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RHEOLOGICAL BEHAVIOR AND MICROSTRUCTURE OF DEVELOPED AND UNDEVELOPED WHEAT DOUGH

By

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ABSTRACT

RHEOLOGICAL BEHAVIOR AND MICROSTRUCTURE OF DEVELOPED AND UNDEVELOPED WHEAT DOUGH

By

Emily Joann Schluentz

A study was conducted to investigate the role of shear and extensional deformation on wheat dough development. Undeveloped dough samples were strained in controlled shear and extensional flow between parallel plate sensors in a Haake RS100 rheometer. After deformation, samples were prepared for structure evaluation using scanning electron microscopy, or rheological testing.

Micrographs of wheat dough were subjected to numerical digital image processing to quantify image texture related to protein development. These results were compared to dynamic rheological properties to evaluate the influence of deformation on microstructure. The viscoelastic behavior of wheat dough showed that developed dough had the greatest amount of structure formation, followed by extensionally strained and shear strained samples, respectively. Undeveloped dough showed the lowest levels of structure development and viscoelastic moduli. Image analysis results were statistically different between developed and undeveloped samples; however, results were not significantly different between shear and extensionally strained samples.

DEDICATION

To my parents, Jack and Joann;
and to my sister, Mandy.

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NOMENCLATURE

a	constant defined by Eq. [4.1], Pa s^b
b	constant defined by Eq. [4.1], dimensionless
G^*	complex modulus, Pa
G'	shear storage modulus, Pa
G''	shear loss modulus, Pa
h	gap width, mm
h_o	initial gap width, mm
R	outer plate radius, mm
δ	phase shift or phase angle, radians
ε_B	biaxial extensional strain, dimensionless
γ_o	strain amplitude, dimensionless
γ_o	maximum shear strain, dimensionless
σ_o	stress amplitude, Pa
Ψ	sweep angle, radians

CHAPTER I

INTRODUCTION

Rheology is the science of deformation and flow of matter. It is how a material responds to applied strains and stresses and is an important tool applicable to process engineering, quality control, shelf life testing, ingredient functionality and sensory analysis. Materials are rheologically classified according to their flow behavior. The two simplest materials are ideal elastic solids and viscous liquids. An ideal solid (Hookean solid) is characterized by a shear stress proportional to strain, indicating the ability of a material to fully recover when the stress is removed. Newtonian fluids are ideal viscous liquids where stress is proportional to strain rate. However, most materials do not exhibit ideal flow behavior. Wheat dough is viscoelastic, exhibiting both liquid-like and solid-like behavior. Rheological characterization of wheat dough is important because the rheological properties of the material continuously change throughout processing stages (Szczesniak, 1988).

Wheat varieties are usually classified as soft or hard. These terms generally correspond to protein content: soft wheat products are associated with cakes, cookies, and crackers; and hard wheat products with bread and pasta. When wheat flour is

combined with water, and energy is applied, a basic dough will form. Traditional mixing systems such as the farinograph and mixograph uniformly distribute flour and water, and add energy by rotating the mixing elements. Water causes the proteins to swell and mechanical energy promotes organization of the proteins into a continuous matrix, giving dough a unique viscoelastic structure (Scholfield and Scott Blair, 1932). In this research, "development" is associated with the energy input element of mixing and "undeveloped" refers to a homogeneous, hydrated flour that has formed in the absence of mechanical energy input.

Traditional mixing systems combine distribution and energy input functions in a manner that provides limited fundamental information on dough development. Qualitative information obtained by these instruments cannot be used for fundamental computations because the results pertain to dough as it is mixed and not to the flow characteristics of mixed dough (Campos, 1996). When using these traditional instruments, the influence of shear and extensional deformation on protein development is uncontrollable.

The current research allows for separate evaluation of shear and extensional flow on dough development. The flour and ice technology developed by Campos et al. (1996) decouples hydration and energy input in dough development allowing the amount of shear and extensional strain to be controlled and the amount of structural development to be rheologically tested. Scanning electron microscopy will provide meaningful images of

dough development after different levels of deformation. Furthermore, digital image analysis techniques will use edge enhancement and thresholding to numerically quantify image texture reflecting the level of protein development. Understanding how the protein matrix develops from controlled deformation will improve equipment design, process design, and process optimization.

Objectives

The objectives of this research are:

a) to use controlled shear and extensional deformation to investigate the role of deformation on dough development;

b) to prepare dough samples for scanning electron microscopy in a way that minimizes artifacts and maintains the integrity of the starch and gluten matrix;

c) to quantitatively determine the degree of image texture related to development (structure formation) of proteins in wheat dough from image analysis of electron micrographs;

d) to evaluate the influence of controlled deformation on the dynamic rheological properties of wheat dough.

CHAPTER II

LITERATURE REVIEW

2.1 Dough Development Process

Understanding the role of mixing is an essential step toward optimizing wheat dough development. However, the processing effects have been difficult to ascertain because there is a lack of instrumentation that measures the amount of protein development. Dough, in the most basic form, is a combination of water, flour, and energy (Campos et al., 1996). Water causes the proteins to swell and mechanical energy organizes the proteins into a continuous matrix, giving dough viscoelasticity (Schofield and Scott Blair, 1932). Lindborg (1995) describes three objectives of dough mixing: blend the ingredients into a homogeneous mass, develop the protein capable of holding gas, and include air cells into the dough. Bloksma and Bushuk (1988) separated mixing into three distinct elements: distribution of materials, hydration, and energy input to stretch and align protein molecules. The energy input phase of mixing, which involves shear and extensional deformation, is an important aspect of protein development. MacRitchie (1986) describes the alignment of the largest gluten molecule in the direction of shear, as peak development time associated with mixing.

Mixing is important because it significantly changes the rheological properties of wheat dough. Dough formation is achieved through unknown amounts of shear and extensional deformation using industrial or laboratory bench top mixers. Typically, the mixed dough is then tested to determine physical properties such as viscosity, strain or shear stress history, or temperature and time dependence. Depending on the type of mixing equipment and mixing time, large deformations stretch and align gluten proteins giving dough the ability to store energy. Lindborg (1995) discusses several dough mixers and characterizes the type of deformation acting on the dough. Single-arm mixers exert pushing and pulling on the dough to develop the proteins. Lindborg (1995) also describes shaking, lifting, folding, and stretching movements in a twin-arm batch mixer, and a lifting and swiveling action in high speed mixers. Besides intensity, mixing time is also important to dough development and ranges from 1.5 min in a high speed mixer to 15-20 min in a single arm mixer.

The farinograph and mixograph are the preferred lab instruments for evaluating wheat flour. They use mechanical energy to distribute the ingredients and develop protein. When using these traditional instruments, the amount of shear and extensional deformation contributing to protein development is uncontrollable. MacRitchie (1992) relates development time to protein composition. High dough mixer shear rates remove the outer layer of flour particles and stretch the components into layers as they become hydrated (Lindborg, 1995); however, Cheng (1979) concluded that the relationship between dough strength, hydration, and energy input from shear and extensional deformation is unclear. Lindborg (1995) agrees that there is little known about dough

mixing and points out the complicated nature of mixing velocity (shear rate) as an example. In describing the effects of mixing on protein development, information in published literature can only relate the characteristics of a piece of equipment (with undefined flow fields) to an optimally developed dough. This correlation does not represent a true optimum, only a crude feature of the mixing system.

Campos et al. (1996) developed a method, using a flour and ice powder technology, to enhance understanding of the mechanical factors involved with protein development. This technology decouples hydration and energy input in dough development. An "undeveloped" dough is a homogeneous, hydrated flour (Campos et al., 1996) that has formed in the absence of mechanical energy (Figure 1.1). The advantage of an undeveloped dough matrix is that the contribution of shear and extensional flow fields can be separately analyzed in controlled deformation experiments. Past research efforts have also tried to produce dough in the absence of mechanical energy (Olcott and Mecham, 1947, Davies et al., 1969); however, these studies only focused on lipid binding. The "undeveloped" dough method of Campos et al. (1996), achieved a uniform distribution of water in the dough system by combining flour and ice particles. This technique allows for separate evaluation of shear and extensional flow fields that are otherwise, undefined in traditional dough mixing systems.

2.2 Rheological Properties of Wheat Dough

Rheological properties of dough prepared in traditional mixing systems may not accurately reflect the dough development process because the amount of shear and

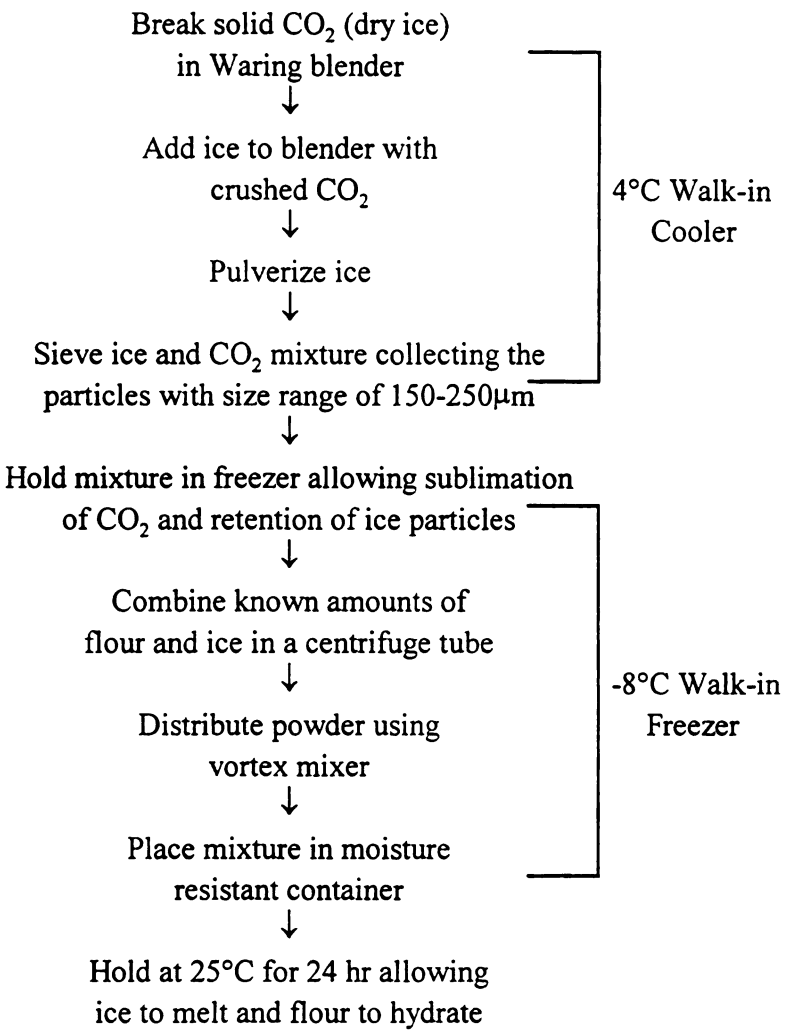

Figure 2.1. Flow diagram of the powder method of making "undeveloped dough." (Campos et al., 1996)

extensional deformations (which may have a significant influence on molecular alignment during processing) are unknown. Mechanical instruments only describe the mechanical properties of dough (Szczesniak, 1988), not the basic rheological properties of stresses and strains related to protein development and structural characteristics of the material. Rheological flow characteristics of wheat dough provide powerful knowledge and understanding to improve wheat dough handling and processing. Some properties can be measured using standard rheological methods (Steffe, 1996).

Wheat flour dough behaves as a viscoelastic material, partly due to unique protein characteristics (Scholfield and Scott Blair, 1932). Viscoelastic materials have both solid and liquid properties. Campos (1996) used the Maxwell, Kelvin, and Burgers mechanical analogs to describe the rheological behavior of wheat dough. These models combine springs and dashpots, representing elastic and viscous behavior, respectively, to better visualize the viscoelastic nature of wheat dough. Muller (1975) also used the spring and dashpot model to describe thixotropy of wheat dough.

