protein Fractions to Flour

Addition of protein fractions to defatted base flour (Jagger and soft) was done at a level of 1% (dry protein) on total flour weight basis.

5.2.10 Test Baking

Test loaves (35 g flour) were baked using a modified rapid bake test (MacRitchie and Gras, 1973). A lean formulation was used with no added shortening; flour (100%), sugar (6%), sodium chloride (1.5%), instant yeast (2.7%), potassium bromate (30ppm), water and mix time (optimized from Mixograph analysis, Table 3.1). Loaf volumes were measured by rapeseed displacement after cooling for 20 min.

5.2.11 Image Analysis

Image analysis of crumb grain of baked loaves was done 12 hours after baking with a C-Cell, an image analyzing software and equipment (Calibre Control International Ltd., UK). Loaves were sliced using a rotary disc blade (unserrated Graef® blade) cutter. Central slices of 15 mm thickness were obtained. After slicing, image analyses were performed as soon as possible to avoid any shrinkage of crumb grain. Image analysis parameters (number of cells and average cell elongation) were used to compare different treatments.

5.2.12 Biaxial Extensional Rheology

Biaxial extensional rheological properties of the doughs were measured with a Stable Micro Systems dough inflation system mounted on a texture analyzer (TAXT 2 Plus) by means of the procedure established by Dobraszczyk (1997). Doughs for rheological testing were mixed in the same mixer as used for bake tests, using the same water absorption, mixing times and sodium chloride addition. After mixing, dough pieces were squashed by hand on a sheeting board without putting too much stress on the dough, and then allowed to relax for 5 min. They were then sheeted, rolled out slowly by several passes and dough pieces were rotated through 90 degrees after each pass. Sheeting was done for 5 min with relaxation of 10 s between each pass. Sheeting in all directions prevents anisotropic effects during dough inflation, allowing dough pieces to expand uniformly into spherical shapes. After sheeting, dough pieces were relaxed for 20 min. They were then cut into circular discs using a 55 mm cookie cutter, squashed to a height of 2.67 mm for 20 s into oiled dough inflation system pots. Sample dough pieces (in pots) were then proofed at 35^0C for 25 min. During sample preparation, dough pieces were protected against loss of moisture using a fine coating of mineral oil (Raybold viscosity 335/358) and covering with shrink wrap film. Mineral oil of lower viscosity seems to penetrate dough pieces and may affect rheological measurements.

Dough pieces were inflated at a flow rate of 500cm^3/min at the strain rate of 0.1/s (unless otherwise stated). Rheological parameters (peak stress, failure strain and strain hardening index) were used to compare different treatments. Strain hardening index was calculated by fitting an exponential curve to the stress-strain (Hencky) curve, after transferring data to Microsoft Excel worksheet.

5.2.13 Statistical Analysis

Results were analyzed using analysis of variance (ANOVA). ANOVA was performed using a general linear model procedure to determine significant differences and interactions for the various treatments. Means were compared by using Fishers LSD procedure ($\alpha = 0.05$). Statistical analysis was performed using proc GLM in SAS (version 9.1; SAS Institute Inc., Cary, NC) software. Duplicates were prepared for each treatment and the order of treatment was not significant.

5.3 Results and Discussion

To understand molecular structure-function relationships for strain hardening of the gluten-starch matrix and to interpret them based on well established polymer theory of entanglements, gluten proteins were fractionated into fractions with different molecular weight distributions (MWD) and reconstitution studies were conducted.

5.3.1 Gluten Fractionation

Gluten obtained from Jagger was subjected to pH fractionation to obtain fractions varying in molecular weight (MW) and MWD. Yield (%) and protein content (%) of each fraction is given in Table 5.1. SE-HPLC analysis (Table 5.2) shows that the percentage of total polymeric protein and its unextractable fraction increased with lowering of pH. Figs 5.1 and 5.2 are overlaid SE-HPLC chromatograms showing the shift in MW and MWD of gluten protein fractions obtained at different pHs. A significant amount of gliadins (monomeric proteins) was extracted in the pH 5.3 fraction. This is in accordance with earlier observations by MacRitchie and co-workers (MacRitchie, 1987; Gupta et al, 1990) where it was observed that, as the pH is lowered, larger sized glutenins (polymeric proteins) become soluble (MacRitchie, 1979) and can be separated by a stepwise reduction of pH (MacRitchie, 1985; Gupta et al, 1990).

Slightly high standard deviations in certain cases (Table 5.2) and some scatter in SE-HPLC chromatograms (Fig 5.1) of the latest fractions were observed. This is probably due to lack of efficiency of solubilization, as the latest fractions which were concentrated with largest MW glutenins may not solubilize completely even on sonication. Another reason could be the small sample size that was required to be taken because of very high protein content of these fractions. This is also a reason why MALLS analysis was not performed on fractions alone.

Cumulative percentage of total polymeric protein (Table 5.3), extracted as a function of pH of a supernatant, was calculated from yield of (Table 5.1), and total polymeric protein (Table 5.2) in, each fraction of Jagger gluten. Fig 5.3 and Table 5.3 show that 83.59% of total polymeric protein was extracted up to pH 3.1 as supernatant and the remaining fraction was obtained as a residue of pH 3.1. Similar extraction trends by lowering pH have been reported by MacRitchie (1987). The calculated value of total polymeric protein (Table 5.3) in gluten obtained from Jagger by hand washing was higher (46.2%) than SE-HPLC estimation of flour (31.6%). This is

Table 5.1 Yield, moisture and protein content (percentages) of gluten protein fractions extracted by pH fractionation.

Fractions	Yield (%)	Moisture Content (%) (% Wet Basis)	Protein Content (%) (14% m.b.)
pH 5.3	50.4	4.5 ± 0.08	83.8 ± 0.05
pH 4.9	20.5	4.6 ± 0.16	78.0 ± 0.08
pH 4.1	10.6	4.1 ± 0.15	79.6 ± 0.12
pH 3.5	4.2	4.2 ± 0.37	78.5 ± 0.07
pH 3.1 Supernatant	3.5	4.3 ± 0.09	73.2 ± 0.14
pH 3.1 Residue	10.9	4.9 ± 0.26	45.8 ± 0.18

Table 5.2 SE-HPLC relative composition (percentages) of polymeric proteins in gluten protein fractions. [A, B]

Fractions	TPP*	EPP*	UPP*
pH 5.3	31.6 ± 1.59 f	76.1 ± 0.15 a	23.9 ± 0.13 d
pH 4.9	52.6 ± 0.08 e	27.7 ± 0.07 b	72.6 ± 1.48 c
pH 4.1	64.1 ± 0.10 d	21.7 ± 0.65 c	78.3 ± 2.85 b
pH 3.5	65.5 ± 0.01 c	22.4 ± 0.11 c	77.6 ± 2.31 b
pH 3.1 Supernatant	68.0 ± 0.21 b	16.9 ± 0.31 d	83.2 ± 0.93 a
pH 3.1 Residue	69.5 ± 0.49 a	13.4 ± 0.22 e	86.6 ± 0.08 a

* TPP - total polymeric protein; EPP - extractable polymeric protein; UPP – unextractable polymeric protein

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Fig 5.1 SE-HPLC chromatograms of total protein of gluten protein fractions.

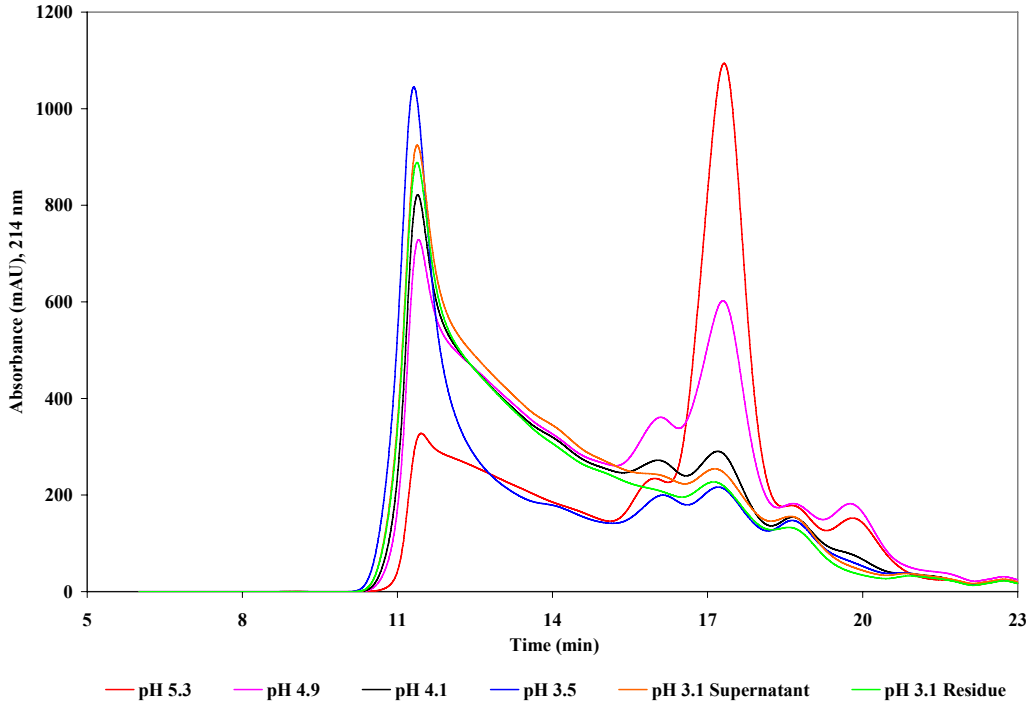


Fig 5.2 SE-HPLC chromatograms of unextractable protein of gluten protein fractions.

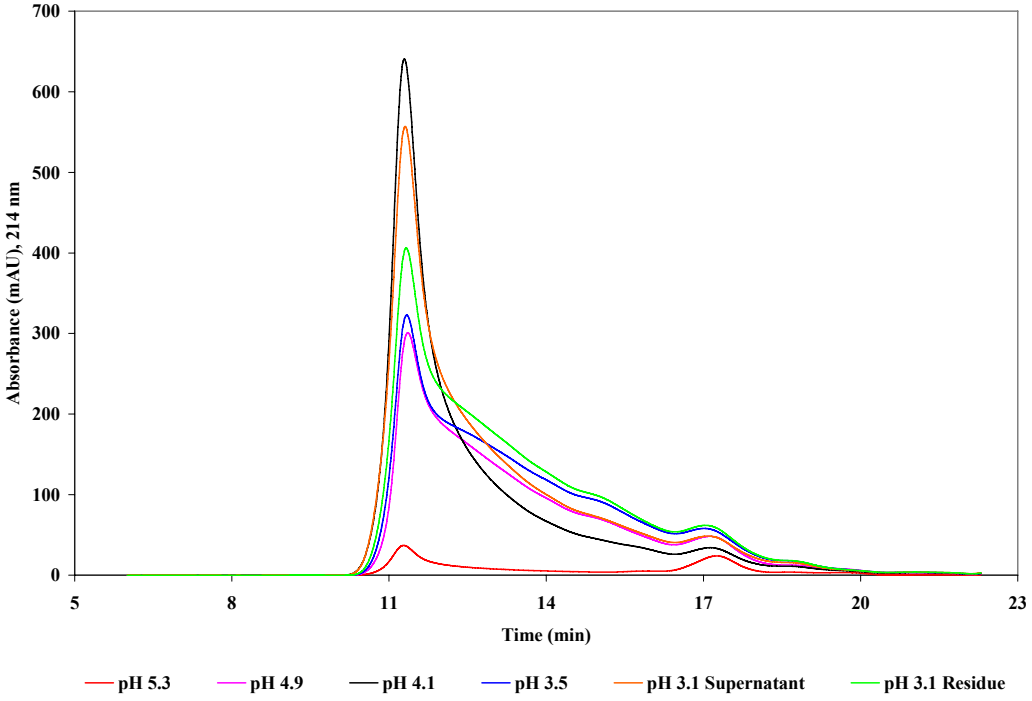


Fig 5.3 Percentage of total polymeric protein extracted as a function of final pH of supernatant of Jagger gluten.

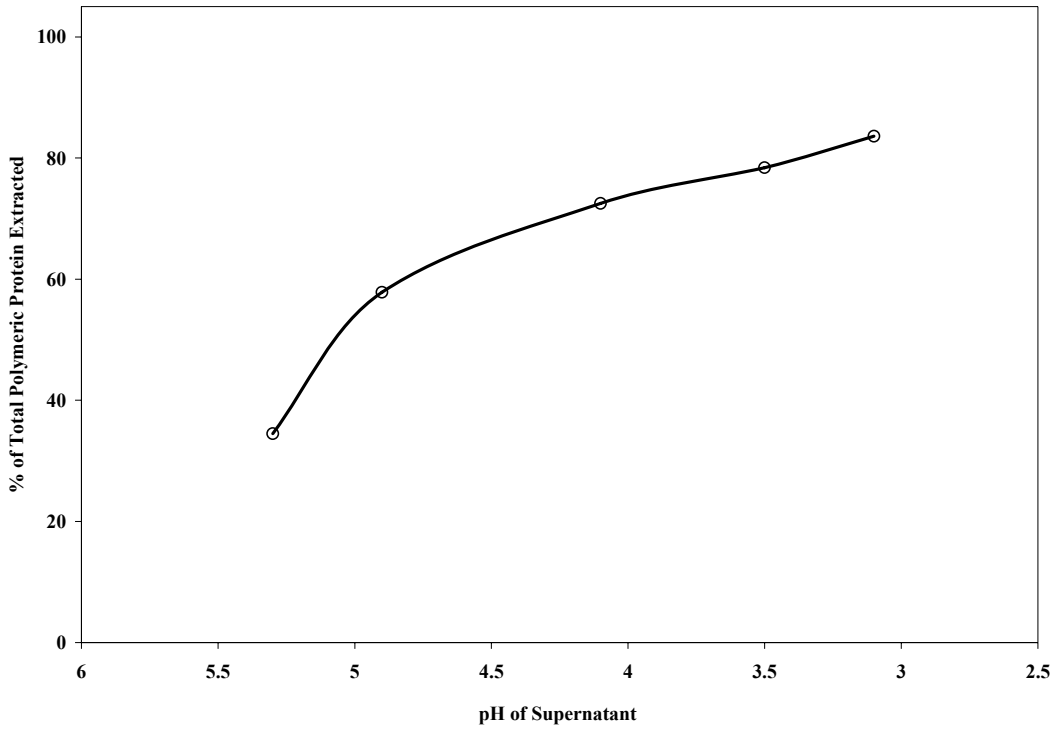


Table 5.3 Data on cumulative percentage of total polymeric protein as a function of final pH of supernatant for Jagger gluten.

Fractions	TPP* (%) (SEC-HPLC)	Yield (%)	Fraction of TPP* of original gluten in each fraction	Cumulative TPP* (Extracted up to pH)	% Cumulative TPP*
pH 5.3	31.6	50.4	15.9	15.9	34.5
pH 4.9	52.6	20.5	10.8	26.7	57.8
pH 4.1	64.1	10.6	6.8	33.5	72.5
pH 3.5	65.5	4.2	2.7	36.2	78.4
pH 3.1 Supernatant	68.0	3.5	2.4	38.6	83.6
pH 3.1 Residue	69.5	10.9	7.6	46.2	100.0
Σ	—	**100.00**	**46.2**	—	—

* TPP - total polymeric protein

due to loss of some gliadins and soluble proteins (albumins/globulins) during gluten washing, as most of the liquid was discarded.

