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Mechanism of gas cell stabilization in bread making. I. The primary gluten–starch matrix☆

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ABSTRACT

Expansion of dough and hence bread making 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). Thin liquid lamellae (films), stabilized by adsorbed surface active compounds, act as an auxiliary to the primary gluten–starch matrix in stabilizing expanding gas cells and this mechanism operates when discontinuities begin to appear in the gluten–starch matrix during later proving and early baking stages. Contributions of the liquid lamellae stability to dough expansion were assessed using flours varying in their lipid content. Incremental addition of natural lipids back into defatted flour caused bread volume to decrease, and, after reaching a minimum, to increase. Strain hardening is a key rheological property responsible for stabilizing the primary gluten–starch matrix. Jagger gave higher test-bake loaf volume than Soft and higher strain hardening index for dough. The different lipid treatments were found to have negligible effects on strain hardening index. Image analysis of crumb grain revealed that differences in number of gas cells and average cell elongation with different lipid treatments were insignificant. The evidence agrees with a dual mechanism to stabilize the gas cells in bread dough. To understand dough rheology at a molecular level, rheological properties of doughs were varied by addition of flour 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 fractionation decreased. Mixograph dough 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 is thought 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 molecular weight higher than the optimum results in a stronger network with reduced slippage through entanglement nodes, whereas a shift to lower molecular weights will decrease the strength of the network due to a lesser number of entanglements per chain.

1. Introduction

During mixing, gas is occluded and concentrated in the liquid phase of dough in the form of small nuclei. 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. Gas nuclei expand during fermentation due to release of fermentation gases, and during baking due to expansion of these gases as temperature increases.

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, 1990; Hoseney, 1992). However, scanning electron micrographs of dough at the end of the proving stage appear to show the existence of intact gas cells even when discontinuities in the gluten–starch matrix begin to appear (Gan et al., 1990). Based on these electron micrographs of

Abbreviations: MALLS, multiangle laser light scattering; MDDT, mixograph dough development time; M_T, threshold molecular weight; MW, molecular weight; M_W, weight average molecular weight; MWD, molecular weight distribution; R_{max}, extensograph maximum resistance to extension; SDS, sodium dodecyl sulfate; SE-HPLC, size-exclusion high-performance liquid chromatography; UPP, unextractable polymeric protein.

☆ Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others not mentioned.

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the dough, Gan and co-workers (Gan et al., 1990, 1995) proposed that the expanding gas cells appear to be stabilized against coalescence and disproportionation by a primary gluten–starch matrix with a secondary liquid lamella on its inner side, enveloping the gas cell. The dual film hypothesis seems plausible in view of evidence from previous studies, which show presence of a continuous liquid phase in dough (MacRitchie, 1976a) and the effect of surface active components on bread loaf volume (DeStefanis and Ponte, 1976; MacRitchie, 1976a,b; MacRitchie and Gras, 1973). In such cases, where there is an independent mechanism of gas cell stabilization apart from the gluten–starch matrix, the dough rheology should not be affected by variations in natural lipid levels. MacRitchie and Gras (1973) reported that Alveograms are not affected by variations in the natural lipid fraction of the flour. A Stable Micro Systems dough inflation system mounted on a texture analyzer (TAXT 2plus), used in this study to understand biaxial extensional rheology of wheat flour dough, works on a principle similar to the Alveograph, with the advantage of inflating bubbles at constant strain rates. The amounts of lipid in the study by MacRitchie and Gras (1973) were as low as 1–1.5% and had significant effects on baking performance. It is probably due to their surface activity that these compounds (lipids and proteins) are adsorbed at the gas–liquid interface of the liquid lamellae and affect stability of the gas cells (Mills et al., 2003; Paternotte et al., 1993; Ross and MacRitchie, 1995). Analogous variations in foaming properties of the dough liquor, and bread loaf volume and crumb structure (MacRitchie, 1976a) provide reasonable evidence for their action at the interface.

Loaf volume can be defined as the extent of dough expansion (Gandikota and MacRitchie, 2005), which depends upon how thin the gluten–starch matrix can be stretched before reaching its expansion limit or point of rupture. Rheology of the gluten–starch matrix is important in the bread making process as this determines extensibility and strength. The proving and baking stages of bread making are characterized by fast biaxial expansion of gas cells, expanding at strain rates of 0.001–0.0001/s and 0.01–0.001/s, respectively (Dobraszczyk, 1997). During expansion, 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 proving, 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 a 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 (Dobraszczyk and Roberts, 1994; van Vliet et al., 1992), and is a necessary rheological property for obtaining good bread volume.