The rheological properties of dough are largely controlled by the molecular weight distribution of the gluten protein (Castell-Perez and Steffe, 1992). Recent developments of dynamic rheometers have allowed the evaluation of storage and loss moduli in dynamic oscillatory flow. Wheat dough samples are subjected to a sinusoidal deformation and the time dependent response of the material is related to the viscoelastic behavior of the material. The amount of energy stored in wheat dough is related to G′ (storage modulus) and the viscous dissipation is related to G″ (loss modulus). Both

moduli are functions of frequency and can be expressed in terms of amplitude ratio and phase shift (Steffe, 1996):

$$G' = \left(\frac{\sigma_o}{\gamma_o}\right)\cos\delta \qquad [2.1]$$

$$G'' = \left(\frac{\sigma_o}{\gamma_o}\right)\sin\delta \qquad [2.2]$$

where σ_0 is stress amplitude, γ_0 is strain amplitude, and δ is phase shift relative to strain. The ratio of viscous to elastic moduli is equal to the tangent of the phase shift:

$$\tan\delta = \frac{G''}{G'} \qquad [2.3]$$

Complex modulus, G^*, is an additional frequency dependent material function:

$$G^* = \frac{\sigma_o}{\gamma_o} = \sqrt{(G')^2 + (G'')^2} \qquad [2.4]$$

Previous research efforts agree that linear viscoelastic behavior of wheat dough occurs only at low strains. Campos (1996) found linear behavior for undeveloped dough up to a shear stress of 50 Pa, corresponding to a 0.2% strain level. The 50 Pa maximum shear stress was applied to the sample in the frequency sweep mode of a dynamic oscillatory test. A frequency dependence of both G′ and G″ was observed. Furthermore, G′ was consistently greater than G″, throughout the frequency range tested, indicating greater elastic properties of wheat dough at 50 Pa. This behavior is typical for structured

materials like wheat dough. Campos (1996) also obtained similar rheological results for dough prepared in a farinograph.

2.3 Electron Microscopy

Electron microscopy (EM) is the science of using an electron beam to produce magnified images of minute specimens (Flegler et al., 1993). Two EM techniques are commonly used: transmission electron microscopy (TEM) and scanning electron microscopy (SEM). An advantage of EM, compared to light microscopy (LM), is an increased resolving power because electrons have a shorter wavelength than photons. The ability to distinguish fine detail for a light microscope is at best 200 nm; for TEM and SEM, the resolving power is 0.2 nm and 3 nm, respectively (Flegler et al., 1993). Two other advantages to EM include a better depth of field, and analytical methods to determine the composition of structures containing various metallic elements. Food microscopy is a practical science which has been applied to flours, dough, bread, proteins, yogurt, and ice cream. Better than any other technique, EM has allowed scientists to collect data based on high resolution images.

Both TEM and SEM systems require high vacuums. There are many reasons a vacuum system is mandatory: to reduce electron beam instability, to reduce reactive gases that could burn out the electron filament, to decrease the occurrence of electron beam scattering which lowers image contrast, and to inhibit specimen-beam interactions that may contaminate or alter the specimens. One disadvantage of EM is that specimens

often require more extensive preparation which is critical to image quality in EM (Flegler et al., 1993), than those required with LM.

2.3.1 Principles of Scanning Electron Microscopy

The first commercial scanning electron microscope was introduced in 1965 (Flegler et al., 1993). Since then the instrument has been recognized by food scientists as the primary source of microstructure information (Aguilera and Stanley, 1990). In SEM, the surface of the sample is scanned with an electron beam and the digital image observed is created by secondary electrons emitted from the specimen.

The microscope's electron gun emits a beam of electrons that traverses through a high vacuum column and is focused by the objective lens on the specimen. Scanning coils create a magnetic field that deflects the electron beam across the specimen surface in a repeated raster format, releasing secondary electron from the specimen surface. The secondary electrons are detected by an Everhart-Thornley scintillator-photomultiplier system, and the cathode-ray tube displays a digital image of the specimen surface (Flegler et al, 1993).

2.3.2 Scanning Electron Microscopy Sample Preparation Methods for Wheat Dough

The advantages to SEM, compared to TEM, include a very large depth of focus, the possibility of viewing larger sample sizes, and easier sample preparation techniques (Aranyi and Hawrylewicz, 1968). There are three SEM requirements for biological samples: 1) the sample must not contain any volatile chemicals that may decrease the

vacuum or contaminate the microscope, 2) the sample must be firmly mounted to a stub, 3) the sample must be electrically conductive. Since wheat dough contains water, it must be dehydrated. After dehydration, the sample must be firmly mounted to an aluminum stub using an epoxy resin or adhesive tabs. Finally, the dough sample must be sputter coated with a thin layer of gold particles to make it conductive.

Several researchers have used the SEM to observe the ultrastructure of bread and bread dough (Aranyi and Hawrylewicz, 1968, 1969; Berglund et al., 1990, 1991; Chabot et al., 1979; Evans et al., 1977, 1981; Khoo et al., 1975; Parades-Lopez and Bushuk, 1982; Pomeranz et al., 1984; Pomeranz and Meyer, 1984; Variano-Marston, 1977). Others have observed the ultrastructure of wheat gluten (Cumming and Tung, 1975; Freeman et al., 1991), wheat flour tortillas (McDonough et al., 1996), and bagels (Umbach et al., 1990). Berglund et al. (1990), reported that even though freeze drying or chemical fixation and dehydration are the most common sample preparation techniques, these methods may produce artifacts, mask surface detail or cause alteration of the microstructure.

Variano-Marston (1977) explored many SEM preparation techniques for wheat dough specimens including four dehydration procedures: freeze drying at -65°C for 48 hours, vacuum desiccation at room temperature for 24 hours, air drying at room temperature for 24 hours, and acetone dehydration followed by critical point drying. The effect of chemical fixation with osmium tetroxide (OsO_4) vapor or buffered glutaraldehyde, postfixed with buffered OsO_4 were considered, as well as frozen and

unfrozen dough samples. Variano-Marston (1977) concluded that freeze drying (frozen dough state) and vacuum desiccation (frozen and unfrozen dough state) gave the best resolution and depth of field. Furthermore, it was concluded that air drying and chemical fixation, followed by critical point drying gave poor results with wheat dough. Latter work by Chabot et al. (1979), however, did not conclude that air drying caused more structural distortions than freeze drying in bread samples. Aranyi and Hawrylewicz (1968) used vacuum desiccation for wheat dough specimens and described the observed image as a veillike networking of protein covering an even distribution of starch. Evans et al. (1981) used freeze drying to observe optimally developed dough and characterized the dough as having a continuous and strong adhering gluten layer covering the starch granules.

Researchers described dough and bread samples exposed to chemical fixatives as not having a continuous protein covering over the starch granules (Aranyi and Hawrylewicz, 1969) and found severe rupturing in gluten sheets at the starch-protein interface (Evans et al., 1977). Chabot et al. (1979) concluded that ethanol dehydration before drying altered the specimens by further compacting the structure causing it to appear dense. Cumming and Tung (1975) also concluded that fixation and dehydration removed the veillike protein from the starch, which permitted evaluation of starch morphology. It was suggested by Variano-Marston (1977) that chemical fixatives caused discontinuities in the protein film.

Water is an important component in wheat dough. Traditional SEM preparation techniques require the removal of water by the aforementioned drying techniques which, unfortunately, cause changes in ultrastructure. Berglund et al. (1990) compared two cryogenic preparation techniques for low temperature SEM on frozen bread dough. Bread dough was frozen at -23°C and sampled using two methods: 1) samples were placed on specimen holders at 22°C to allow partial thawing, 2) samples were kept frozen with dry ice during mounting on specimen holders (Berglund et al., 1990). The cryogenic preparation used by Berglund et al. (1990), involves plunging the specimen and holder into a nitrogen slush at -196°C in the freezing chamber, transferring the specimen under vacuum to the cold stage (-180°C) using a shroud (prevents warming and contamination), fracturing the sample with a cooled knife and observing the fractured samples in the scanning electron microscope as it sublimes on the cryostage. Berglund et al. (1990) concluded that thawed specimens produced patterns due to recrystalization of water: samples kept frozen during mounting did not exhibit these patterns. Berglund et al. (1990) also found that smaller samples had a greater tendency to thaw during mounting, causing recrystalization upon refreezing in the nitrogen slush.

2.3.3 Image Analysis of Electron Micrographs

Digital image processing and analysis is a powerful technique applicable to manufacturing, medicine, military, and agriculture. Digital image processing and machine vision have been used in plant identification (Guyer et al., 1986), bread crumb grain evaluation (Zayas, 1993), extruded yellow corn puff evaluation (Gao and Tan,

1993), raisin grading (Okamura et al., 1993), snack quality evaluation (Sayeed et al., 1995), and wheat classification (Zayas et al., 1996).

Whittaker et al. (1984) describes digital image processing in steps: capture of a digital image with a sensor, segmentation, description, recognition, and interpretation. An image is a source of information about the physical object it represents (Whittaker et al, 1984). Physical properties may include size, shape, color, and texture of a specimen whereby, meaningful data can be derived from the image using image analysis techniques. A monochrome digital image is a two dimensional array of pixels (picture elements). Each pixel represents the gray level or brightness at each specific location of the image. In SEM, a photomultiplier tube detects or senses secondary electrons emitted from the specimen under observation. The digital image of the specimen, subsequently produced on a monitor, is ready for digital image processing. Castleman (1979) defines digital image processing as subjecting numerical representations of objects to a series of operations in order to obtain a desired result. In this research, the result will be a quantification of image texture reflecting the level of protein development.

There are 256 gray levels in a digital image, where 0 is black and 255 is white. Segmentation, the first processing technique, includes the following: edge detection, thresholding, contrast stretching, and smoothing (Whittaker et al., 1984). Scanning electron micrographs can be subjected to edge detectors which identify gradients in gray levels. Gradient operations compute an angle indicating the direction of maximum gray level change, and a magnitude to describe the steepness of change. Thresholding uses

contour lines of the gray level intensities as boundaries for segmentation (Whittaker et al., 1984).

After segmentation, image properties such as percent area, can be computed to describe the image. Typically, algorithms are used to calculate shape features from the segmented image. The final two steps in digital image processing are recognition and interpretation. Recognition assigns labels to an object and interpretation is the final decision based on derived information about the specimen (Whittaker et al., 1984).

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Flour

Two types of commercial wheat flours were used in this research study: soft white winter and hard red winter (King Milling Co., Lowell, MI). These flours have distinct physical and chemical characteristics. Typically, soft wheat flours are used in cakes, cookies, and crackers; and hard wheat flours are used in breads and pasta (durum wheat). Campos (1996) characterized the same flour types (King Milling Co. soft white and hard red winter wheat flour) for their protein content, moisture content, ash content, and falling number. Since the same lot of flour was used in this research, only moisture content and falling number were retested and compared to the Campos (1996) results to determine if there were any significant chemical changes in the materials. A farinograph test following standard American Association of Cereal Chemists (AACC) procedures was also conducted (AACC, 1995).

Campos (1996) reported higher protein levels for hard wheat flour (12.36%) than soft wheat flour (10.73%). The higher protein content in hard wheat flour is usually

expected, and is responsible for the elevated viscoelastic structure of the dough. The ash content does not affect physical characteristics of flours (Hoseney, 1994) but does indicate the presence of bran. Again, the ash content of hard wheat flour (0.52%) was slightly greater than soft white flour (0.45%) (Campos, 1996).

The moisture content of the flours was determined by adding ten grams of flour to an aluminum weighing dish. The dish was placed on an Ohaus Moisture Determination Balance (Ohaus Scale Corporation, Florham Park, NJ) and the weight was tared. The scale was placed under intense heating until the scale reached equilibrium (approximately five minutes) and a final weight could be recorded. The final weight on the scale corresponded to the moisture percentage of the flour sample. The average of three replications was the moisture content for hard wheat (14.00%) and soft wheat (14.70%) flours. Campos (1996) reported a moisture content of 14.24% and 14.84% for hard wheat and soft wheat flours, respectively.

The falling number test measures α-amylase enzymatic activity and indicates the degree of sprouting in wheat grains. A significant degree of enzymatic activity will change the physical properties of wheat flour over time. The falling number test is the total time in seconds from the immersion of the viscometer tube into the water bath until the viscometer stirrer has fallen the prescribed distance through the gelatinized suspension (AACC, 1995). Low α-amylase activity is indicated by a falling number value above 300 s; and a high α-amylase activity is indicated by a falling number value

below 150 s. The approved AACC Method 56-81B (AACC, 1995) was used to obtain an average (three replications) falling number value of 383 s and 249 s for hard and soft wheat flours, respectively. Campos (1996) reported falling number a value of 478 s for hard wheat flour, and 259 s for soft wheat flour. Rheologically, a high falling number value (low α-amylase activity) is indicative of viscosity of the gelatinized suspension. An increase in activity enzymatically decreases the ability of starch to gelatinize, therefore becoming less viscous.

Farinograph tests were also conducted for the hard and soft wheat flours following the approved AACC Method 54-21 (AACC, 1995). Fifty grams of flour were placed into the 50 g mixing bowl of a Brabender Farinograph (C.W. Brabender Instruments Inc., South Hackensack, NJ). Water absorption, development time, arrival time, stability time, departure time, and mixing tolerance results are reported in Table 3.1.