5.3.2 Reconstitution Studies

Extracted fractions were added to defatted base flour (Jagger and soft) as 1% (dry protein) on a total flour weight basis. The reconstituted flours were analyzed for their MWD, dough mixing properties, baking performance and biaxial extensional rheology.

5.3.2.1 SE-HPLC and MALLS (SEC-MALLS) Analysis

Areas under SEC-HPLC and MALLS chromatograms for all samples of Jagger and soft wheat flours (base and reconstituted) were integrated in two ways. First they were integrated into three peaks to determine the relative proportion of polymeric proteins (mainly glutenins), gliadins (monomeric proteins) and albumins/globulins, as shown in Fig 5.4. Second, integration of the area under chromatograms was made at intervals of 0.4 min from the beginning of the polymeric peak to 15.2 min (Fig 5.5); the first cutoff was from the beginning to 11.2 min. In total, there were 11 cutoffs in the polymeric part of the chromatograms and each cutoff was referred by a number from P1 to P11.

Percentages of total (TPP), extractable (EPP) and unextractable (UPP) polymeric protein in base Jagger and soft wheat flours and on reconstitution with gluten fractions are given in Table 5.4. Significant differences were observed on addition of different fractions to base flours (Table 5.4, Fig 5.6, Fig 5.8). Decrease in TPP and UPP was observed on addition of pH 5.3 fraction. However, for addition of later fractions (pH 4.9 to pH 3.1 residue), progressive increase in both TPP and UPP was observed, except for some deviations in soft flour. Addition of the latest fractions gave the highest percentages of TPP and UPP. Similar trends for weight average molecular weight (M_w) of polymeric proteins were observed from MALLS data (Table 5.5, Table 5.6). Reverse trends were recorded for monomeric to polymeric ratio (Mon/Pol). Relatively higher M_ws' were recorded for soft flour compared to Jagger.

Based on observations of Bangur et al (1997), that the fraction of polymeric proteins with $M_w \geq 250{,}000$ confers strength to dough, the percentage of this fraction (on a total polymeric protein basis) was calculated from cutoff analysis of SEC-MALLS chromatograms. The percentage of this fraction (i.e. percentage of polymeric $\geq$ MW 250,000) for all treatments is

Fig 5.4 SE-HPLC chromatogram of total protein of Jagger, extracted using 50 mM NaPhos buffer, pH 6.9, + 0.5% SDS. sonicated at 6 W for 15 s. Eluted with 50 mM NaPhos buffer, pH 7.0, + 1% SDS, as mobile phase. Peak (I) 0-15.2 min (mostly polymeric proteins / glutenins), peak (II) 15.2-19.3 min (mostly monomeric proteins / gliadins), and peak (III) 19.3-22.0 min (albumins/globulins).

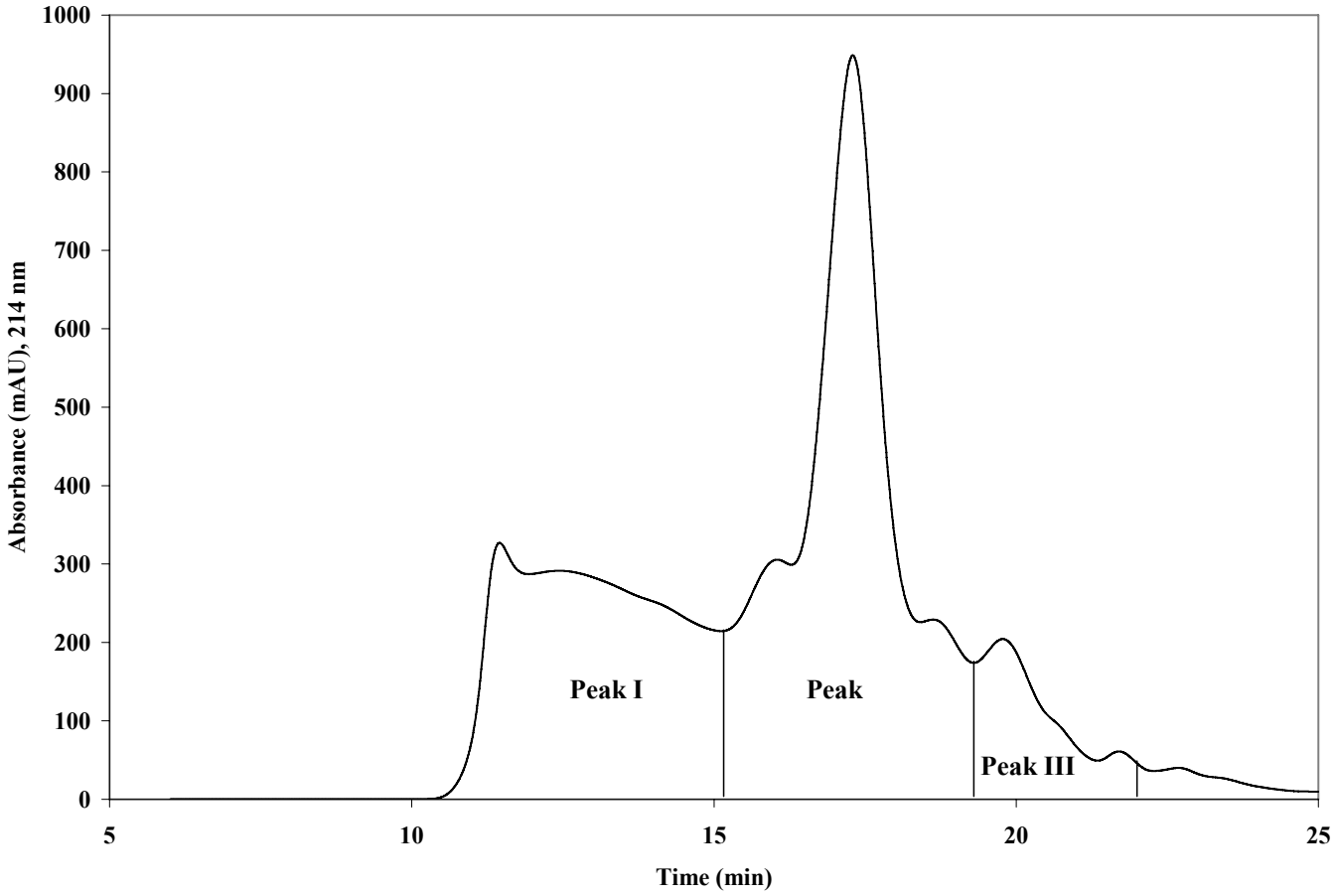


Fig 5.5 SEC-MALLS protein profile of one of the samples illustrating division of the chromatogram at elution time intervals of 0.4 min.

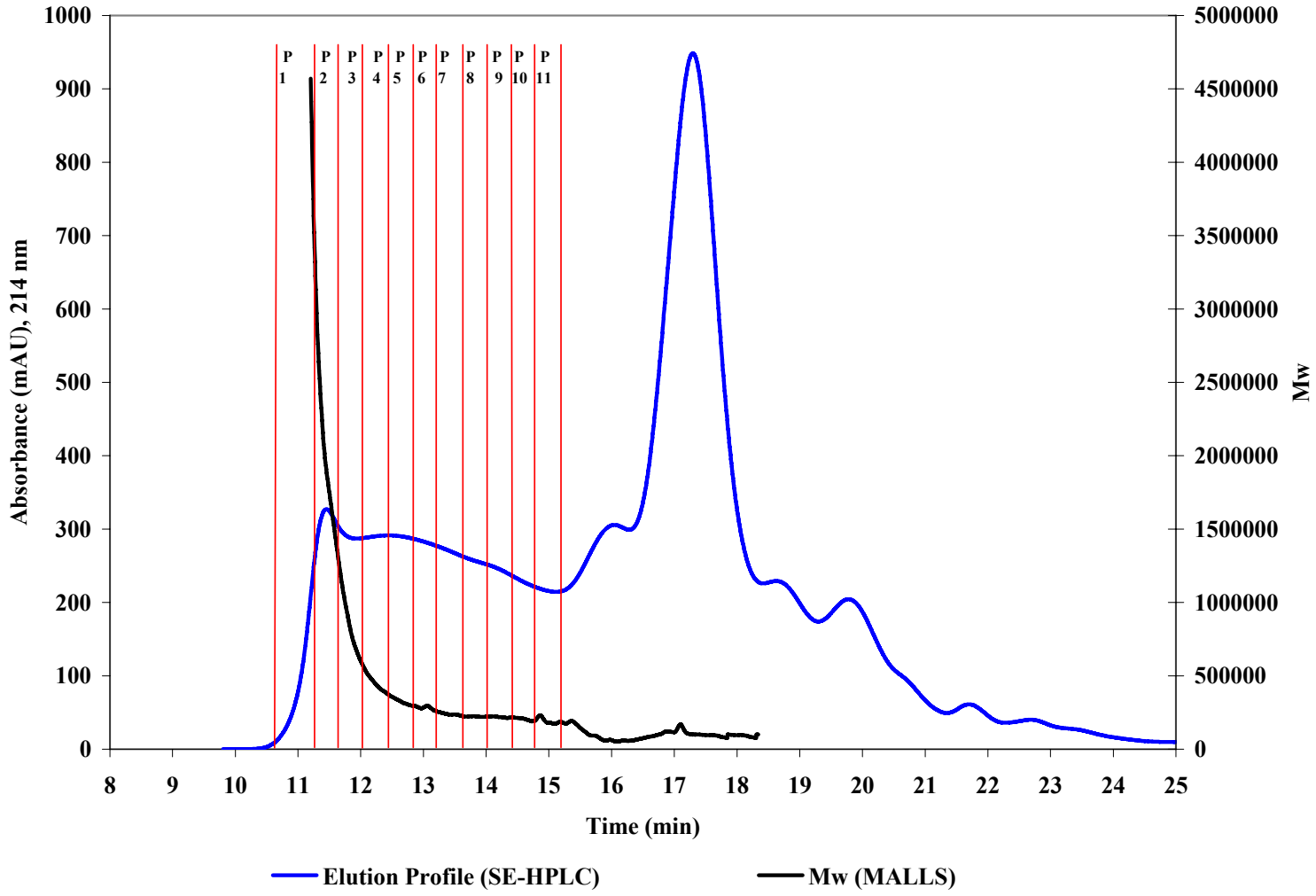


Table 5.4 SE-HPLC relative composition (percentages) of polymeric proteins in Jagger and Soft wheat flours with addition of gluten protein fractions, added at 1% (dry protein) level (on flour weight basis). [A, B]

Fraction Added	Jagger Wheat Flour			Soft Wheat Flour		
	TPP*	EPP*	UPP*	TPP*	EPP*	UPP*
None (Control)	36.4 ± 0.01d	43.6 ± 0.02 b	56.4 ± 2.77 c	37.0 ± 0.05 c	39.0 ± 0.11 b	61.1 ± 3.40 b,c,d
pH 5.3	36.3 ± 0.14 d	46.8 ± 0.23 a	53.2 ± 0.51 d	36.8 ± 0.46 c	42.3 ± 0.15 a	57.7 ± 1.27 d
pH 4.9	38.1 ± 0.19 c	41.4 ± 0.08 c	58.6 ± 0.42 b,c	39.5 ± 1.20 b	35.8 ± 0.08 c,d	64.2 ± 0.54 a,b
pH 4.1	38.6 ± 0.02 b	40.0 ± 0.00 c,d	59.9 ± 0.24 a,b	39.4 ± 0.15 b	39.5 ± 0.06 b	60.5 ± 0.34 c,d
pH 3.5	38.0 ± 0.01 c	39.3 ± 0.00 d	60.6 ± 1.26 a,b	39.1 ± 0.18 b	37.5 ± 0.00 c	62.4 ± 0.42 a,b,c
pH 3.1 Supernatant	38.8 ± 0.04 a,b	39.1 ± 0.09 d,e	60.9 ± 0.75 a,b	41.6 ± 0.12 a	34.9 ± 0.37 d	65.1 ± 0.26 a
pH 3.1 Residue	38.9 ± 0.01 a	38.0 ± 0.24 e	62.0 ± 1.10 a	41.6 ± 0.30 a	35.4 ± 0.01 d	64.6 ± 0.62 a

* TPP - total polymeric protein; EPP - extractable polymeric protein; UPP – unextractable polymeric protein

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Fig 5.6 SE-HPLC chromatograms of total protein of Jagger wheat flour with added gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

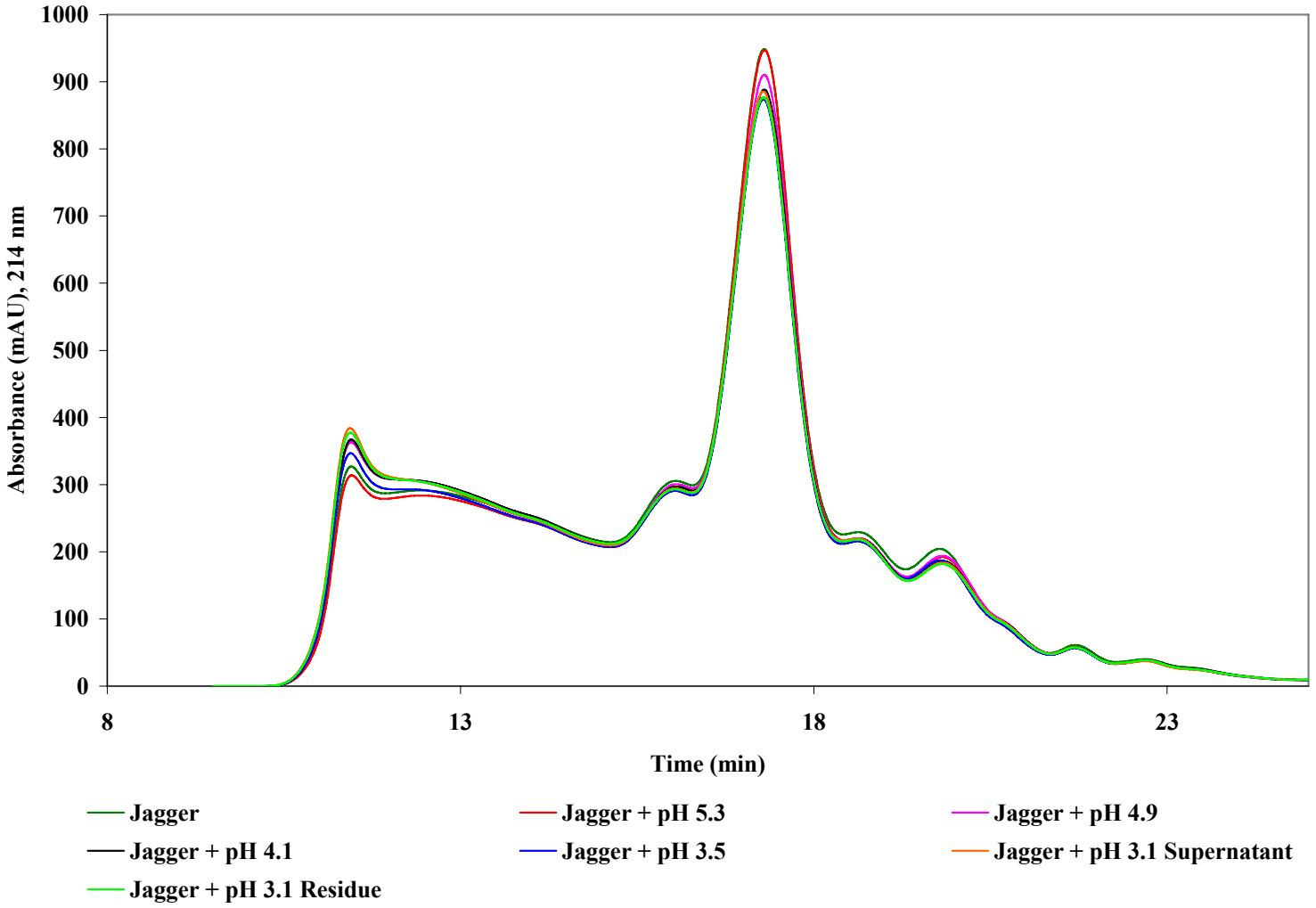


Fig 5.7 Effect on M_w (as determined by MALLS) of total protein of Jagger wheat flour with on addition of gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