A power law relation between stress and Hencky strain, as described by the equation below, was used by Dobraszczyk and Roberts (1994) to determine strain hardening.

$$\sigma = K\varepsilon^{n}$$

where, σ is the true stress, K is a power law constant, ε is strain and n is a strain hardening index. The index n must be greater than 1 in order to have a curved relation between stress and Hencky strain (Fig. 1). Doughs with a strain hardening index of 1 or higher have the potential to give good loaf volume by allowing gas cells to expand without undergoing disproportionation and coalescence (Dobraszczyk and Roberts, 1994). In later studies, Dobraszczyk and co-workers (Dobraszczyk and Salmanowicz, 2008; Dobraszczyk et al., 2003) found that an exponential relationship shows better fit to data, particularly in the case of doughs for which bubbles inflate

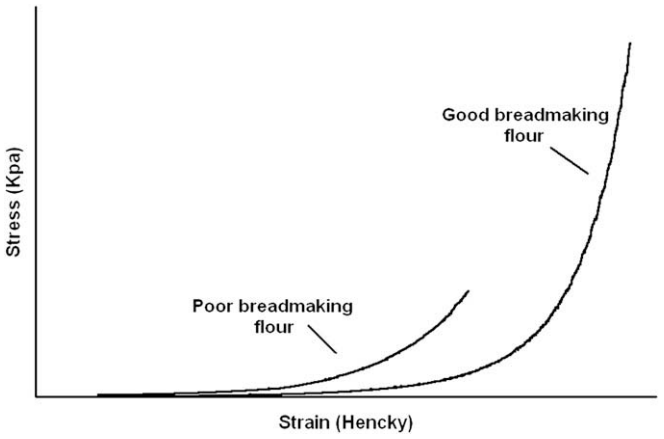

Fig. 1. Stress vs. strain (Hencky) curves of Soft and Jagger wheat flours showing differences in their tendencies to strain harden. The Soft wheat flour with poor bread making potential fails at relatively lower strains.

to strains greater than 2. Chin and Campbell (2005) also used an exponential relationship to study dough rheology by bubble inflation. Strain hardening index is a reliable criterion for differentiating flours based on bread making potential (Dobraszczyk and Roberts, 1994; Dobraszczyk and Salmanowicz, 2008; Dobraszczyk et al., 2003; Tronsmo et al., 2003;). This is illustrated in Fig. 1 for the two flours used in this study.

At a molecular level, strain hardening can be explained by a well established polymer entanglement network theory (MacRitchie and Lafiandra, 1997; Singh and MacRitchie, 2001). Strain hardening is believed to originate from entanglement coupling of large glutenin molecules (Singh and MacRitchie, 2001), with molecular weight (MW) greater than a threshold MW (M_T), M_T being the minimum molecular weight at which stable entanglements are formed (Bersted and Anderson, 1990). Various studies substantiate this theory. Dough strength in terms of R_{max} (extensograph maximum resistance to extension) shows positive correlation of the glutenin fraction with MW greater than M_T, which is estimated to be approximately 250,000 Da (Bangur et al., 1997). 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 an increase in strength (Gupta et al., 1990). A similar phenomenon is observed in wheat flour doughs, where the strain hardening index (n) seems to have a positive curvilinear relation with failure strain. As n approaches higher limits, the failure strain seems to become constant (Dobraszczyk and Roberts, 1994; Dobraszczyk et al., 2003). 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 (Lundh and MacRitchie, 1989; MacRitchie, 1987; MacRitchie et al., 1991). The optimum here possibly indicates a balance between strength and extensibility, beyond which high entanglement network density, resulting in decrease in failure strain, might cause lower loaf volumes (Singh and MacRitchie, 2001; Termonia and Smith, 1988).

The objectives of this study were: firstly, 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 is plausible; secondly, to understand the rheology of the gluten–starch matrix required to stabilize the expanding gas cells and to understand the underlying molecular structure–function relationship as explained by polymer entanglement network theory (MacRitchie and Lafiandra, 1997; Singh and MacRitchie, 2001).

2. Experimental

2.1. Materials

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 a Buhler mill (73% milling extraction rate) and were evaluated for certain quality characteristics which are shown in Table 1. These flours were stored at −20 °C until use. For the sake of brevity, Jagger wheat flour and soft wheat flour have been referred to as Jagger and Soft, respectively. Reagents were purchased from Sigma–Aldrich, USA. Chloroform was of HPLC grade, whereas all other chemicals used were of ACS grade. Distilled deionized water, sterilized in an autoclave, was used in all stages of the experiments.

2.2. Analytical procedures

Moisture content was determined as per AACC method 44-15 A (AACC, 2001). Protein content was determined by the nitrogen combustion method using a 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 the procedure of MacRitchie and Gras (1973).