3.1.2 Dough Preparation

"Undeveloped" dough, prepared using the method of Campos et al. (1996) described in Figure 2.1, achieved a uniform distribution of water in the dough system by combining flour and ice of similar particle size (150-250 μm). The powder mixtures were prepared and stored in a -8°C freezer. Campos et al. (1996) allowed samples to thaw for 24 hours; however, Campos et al. (1997) later found that holding flour and ice mixtures at room temperature for at least 3 hours was adequate to achieve an equilibrium condition. In this study powder samples were thawed for approximately 3 to 8 hours,

Table 3.1 Farinograph properties of soft white and hard red winter wheat flour.

Farinograph Properties	Soft White Winter Wheat Flour	Hard Red Winter Wheat Flour
water absorption (% wt. basis)	50.0	53.9
arrival time (min)	0.25	0.75
dough development time (min)	0.50	2.0
stability time (min)	1.75	9.25
mixing tolerance index (BU)	160	40
departure time (min)	2.00	10.0

forming a dough in the absence of mechanical energy. This technique allows for separate evaluation of shear and extensional flow fields that are otherwise undefined in a traditional dough mixing system.

3.2 Experimental Procedure to Develop Dough

3.2.1 Shear Deformation

A Haake Model RS100 RheoStress (Haake, Paramus, NJ) controlled-stress rheometer was used to rheologically test dough samples. Serrated parallel plates, 20 mm in diameter, were used in all measurements and connected to a load cell with a 5 N-cm torque capacity. The rheometer was interfaced with a computer for measurement control and data acquisition, using software developed by Haake. Shear deformation was created by rotating parallel plates to a maximum predefined strain in a creep test. Maximum strain at the outer rim of the plates, was determined from the following equation (Steffe, 1996):

$$\gamma_o = \frac{R\psi}{h} \qquad [3.1]$$

where γ_0 is maximum strain, R (mm) is outer radius of plate, ψ is the sweep angle in radians, and h is the distance between the parallel plates (mm). For this study, R=10 mm, $\psi=\pi$ radians, and h=2 mm. Following Eq. [3.1], the maximum shear deformation achieved was 1570% strain. Preliminary experiments established π radians (180°) of plate rotation as the maximum possible movement before dough samples "rolled out" of

the gap between the plates. To eliminate artifacts due to the edge of the plate, dough samples for SEM were taken at R/2, resulting in a 785% shear strain for each sample.

Flour and ice powder mixtures were leveled and thawed flat in a small petri dish lined with parafilm, for 3 to 8 hours at 25°C, prior to testing. After the powder mixture thawed, the parafilm and dough were lifted from the petri dish and cut into quarters with scissors. A quartered section of dough was removed from the parafilm with a spatula, and placed on the stationary plate of the rheometer. To prevent dough from sticking to the parallel plates, they were coated with a thin layer of corn oil. It is important to note that this technique did not create slip because the dough sample was firmly engaged by the plate serrations. Once the bottom stationary plate moved to measurement position (2 mm gap width), a thin layer of petroleum jelly was applied around the outside of the sample to prevent drying during measurement.

To initiate sample deformation, a controlled stress testing program was loaded into the Haake RS100, serrated plates were attached to the instrument, and the torque sensor was zeroed. The gap between the plates was maintained at 2 mm. A creep program was set to shear the dough sample at 600 Pa until the predefined breaking strain of 1570%, equivalent to 180° rotation, was achieved. Once the shear test was complete and the sample had reached maximum strain, the sample was allowed to relax for 3 minutes in the rheometer to prevent recoil and uncontrolled deformation. After the sample relaxed, the dynamic rheological properties were immediately measured, or the dough was prepared for scanning electron microscopy.

Samples deformed for observation in the scanning electron microscope were rapidly frozen by pouring crushed dry ice particles over the parallel plates. This hardened the dough allowing easier handling during SEM preparation, and further prevented dough from sticking to the plates causing undesirable extensional sample deformation during plate separation. After the bottom plate was lowered, a portion of the sheared dough sample was cut with a razor blade at R/2 and immediately submerged in ethanol to initiate dehydration.

3.2.2 Extensional (Biaxial) Deformation

The Haake Model RS100 RheoStress (Haake, Paramus, NJ) was also used to induce lubricated squeezing flow between parallel plates and create extensional (biaxial) deformation. In biaxial extension, the upper plate is fixed and the lower plate moves vertically upward. The diameter of the dough sample in contact with the 20 mm stainless steel plates, increases as the height decreases. The extensional (biaxial) strain, for an incompressible material with a partially full gap is (Steffe, 1996)

$$\varepsilon_B = -\frac{1}{2}\ln\left(\frac{h}{h_0}\right) \qquad [3.2]$$

where extensional strain, ε_B, is a function of the initial (h_0) and final height (h). If a sample is not properly lubricated, shear flow at high strain levels is introduced (Steffe, 1996). Extensional deformation is uniform throughout the sample.

Flour and ice powder mixtures were leveled and thawed flat in a small petri dish lined with parafilm, for 3 to 8 hours at 25°C, prior to testing. After the powder mixture thawed, the parafilm and dough were lifted from the petri dish and cut into quarters with scissors. A quartered section of dough was removed from the parafilm with a spatula, and placed on the stationary plate of the rheometer lubricated with corn oil. The stationary plate moved vertically upward at 5 mm/min until the sample was in contact with the lubricated upper parallel plate and a gap width of 2.5 mm was obtained. Once the measurement position was attained, the lower parallel plate moved vertically upward and induced lubricated squeezing flow at 1.5 mm/min until a gap width of 0.5 mm was obtained. Following Eq. [3.2], the computed extensional strain is 80.5% when h=0.5 mm and h_o=2.5 mm.

After biaxial deformation, the dynamic rheological properties of the cylindrical sample were measured, or the dough was prepared for scanning electron microscopy. Samples deformed for observation in the microscope were rapidly frozen between the plates by placing dry ice particles on the rheometer. After the sample was frozen, the lower parallel plate was lowered, and the disk shaped dough sample was placed in a petri dish. No tensile extensional flow was observed when the plates were separated. A small sample of dough was cut with a razor blade at the center of the disk and immediately submerged in ethanol for dehydration.

3.2.3 Oscillatory Testing After Shear and Extensional (Biaxial) Deformation

The structural development of wheat dough was determined from an oscillatory test on the Haake RS100. Following shear deformation and relaxation, the complex modulus G* (Pa), storage modulus G' (Pa), and loss modulus G" (Pa) were measured over a frequency range of 0.1 to 100 rad/s at a constant shear stress of 50 Pa. The frequency range was selected because it represents three decades of change commonly used to test the viscoelastic behavior of food. The shear stress level of 50 Pa was established from previous research efforts by Campos (1996) and corresponds to a 0.2% strain level which maintains a linear viscoelastic material response. Rheograms reflect the mechanical strength, due to structural development, of wheat dough at the specified strain level.

3.3 Dough Preparation for Scanning Electron Microscopy

Sample preparation is the most critical step in SEM. Poor technique can cause artifacts and produce variable results among images of the same sample. In this work, five sample preparation techniques were studied for both developed and undeveloped dough. Following each preparation technique, dehydrated samples were mounted with epoxy resin on standard aluminum stubs. After mounting, samples were sputter coated with gold particles (EMSCOPE SC500, T55-29173, Ashford, Kent, England) at 20 mA for 4 minutes.

The effect of fracturing versus not fracturing a sample was also studied. Fracturing was done by shattering a sample with sharp pointed tweezers to expose inner

surfaces, and mounting a fractured piece on an aluminum stub for metallic coating. Both non-fractured and fractured samples were mounted and scanned.

3.3.1 Chemical Fixation and Ethanol Dehydration

Chemical fixation crosslinks proteins in biological samples while ethanol dehydration, followed by critical point drying with CO_2, allows for complete water removal. Developed and undeveloped dough samples, approximately 4 mm^3, were cut from frozen samples. Dough was fixed by submergence in 4% glutaraldehyde, buffered with 0.1M phosphate buffer (pH 7.4), for 0.5 hr at room temperature. Fixed samples were rinsed with phosphate buffer for 10. to 15 minutes. After buffering, samples were submerged in a graded ethanol series (25%, 50%, 75%, 95%, 100%) for 20 minutes at each gradation; then submerged in 100% ethanol for three consecutive 20 minute intervals to ensure full dehydration. Since CO_2 is miscible with ethanol, dough samples were critical point dried using a Balzers Critical Point Dryer (Balzers Union, FL-9496, Furstentum, Liechtenstein). Critical point drying allows ethanol removal in CO_2 without large surface tension forces that may distort the sample.

3.3.2 Ethanol Dehydration Without Chemical Fixation

Previous research efforts included chemical fixation prior to ethanol dehydration. In this research, the same preparation procedure mentioned above, without the chemical fixation in 4% glutaraldehyde, was followed. No published record of this technique could be found.

3.3.3 Vacuum Desiccation

Vacuum desiccation did not use chemicals or rapid freezing; rather, the sample was allowed to dry in a vacuum desiccator. Small samples (5-7 mm^3), were placed on parafilm in a desiccator containing Drierite (anhydrous calcium sulfate) desiccant. Samples were dehydrated for 24 hours at room temperature in the desiccator.

3.3.4 Freeze Drying

Samples were placed in scintillator vials, previously chilled in an ethanol and dry ice slush at approximately -70°C, for transfer to the freeze dryer (Labconco Corporation, Kansas City, MS). Once the dryer reached -40°C and $12x10^{-3}$ mbar, samples were freeze-dried for 24 hours. After drying, the vials were capped, wrapped in parafilm and placed in a freezer (Forma Scientific Freezer, Marietta, OH) maintained at -86°C for storage.

3.3.5 Cryogenic Techniques

Dough samples were mounted on a special cryo stub with Tissue-Tek II O.C.T. (Lab-Tek Products, Naperville, IL) compound. The sample and holder were submerged into a liquid nitrogen slushing chamber and transferred to the JEOL JSM-35CF Cryo-SEM chamber (JEOL LTD., Tokyo, Japan) maintained at approximately -100°C. Etching heated the scanning electron microscope chamber to -65°C, allowing the water in the sample to sublime over a period of 20-30 minutes. After etching, the sample was transferred back to the EMSCOPE SP2000 (T8-84442, Ashford, Kent, England) working

chamber for sputter coating with gold particles. Samples were viewed on the scanning electron microscope stage at -90°C since thermal stress caused the sample to crack at -140°C.

3.4 Data Collection

3.4.1 Scanning Electron Microscopy Images

A JEOL JSM-6400V Scanning Electron Microscope (JEOL, Tokyo, Japan) scanned soft white and hard red developed, partially developed (in shear or extensional deformation), and undeveloped dough samples using an accelerating voltage of 13 kV, 15 mm working distance, and a condenser lens setting of 10. Analog images from the scanning electron microscope are produced on a cathode-ray tube with a 2,500-line resolution capacity (Flegler et al., 1993) and converted to a digital image. The 1024 x 910 pixel size, black and white, images were magnified at 2,000 X and subjected to pixel averaging data image acquisition. While pixel averaging is slow, the resultant image is better because the beam dwells at each pixel longer and the gray level is a time averaged value. The final image was saved as a tagged image file (TIF) on a 3.5 inch floppy disk. Five SEM images were taken for each replication of a dough sample treatment. At least ten different sample replications were observed under the microscope, resulting in fifty images per sample treatment, providing sufficient information for complete statistical analysis.

3.4.2 Image Analysis

Visual distinction between starch and protein texture was evident in scanning electron micrographs of developed, partially developed (controlled shear and extensional deformation), and undeveloped soft white and hard red wheat dough. Image analysis software was used to compare images and quantify relative texture using edge detection and thresholding. All images subjected to numerical image analysis were from samples prepared using the technique involving chemical dehydration in ethanol and critical point drying in CO_2. Results were compared to rheological data of these doughs tested on the Haake RS100 rheometer.

Numerical digital image analysis software was used to quantify relative protein texture present in SEM images. The Optimas 4.10 (Optimas Corporation, Edmonds, WA) software program contains image processing tools which can be used to assess texture edges. The software program was loaded onto a computer interfaced with a black and white frame grabber. Before the scanning electron micrographs were analyzed, TIF images having a known amount of black and white area were used to calibrate processing tools in the software program. After calibration, micrographs in standard TIF format were loaded into the program and displayed on the frame grabber.

The Sobel operator is a gradient operator that detects gradients in gray levels and computes an angle indicating the direction and steepness of maximum gray level change. A SEM image was loaded onto the computer and displayed on the frame grabber. The

Sobel operator defined the edges in the image related to protein texture and changed the image to a gray level edge intensity image. Figures 3.1a and 3.2a are original SEM images that appear on the frame grabber screen. Figures 3.1b and 3.2b illustrate the gray level edge intensity detected by the Sobel operator for developed and undeveloped soft wheat dough, respectively. A pixel count routine analyzed each pixel and determined if the value was within the predefined threshold. Pixel values between 129 and 255 (inclusive) were used to compute a protein texture value percentage (PTV%):

$$\mathbf{PTV\%} = \frac{\mathbf{number\ of\ texture\ pixels} \geq \mathbf{129}}{\mathbf{total\ number\ of\ pixels}} \times \mathbf{100\%} \qquad [3.3]$$

The more texture detected in SEM, the greater the protein texture value. PTV% results were compared to rheological data.