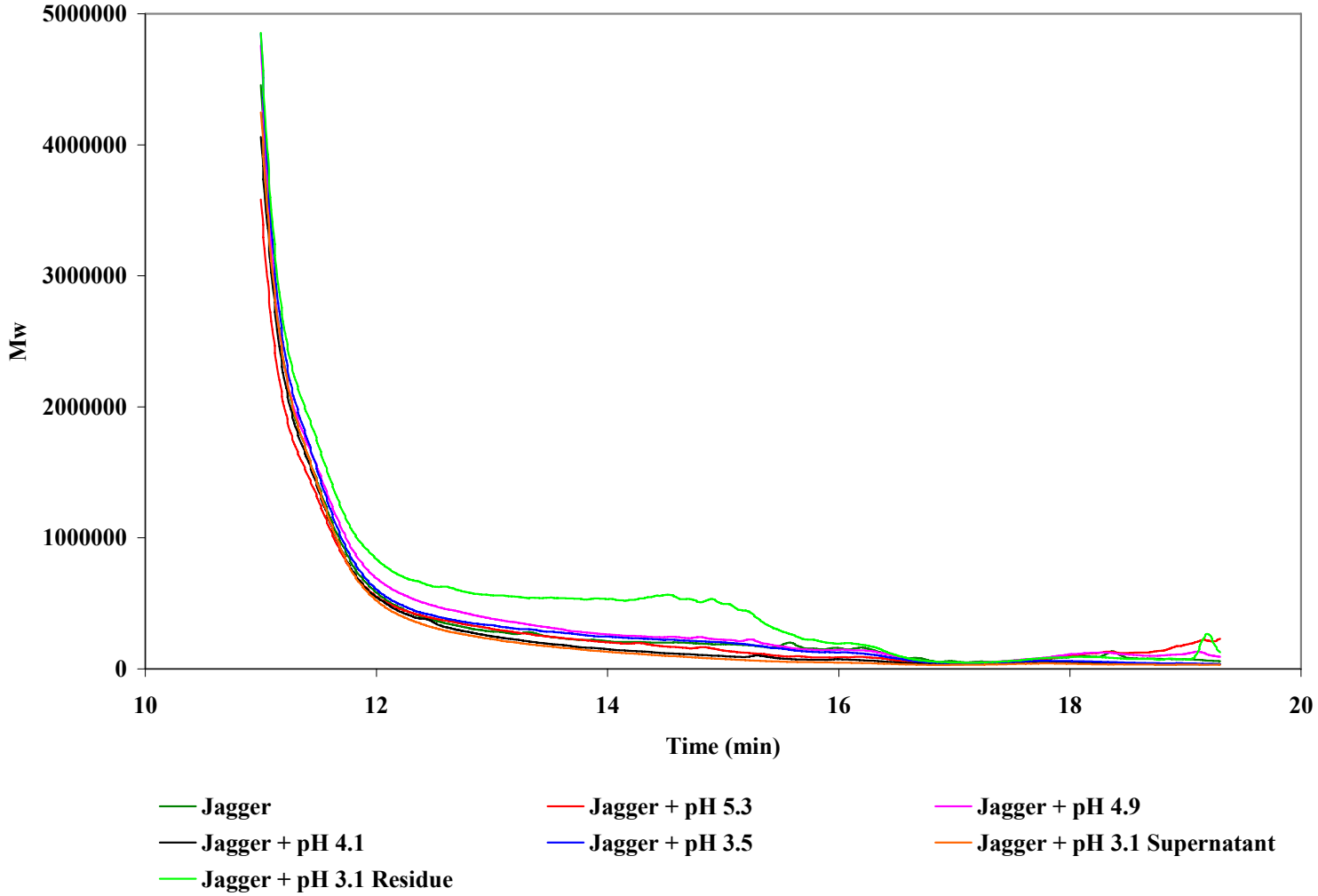


Fig 5.8 SE-HPLC chromatograms of total protein of Soft wheat flour with added gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

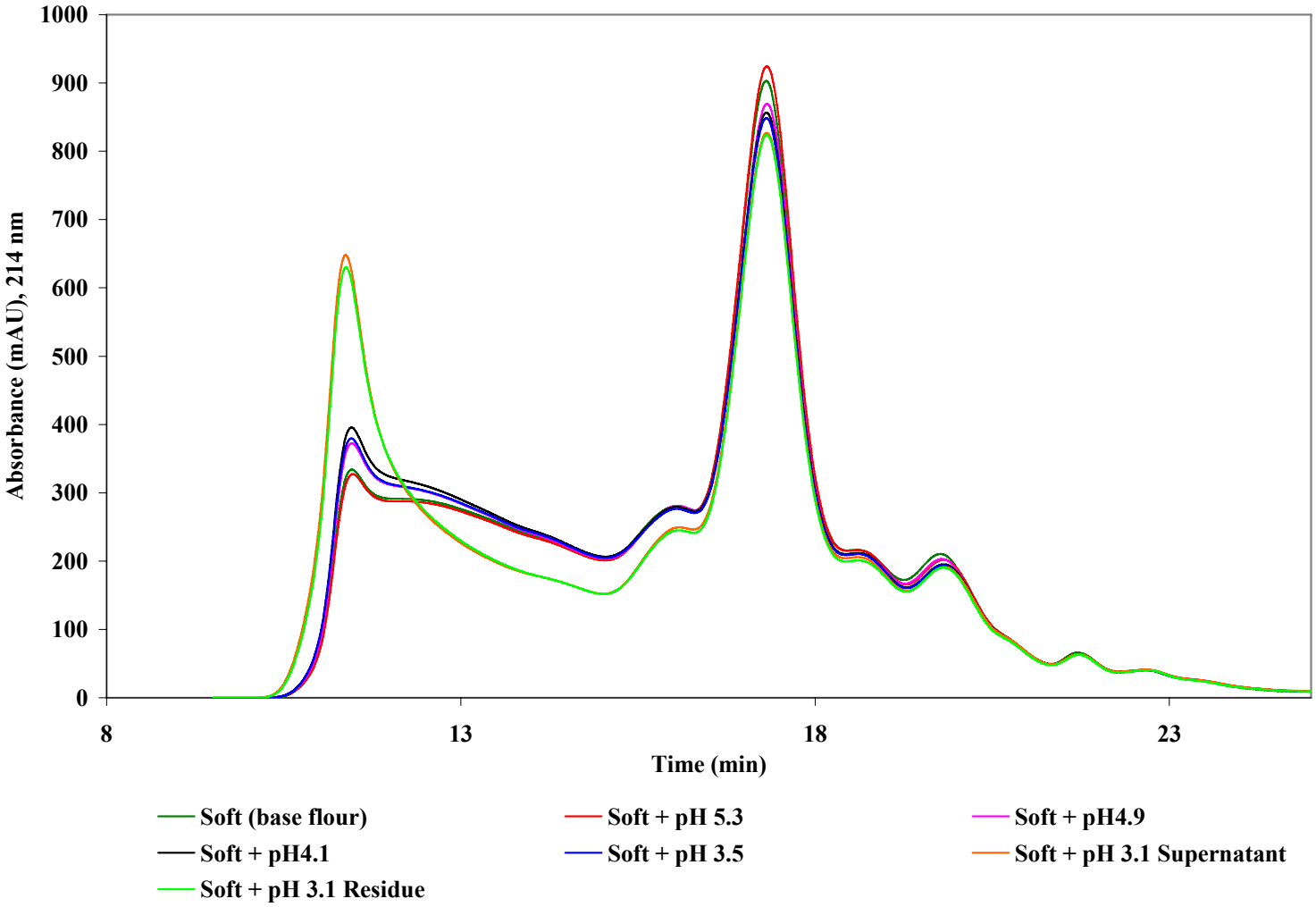


Fig 5.9 Effect on M_w (as determined by MALLS) of total protein of Soft wheat flour on addition of gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

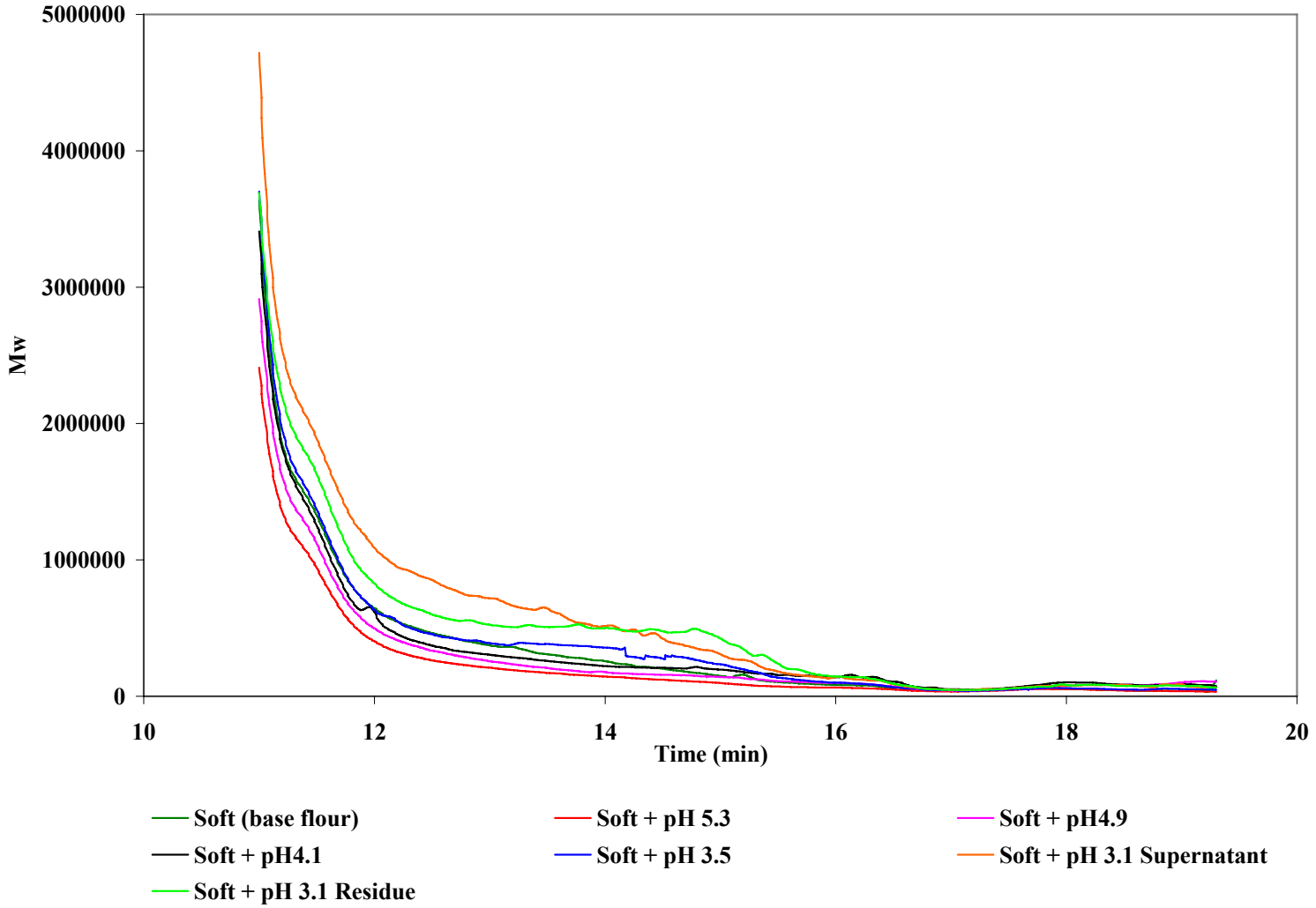


Table 5.5 Parameters calculated from SEC-MALLS chromatograms of Jagger wheat flour with addition of gluten protein fractions, added at 1% (dry protein) level (on flour weight basis). [A, B]

Fraction Added	MON/POL*	% POL > 250,000**	Mw POL***
None (Control)	1.47 ± 0.00 a	65.9 ± 0.01 d	643500 ± 158632 a
pH 5.3	1.48 ± 0.01 a	35.0 ± 0.05 f	278600 ± 20536 b
pH 4.9	1.36 ± 0.01 b,c	66.9 ± 0.18 c	758200 ± 86945 a
pH 4.1	1.34 ± 0.00 c,d	58.0 ± 0.04 e	530900 ± 106585 a,b
pH 3.5	1.37 ± 0.00 b	67.1 ± 0.02 c	631700 ± 78946 a
pH 3.1 Supernatant	1.33 ± 0.01 d	85.1 ± 0.12 b	822200 ± 164926 a
pH 3.1 Residue	1.32 ± 0.00 d	100 ± 0.00 a	829000 ± 245300 a

* Monomeric to polymeric protein ratio

** Percentage of polymeric proteins ≥ 250,000

*** Weight average molecular weight of polymeric fraction

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Table 5.6 Parameters calculated from SEC-MALLS chromatograms of Soft wheat flour with addition of gluten protein fractions, added at 1% (dry protein) level (on flour weight basis). [A, B]

Fraction Added	MON/POL*	% POL > 250,000**	Mw POL***
None (Control)	1.41 ± 0.01 a	76.8 ± 0.07 d	681800 ± 182230 b,c
pH 5.3	1.43 ± 0.03 a	46.7 ± 0.10 f	425000 ± 95377 c
pH 4.9	1.27 ± 0.07 b	68.1 ± 0.22 e	659400 ± 120788 b,c
pH 4.1	1.28 ± 0.01 b	99.7 ± 0.14 b,c	759000 ± 159216 b,c
pH 3.5	1.30 ± 0.01 b	100.0 ± 0.00 a	861400 ± 102356 b,c
pH 3.1 Supernatant	1.16 ± 0.00 c	96.1 ± 0.04 c	991400 ± 134584 a,b
pH 3.1 Residue	1.16 ± 0.02 c	100.0 ± 0.00 a	1378000 ± 388246 a

* Monomeric to polymeric protein ratio

** Percentage of polymeric proteins ≥ 250,000

*** Weight average molecular weight of polymeric fraction

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Table 5.7 Weight average molecular weights (M_w) of different peak regions corresponding to elution time intervals of 0.4 min for Jagger wheat flour with addition of gluten protein fractions, added at 1% (dry protein) level (on flour weight basis). [A, B]

Fraction Added	Mw P1	Mw P2	Mw P3	Mw P4
None (Control)	5235000 ± 613768 a,b	1742000 ± 89802 a,b,c	845300 ± 87964 a	488800 ± 67670 b
pH 5.3	2652000 ± 113844 c	1096000 ± 33446 d	479600 ± 22160 a	257000 ± 16129 c
pH 4.9	6268000 ± 552250 a	1931000 ± 11667 a,b	1000000 ± 49992 a	617900 ± 29769 a
pH 4.1	4603000 ± 116601 b	1617000 ± 28920 c	766300 ± 20697 a	443900 ± 16206 b
pH 3.5	4618000 ± 941159 b	1666000 ± 137171 b,c	793400 ± 32880 a	458000 ± 2474 b
pH 3.1 Supernatant	6032000 ± 614475 a	2001000 ± 185261 a	997800 ± 146583 a	595500 ± 11631 a
pH 3.1 Residue	5172000 ± 409414 a,b	1815000 ± 157684 a,b,c	878100 ± 166099 a	512000 ± 15895 b