2.3. Dough mixing properties

Mixing properties were evaluated using a 10 g 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 (AACC, 2001) except that sodium chloride (1.5% w/w on a flour weight basis) was added.

2.4. Preparation of gluten proteins

Gluten protein was prepared from defatted Jagger flour following the procedure used by MacRitchie (1985). The wet gluten mass was freeze-dried and ground to a particle size less than 150 microns. Powdered gluten was stored at a temperature of −20 °C until its use.

2.5. pH fractionation of gluten protein

Powdered gluten prepared from Jagger was subjected to pH fractionation, using the procedure of Gupta et al. (1990). Gluten was stirred in water at pH 5.3 (35 g/1000 ml) for 10 min and centrifuged at 2300 × *g* for 10 min. The residue, after collection of supernatant, was subjected to two more extractions at the same pH. The total combined supernatant was named as pH 5.3 gluten fraction. The residue was subjected to further fractionation in a similar manner by lowering of pH to 4.9 by addition of HCl. The gluten protein was thus 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, obtaining two fractions at pH 3.1 i.e. supernatant of pH 3.1 and residue of pH 3.1. All fractions were then freeze-dried. Freeze-dried fractions were ground to particle sizes less than 150 microns. The fractions were stored in polyethylene bags in containers with desiccant at a temperature of −20 °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).

Table 1
Physico-chemical analysis and SE-HPLC relative composition of polymeric proteins of Jagger and Soft wheat flours

	Jagger wheat flour	Soft wheat flour
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
Mixograph absorption (%)	63	60
Area (%) under chromatogram curve		
TPP	36.4 ± 0.01	37.0 ± 0.05
EPP	43.6 ± 0.02	39.0 ± 0.11
UPP	56.4 ± 2.77	61.1 ± 3.40

TPP—total polymeric protein; EPP—extractable polymeric protein; UPP—unextractable polymeric protein.

2.6. 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 a total flour weight basis.

2.7. 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). The basic procedure for SE-HPLC is described elsewhere (Batey et al., 1991). Proteins were fractionated with a Biosep SEC-4000 column (Phenomenex, Torrance, CA) using; 50 mM disodium orthophosphate 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. 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 issues in MALLS analysis due to the 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 the d*n*/d*c* value, as per procedure by Bean and Lookhart (2001).

2.8. Sample preparation for SE-HPLC

The basic procedure for SE-HPLC is based on the method of (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 the quantity for Jagger being 10.0 mg ± 0.1 mg. Weighed samples were suspended in 50 mM 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 W for 15 s. No sonication step was included 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 12,000 × *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 °C 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 25 s at an output of 6 W.

2.9. Lipid extraction from flour

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 h to allow evaporation of solvent.

2.10. Addition of lipids to defatted flour

For incorporation of intact natural 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.

2.11. 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 (30 ppm), water and mix time (as optimized from Mixograph analysis). Loaf volumes were measured by rapeseed displacement after cooling for 20 min.

2.12. Image analysis

Image analysis of crumb grain of baked loaves was carried out 12 h 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. Image analysis was performed on central slices of 15 mm thickness, 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.

2.13. 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. Sample dough pieces (in pots) were then proved at 35 °C 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 500 cm^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.

2.14. 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. Results and discussion

This section is further divided in two sub-sections in accordance with objectives of the study.

3.1. General mechanism of gas cell stability (investigating dual film hypothesis)

3.1.1. 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 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). Higher percentage of unextractable polymeric protein (UPP) (Table 1) in Soft 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 wheat flour was milled from a 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.

3.1.2. Effect of natural flour lipid level variation on baking performance

3.1.2.1. Bread making. Significant differences ($P < 0.0001$) in loaf volumes of the two flours were observed in response to varying levels of flour lipids (Fig. 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 compared to Jagger. For Soft, minimum volume is reached at a relatively lower percentage of natural flour lipids (~30%) in comparison to Jagger (~50%) (Fig. 2).

The results of loaf volume variations in response to natural flour lipid level variations agreed qualitatively with those of MacRitchie and co-workers (MacRitchie, 1976a; MacRitchie and Gras, 1973; McCormack et al., 1991). Loaf volume seems to be governed by expansion capacity of the gas cells. The expansion capacity of the gas cells can be defined as the extent to which gas cells can expand