3.4.3 Rheograms

Rheograms of dynamic moduli (G*, G′, G″) versus frequency were obtained from oscillatory testing using the Haake RS100 rheometer. Each data set was replicated at least four times for each sample treatment (soft and hard wheat dough: developed, shear deformation, extensional deformation, undeveloped) and statistical analyses were performed to model the rheological behavior of the dough. Linear viscoelastic behavior was plotted over a frequency range of 0.1 to 100 rad/s, and the structural effect of each deformation was compared for soft white and hard red wheat dough.

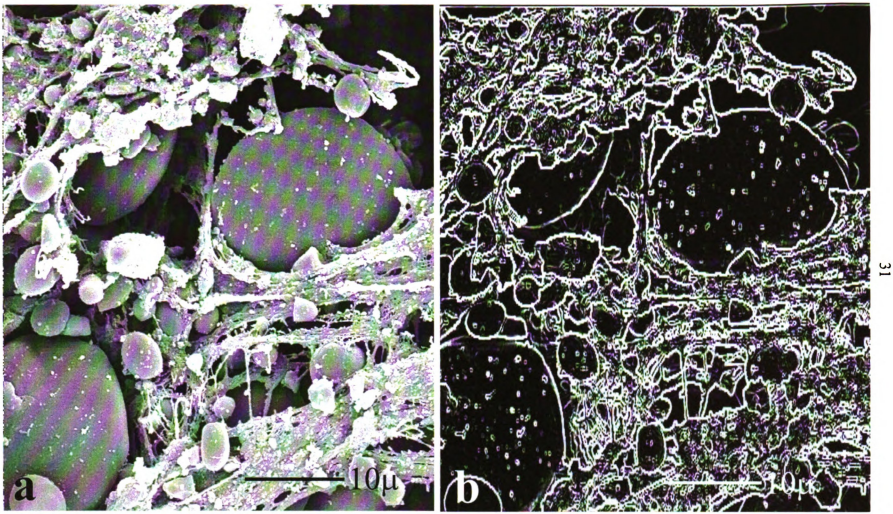

Figure 3.1a. Developed soft white winter wheat dough. 3.1b. Edges of developed soft white winter wheat dough detected by the Sobel operator.

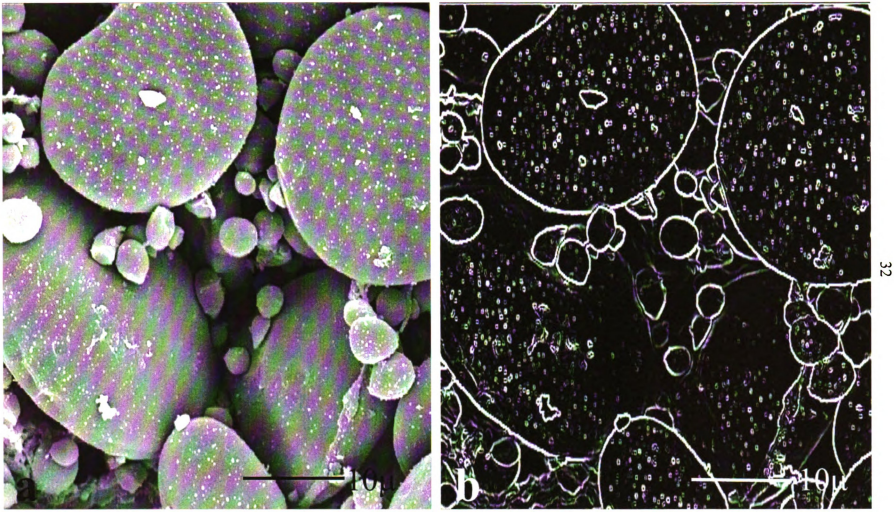

Figure 3.2a. Undeveloped soft white winter wheat dough. 3.2b. Edges of undeveloped soft white winter wheat dough detected by the Sobel operator.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Scanning Electron Microscopy Sample Preparation Techniques

The purpose in testing various specimen preparation methods was to determine which technique gave the best results for visual image analysis by distinguishing protein and starch. The most common preparation method for biological samples is chemical fixation and dehydration, followed by critical point drying. Micrographs of soft white winter wheat developed dough not fixed, or fixed with 4% buffered glutaraldehyde, followed by ethanol dehydration and critical point drying with CO_2, are shown in Figure 4.1 and Figure 4.2, respectively. Figure 4.1 shows protein continuity and definite contrast between starch and protein. The continuous protein is not evident in Figure 4.2, and the contrast between starch and protein is poor. Undeveloped dough (Figure 4.3) chemically prepared without fixation illustrates a homogeneous, undeveloped, hydrated dough that formed with the absence of mechanical energy input.

It was difficult to distinguish developed freeze-dried dough samples from undeveloped freeze-dried dough samples shown in Figures 4.4a and 4.4b, respectively.

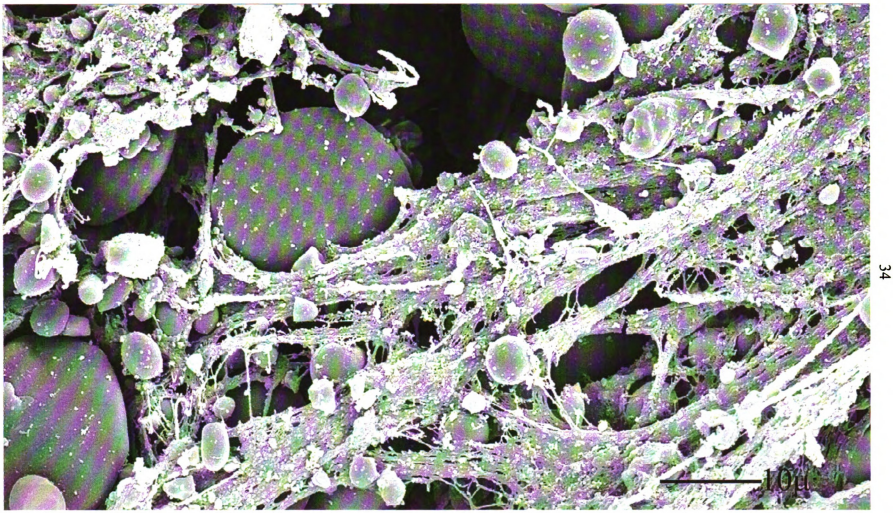

Figure 4.1. Developed soft white winter wheat dough chemically dehydrated followed by critical point drying.

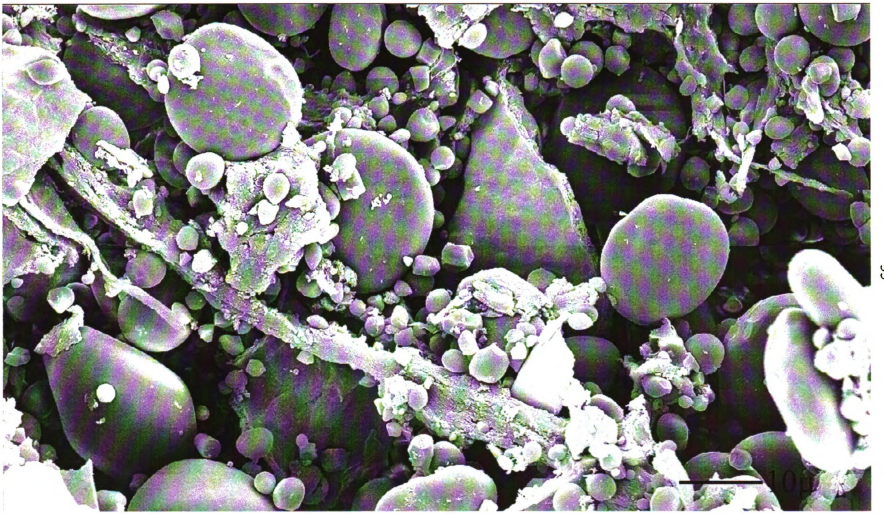

Figure 4.2. Developed soft white winter wheat dough chemically fixed and dehydrated followed by critical point drying.

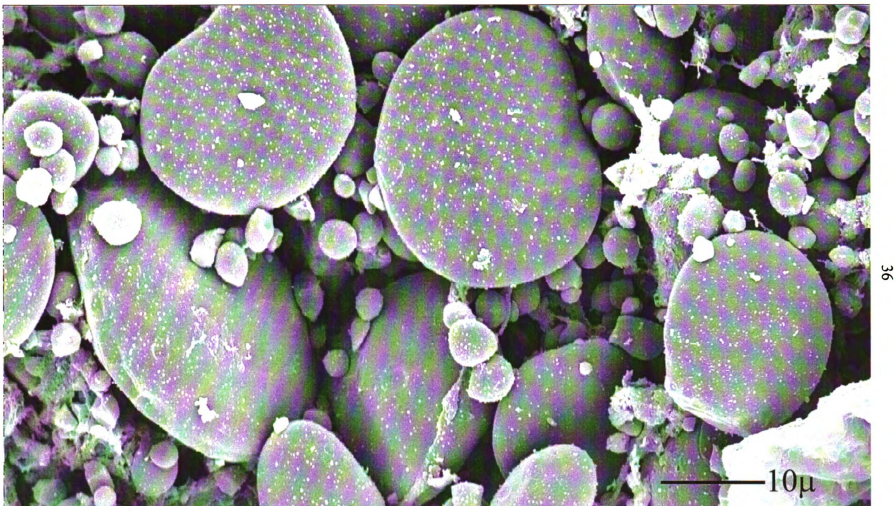

Figure 4.3. Undeveloped soft white winter wheat dough chemically dehydrated followed by critical point drying.

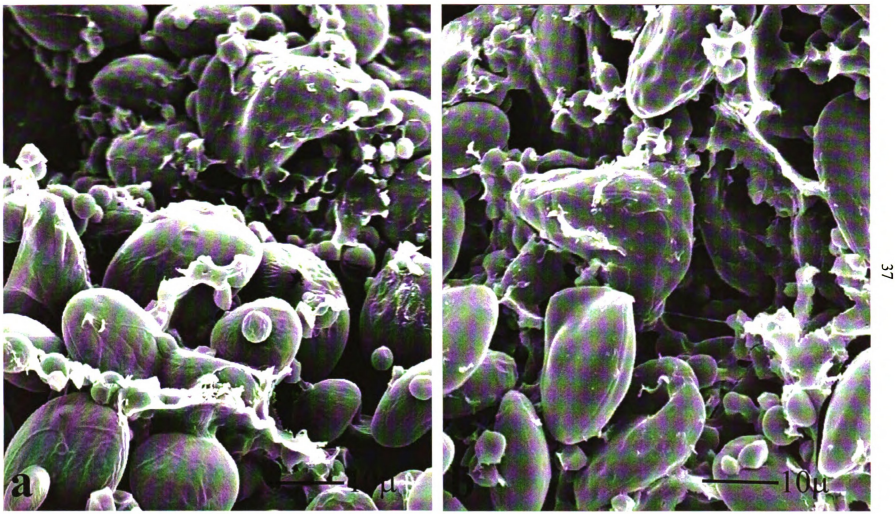

Figure 4.4a. Freeze-dried developed soft white winter wheat dough. 4.4b. Freeze-dried undeveloped soft white winter wheat dough.

Although freeze drying allows sublimation of H_2O occurring in the absence of surface tension, ice crystal formation may damage specimen morphology.

Figure 4.5a shows a developed dough sample dehydrated in a vacuum desiccator for 24 hours, and Figure 4.5b is an undeveloped dough sample prepared in the same manner. In these images, protein envelopes the starch particles, covering them like a blanket making it difficult to distinguish starch and protein based on contrast. Furthermore, samples were not fully dehydrated which caused a low vacuum in the microscope, resulting in a grainy image texture that lacked good clarity.

It was difficult to distinguish protein and starch for both developed and undeveloped samples subjected to cryogenic preparation. The microscopic images produced by this method could only be obtained with a Polaroid camera and saved as an analog image on a negative, making numerical digital image analysis difficult. Furthermore, cryogenic preparation required a significant amount of time for an individual sample.