Fraction Added	Mw P5	Mw P6	Mw P7	Mw P8
None (Control)	361700 ± 53669 b,c	297200 ± 42992 c	277500 ± 22061 a	234700 ± 18809 b
pH 5.3	178800 ± 14481 d	135600 ± 13010 e	107500 ± 11391 b	85110 ± 10188 d
pH 4.9	466400 ± 12515 a	367500 ± 2969 a,b	291100 ± 17606 a	229700 ± 26587 b
pH 4.1	324100 ± 14898 c	246200 ± 15902 d	198400 ± 17225 a,b	162900 ± 20937 c
pH 3.5	349000 ± 12657 b,c	293100 ± 17041 c,d	254600 ± 15768 a,b	219800 ± 14566 b
pH 3.1 Supernatant	463000 ± 11349 a	390500 ± 11009 a	334600 ± 10451 a	280200 ± 9093 a
pH 3.1 Residue	390500 ± 16475 b	318700 ± 17196 b,c	276800 ± 181938 a	254400 ± 18809 a,b

Fraction Added	Mw P9	Mw P10	Mw P11
None (Control)	219200 ± 5020 b,c	216400 ± 18384 a,b	206400 ± 3903 b
pH 5.3	71590 ± 9490 e	60800 ± 82377 e	52280 ± 7362 f
pH 4.9	193300 ± 31819 c	149300 ± 5388 c,d	114800 ± 6498 d
pH 4.1	135600 ± 2721 d	113500 ± 4092 d,e	95880 ± 7135 e
pH 3.5	193900 ± 12445 c	173700 ± 7778 b,c,d	153200 ± 8061 c
pH 3.1 Supernatant	334600 ± 15026 a	189100 ± 6396 a,b,c	146500 ± 4857 c
pH 3.1 Residue	249100 ± 18186 b	252900 ± 17896 a	268500 ± 13300 a

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Table 5.8 Weight average molecular weights (M_w) of different peak regions corresponding to elution time intervals of 0.4 min for Soft wheat flour with addition of gluten protein fractions, added at 1% (dry protein) level (on flour weight basis). [A, B]

Fraction Added	Mw P1	Mw P2	Mw P3	Mw P4
None (Control)	4336000 ± 330219 a,b	1493000 ± 38890 b,c	840100 ± 33022 c	548300 ± 36982 b,c
pH 5.3	3324000 ± 987828 b	1177000 ± 232638 d	607500 ± 17218 d	358700 ± 13399 d
pH 4.9	3898000 ± 4242 a,b	1474000 ± 82731c	815500 ± 68872 c	485800 ± 31961 c
pH 4.1	4448000 ± 337289 a	1593000 ± 104651 b,c	885800 ± 82943 b,c	554800 ± 61306 b,c
pH 3.5	4254000 ± 333754 a,b	1590000 ± 79903 b,c	876700 ± 65902 b,c	569100 ± 68589 b,c
pH 3.1 Supernatant	4320000 ± 236173 a,b	1737000 ± 53033 a,b	982300 ± 56356 a,b	595900 ± 55366 b
pH 3.1 Residue	4821000 ± 13435 a	1874000 ± 2121 a	1099000 ± 707 a	715500 ± 19940 a

Fraction Added	Mw P5	Mw P6	Mw P7	Mw P8
None (Control)	437300 ± 46952 b	371800 ± 7852 b	320300 ± 10119 b,c	270800 ± 13484 b,c
pH 5.3	270700 ± 11914 c	224700 ± 5217 c	191300 ± 56237 d	166100 ± 8394 c
pH 4.9	345900 ± 6930 c	263200 ± 6364 c	211300 ± 11243 d	173500 ± 15061 c
pH 4.1	436300 ± 4279 b	369000 ± 52821 b	310000 ± 38537 b,c	273000 ± 36770 b,c
pH 3.5	451300 ± 31801 b	403500 ± 32691 b	392100 ± 22914 b	376100 ± 63710 a,b
pH 3.1 Supernatant	451200 ± 45679 b	370200 ± 27365 b	309000 ± 19233 c	266800 ± 3394 b,c
pH 3.1 Residue	576800 ± 38608 a	517500 ± 61094 a	491700 ± 80610 a	478200 ± 100833 a

Fraction Added	Mw P9	Mw P10	Mw P11
None (Control)	227400 ± 15083 c	186500 ± 18710 c	151100 ± 160230 b
pH 5.3	144200 ± 7411 d	123800 ± 7396 c	101700 ± 75378 b
pH 4.9	150300 ± 18243 d	134100 ± 21142 c	119700 ± 20789 b
pH 4.1	249900 ± 37406 c	251600 ± 4538 b,c	254900 ± 59114 a,b
pH 3.5	773000 ± 39035 a	385700 ± 119784 a,b	243100 ± 13576 a,b
pH 3.1 Supernatant	249700 ± 8556 c	244700 ± 43558 b,c	218500 ± 59185 a,b
pH 3.1 Residue	444200 ± 8959 b	429400 ± 96874 a	370100 ± 88247 a

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

given in Table 5.5 for Jagger and in Table 5.6 for soft wheat flour. The calculations were based on M_w analysis presented in Tables 5.7 and 5.8, and TPP analysis in Table 5.4.

MALLS recorded high standard deviations for M_w analysis. Lemelin et al (2005) also reported large deviations in duplicate analyses of wheat flours, performed on MALLS. Statistically this is not favorable for quantitative conclusions and comparisons; nevertheless, variations between treatments are clear enough to make qualitative conclusions.

5.3.2.2 Dough Mixing Properties

To observe variations in mixing properties of the flours on addition of fractions, Mixograph analysis was performed with constant water absorption i.e. 63% for Jagger and 60% for soft wheat flour. Midline peak times (MPT) and weakening angles calculated from Mixographs traces of Jagger (Fig 5.10) and soft (Fig 5.11) wheat flours are given in Table 5.9.

The trends observed for MPTs and weakening angles were in accordance with those of SEC-MALLS data (Table 5.4, Table 5.5, Table 5.6). Mixing requirements decreased with addition of the pH 5.3 fraction which represented the first 35% of the polymeric protein extracted and a major part of gliadins that were extracted. This fraction also has the lowest percentage of polymeric proteins ≥ MW 250,000 (Table 5.5, Table 5.6). Addition of subsequent fractions caused successive increase in MPTs and a decrease in weakening angles (Table 5.9, Fig 5.12, Fig 5.13). This increase in dough strength could be attributed to an increase in the percentage of polymeric proteins ≥ MW 250,000 and a decrease in Mon/Pol.

MacRitchie and co-workers (MacRitchie, 1987; Lundh and MacRitchie, 1989; MacRitchie et al, 1991) observed a decrease in MPTs for the very latest fractions. This could be attributed to under mixing of flours with these latest fractions, as we observed that mixing requirements increase significantly with increase in M_w of the polymeric fraction and more so as percentage of polymeric proteins ≥ MW 250,000 increased. Gupta et al (1993) also showed that with increase in UPP, the mixing requirements of flours increased. Fig 5.10 and Fig 5.11 show Mixograph charts of Jagger and soft wheat flour respectively. Mixing was done with the same mixer; i.e. at constant rpm. With addition of the latest fractions the Mixogram peak became less well defined. The Mixograms in these cases resembled those of high intensity requirement flours/very-strong flours, as represented by MacRitchie (1986) based on the work by Kilborn and Tipples (1972) and Tipples and Kilborn (1975).

Fig 5.10 Mixographs of Jagger wheat flour with added protein fractions (A) Jagger (control), (B) Jagger + pH 5.3, (C) Jagger + pH 4.9, (D) Jagger + pH 4.1, (E) Jagger + pH 3.5, (F) Jagger + pH 3.1 supernatant, (G) Jagger + pH 3.1 residue. Fractions added at a 1% (dry protein) level (on flour weight basis).

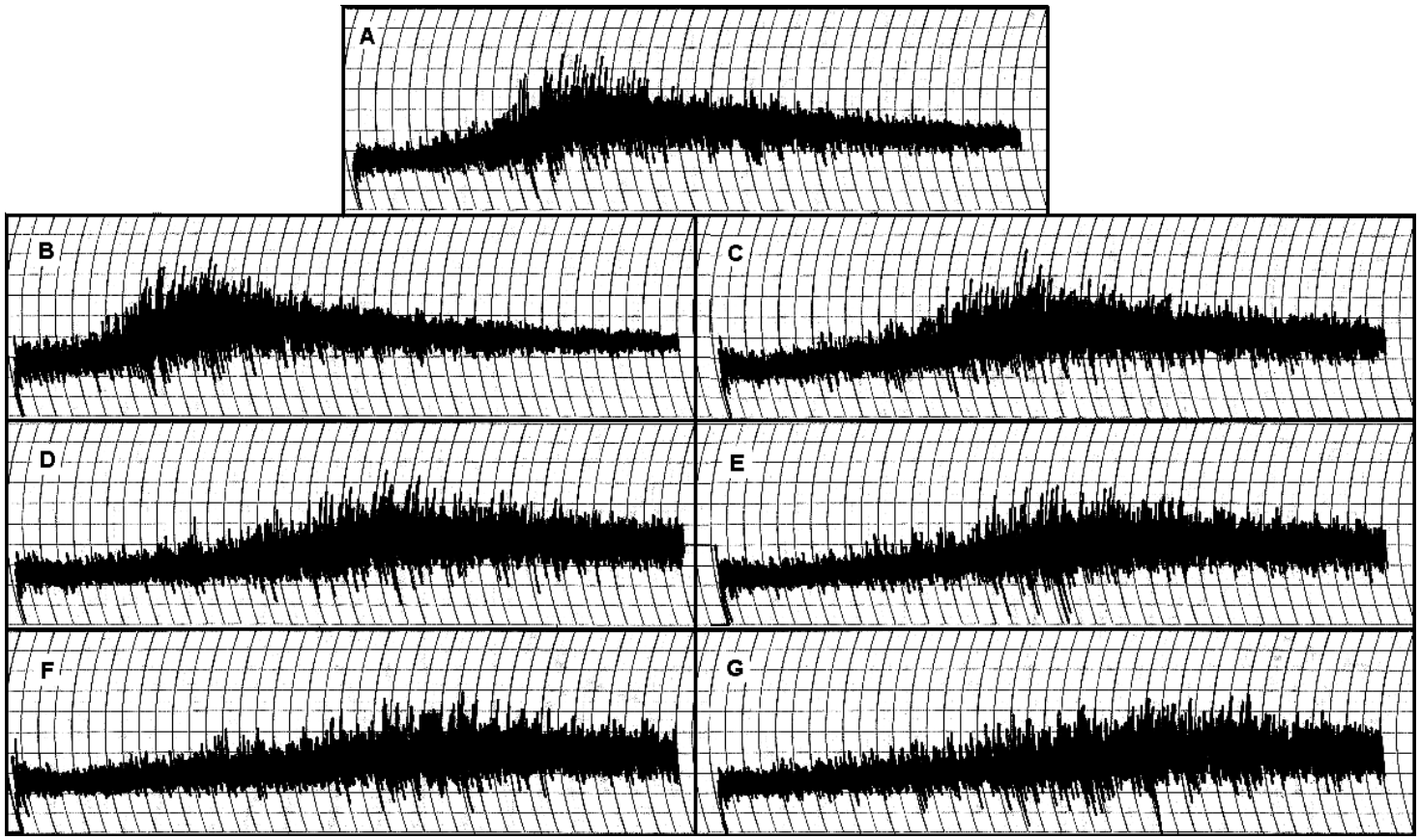


Fig 5.11 Mixographs of Soft wheat flour with added protein fractions (A) Soft (control), (B) Soft + pH 5.3, (C) Soft + pH 4.9, (D) Soft + pH 4.1, (E) Soft + pH 3.5, (F) Soft + pH 3.1 supernatant, (G) Soft + pH 3.1 residue. Fractions added at a 1% (dry protein) level (on flour weight basis).

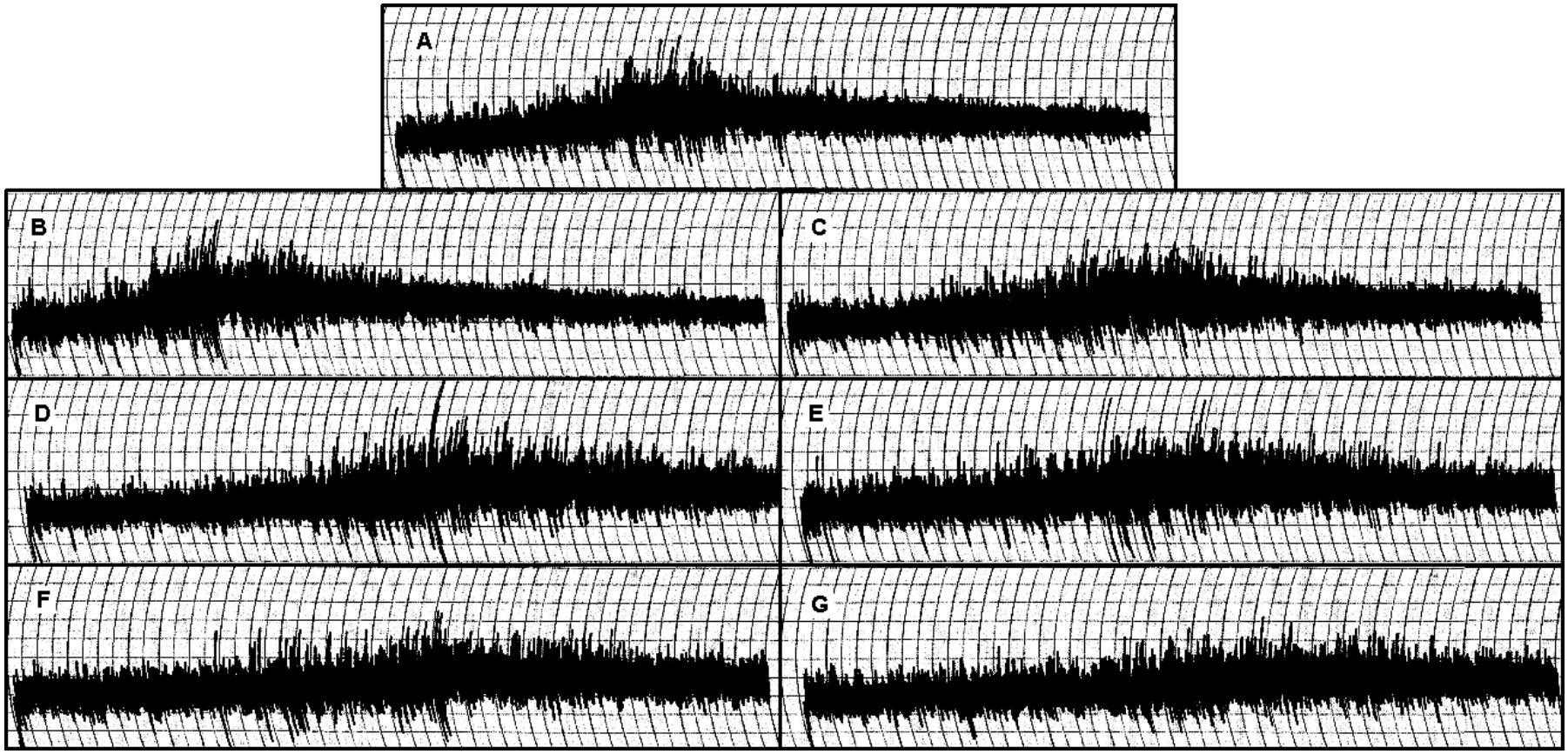


Table 5.9 Mixograph peak time and weakening angles for Jagger and Soft wheat flour with addition of gluten protein fractions, added at 1% (dry protein) level (on flour weight basis). [A, B]