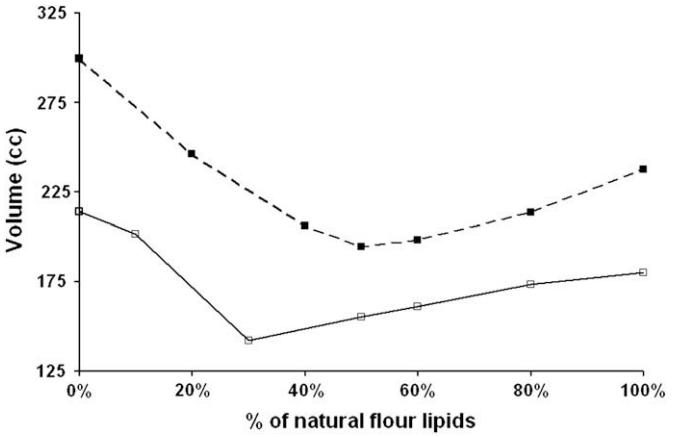

Fig. 2. Loaf volume vs. intact natural flour lipids, added to defatted Jagger (- - -) and Soft (—) wheat flours as percentage of natural flour lipids.

without failure; i.e. the value reached after which the loaf does not increase in volume. This maximum for doughs giving lower loaf volumes is achieved during the late proving stage, and for those giving higher loaf volumes is achieved during the early baking stage. This was determined by measuring oven spring (data not shown). Oven spring was observed only in doughs giving higher loaf volume.

Scanning electron micrographs by Gan et al. (1990) appeared to show the presence of intact gas cells with discontinuities in gluten–starch matrix at advanced stages of proving. 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. When liquid lamellae also fail, the presence of discontinuities in the gluten–starch matrix leads to coalescence of gas cells, thus decreasing volume. It is quite possible that lipids, due to their surface action, will be affecting stability of liquid lamellae, thus causing variation in loaf volume. However, this needs to be investigated further in order to make sure that the action of flour lipids is independent of the rheological properties of the gluten–starch matrix.

Relatively lower loaf volumes at all levels of lipid addition in Soft, could be attributed to inherent differences in gluten quality of the two flours, as previously suggested by MacRitchie (1978). The differences in the two flours are clear from SEC-MALLS data (Table 5). A new desirable relative proportion of polymeric proteins greater than 250,000 Da in Jagger is probably responsible for better loaf volumes as explained in Section 3.2.2.3.

3.1.2.2. Crumb structure. Image analysis of bread crumb (Table 2) showed that addition of different levels of flour lipids resulted in insignificant variations in number of gas cells ($P = 0.02$) and average cell elongation ($P = 0.94$ for Jagger and $P = 0.12$ for Soft). These non-significant differences in number of gas cells further make it evident that differences in loaf volumes are due to differences in expansion capacity of the gas cells (Table 2), and factors like gas cell concentration are not involved. 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).

3.1.3. Effect of natural flour lipid level variation on biaxial extensional rheology of gluten–starch matrix

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

Table 2
Crumb structure responses of Jagger and Soft wheat flour breads to natural flour lipid levels

% of natural flour lipids	Jagger wheat flour		Soft wheat 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

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

Biaxial extensional rheological parameters (maximum stress, failure strain and strain hardening index) were higher for Jagger doughs (Table 3) in comparison to Soft doughs. Different lipid treatments did not influence these parameters within doughs prepared from each flour type (Table 3). Though 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 bread making flour. Higher strain hardening index and failure strains ensure that gas cells in Jagger doughs expand to a greater extent than Soft doughs. Similar results that differentiate between bread making potential of flours have been reported by Dobraszczyk and co-workers (Dobraszczyk and Roberts, 1994; Dobraszczyk and Salmanowicz, 2008; Dobraszczyk et al., 2003;). On the other hand, variations in natural lipid levels in a particular flour that produce significant variations in loaf volume (Fig. 2) made no difference to biaxial extensional rheology of the dough from that flour. This provides clear evidence of the role of lipids as surface active compounds stabilizing liquid lamellae, a phenomenon independent of dough rheology.

This study, for the first time provides clear evidence on the presence of liquid lamellae as an independent stabilizing mechanism working auxiliary to the gluten–starch matrix in stabilizing expanding gas cells during bread making. The study also demonstrates that lipids at their natural levels do not affect biaxial extensional rheology as determined by dough inflation.

Table 3
Mean bubble inflation rheological response of Jagger and Soft wheat flour doughs to natural flour lipid levels

% of natural 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

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

3.2. Mechanism of stability of the gluten–starch matrix

3.2.1. Gluten fractionation

Yield (%) and protein content (%) of each fraction from the pH fractionation are given in Table 4. Cumulative percentage of total polymeric protein extracted is plotted as a function of pH of solution in Fig. 3. This was calculated using yield data and percentage total polymeric protein of each fraction as given in Table 4. Approximately 35% of total polymeric proteins and most of the gliadins were solubilized and extracted at pH 5.3. The relative proportion of total polymeric proteins and unextractable polymeric proteins increased in subsequent fractions (Table 4), with the highest values in the pH 3.1 residue. SE-HPLC analyses of individual fractions (Fig. 4) also show the subsequent shift in MWD of the fractions to higher MW. This is clear in Table 5, where addition of these fractions to the base flours causes similar variations in percentage of polymeric protein greater than 250,000 Da. The solubility of gluten proteins depends on their molecular weight (Singh and MacRitchie, 2001). As the pH is lowered, relatively larger sized gluten proteins become soluble, and can be separated into different fractions by stepwise reduction of pH (Gupta et al., 1990; MacRitchie, 1985).