The effect of sample fracturing to expose the inner surfaces was also studied. Fracturing was done by shattering a dough sample with sharply pointed tweezers, or by cutting the dough sample with a razor blade. The tweezer method distorted the proteinaceous material surrounding the starch disconnecting the protein network. It appears that the force of fracturing, causes weakly bound proteins to shatter (Figure 4.4). This trend was found in both developed and undeveloped dough samples subjected to chemical fixation with glutaraldehyde, freeze drying, vacuum desiccation, and cryogenic

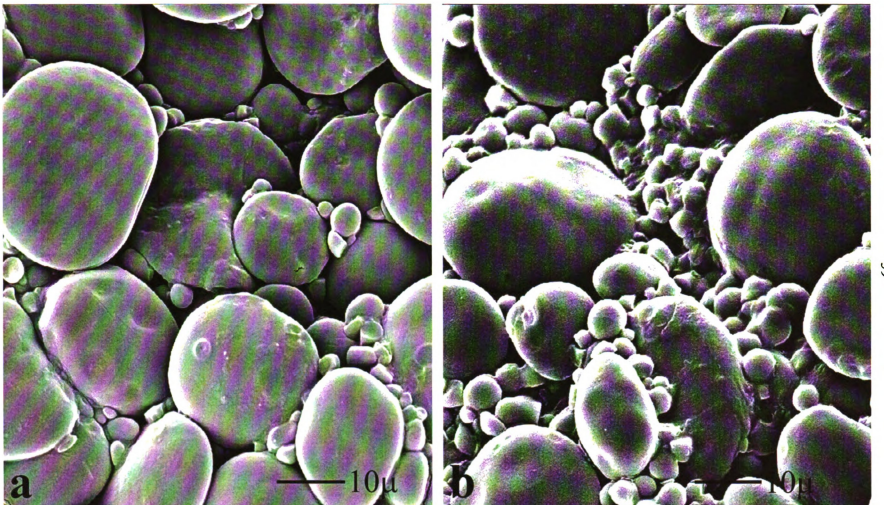

Figure 4.5a. Vacuum-desiccated developed soft white winter wheat flour. 4.5b. Vacuum-desiccated undeveloped soft white winter wheat dough.

preparation techniques. Slicing samples with a razor blade at freezing temperatures also produced unacceptable results because the samples appeared sliced and lacked contrast.

The contrast in SEM images created by chemical dehydration and critical point drying produced the best visual distinction between starch and protein in wheat dough samples. Dough preparation was the least time consuming for freeze drying and vacuum desiccation methods; however, visual distinction between developed and undeveloped dough was difficult. Furthermore, vacuum desiccated samples were not fully dehydrated and produced grainy microscopic images. Cryogenic preparation is advantageous because dough samples are observed as water sublimes; however, this method is the most laborious and only produces analog images.

4.2 Development of the Protein Matrix in Dough

Scanning electron micrographs of soft white and hard red wheat dough revealed significant visual distinctions between developed and undeveloped dough. Figure 4.6 is a micrograph of soft wheat dough developed in the farinograph. The figure shows a weblike protein structure surrounding starch particles. Figure 4.7 illustrates soft wheat undeveloped dough which was prepared in the absence of mechanical energy. The figure shows large round starch particles and minimal protein development. Figure 4.8 is developed hard wheat dough and Figure 4.9 is undeveloped hard wheat dough. Similarly, the developed wheat dough micrograph shows an abundance of starch particles surrounded by a developed protein network while the micrograph for undeveloped dough

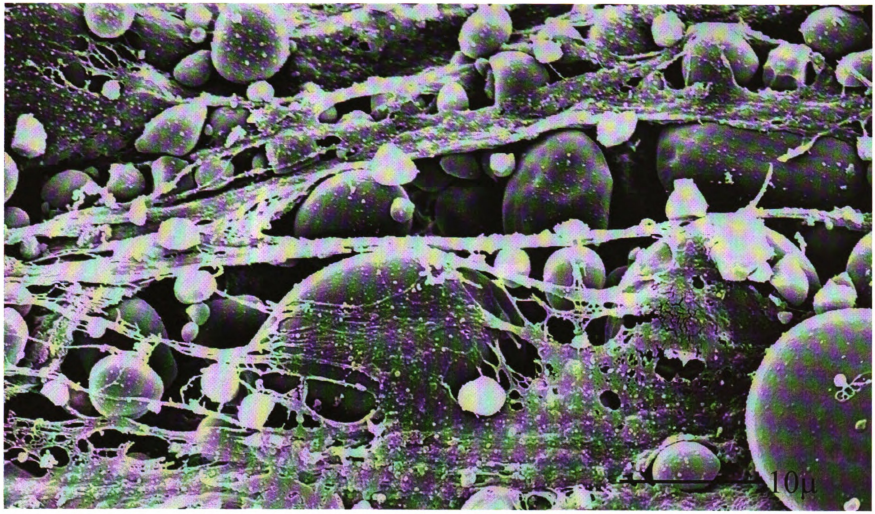

Figure 4.6. Developed soft white winter wheat dough.

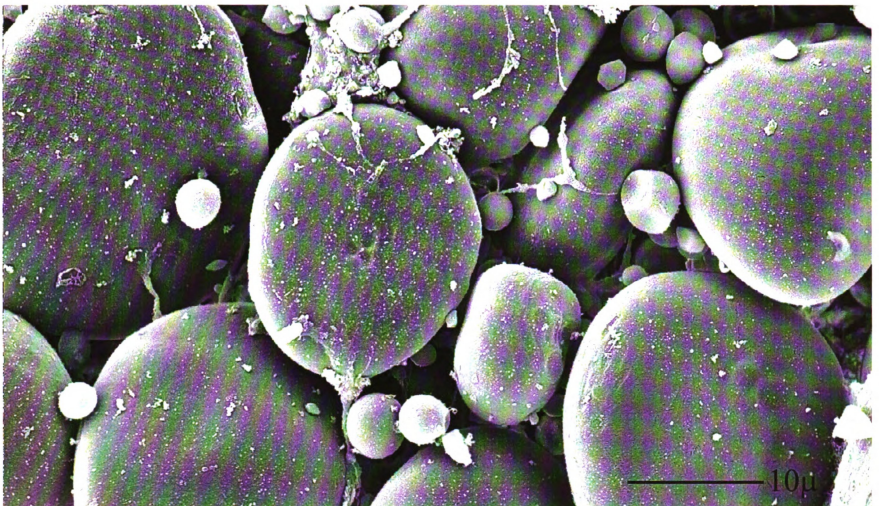

Figure 4.7. Undeveloped soft white winter wheat dough.

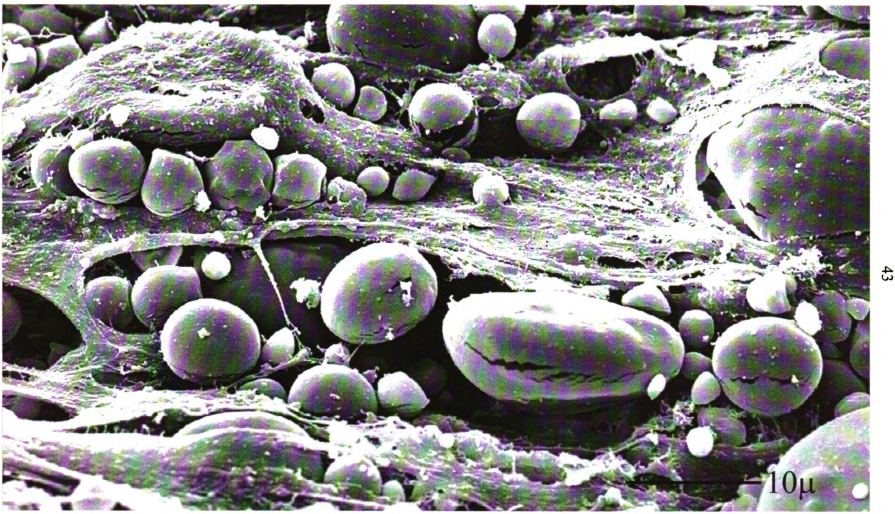

Figure 4.8. Developed hard red winter wheat dough.

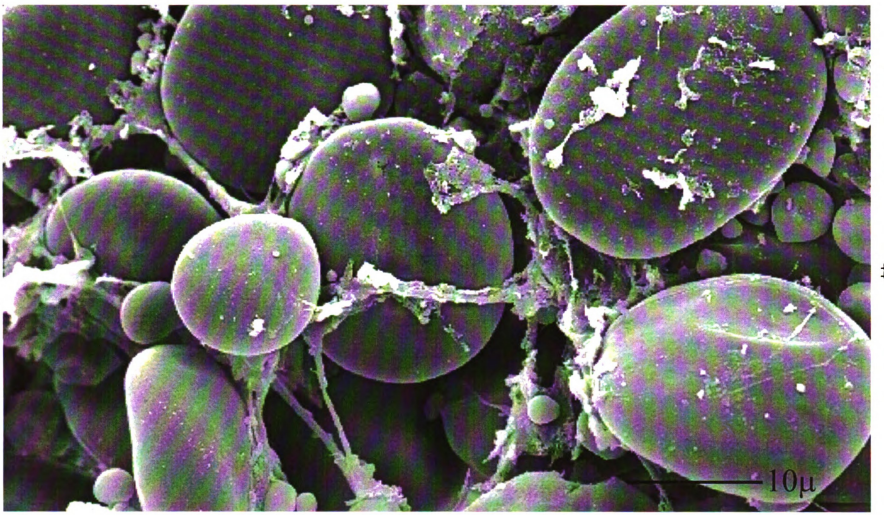

Figure 4.9. Undeveloped hard red winter wheat dough.

shows starch particles with a minimal protein network. Figures A.1 to A.8 are additional images of developed and undeveloped, soft and hard wheat dough.

It was not as easy to visually distinguish scanning electron micrographs of soft wheat dough from hard wheat dough. Generally, hard wheat dough contains more protein than soft wheat dough; however, this generalization could not be verified from simple visual observation of micrographs.

4.2.1 Dough Development in Shear Flow

Images of soft and hard wheat dough subjected to 785% shear deformation were also taken in the scanning electron microscope. Figure 4.10 is a micrograph of soft wheat dough and Figure 4.11 is of hard wheat dough. In both images, protein forms a weblike network with the starch particles. Since samples were prepared perpendicular to the shear field, the protein network appears to form in the direction of the shear force. Figures A.9 to A.12 are additional images of soft and hard wheat dough subjected to shear deformation.

4.2.2 Dough Development in Extensional (Biaxial) Flow

Soft and hard wheat dough was partially developed in extensional deformation using squeezing flow displacement. An image of extensionally developed soft wheat dough is found in Figure 4.12 and hard wheat dough in Figure 4.13. Figures A.13 to A.16 are additional images of biaxially strained soft and hard wheat dough. It was not easy to distinguish soft wheat dough from hard wheat dough; however, protein

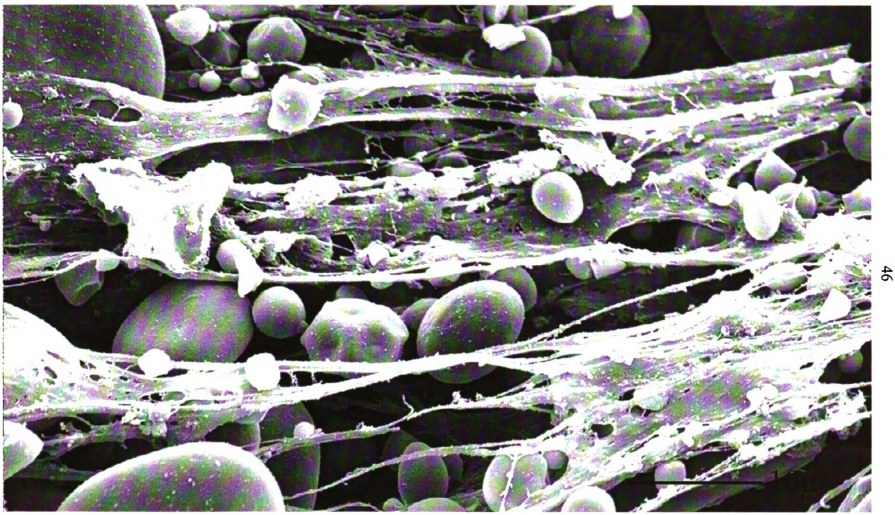

Figure 4.10. Soft white winter wheat dough subjected to shear deformation.