Fraction Added	Jagger Wheat Flour		Soft Wheat Flour	
	Midline Peak Time (MPT) (min)	Weakening Angle	Midline Peak Time (MPT) (min)	Weakening Angle
None (Control)	7.21 ± 0.06 e	5.0 ± 0.00 b	9.25 ± 0.00 e	3.0 ± 0.00 b
pH 5.3	6.09 ± 0.12 f	6.1 ± 0.18 a	7.13 ± 0.18 f	4.3 ± 0.35 a
pH 4.9	9.50 ± 0.00 d	3.4 ± 0.18 c	12.25 ± 0.35 d	3.0 ± 0.00 b
pH 4.1	11.67 ± 0.12 c	2.0 ± 0.00 d	14.25 ± 0.35 b	1.1 ± 0.18 c
pH 3.5	11.71 ± 0.18 c	1.6 ± 0.18 d,e	13.50 ± 0.24 c	1.0 ± 0.00 c
pH 3.1 Supernatant	13.38 ± 0.18 b	1.3 ± 0.35 e,f	14.42 ± 0.23 b	0.5 ± 0.00 d
pH 3.1 Residue	14.17 ± 0.23 a	0.9 ± 0.18 f	15.34 ± 0.23 a	0.5 ± 0.00 d

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Fig 5.12 Effect on mixograph peak times of Jagger and Soft wheat flours on addition of gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

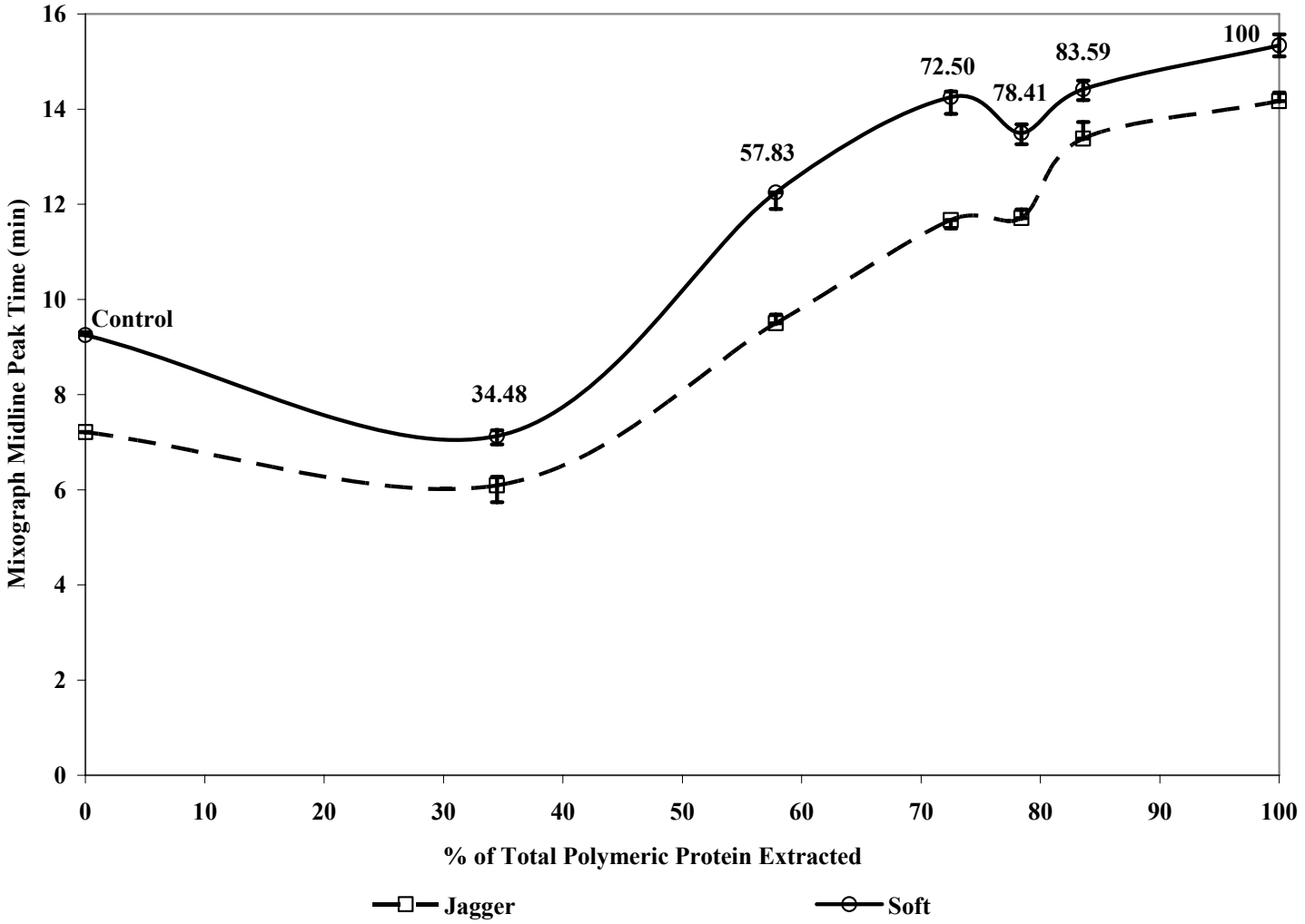
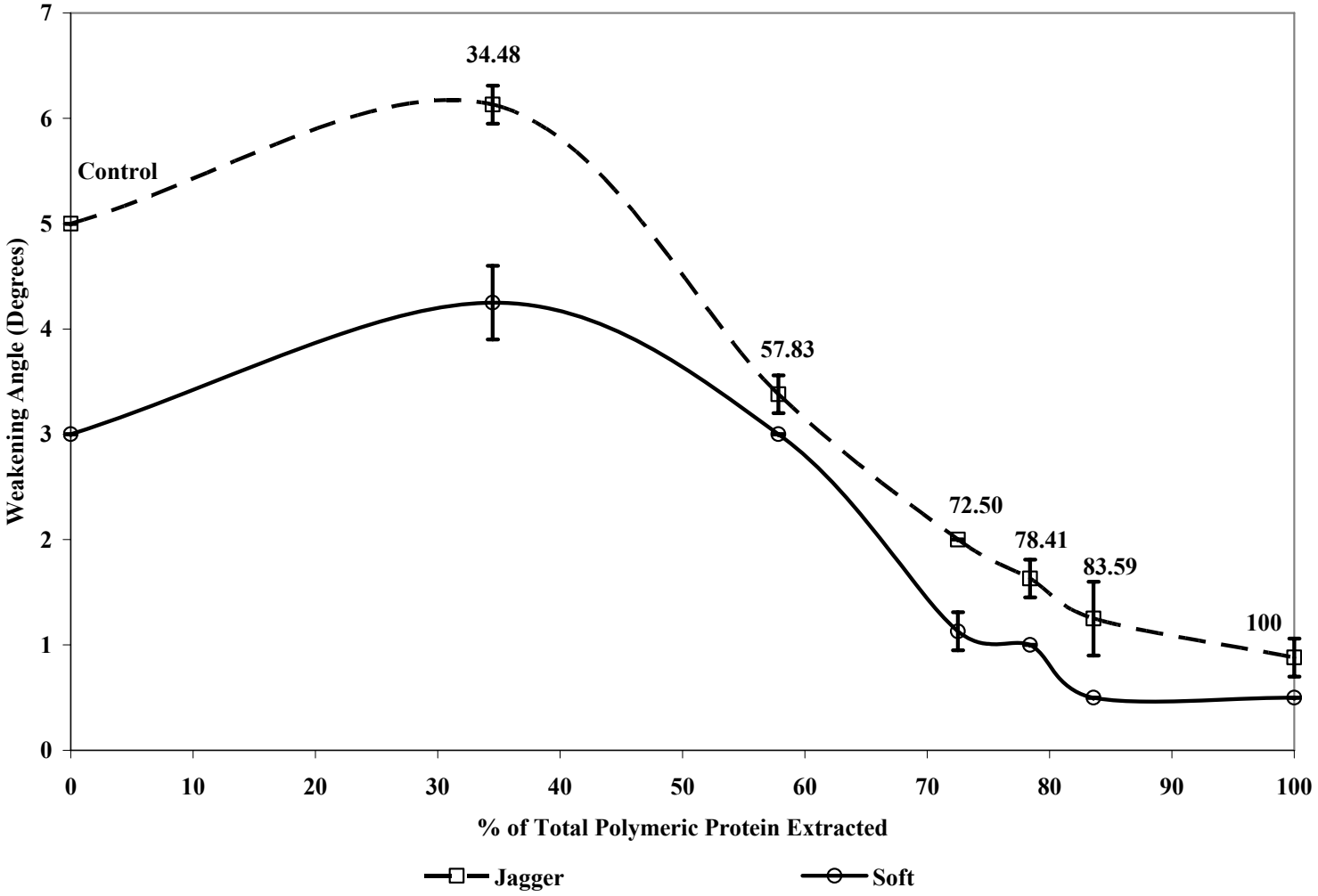


Fig 5.13 Effect on mixograph weakening angles of Jagger and Soft wheat flours on addition of gluten protein fractions at a 1% (dry protein) level (on flour weight basis).



In this study, since the peaks were attained, it can be concluded that the doughs had been mixed to peak development.

5.3.2.3 Baking Performance

Loaf volumes (Table 5.10) and image analysis parameters (number of gas cells and average cell elongation) (Table 5.11) decreased on addition of the pH 5.3 fraction. However, on addition of subsequent fractions that lead to increase in mixing strength (Mixograph analysis), increase in loaf volume and image analysis parameters was observed, which after reaching maximum, again decreased. This maximum was achieved; in the case of Jagger on addition of total polymeric proteins that were extracted after the first 60% and before the first 80%, corresponding to pH 4.1 fraction, and in the case of soft on addition of polymeric proteins that were extracted after the first 35% and before the first 70%, corresponding to pH 4.9 fraction (Fig 5.14, Fig 5.15, Fig 5.16). It seems that the optimum balance of strength and extensibility was achieved on addition of these fractions or ranges of polymeric proteins to base flours, thus giving best baking performance (Fig 5.17, Fig 5.18). Differences in volumes could also be attributed to differences in gas cell concentrations (Fig 5.15) apart from expansion of gas cells, unlike previous results on lipid variations (Chapter 3, Chapter 4) where differences in volume were mainly due to differences in expansion capacity of gas cells.

Table 5.5 and Table 5.6 show that addition of these fractions to both flours (pH 4.1 for Jagger and pH 4.9 for soft) caused 60% (approximately) of total polymeric fraction to be ≥ 250,000. This fraction is thought to confer strength (Bangur et al, 1997). The remaining 40% is of M_w less than this threshold molecular weight (M_T) (Equation 2.1), and confers extensibility. This balance of 60:40 of strength and extensibility contributions of polymeric fraction seems to be optimum. Similar observations on relative proportions of strength and extensibility conferring factors were concluded by MacRitchie (1998) from results of Bangur et al (1997). For soft flour this balance was achieved on addition of pH 4.9 fraction, this was nearest to the 60:40 figure although bit skewed towards strength.

5.3.2.4 Biaxial Extensional Rheology

Significant differences ($P < 0.0001$) in biaxial extensional rheological parameters (maximum stress, failure strain and strain hardening index) were recorded on addition of fractions to the base flours (Table 5.12, Table 5.13, Fig 5.21, Fig 5.22). Variations in strain

Table 5.10 Loaf volume responses of Jagger and Soft wheat flour with addition of gluten protein fractions, added at 1% (dry protein) level (on flour weight basis). [A, B]

Fraction Added	Loaf Volume (cc)	
	Jagger Wheat Flour	**Soft Wheat Flour**
None (Control)	247.0 ± 1.41 b,c	214.0 ± 1.41 b
pH 5.3	232.5 ± 2.12 d	201.0 ± 1.41 c
pH 4.9	253.5 ± 2.12 b	222.5 ± 3.54 a
pH 4.1	284.5 ± 3.54 a	196.0 ± 1.41 c
pH 3.5	245.0 ± 7.07 c	170.5 ± 0.71 d
pH 3.1 Supernatant	—	163.0 ± 4.24 e
pH 3.1 Residue	196.0 ± 1.41 e	146.5 ± 2.12 f

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Fig 5.14 Effect on loaf volumes of Jagger and Soft wheat flours on addition of gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

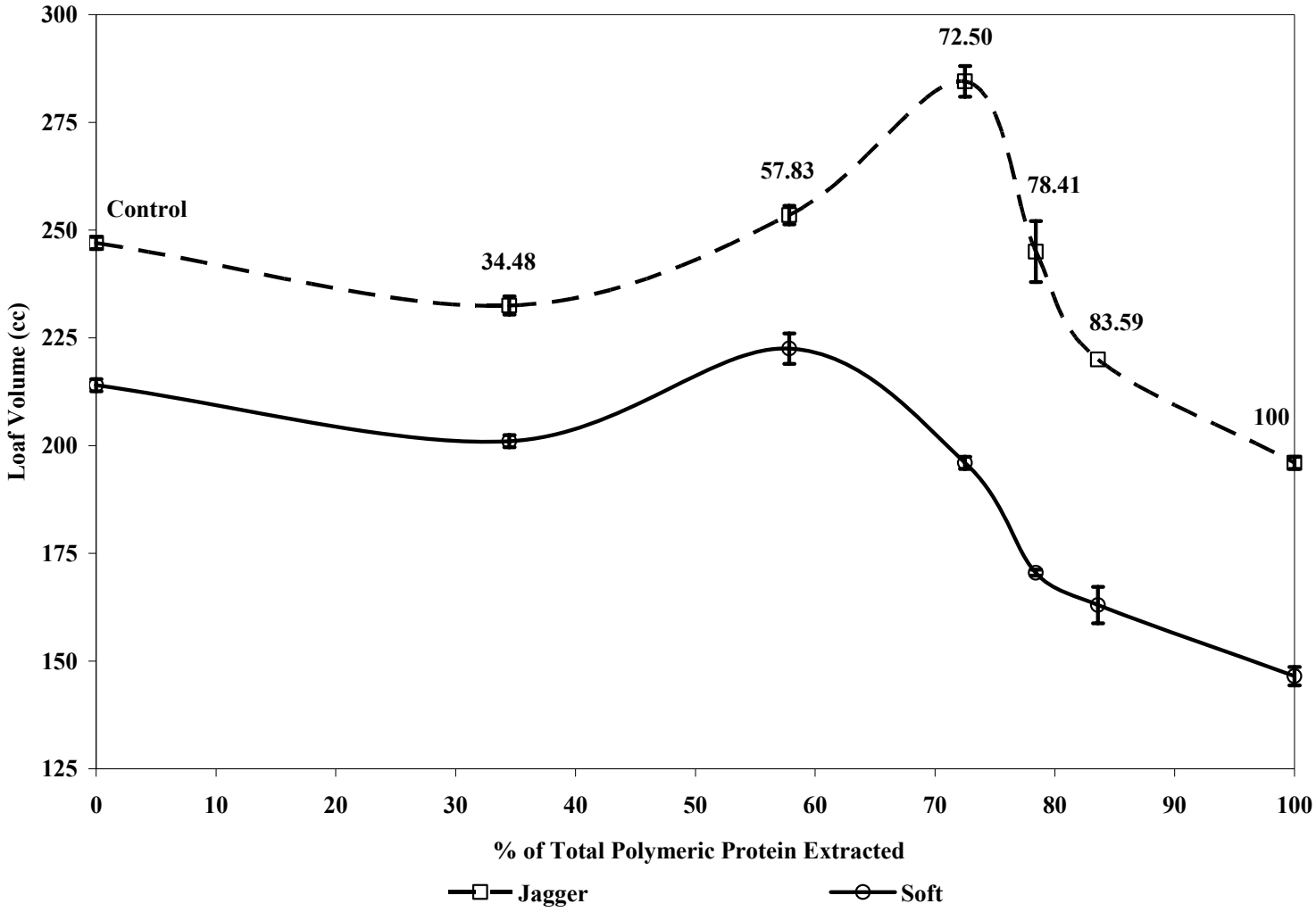


Table 5.11 Crumb structure responses of Jagger and Soft wheat flour with addition of gluten protein fractions, added at 1% (dry protein) level (on flour weight basis). [A, B]