3.2.2. Reconstitution studies

Extracted fractions were added to defatted base flours (Jagger and Soft) at a level of 1% (dry protein) on a total flour weight basis. MacRitchie (1987) found significant rheological differences at these levels of addition of protein fractions. The reconstituted flours were analyzed for their MWD, dough mixing properties, baking performance and biaxial extensional rheology.

3.2.2.1. SE-HPLC and MALLS (SEC-MALLS) analysis. Cut-off analysis, as described elsewhere (Bangur et al., 1997), of SE-HPLC chromatograms overlaid with MALLS signal, of all Jagger and Soft wheat flours (base and reconstituted) at 4 min intervals, helped to determine the elution time at which the polymers with M_W of 250,000 Da were being eluted. The chromatograms were integrated to determine the monomeric (mainly gliadins) to polymeric (mainly glutenins) ratio, and relative proportion of polymeric proteins greater than 250,000 Da (Table 5). Addition of the gliadin rich (pH 5.3) fraction caused a decrease in the relative proportion of polymeric proteins greater than 250,000 Da. However, with addition of later fractions (pH4.9 to pH 3.1 residue), progressive increase in the proportion of polymeric proteins greater than 250,000 Da was observed. Use of the relative proportion of polymeric proteins greater than M_W of 250,000 is based on a previous estimate by Bangur et al. (1997), which also seems to hold true in this study and is explained in Section 3.2.2.3.

Table 4
Yield, moisture and protein content of gluten protein fractions extracted by pH fractionation, and their SE-HPLC relative composition of polymeric proteins (all numbers in percentages)

Fractions	Yield (%)	Moisture content (% wet basis)	Protein content (%) (14% m.b.)	Area (%) under chromatogram curve	
				TPP	UPP
pH 5.3	50.4	4.5 ± 0.08	83.8 ± 0.05	31.6 ± 1.59 f	23.9 ± 0.13 d
pH 4.9	20.5	4.6 ± 0.16	78.0 ± 0.08	52.6 ± 0.08 e	72.6 ± 1.48 c
pH 4.1	10.6	4.1 ± 0.15	79.6 ± 0.12	64.1 ± 0.10 d	78.3 ± 2.85 b
pH 3.5	4.2	4.2 ± 0.37	78.5 ± 0.07	65.5 ± 0.01 c	77.6 ± 2.31 b
pH 3.1 supernatant	3.5	4.3 ± 0.09	73.2 ± 0.14	68.0 ± 0.21 b	83.2 ± 0.93 a
pH 3.1 residue	10.8	4.9 ± 0.26	45.8 ± 0.18	69.5 ± 0.49 a	86.6 ± 0.08 a

TPP—total polymeric protein; UPP—unextractable polymeric protein
Values represent mean ± standard deviation for duplicate determinations.
Means with the same letter within columns are not significantly different ($p > 0.05$).

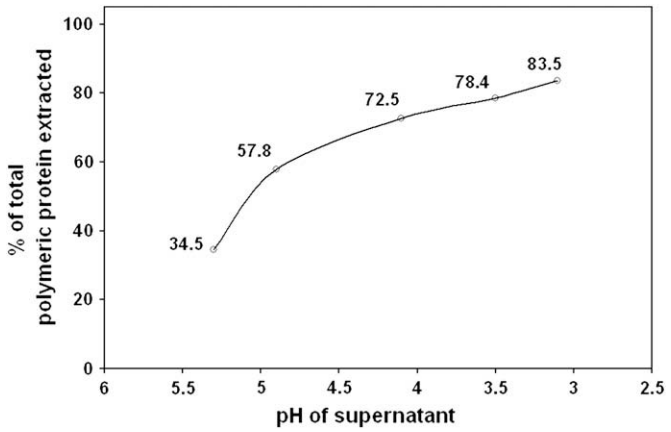

Fig. 3. Cumulative percentage of total polymeric protein extracted as a function of final pH of supernatant of Jagger gluten.

Elution of proteins through a SE-HPLC column is based on their hydrodynamic radii. Proteins with similar M_W may vary in the conformation and thus the hydrodynamic radii in solution. Such proteins will not elute at the same time through the SE-HPLC column. An overlaid MALLS signal provides a true measure of M_W at different elution times (Bean and Lookhart, 2001), providing nearly precise estimation of the relative proportion of polymeric proteins greater than 250,000 Da.