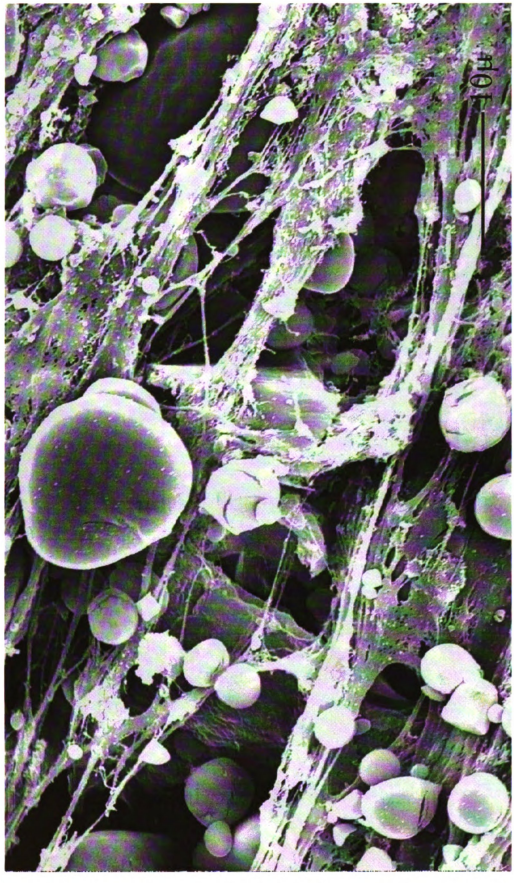

Figure 4.11. Hard red winter wheat dough subjected to shear deformation.

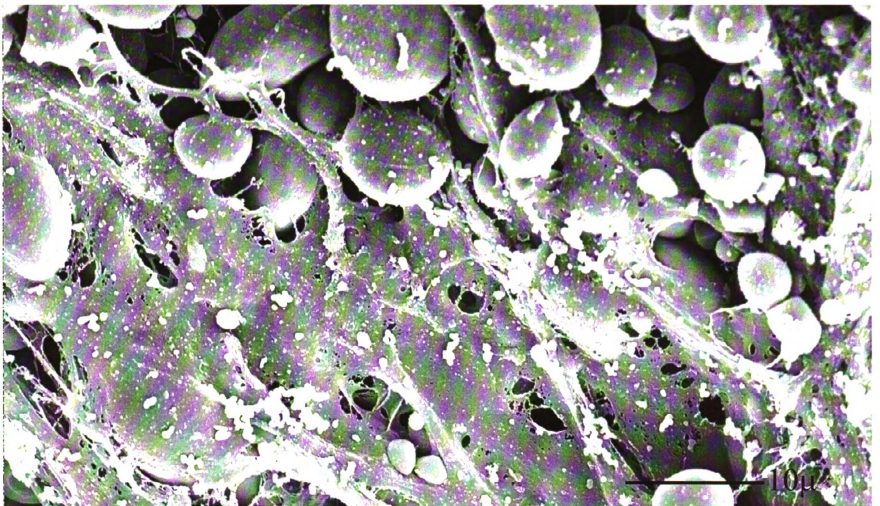

Figure 4.12. Soft white winter wheat dough subjected to extensional deformation.

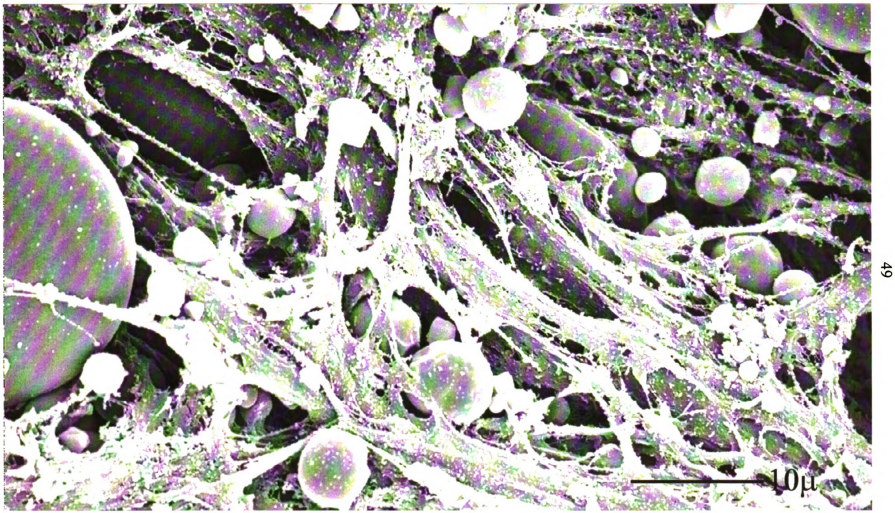

Figure 4.13. Hard red winter wheat dough subjected to extensional deformation.

development characterized by squeezing flow was evident. Protein development from extensional deformation appears to cover more surface area compared to developed and undeveloped wheat dough. The protein does not have a "stringy" appearance and covers the starch particles like a blanket.

4.2.3 Dynamic Rheological Properties after Shear and Extensional (Biaxial) Deformation

Dynamic rheological measurements were used to determine the structural characteristics of soft and hard wheat dough. Developed and undeveloped samples, as well as samples subjected to shear and extensional deformation, were tested on the Haake RS100 rheometer. Dynamic moduli (G*, G′, G″) described the linear viscoelastic behavior of dough at constant shear stress of 50 Pa over a frequency range of 0.1 to 100 rad/s. At least four data sets were taken of soft and hard wheat dough at each deformation, and data from these replicates were pooled because there was no statistical difference between curves.

Dynamic rheological behavior of wheat dough followed a power law model:

$$G^* = a\omega^{b} \qquad [4.1]$$

where the constants, a and b, were determined from linear regression analysis after a logarithmic transformation of the raw data. Dynamic variables G′ and G″ also showed power law behavior with respect to frequency. Tables 4.1-4.3 contain values of a and b from regression analysis for G*, G′ and G″, respectively.

Table 4.1. Power law constants for G*, determined from linear regression analysis.

development	Soft White Winter Wheat Dough			Hard Red Winter Wheat Dough		
	a	b	R^2	a	b	R^2
developed	8905	0.27	0.90	20320	0.23	0.97
extensional deformation	6415	0.27	0.72	10490	0.23	0.94
shear deformation	2263	0.40	0.83	5758	0.32	0.98
undeveloped	1326	0.42	0.88	5305	0.27	0.87

Table 4.2 Power law constants for G′, determined from linear regression analysis.

development	Soft White Winter Wheat Dough			Hard Red Winter Wheat Dough		
	a	b	R^2	a	b	R^2
developed	7806	0.28	0.89	18682	0.23	0.97
extensional deformation	5484	0.28	0.71	9584	0.24	0.94
shear deformation	1706	0.40	0.84	4581	0.34	0.97
undeveloped	973	0.40	0.84	4749	0.26	0.86

Table 4.3 Power law constants for G″, determined from linear regression analysis.

	Soft White Winter Wheat Dough			Hard Red Winter Wheat Dough		
development	**a**	**b**	**R^2**	**a**	**b**	**R^2**
developed	4246	0.27	0.91	7909	0.22	0.95
extensional deformation	3299	0.25	0.71	3245	0.29	0.97
shear deformation	1476	0.38	0.81	4214	0.22	0.93
undeveloped	889	0.44	0.91	2358	0.28	0.90

Data analysis showed G′ (storage modulus) to be consistently greater than G″ (loss modulus), indicating dough to behave more as a solid than a liquid over the entire frequency range studied. Moduli were greatest for developed dough, followed by dough subjected to extensional deformation and shear deformation. As expected, undeveloped dough had the smallest moduli. Figures 4.14 and 4.15 are rheograms showing the structural characteristics of soft and hard wheat dough, respectively. Error bars to indicate the 95% confidence interval were very small and could not be clearly displayed in the figures. Figures 4.14 and 4.15 clearly indicate independent structural development of wheat dough in which the type of deformation or development are unrelated. An F-test further attested that the four models in Figures 4.14 and 4.15 do not correlate with each other. Figures 4.14 and 4.15 show greater structural development for extensional deformation than shear deformation even though the strain percentage was 80.5% and 1570% for extensional strain and shear strain, respectively. The difference in structure indicates greater molecular alignment and disulfide bonding in extensional flow fields compared to shear flow fields.

Campos (1996) also tested structure development for soft white and hard red, developed and undeveloped wheat dough; however, moduli in the current study were lower. The difference was expected because enzymatic activity increased over time as indicated by the falling number test, and decreased viscoelasticity. Image analysis techniques identified a protein texture value allowing for comparison with structural development determined from rheological measurements.

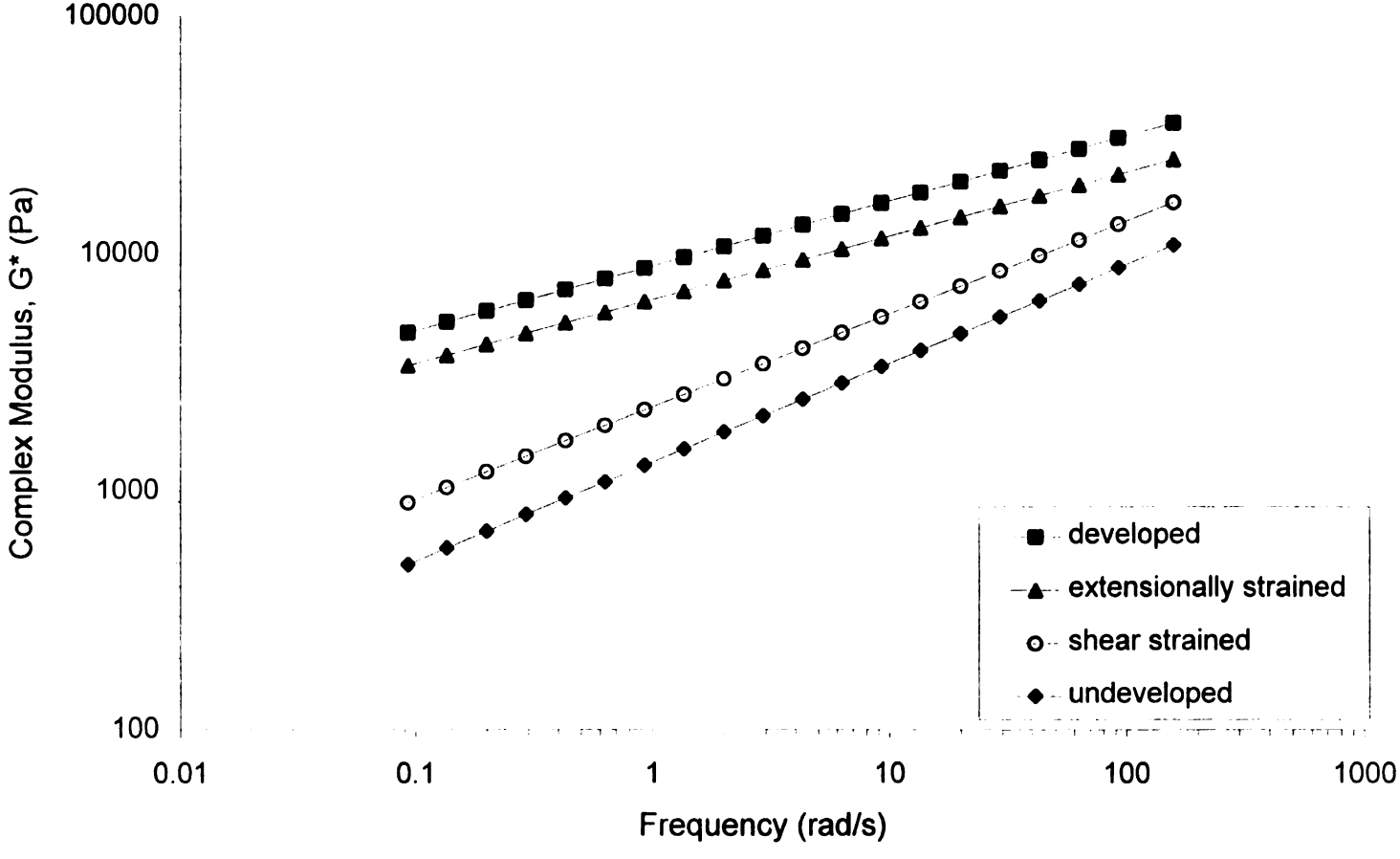

Figure 4.14. Oscillatory behavior of developed, extensionally strained, shear strained, and undeveloped soft white winter wheat dough.