Fraction Added	Jagger Wheat Flour		Soft Wheat Flour	
	Number of Cells	Average Cell Elongation (C-Cell Score)	Number of Cells	Average Cell Elongation (C-Cell Score)
None (Control)	2253.5 ± 24.75 b	1.64 ± 0.02 c,d	1958.0 ± 60.81 c	1.57 ± 0.03 c,d
pH 5.3	1998.5 ± 9.19 c	1.59 ± 0.03 d	1951.5 ± 122.33 c	1.52 ± 0.00 d
pH 4.9	2378.5 ± 98.29 b	1.75 ± 0.06 b	2427.5 ± 41.72 a	1.83 ± 0.04 a
pH 4.1	2762.0 ± 66.47 a	1.92 ± 0.01 a	2174.0 ± 43.84 b	1.70 ± 0.01 b
pH 3.5	2366.5 ± 88.39 b	1.69 ± 0.00 b,c	1906.5 ± 94.05 c	1.67 ± 0.02 b
pH 3.1 Supernatant	—	—	1919.0 ± 21.21 c	1.60 ± 0.01 c
pH 3.1 Residue	1982.0 ± 57.98 c	1.60 ± 0.01 d	1887.5 ± 3.54 c	1.59 ± 0.02 c

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Fig 5.15 Effect on number of gas cells of Jagger and Soft wheat flours on addition of gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

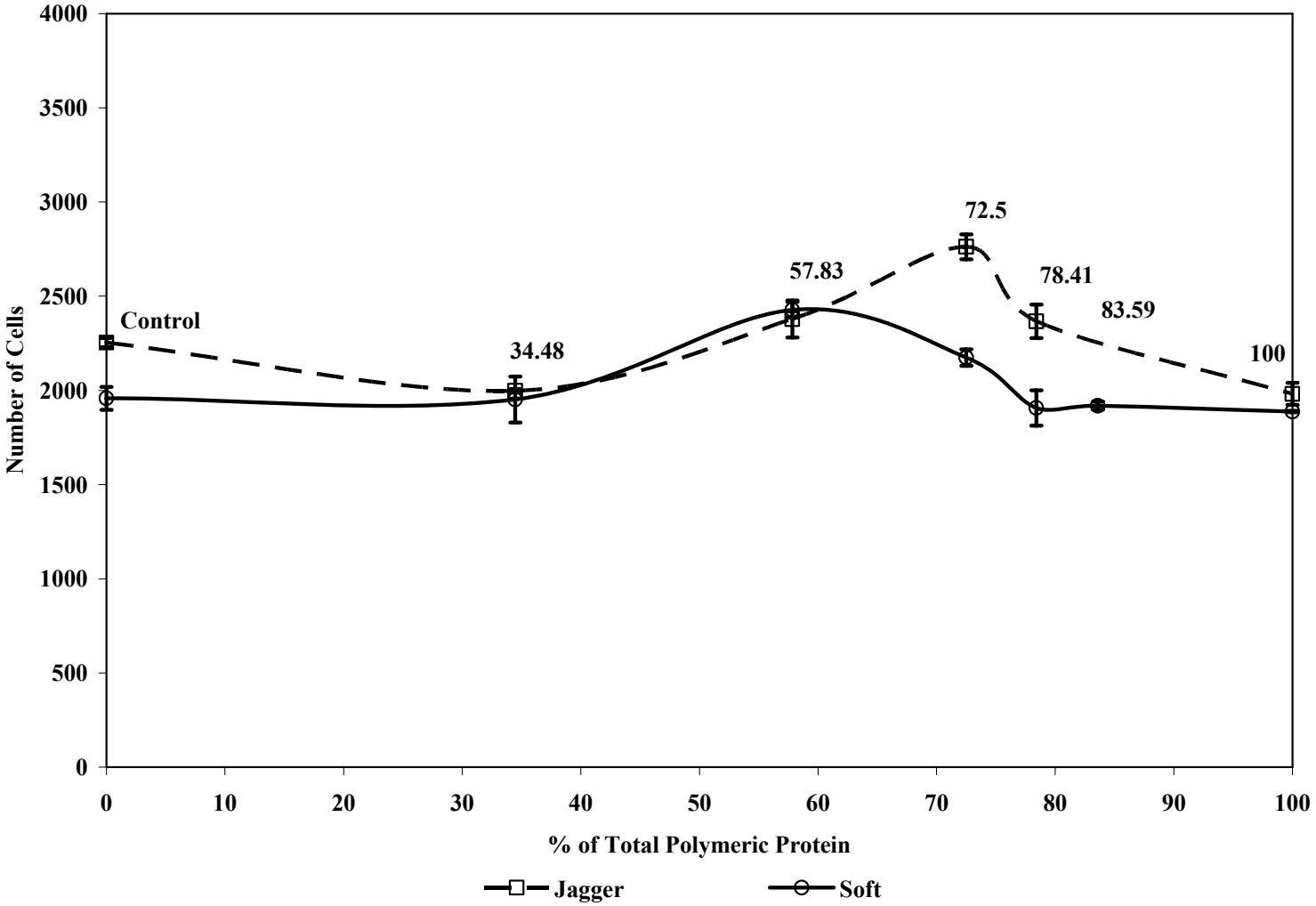


Fig 5.16 Effect on average cell elongation of Jagger and Soft wheat flours on addition of gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

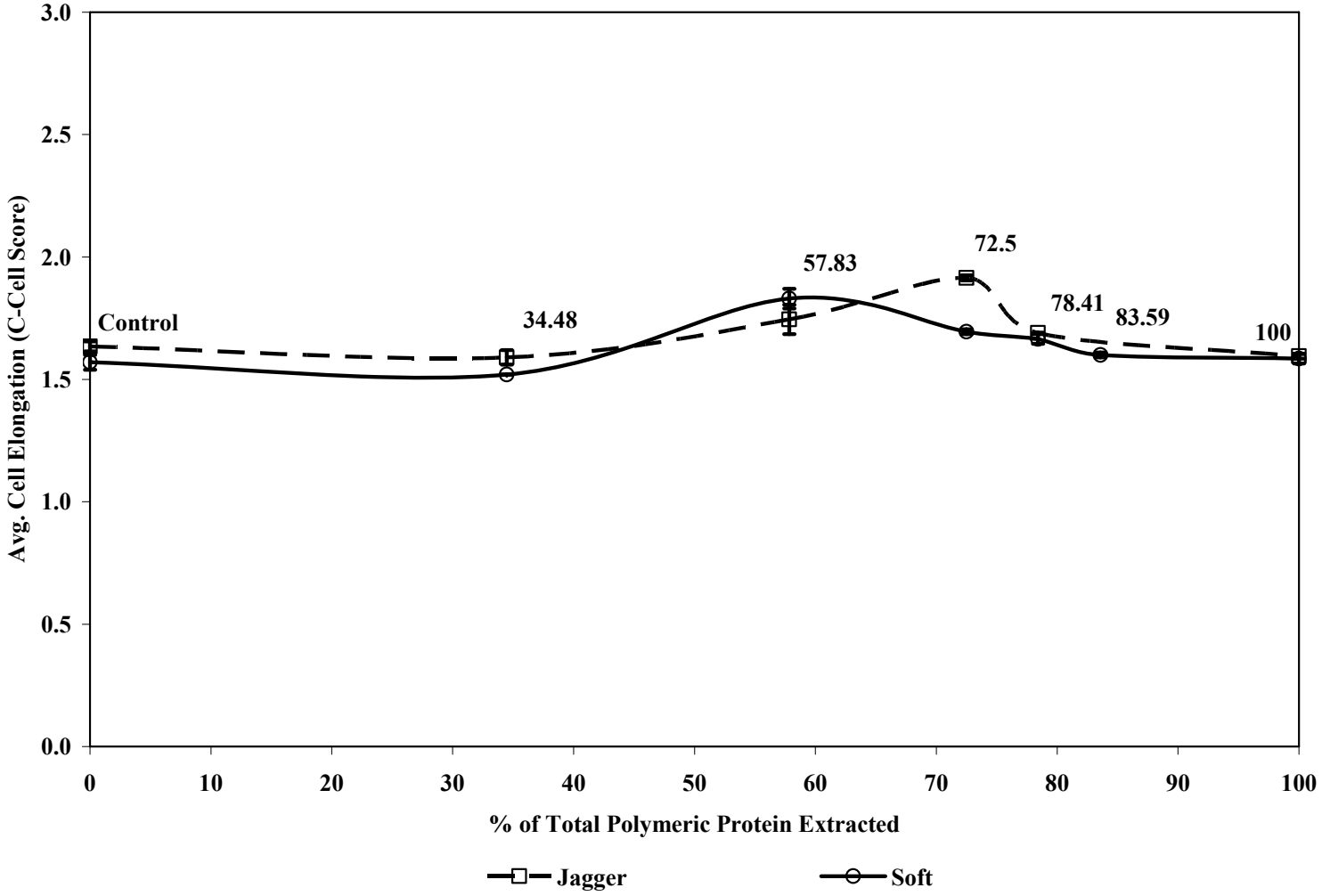


Fig 5.17 C-Cell images of Jagger wheat flour bread slices showing differences in volumes due to concentration of gas cells on addition of different gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

Fig 5.18 C-Cell images of Soft wheat flour bread slices showing differences in volumes due to concentration of gas cells on addition of different gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

Table 5.12 Mean bubble inflation rheological responses of Jagger and Soft wheat flour doughs with addition of gluten protein fractions, added at 1% (dry protein) level (on flour weight basis). [A, B]

Fraction Added	Max. Stress (Kpa)	Failure Strain (Hencky)	Strain Hardening Index
None (Control)	476.41 ± 45.01 d,e	2.51 ± 0.05 c,d	2.10 ± 0.03 c
pH 5.3	650.56 ± 160.65 c,d	2.42 ± 0.06 d	2.01 ± 0.01 d
pH 4.9	1099.45 ± 6.57 b	2.69 ± 0.00 b	2.22 ± 0.01 b
pH 4.1	1981.42 ± 172.72 a	2.91 ± 0.02 a	2.52 ± 0.09 a
pH 3.5	877.91 ± 196.55 b,c	2.60 ± 0.02 b,c	2.20 ± 0.03 b
pH 3.1 Supernatant	257.17 ± 48.82 e,f	1.99 ± 0.03 e	1.89 ± 0.00 e
pH 3.1 Residue	138.33 ± 20.29 f	1.59 ± 0.04 f	1.77 ± 0.00 f

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Table 5.13 Mean bubble inflation rheological responses of Jagger and Soft wheat flour doughs with addition of gluten protein fractions, added at 1% (dry protein) level (on flour weight basis). [A, B]

Fraction Added	Max. Stress (Kpa)	Failure Strain (Hencky)	Strain Hardening Index
None (Control)	641.29 ± 39.67 b	2.49 ± 0.03 b	2.03 ± 0.02 b
pH 5.3	688.14 ± 133.49 b	2.31 ± 0.07 c	1.97 ± 0.01 c
pH 4.9	1794.41 ± 123.12 a	2.83 ± 0.00 a	2.22 ± 0.04 a
pH 4.1	349.02 ± 28.63 c	1.96 ± 0.00 d	1.92 ± 0.00 d
pH 3.5	145.95 ± 18.20 d	1.51 ± 0.01 e	1.83 ± 0.01 e
pH 3.1 Supernatant	107.27 ± 5.20 d	1.39 ± 0.01 f	1.78 ± 0.00 f
pH 3.1 Residue	96.55 ± 2.66 d	1.34 ± 0.01 f	1.78 ± 0.00 f

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

Fig 5.19 Effect on strain hardening index of Jagger and Soft wheat flour doughs on addition of gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

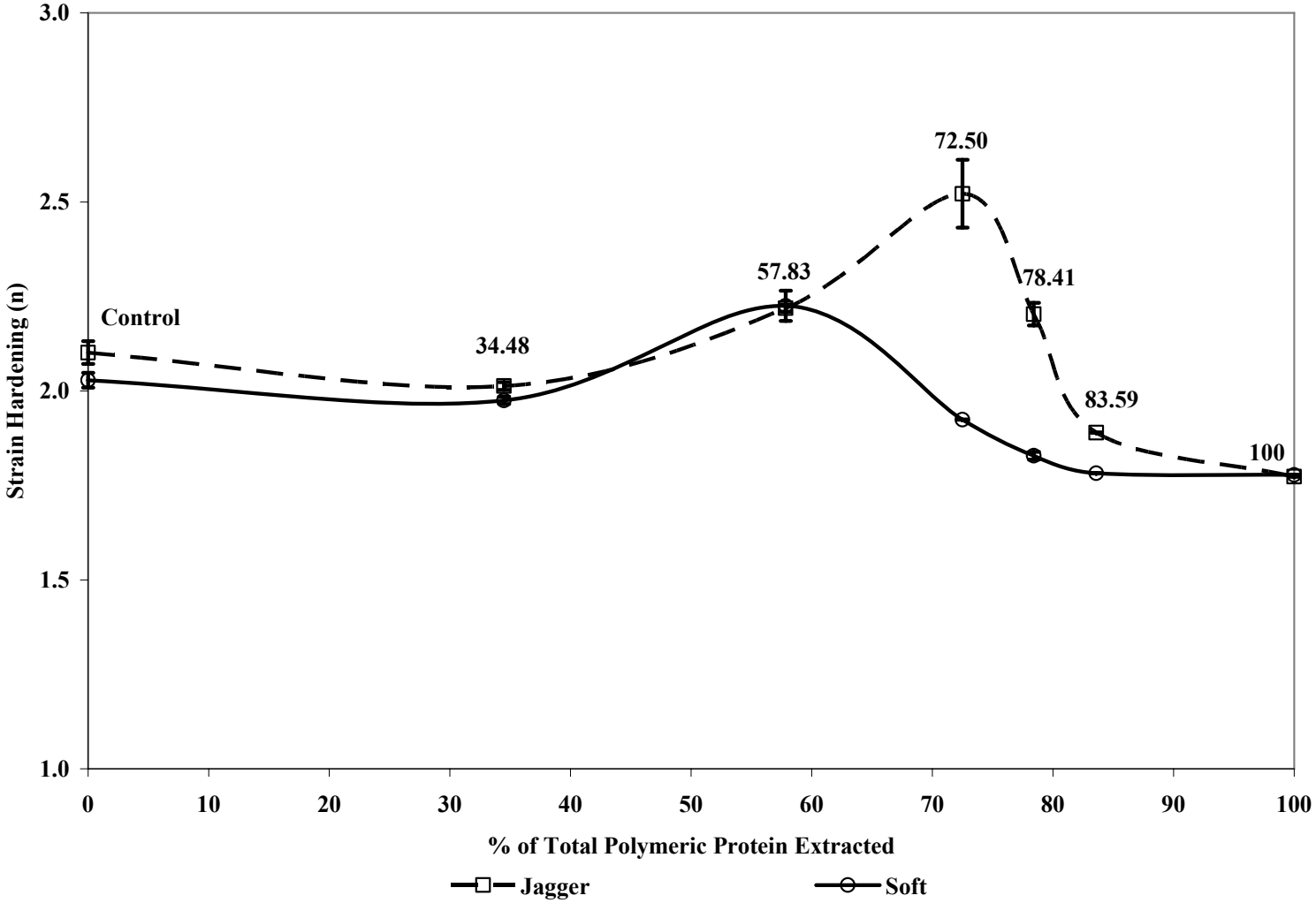


Fig 5.20 Effect on failure strain of Jagger and Soft wheat flour doughs on addition of gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