3.2.2.2. 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 wheat flour and 60% for Soft wheat flour. Mixograph dough development time (MDDT) was recorded from Mixograph traces of Jagger and Soft wheat flours. MDDT is plotted for each fraction as a function of the total protein that has been extracted (Fig. 5). Addition of the first 35% (approximately) of total polymeric protein extracted from Jagger gluten (corresponding to the pH 5.3 fraction) caused a decrease in mixing requirements. In contrast, addition of subsequent fractions caused an increase in mixing requirements.

These trends for MDDTs can be explained on the basis of SEC-MALLS data (Table 5). As expected, the decrease in the monomeric to polymeric ratio and increase in relative proportion of polymeric proteins greater than 250,000 Da lead to an increase in MDDT and corresponding decrease in breakdown. At a molecular level, mixing

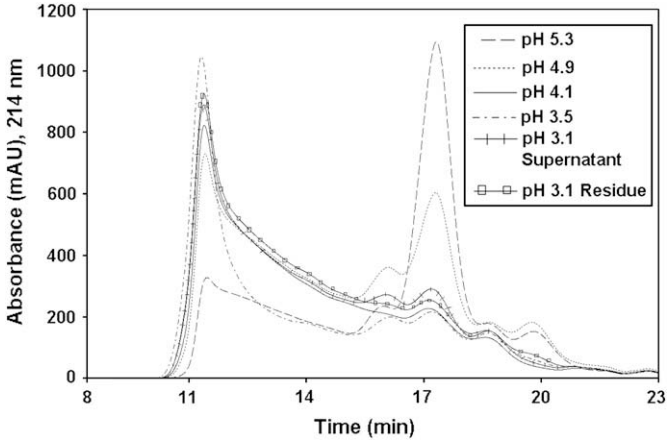

Fig. 4. SE-HPLC chromatograms of total protein of Jagger gluten protein fraction. As pH is reduced, the percentage of polymeric protein increases and MWD shifts towards higher molecular weight.

Table 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)

Fraction added	Jagger wheat flour		Soft wheat flour	
	MON/POL[a]	% POL > 250,000[b]	MON/POL[a]	% POL > 250,000[b]
None (Control)	1.47 ± 0.00 a	65.9 ± 0.01 d	1.41 ± 0.01 a	76.8 ± 0.07 d
pH 5.3	1.48 ± 0.01 a	35.0 ± 0.05 f	1.43 ± 0.03 a	46.7 ± 0.10 f
pH 4.9	1.36 ± 0.01 b,c	66.9 ± 0.18 c	1.27 ± 0.07 b	68.1 ± 0.22 e
pH 4.1	1.34 ± 0.00 c,d	58.0 ± 0.04 e	1.28 ± 0.01 b	99.7 ± 0.14 b,c
pH 3.5	1.37 ± 0.00 b	67.1 ± 0.02 c	1.30 ± 0.01 b	100.0 ± 0.00 a
pH 3.1 supernatant	1.33 ± 0.01 d	85.1 ± 0.12 b	1.16 ± 0.00 c	96.1 ± 0.04 c
pH 3.1 residue	1.32 ± 0.00 d	100 ± 0.00 a	1.16 ± 0.02 c	100.0 ± 0.00 a

Values represent mean ± standard deviation for duplicate determinations.
Means with the same letter within columns are not significantly different ($p > 0.05$).
[a] Monomeric to polymeric protein ratio.
[b] Percentage of polymeric proteins ≥ 250,000 Da.

is characterized by extension (stretching) of glutenin polymers and entangling of these stretched polymers (MacRitchie, 1986). This is achieved by work input and mixing intensity that must be above certain minimum critical levels (Kilborn and Tipples, 1972; Tipples and Kilborn, 1975). At a given work input and intensity of mixing, MDDT probably relates to the extension and entangling of the largest glutenin molecules (Singh and MacRitchie, 2001). The work input and mixing intensity of the flour is therefore a 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 increases, as requirements for work input increase (Gupta et al., 1993).

3.2.2.3. Baking performance. Loaf volumes (Fig. 6) decreased on addition of the pH 5.3 fraction. However, on addition of subsequent fractions that lead to increase in MDDT, increase in loaf volume was observed, which after reaching a 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 the pH 4.1 fraction. In the case of Soft, the maximum occurred on addition of total polymeric proteins that were extracted after the first 35% and before the first 70%, corresponding to the pH 4.9 fraction (Figs. 3 and 6). It seems that the optimum balance of strength and extensibility was achieved on addition of these fractions to base flours, thus giving best baking performance.