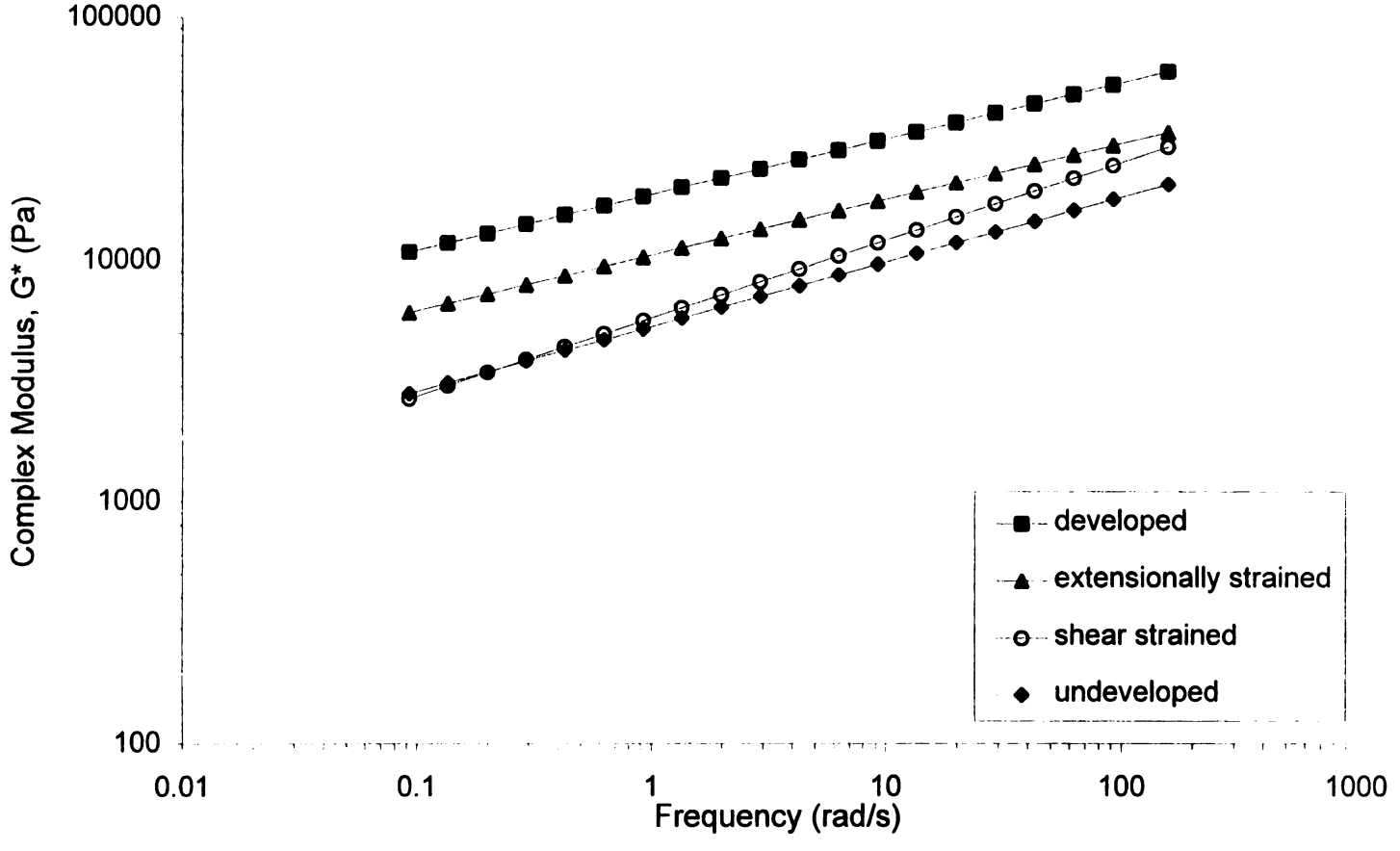

Figure 4.15. Oscillatory behavior of developed, extensionally strained, shear strained, and undeveloped hard red winter wheat dough.

Table 4.4 gives the average protein texture value percentage (PTV%) and standard deviation determined from image analysis of soft and hard wheat dough. A comparison of the means and standard deviations in Table 4.4 statistically indicates that both shear and extensional deformation treatments resulted in similar protein development compared to developed dough. However, rheological data indicate that the type of deformation results in different levels of structural development. Reasons the results from image analysis do not correspond with rheological data include daily contrast and brightness variability in the microscope, and microscope contamination by volatile materials. Furthermore, visual observation of extensionally strained samples do not reveal distinct edges in the protein webbing, rather the protein appears flat. The Sobel operator would not detect these edges and subsequently, the protein texture value calculated for the image would be lower.

Figures 4.16 (a-d) and 4.17 (a-d) allow a visual comparison of the effect of deformation type on soft and hard wheat dough, respectively. Images of developed wheat dough have a protein web in which edges are clearly detected and undeveloped dough images show less protein texture. Images of extensionally strained dough show protein development in the x and y direction whereas, protein development is primarily in the x direction in shear strained dough samples. Protein development in shear strained samples is clearly detectable by the Sobel operator.

Table 4.4 Protein texture values from numerical digital image analysis.

development	Soft White Winter Wheat Dough		Hard Red Winter Wheat Dough	
	PTV%	standard deviation	PTV%	standard deviation
developed	35.3	5.7	31.9	5.7
extensional deformation	28.0	7.0	32.8	7.0
shear deformation	32.3	4.6	32.6	5.7
undeveloped	23.4	3.8	18.8	6.6

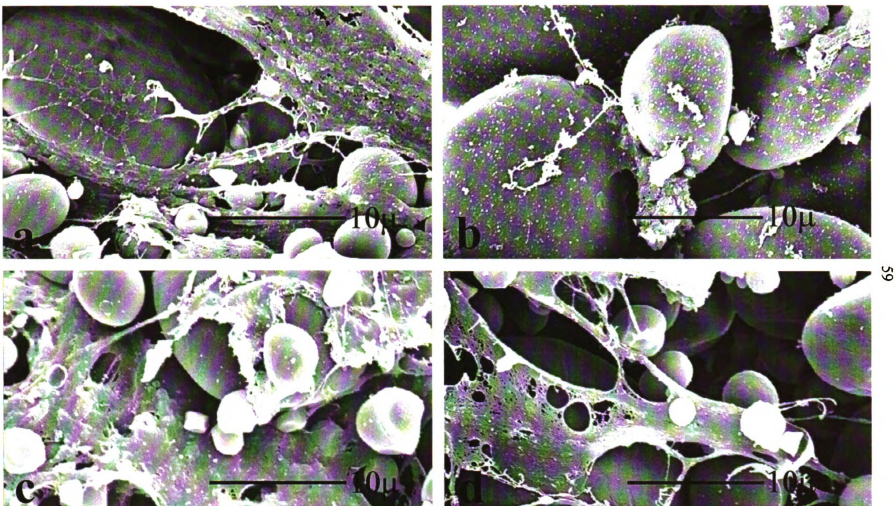

Figure 4.16. Soft white winter wheat dough. a. Developed. b. Undeveloped. c. Extensional deformation. d. Shear deformation.

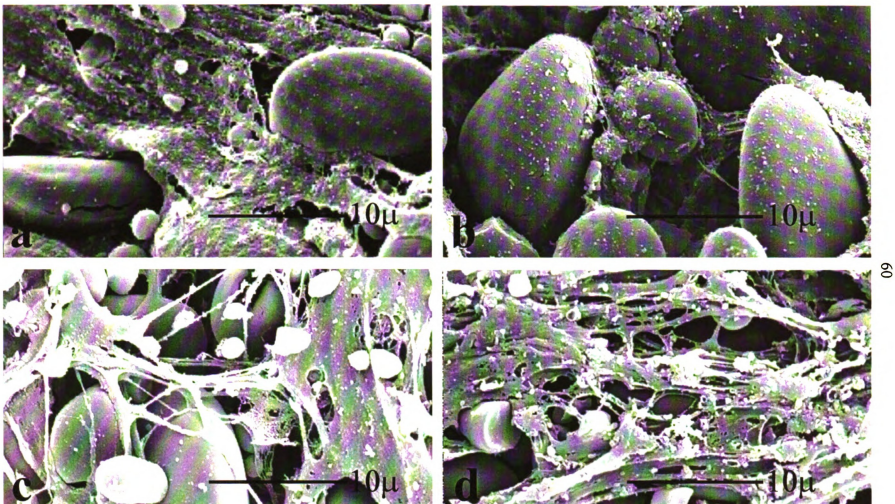

Figure 4.17. Hard red winter wheat dough. a. Developed. b. Undeveloped. c. Extensional deformation. d. Shear deformation.

CHAPTER V

SUMMARY AND CONCLUSIONS

The undeveloped dough concept of Campos et al. (1996) achieved a uniform distribution of water in a wheat dough system by combining flour and ice particles. The advantage of an undeveloped dough matrix is that the contribution of shear and extensional flow fields can be separately analyzed in subsequent controlled deformation experiments. In traditional instruments such as the farinograph and mixograph, shear and extensional deformation are combined, but the relative contribution of each flow field is undefined.

In this study, the development of soft white and hard red winter wheat dough was controlled. Four development treatments were analyzed: fully developed, controlled shear deformation, controlled extensional (biaxial) deformation, undeveloped. Shear and extensional deformation were controlled between parallel plate sensors of a Haake RS100 Controlled Stress Rheometer. Once samples were partially developed, either the dynamic rheological behavior was characterized, or the sample was prepared for imaging in a scanning electron microscope.

Curves describing viscoelastic behavior of wheat dough were fit to a power law model using linear regression analysis. Rheological testing revealed that G′ was consistently greater than G″ over the frequency range of 0.1 to 100 rad/s, indicating that the behavior of wheat dough was more solid-like than liquid-like. Rheograms further depicted greater viscoelasticity for developed doughs, followed by samples subjected to extensional deformation and shear deformation, respectively. Undeveloped samples showed the lowest moduli, reflecting a low level of structural development. A statistical comparison of regression lines clearly indicated significant differences between the developed, shear strained, extensionally strained and undeveloped dough models.

Scanning electron microscopy is a powerful method for visualizing wheat dough samples and investigating structural development related to rheological data. Dough samples were submerged in a graded ethanol series, critical point dried, and sputter coated with evaporated gold particles to create a conductive surface. Although four other preparation methods were investigated, this preparation technique provided best visual distinction between starch and protein for numerical digital image analyses.

Numerical digital image analysis was used to quantitatively determine protein texture values in dough images. A Sobel operator was used as an edge detector. After the Sobel operator passed through the image, processing tools computed the percentage of protein texture related pixels within a predefined threshold. Statistical analysis of the protein percentages showed there was no significant difference in protein texture between

developed, shear strained, and extensionally strained wheat dough. There was however, a difference between developed wheat dough and undeveloped wheat dough. Although it was difficult to select and generate consistent images in the microscope, numerical image analysis is an excellent tool for investigating the physical structure of wheat dough.

Based on the results of this study, the following conclusions are drawn:

1. Shear and extensional deformation can be controlled using undeveloped dough as the starting point for induced deformation. Shear deformation was controlled using serrated parallel plates attached to the Haake RS100 rheometer. Samples were sheared until a breaking strain was obtained. Biaxial deformation was also controlled using the rheometer by placing samples between parallel plates and squeezing them to a predetermined extensional strain. Both techniques to control deformation, and further investigate the influence of deformation on dough development, were successful.

2. Scanning electron micrographs provide meaningful information about the nature of dough deformation, and can be used to investigate the structural development of a wheat dough samples. The best method (one that minimizes artifacts and maintains sample integrity) to prepare samples for microscopic analysis involves soaking samples in sequential graded ethanol solutions, critical point drying, and sputter coating with gold. Images prepared in this way revealed a clear network of starch and protein in wheat dough samples.

3. Numerical digital image analysis is a unique tool to determine image texture related to protein development; however, it cannot serve as the only analytical tool in a controlled deformation experiment. The electron microscope is a sensitive instrument that may cause grainy and dark images, due to contamination. Poor images make edge detection difficult using the Sobel operator and subsequently, to calculate the percentage of foreground pixels. Further investigations in this area are necessary.

4. The oscillatory behavior of developed, shear strained, extensionally strained, and undeveloped wheat dough showed the rheological properties of each were statistically different. Fully developed dough exhibited the greatest moduli followed by extensionally strained and shear strained dough samples, respectively. Undeveloped dough exhibited the lowest moduli because protein development was smaller compared to the other deformation treatments.

CHAPTER VI

RECOMMENDATIONS FOR FUTURE RESEARCH

The following recommendations are for further research and potential industry applications:

1. To better distinguish starch and protein, scanning electron micrographs should be compared to laser scanning confocal micrographs of samples containing a protein specific stain. This would clearly distinguish starch from protein and ensure more accurate protein texture values in numerical image analysis.

2. Apply information regarding the structural characteristics of a partially strained dough to optimize engineering design. The protein development of dough as influenced by mixing or sheeting, could be investigated by taking samples at one consistent location to determine optimal protein development, specific to a process or product.

3. Compare baked product properties of shear and extensional deformed dough samples.

4. Compare protein development in wheat dough subjected to the same amount of shear and extensional strain.

5. Compare wheat dough samples subjected to a combination of shear and extensional deformation. Samples could be deformed in an extensional flow field and then deformed in a shear flow field.