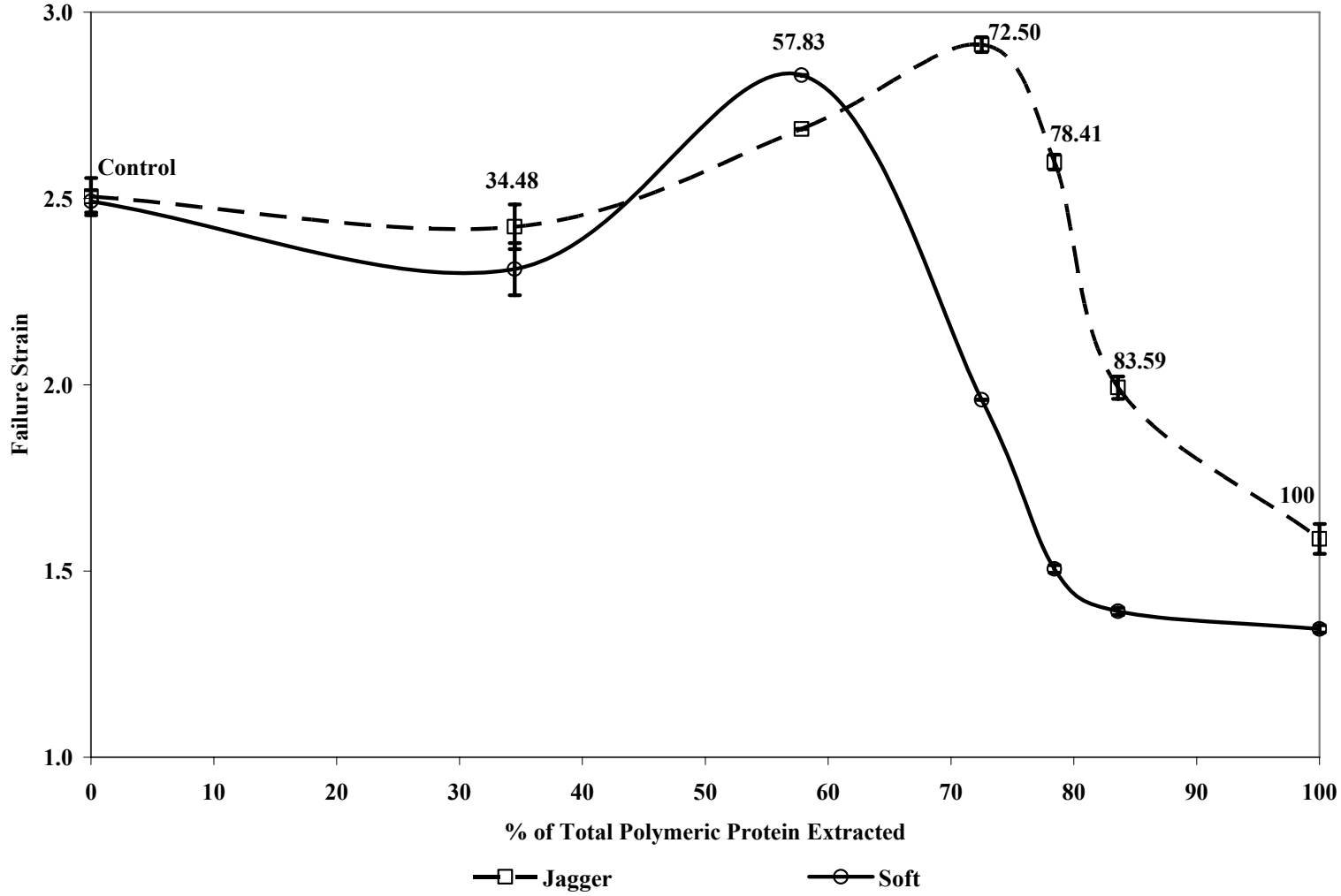


Fig 5.21 Stress vs. strain (Hencky) curves of Jagger flour doughs on addition of gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

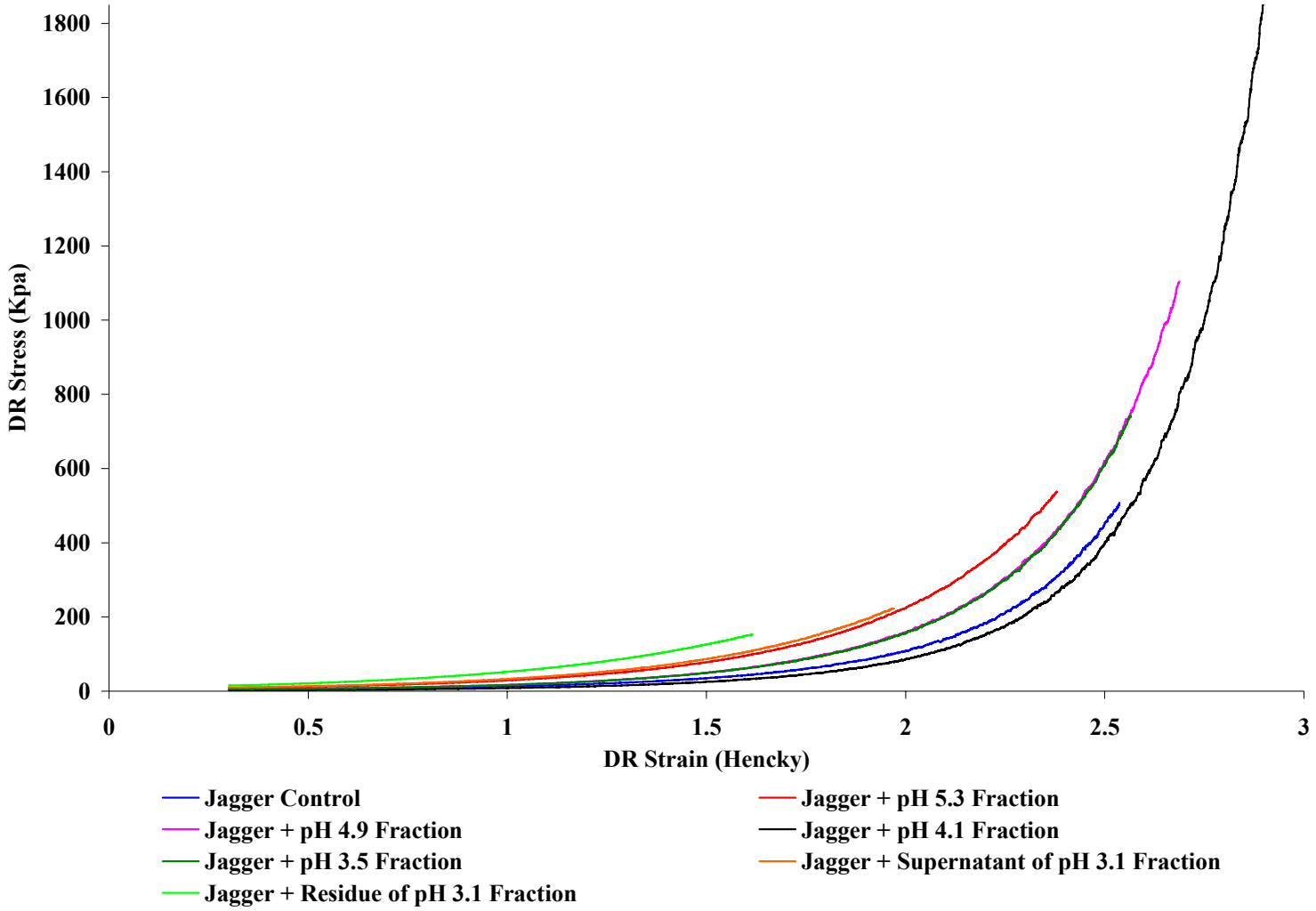


Fig 5.22 Stress vs. strain (Hencky) curves of Soft flour doughs on addition of gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

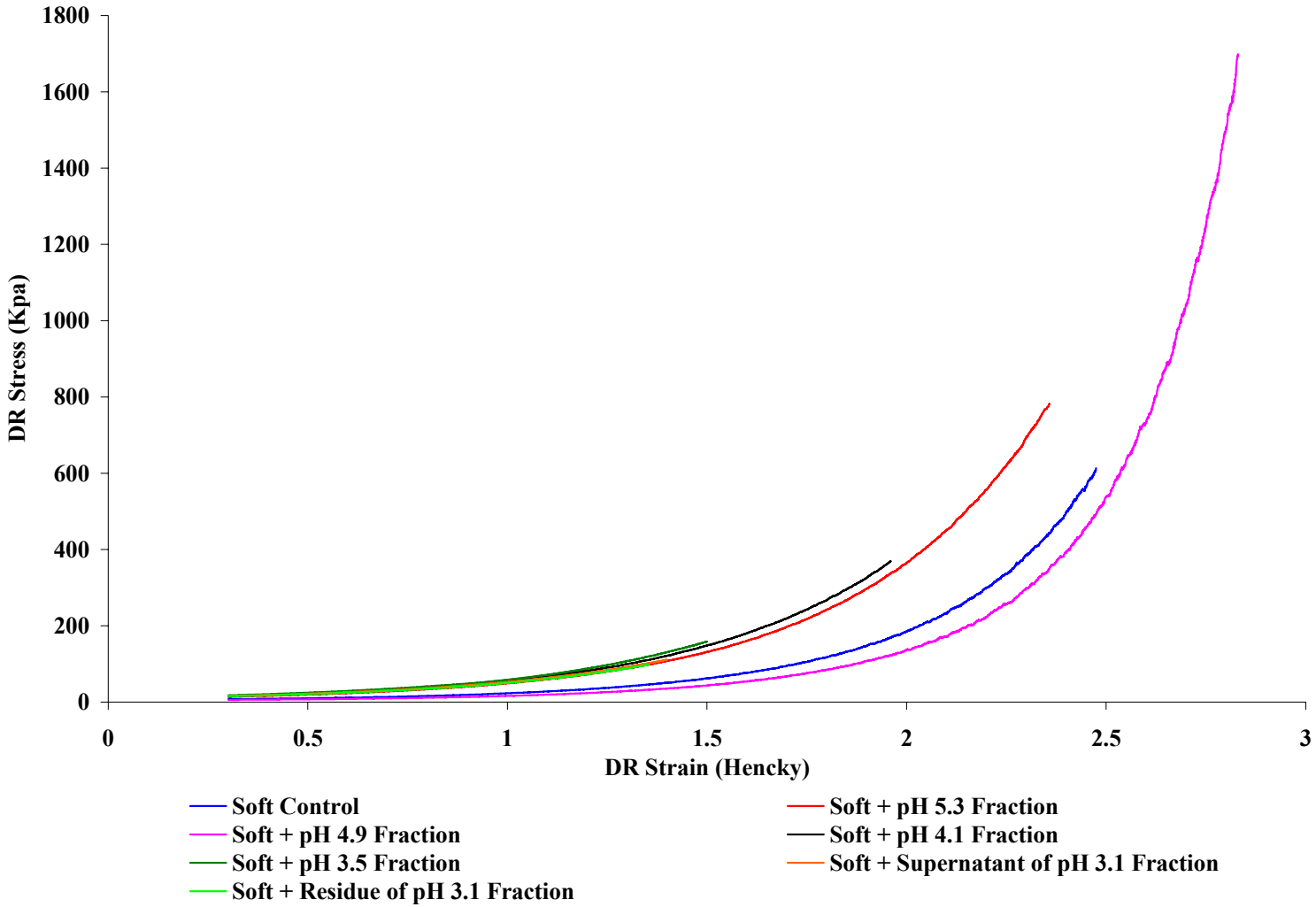


Table 5.14 Mean bubble inflation rheological responses at different strain rates of Jagger wheat flour doughs with addition of gluten protein fractions, added at 1% (dry protein) level (on flour weight basis). [A, B]

Fraction Added	Strain Hardening Index		Failure Strain (Hencky)	
	Strain Rate 0.1%	Strain Rate 0.01%	Strain Rate 0.1%	Strain Rate 0.01%
None (Control)	2.12 ± 0.03 b	2.06 ± 0.01 b	2.59 ± 0.06 b	2.50 ± 0.06 b
pH 5.3	2.28 ± 0.02 a	2.08 ± 0.01 a,b	2.83 ± 0.00 a	2.76 ± 0.04 a
pH 4.9	2.05 ± 0.02 c	2.11 ± 0.00 a	2.59 ± 0.12 b	2.57 ± 0.07 b
pH 4.1	2.01 ± 0.00 c,d	2.07 ± 0.02 a,b	2.48 ± 0.03 b	2.34 ± 0.01 c
pH 3.5	1.91 ± 0.03 e	—	2.16 ± 0.10 c	—
pH 3.1 Supernatant	1.97 ± 0.04 d	—	2.23 ± 0.01 c	—
pH 3.1 Residue	1.81 ± 0.02 f	1.93 ± 0.01 c	1.76 ± 0.05 d	1.62 ± 0.04 d

[A] Values represent mean ± standard deviation for duplicate determinations

[B] Means with the same letter within columns are not significantly different ($p > 0.05$)

hardening index paralleled those of loaf volume and image analysis parameters, for both the flours. (Fig 5.19, Fig 5.20). These analogous variations in strain hardening index and loaf volume show that the biaxial extensional rheological procedure is a good indicator of baking performance as affected by rheology of the gluten-starch matrix.

At a molecular level, these changes can be explained by the linear polymer entanglement model (Fig 2.10) illustrated in section 2.4.2 of chapter 2. On addition of the first 35% of extracted polymeric proteins (pH 5.3 fraction), decrease in strain hardening and loaf volume is probably due to a shift from condition II to condition I in the model (Fig 2.10), as the percentage of polymeric proteins ≥ 250000 decreases much lower than the desired 60%. Subsequent augmentation of these parameters might be due to a shift back to condition II, as the percentage of polymeric proteins ≥ 250000 approaches the desired balance. Addition of the latest fractions i.e. after the optimum balance between strength and extensibility was achieved, possibly shifted the balance towards strength, causing strain hardening and loaf volume to decrease, as explained by condition III of the model (Fig 2.10). In other words, progressive addition of fractions (from pH 5.3 to pH 3.1 residue) to the base flours caused a shift in balance from extensibility to optimum (60:40), and, then skewed it to strength. This also explains why soft wheat flour has poor bread making performance despite higher UPP content than Jagger.

Another experiment was conducted with Jagger as base flour, to see the effect of strain rate variations on biaxial extensional rheological parameters (failure strain and strain hardening index). Since the doughs for this experiment (Table 5.14) were mixed in a 100 g mixer, they are expected to be rheologically different from the 35 g mixings (Table 5.12) and could not be compared with baking performance (Table 5.10, Table 5.11). In this test, it was observed that on decreasing strain rate, the maximum in terms of strain hardening index and failure strain was shifted towards stronger doughs or those with the higher percentage of polymeric fraction ≥ MW 250,000 / M_T. Similar results have been reported by Termonia et al (1988) for synthetic polymers.