Bangur et al. (1997) reported that the ratio of polymeric proteins with MWs above the threshold molecular weight (M_T) to those (i.e. polymeric proteins) with MWs below M_T was approximately 60:40. The M_T for polymeric proteins was estimated to be 250,000 Da. Polymeric proteins greater in molecular weight than 250,000 Da confer strength to the entangled gluten protein network and those with molecular weight less than this may counter the strength by acting as diluents (or plasticizers). This 60:40 proportion is nearly achieved on addition of the pH 4.1 fraction in Jagger wheat flour and pH 4.9 fraction in Soft wheat flour (Table 5) giving maximum loaf volumes and strain hardening index. Any shift in the proportion shows deleterious effects on baking performance (Fig. 6).

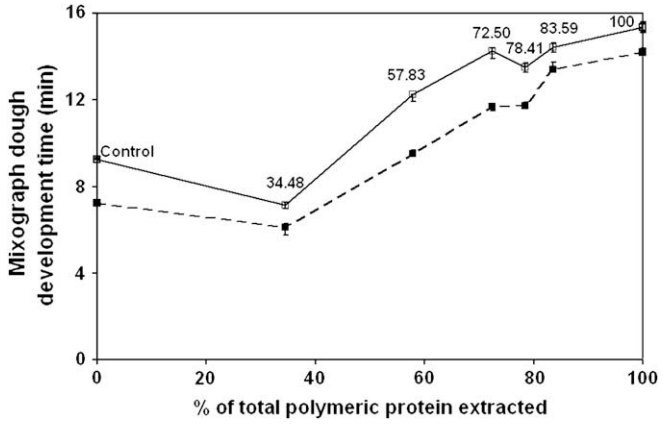

Fig. 5. Effect on Mixograph peak times of Jagger (- - -) and Soft (—) wheat flours on addition of gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

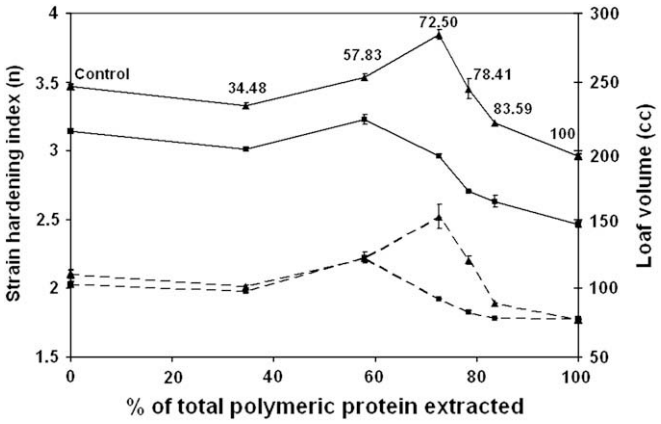

Fig. 6. Effect on loaf volumes (—) and strain hardening index (- - -) of Jagger (▲) and Soft (■) wheat flours on addition of gluten protein fractions at a 1% (dry protein) level (on flour weight basis).

3.2.2.4. Biaxial extensional rheology. Significant differences ($P < 0.0001$) in biaxial extensional rheological parameters (strain hardening index and failure strain) were recorded for addition of fractions to the base flours. Variations in strain hardening index paralleled those of loaf volume, for both flours (Fig. 6). Analogous variations in strain hardening index and loaf volume show that this parameter is a good determinant of rheological requirements for the gluten–starch matrix in terms of bread making. As reported in some previous studies (Dobraszczyk and Roberts, 1994; Dobraszczyk et al., 2003; Tronsmo et al., 2003), in this case also, strain hardening shows a high degree of correlation with loaf volume and failure strain (Fig. 7). The Mixograph parameter (MDDT), on the other hand, correlates well with MWD but does not predict baking performance equally well.

The concept of strain hardening explains the requirements for maximum inflatability of gas cells in bread making without failure, to give good loaf volume and crumb grain. In a gluten–starch matrix, gluten proteins form a continuous network and the rheology of the dough is that of a continuous gluten protein network. It is only the fraction of glutenins with MW greater than M_T that confers strength, as suggested by Bangur et al. (1997), whereas smaller ones act as diluents, preventing additional physical constraints or entanglements and enhancing extensibility (Bersted and Anderson, 1990; Singh and MacRitchie, 2001). To get maximum inflatability of the gas cells, the gluten–starch matrix around them must stretch to its maximum extensibility without breaking, i.e. the glutenins/polymeric proteins (mainly with MW > M_T; i.e. 250,000 Da) must be stretched to their maximum length through entanglements, as described by entanglement network theory (MacRitchie and Lafiandra, 1997; Singh and MacRitchie, 2001). Addition of the gliadin rich fraction, i.e. the pH 5.3 fraction (Fig. 4), significantly increases diluent concentration that will lead to disentanglement of the gluten protein network without sufficient elongation when subjected to extensional forces, thus leading to earlier failure of the gas cells. As MWD shifts

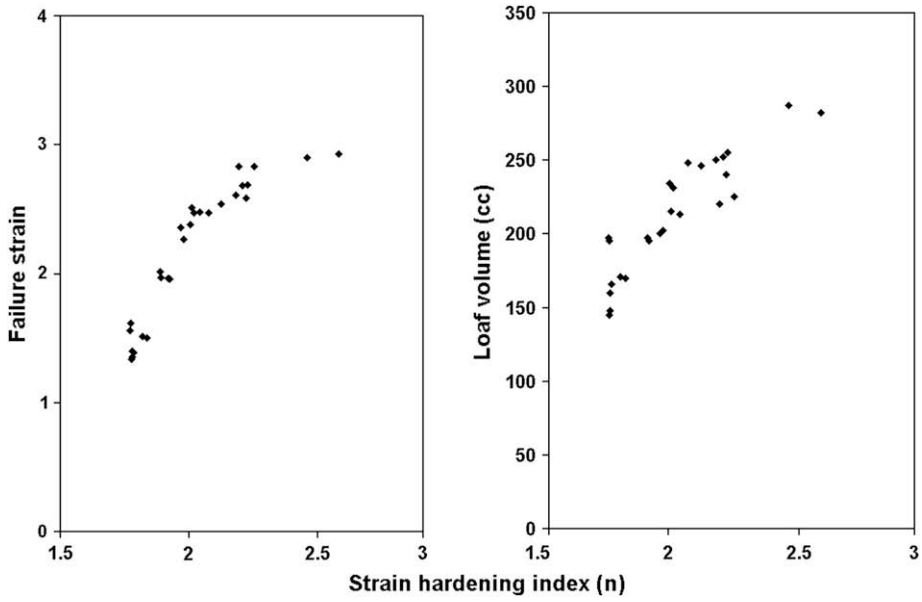

Fig. 7. Scatter plots of strain hardening index vs. failure strain and loaf volume (cc) for different Jagger and Soft wheat flour doughs exhibiting high degree of correlation.

towards larger glutenins, the strength increases (Gupta et al., 1993). On doing so, it passes through maximum extensibility or strain (Hencky), beyond which the balance shifts towards strength and the gluten protein network is no longer as extensible. This leads to decrease in strain hardening index as well as loaf volume upon addition of the latter extracted fractions (Fig. 6).

4. Conclusion

Results of this study provide clear evidence for the presence of liquid lamellae, thus supporting the dual film hypothesis. The liquid lamellae act as a secondary stabilizing mechanism and is on the inner side of the gluten–starch matrix enveloping the gas cells (Gan et al., 1995).

Stability of the gluten–starch matrix, which is the primary stabilizing factor for expanding gas cells against disproportionation and coalescence, depends on its tendency to strain harden. 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. Extensibility ensures slippage of the maximum number of statistical segments between entanglements (Singh and MacRitchie, 2001), whereas strength prevents disruption of the entangled network of polymeric proteins. Thus, to ensure stability of gas cells, the dough needs to be sufficiently extensible to respond to gas pressure but also strong enough to resist collapse. Differences in gluten quality, as demonstrated in this study can significantly affect the bread making potential. 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 may counter the strength by acting as diluents. The optimum balance seems to exist when the relative proportions of polymeric proteins greater and smaller than M_T are roughly 60:40. Shift in the balance to either side will decrease loaf volume. Increase in smaller proteins (less than M_T) may decrease stability of the gluten–starch matrix due to a lesser number of entanglements per chain. On the other hand, increase in strength conferring proteins may prevent sufficient expansion of the gluten–starch matrix required to increase loaf volume due to reduced slippage of gluten polymers through entanglement nodes as a result of increase in number of entanglements per chain.

The secondary stabilizing mechanism involves thin liquid lamellae stabilized by adsorbed surface active compounds (lipids and proteins) at the gas–liquid interface. Liquid lamellae prevent coalescence and disproportionation of gas cells when they come in close contact with each other during the late proving and early baking stages of bread making i.e. when discontinuities begin to appear in the gluten–starch matrix. The study demonstrates that the flour lipids at their natural levels do not influence rheological properties of the gluten–starch matrix surrounding the gas cells, as measured by the dough inflation system. Nevertheless, the small amounts in which these lipids are naturally present are sufficient to influence surface properties. The exact mechanism by which these lipids and other surface active components (proteins) stabilize liquid lamellae is discussed in the succeeding paper (Part II).

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