It seems that as the strain rate is decreased, the stress on polymer chains increases slowly causing non-covalent interactions to break first and then reaches the activation energy level required for slippage of chains through entanglements. Lower strain rate assures that this process continues for a sufficient period in order to get the maximum number of statistical segments between entanglements to slip through an entanglement node. This leads to increased expansion

of the gluten-starch matrix. Increase in strain rate will reduce the time period for the elongation process as energy levels will probably approach those required to break covalent bonds therefore causing disruption of the entangled network and rupture of the gluten-starch matrix. Increase in strain rate, keeping other parameters constant, will result in reduced failure strain and strain hardening index. A linear positive relation between strain hardening index and critical strain at which failure occurs has been reported by Dobraszczyk and Roberts (1994).

4.4 Conclusion

Stability of the gluten-starch matrix, which is the primary protection for expanding gas cells against disproportionation and coalescence, depends on its tendency to strain harden. Doughs with higher strain hardening index, as determined by the procedure developed by Dobraszczyk (1997), are sufficiently extensible to respond to gas pressure but also have sufficient strength to resist collapse. The phenomenon of strain hardening appears to depend on the balance between strength and extensibility of the entangled network of polymeric proteins of wheat flour. At a molecular level, the linear polymer entanglement model illustrated in section 2.4.2 of chapter 2 could explain the phenomenon of strain hardening. Extensibility ensures slippage of the maximum number of statistical segments between entanglements, whereas strength prevents disruption of the entangled network of polymeric proteins.

Strength is conferred by the fraction of polymeric proteins having molecular weight greater or equivalent to M_T (250,000), and the fraction of gluten protein smaller than M_T will confer extensibility. The optimum balance seems to exist when the relative proportions of polymeric proteins greater and smaller than M_T are 60:40. Shift in the balance to either side will decrease the stability of the gluten-starch matrix and thus that of the gas cells. Changes in monomeric to polymeric ratio will change the diluent concentration, affecting the balance. The rate at which the entangled network of gluten proteins (gluten-starch matrix) is stretched (i.e. strain rate) will also affect its tendency to strain harden.

5.4 References

AACC. 2001. Approved Methods of the AACC, Tenth Edition. American Association of Cereal Chemists, St. Paul, Minnesota, USA.

Bangur, R., Batey, I.L., McKenzie, E., MacRitchie, F. 1997. Dependence of extensograph parameters on wheat protein composition measured by SE-HPLC. Journal of Cereal Science 25, 237-241.

Batey, I.L., Gupta, R.B., MacRitchie, F. 1991. Use of size-exclusion high-performance liquid chromatography in the study of wheat flour proteins: an improved chromatographic procedure. Cereal Chemistry 68, 207-209.

Bean, S.R., Lookhart, G.L. 2001. Factors influencing the characterization of gluten proteins by size-exclusion chromatography and multiangle laser light scattering (SEC-MALLS). Cereal Chemistry 78, 608-618.

Bersted, B.H., Anderson, T.G. 1990. Influence of molecular weight and molecular weight distribution on the tensile properties of amorphous polymers. Journal of Applied Polymer Science 39, 499-514.

Dobraszczyk, B.J. 1997. Development of a new dough inflation system to evaluate doughs. Cereal Foods World 42, 516-519.

Dobraszczyk, B.J., Roberts, C.A. 1994. Strain hardening and dough gas cell-wall failure in biaxial extension. Journal of Cereal Science 20, 265-274.

Gandikota, S., MacRitchie, F. 2005. Expansion capacity of doughs: methodology and applications. Journal of Cereal Science 42, 157-163.

Gupta, R.B., Bekes, F., Gras, P.W., MacRitchie, F. 1990. Functionality of glutenin, gliadin and secalin fractions as measured by extensograph and mixograph. In: Gluten proteins. Association of Cereal Research, Detmold. pp 550-559.

Gupta, R. B., Khan, K., MacRitchie, M. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quality and size distribution of polymeric protein. Journal of Cereal Science 18, 23-41.

Kilborn, R.H., Tipples, K.H. 1972. Factors affecting mechanical dough development. I. Effect of mixing intensity and work input. Cereal Chemistry 49, 34-47.

Lemelin, E., Branlard, G., Salvo, L., Lein, V., Aussenac, T., Dayde, J. 2005. Breadmaking stability of wheat flours: relation between mixing properties and molecular weight distribution of polymeric glutenins. Journal of Cereal Science 42, 317-326.

Lundh, G., MacRitchie, F. 1989. Size exclusion HPLC characterization of gluten protein fractions varying in bradmaking potential. Journal of Cereal Science 10, 247-253.

MacRitchie, F. 1978. Differences in baking quality between wheat flours. Journal of Food Technology 13, 187-194.

MacRitchie, F. 1979. A relation between gluten protein amide content and baking performance of wheat flours. Journal of Food Technology 14, 595-601.

MacRitchie, F. 1985. Studies of the methodology for fractionation and reconstitution of wheat flours. Journal of Cereal Science 3, 221-230.

MacRitchie, F. 1986. Physicochemical processes in mixing. In: Blanshard, J.M.V., Frazier, P.J., Galliard, T. (Eds.), Chemistry and Physic of Baking, Royal Society of Chemistry, London, UK, pp 132-146.

MacRitchie, F. 1987. Evaluation of contributions from wheat protein fractions to dough mixing and breadmaking. Journal of Cereal Science 6, 259-268.

MacRitchie, F. 1998. Protein composition and physical properties of wheat flour doughs. In: Gueguen, J., Popineau, Y. (Eds.), Plant Proteins from European Crops – Food and Non-Food Applications, Springer-Verlag, Heidelberg, Germany, pp 113-119.

MacRitchie, F., Gras, P.W. 1973. The role of flour lipids in baking. Cereal Chemistry 50, 292-302.

MacRitchie, F., Kasarda, D.D., Kuzmicky, D.D. 1991. Characterization of wheat protein fractions differing in contributions to breadmaking quality. Cereal Chemistry 68, 122-130.

Singh, N.K., Donovan, G.R., Batey, I.L., MacRitchie, F. 1990. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins.

I. Dissolution of total proteins in the absence of reducing agents. Cereal Chemistry 67, 150-161.

Singh, H., MacRitchie, F. 2001. Application of polymer science to properties of gluten. Journal of Cereal Science 33, 231-243.

Termonia, Y., Allen, S.R., Smith, P. 1988. Kinetic model for tensile deformation of polymers. 3. Effects of deformation rate and temperature. Macromolecules 21, 3485-3489.

Termonia, Y., Smith, P. 1988. Kinetic model for tensile deformation of polymers. 2. effect of entanglement spacing. Macromolecules 21, 2184-2189.

Tipples, K.H., Kilborn, R.H. 1975. Unmixing – the disorientation of developed bread doughs by slow speed mixing. Cereal Chemistry 52, 248-262.

van Vliet, T., Janssen, A.M., Bloksma, A.H., Walstra, P. 1992. Strain hardening of dough as a requirement for gas retention. Journal of Texture Studies 23, 439-460.

CHAPTER 6 - Conclusions and Further Studies

6.1 Conclusion

The first step of breadmaking (i.e. mixing) occludes air in the form of small gas cells into the liquid phase of dough. The liquid phase of dough becomes continuous (MacRitchie, 1976a) as dough approaches peak development and, at this stage, the rate of air occlusion is a maximum (Baker and Mize, 1946). A liquid film in a network of a more viscous gluten-starch matrix thus surrounds these gas cells. Surface properties of the gas-liquid interface, due to adsorption of surface active components (proteins and lipids) of the liquid phase of dough, will determine the ease with which air is occluded i.e. the initial stability of gas cells. During the entire breadmaking process, a dual film (Fig 2.5) (Gan et al, 1990 and 1995) i.e. a primary-gluten starch matrix with secondary liquid lamellae on their inner side enveloping them stabilizes these gas cells. Rheology of the gluten-starch matrix and surface properties of the gas-liquid interface play important roles in maintaining the stability and cell size distribution of the gas cells. Rheology of the gluten-starch matrix is the primary factor determining their stability.

As gas cells expand due to release of carbon dioxide during proofing and thermal expansion of gases during baking, the gluten-starch matrix around them stretches and becomes thin. During advanced stages of proofing or initial stages of baking (oven rise) when cells are no longer spherical, discontinuities may begin to appear in the gluten-starch matrix. However, stable liquid lamellae assure their further expansion, thus avoiding failure. Stability of liquid lamellae depends on the ability of surface active compounds (proteins and lipids) present at the gas-liquid interface to form monolayers. Surface active compounds like lipids, depending on their structure i.e. degree of saturation of the hydrocarbon chain, hydrocarbon chain length and size of the polar head groups, will either form condensed or expanded monolayers (Fig 2.11) (MacRitchie, 1990). Condensed monolayers are characterized by close packing of surface active molecules due to their saturated hydrocarbon chains. These monolayers have low compressibility (Fig. 2.11) because of close molecular packing that also leads to greater attraction between hydrocarbon chains, making the monolayer more stable and not easily desorbed. The surface viscosity of such monolayers is high and with any change in interfacial area, large elastic restoring forces are

generated preventing coalescence and disproportionation (MacRitchie, 1976b). On the other hand, unsaturated hydrocarbons or other foam destabilizers will form expanded monolayers (Fig. 2.11). Molecular packing of expanded monolayers is loose, leading to greater compressibility. These are not so elastic in response to changes in the interfacial area and may be desorbed in the process. Monolayer desorption at the point of contact of two gas cells will lead to coalescence.

Surface active compounds of wheat flour such as phospholipids, DGDG and saturated free fatty acids (stearic acid, palmitic acid) are known to give condensed monolayers. Unsaturated free fatty acids like linoleic acid give expanded monolayers. Any modification in the structure of a hydrocarbon chain such as inclusion of a double bond or decrease in the chain length will lead to transformation of condensed monolayers to expanded ones. With presence of a variety of surface active compounds in the liquid phase of the dough, it is quite possible that some of these molecules may posses a certain degree of mutual solubility. The monolayers of such surface active compounds will be of intermediate type, depending whether individual compounds give expanded or condensed monolayers. The use of surface active components capable of forming condensed monolayers as bakery additives can help improve bread loaf volume.

On the other hand, dough (gluten-starch matrix) surrounding the gas cells must have sufficient extensibility to respond to the gas pressure but also sufficient strength to resist collapse. Strain hardening has been shown to be a necessary rheological property for obtaining good bread volume. Doughs with better bread making potential exhibit a higher strain hardening index. The phenomenon of strain hardening appears to depend on the balance between strength and extensibility of an entangled network of polymeric proteins of wheat flour. At a molecular level, a linear polymer entanglement model illustrated in section 2.4.2 of chapter 2 could explain the phenomenon of strain hardening. Extensibility ensures slippage of a maximum number of statistical segments between entanglements, whereas strength prevents disruption of an entangled network of polymeric proteins. M_T is a threshold MW for stable entanglements, polymers with MW greater than M_T confer strength, and M_T is estimated to be 250,000 for wheat proteins (Bangur et al, 1997). Extensibility, on the other hand, is increased by the fraction smaller than M_T. The right balance seems to exist when relative proportions of polymeric proteins greater and smaller than M_T are 60:40. Shift in balance to either side will decrease the stability of the gluten-starch matrix and thus that of gas cells. Changes in monomeric to polymeric ratio will change the

diluent concentration, affecting the balance. The rate at which the entangled network of gluten proteins (gluten-starch matrix) is stretched will also affect its tendency to strain harden.

6.2 Significance

The study provides explanation of the mechanism of gas cell stability at a basic level and therefore assumes great significance in bakery industry and academic research. One of the biggest problems for processors of bakery products is the variation in consistency of wheat flour consignments and other raw materials, which lead to inconsistent quality of finished product. These variations appear to be mainly environmentally governed. Understanding of the mechanism of gas cell stability will help overcome these inconsistencies in the quality of finished product and help avoid disruption to plant schedules, due to necessitated adjustments to processing that can be costly in terms of money and time.

Unique quality of wheat to give visco-elastic dough has always been of interest to cereal chemists. It is due to this visco-elasticity that it retains gas cells providing consumer attractive aerated products such as bread. The study provides understanding on requirements for non-gluten aerated products. This will assist in development of new bakery products such as health breads (high fiber, gluten free, etc.), which has a big market in U.S.A and abroad. The study can be suitably applied to promote utilization of underutilized cereals (such as sorghum), this may assist in achieving desired agricultural diversification in rainfed regions of Kansas and other places around the globe. In the long run this may help develop arid agrarian African economies where drought resistant crops like sorghum are only choice.

The study can be suitably employed by breeders to develop strategies that will help focus on specific genetic manipulation that will assist in developing wheat varieties capable of producing ideal breads with stable cell structure. The things that the breeder needs to keep in mind are: optimum ratio of strength and extensibility conferring factors of gluten proteins, and presence of more polar lipids and saturated free fatty acids. Lipids are vital for various biochemical reactions and therefore it would be a challenge to induce suitable mutation of their composition and content for best breadmaking performance without affecting plant growth.

6.3 Further Studies

1. Effects of lipids and other surface active components like proteins resemble those of results from film balance studies. However due to the presence of numerous surface active compounds in the liquid dough phase, many of which possess a certain degree of mutual solubility, monolayers formed are of intermediate type. The presence of small amounts of more surface active impurities can mask the surface action of a major compound, as seen in the case of myristic acid. Studies need to be carried out by preparing 'surface chemically pure' surfactant solutions to examine the exact way in which they will affect lamella stability during the entire baking process. Study by replacing the liquid phase of dough, extracted by centrifugation, with solution containing 'surface chemically pure' surfactant will help elucidate the exact mechanism of individual components.
2. Study of dough mixing properties of flours reconstituted with gluten fractions revealed that with increase in molecular weight i.e. a shift of molecular weight distribution to higher molecular weight and decrease in proportion of the monomeric fraction, dough strength increased significantly. Mixographs of such doughs resembled those of under mixed doughs. Further studies can be conducted to see the effect of mixing intensity variation on rheology of doughs i.e. their ability to stabilize gas cells.
3. The study showed effect of liquid lamellae stability and that of gluten-starch matrix on final crumb structure. However, it will be more informative if real time changes in gas cell concentration are tracked using X-ray tomographic procedures.

6.4 References

Baker, J.C., Mize, M.D. 1946. Gas occlusion during dough mixing. Cereal Chemistry 23, 39-51.

Bangur, R., Batey, I.L., McKenzie, E., MacRitchie, F. 1997. Dependence of extensograph parameters on wheat protein composition measured by SE-HPLC. Journal of Cereal Science 25, 237-241.

Gan, Z., Angold, R.E., Williams, M.R., Ellis, P.R., Vaughan, J.G., Galliard, T. 1990. The microstructure of gas retention of bread dough. Journal of Cereal Science 12, 15-24.

Gan, Z., Ellis, P.R., Schofield, J.D. 1995. Gas cell stabilization and gas retention in wheat bread dough. Journal of Cereal Science 21, 215-230.

MacRitchie, F. 1976a. The liquid phase of dough and its role in baking. Cereal Chemistry 53, 318-326.

MacRitchie, F. 1976b. Monolayer compression barrier in emulsion and foam stability. Journal of Colloid and Interface Science 56, 53-56.

MacRitchie, F. 1990. Chemistry at Interfaces. Academic Press, Inc., San Diego, CA, USA.