6. Compare wheat dough samples strained to various levels of shear and extensional deformation.

7. Study the effects of temperature on partial dough development. Once an optimum temperature is established, prepare products of controlled deformation to determine baking quality.

8. Investigate shear stress effects on samples subjected to shear deformation, as well as the effect of squeezing flow rate on samples subjected to extensional deformation.

9. Develop more sophisticated image analysis algorithms allowing better distinction between large round starch particles and protein webbing.

APPENDICES

APPENDIX A

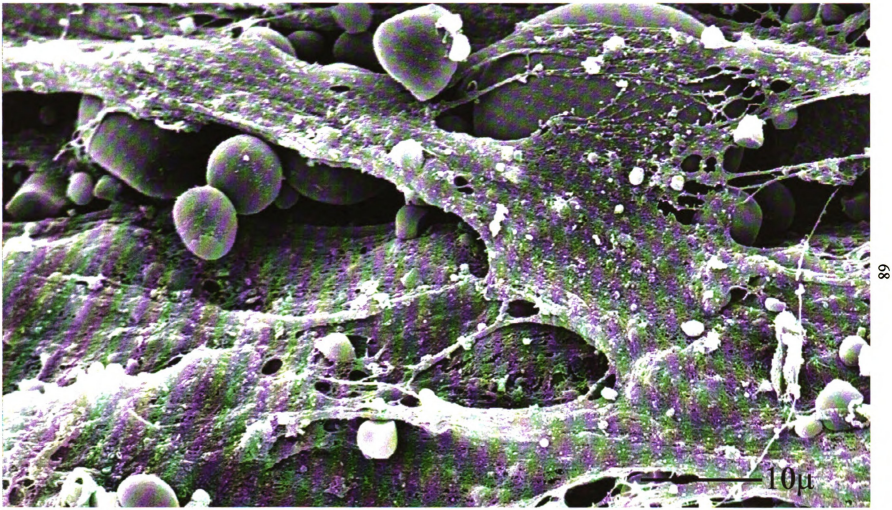

Figure A.1. Developed soft white winter wheat dough.

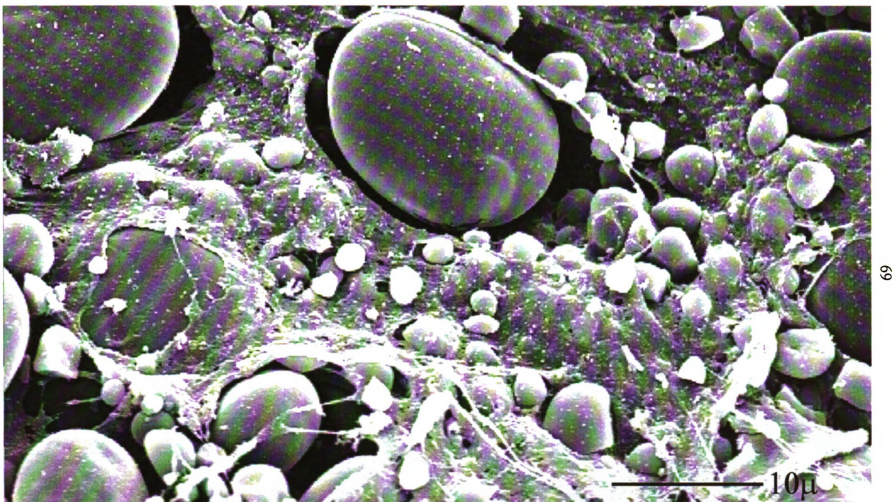

Figure A.2. Developed soft white winter wheat dough.

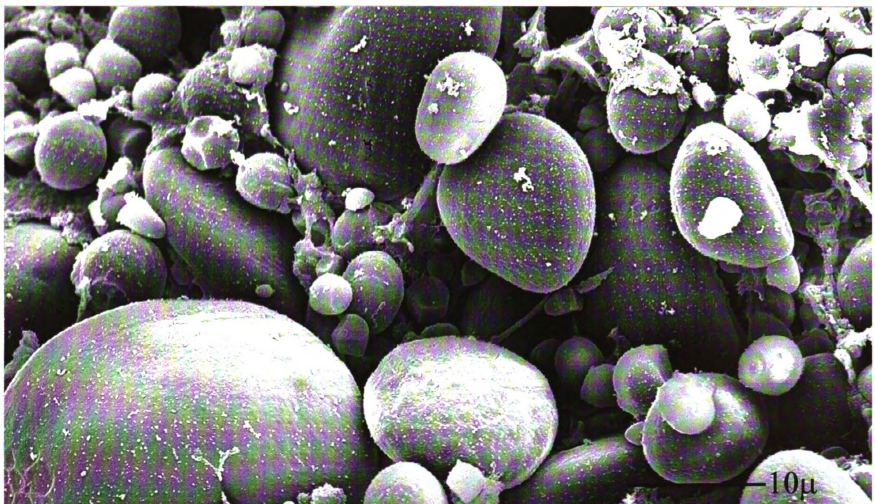

Figure A.3. Undeveloped soft white winter wheat dough.

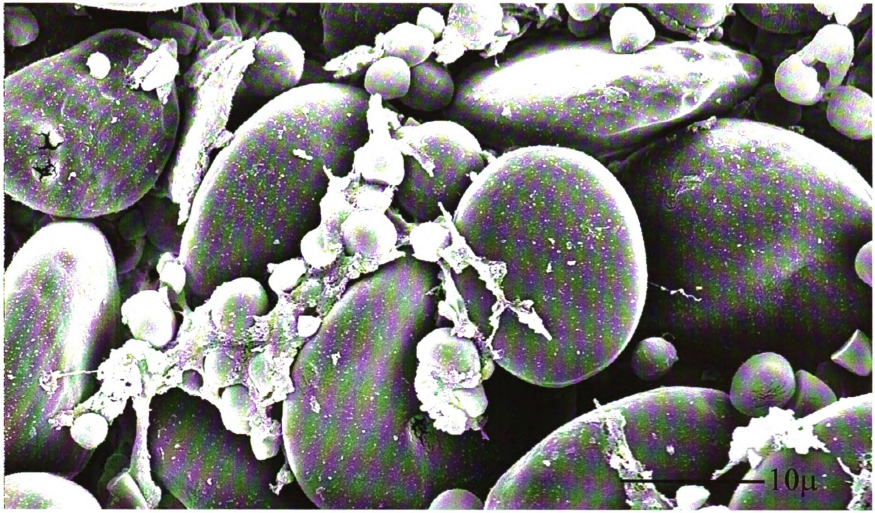

Figure A.4. Undeveloped soft white winter wheat dough.

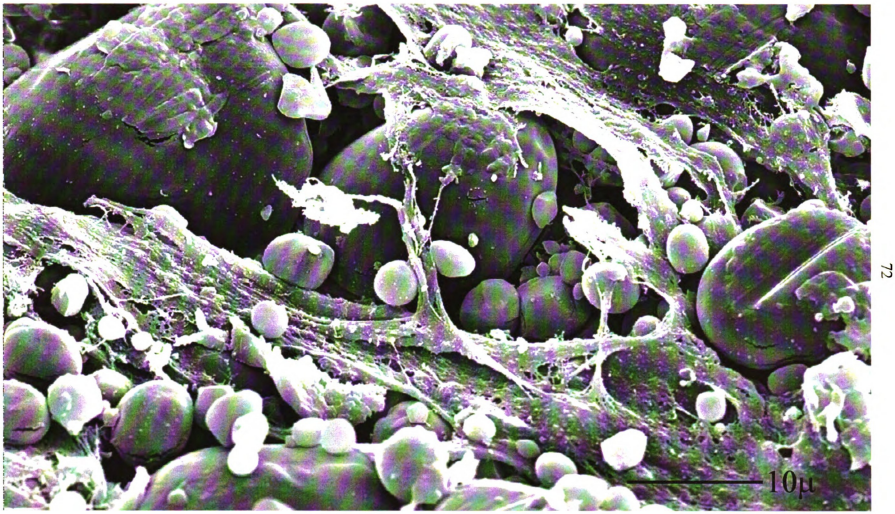

Figure A.5. Developed hard red winter wheat dough.

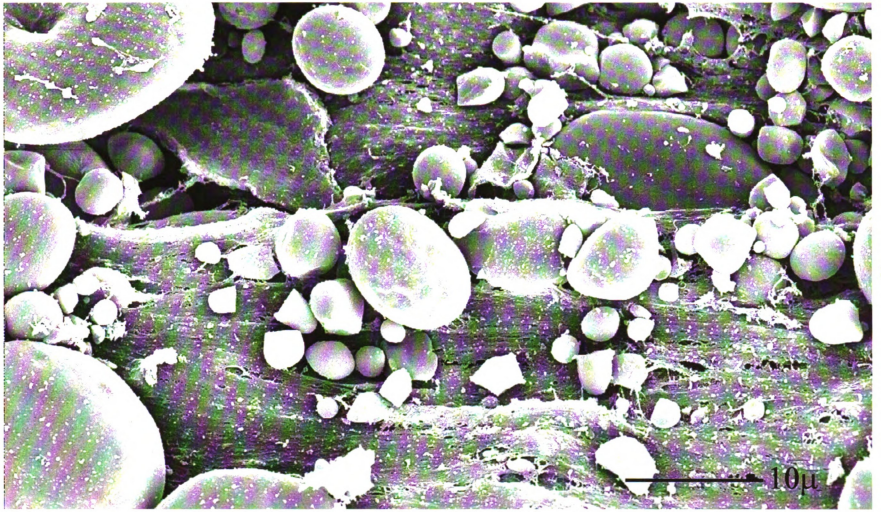

Figure A.6. Developed hard red winter wheat dough.

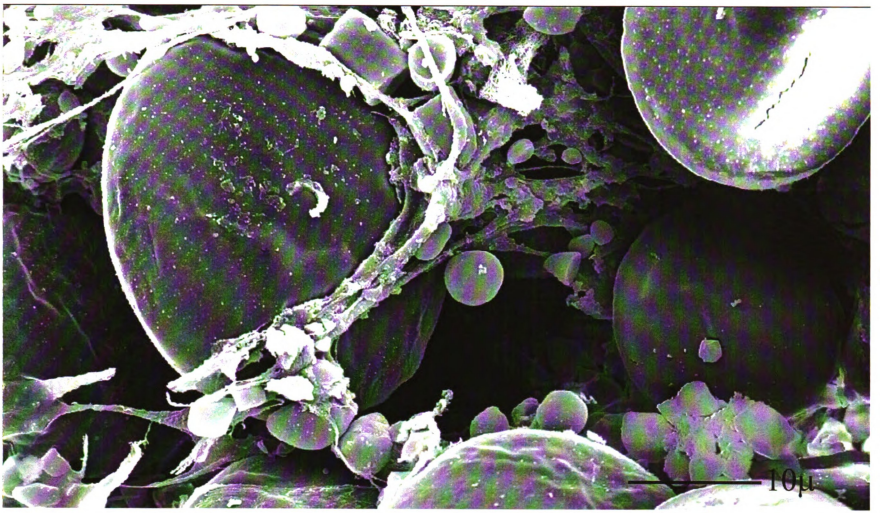

Figure A.7. Undeveloped hard red winter wheat dough.

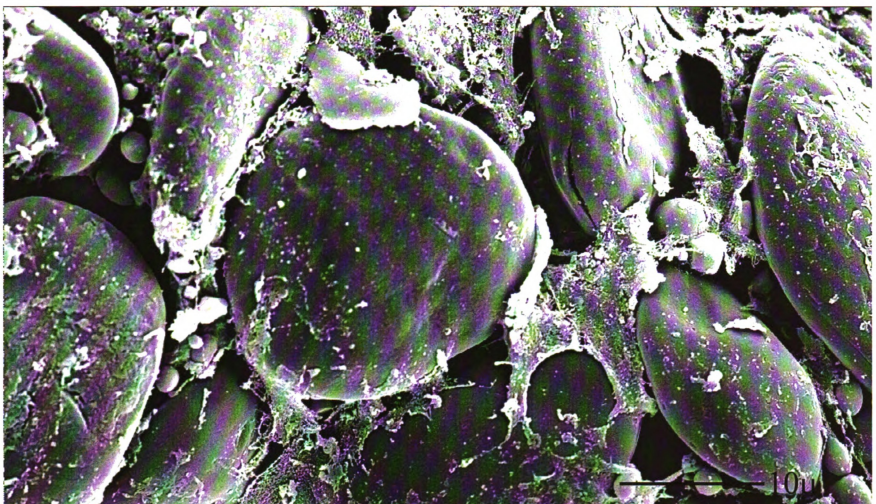

Figure A.8. Undeveloped hard red winter wheat dough.

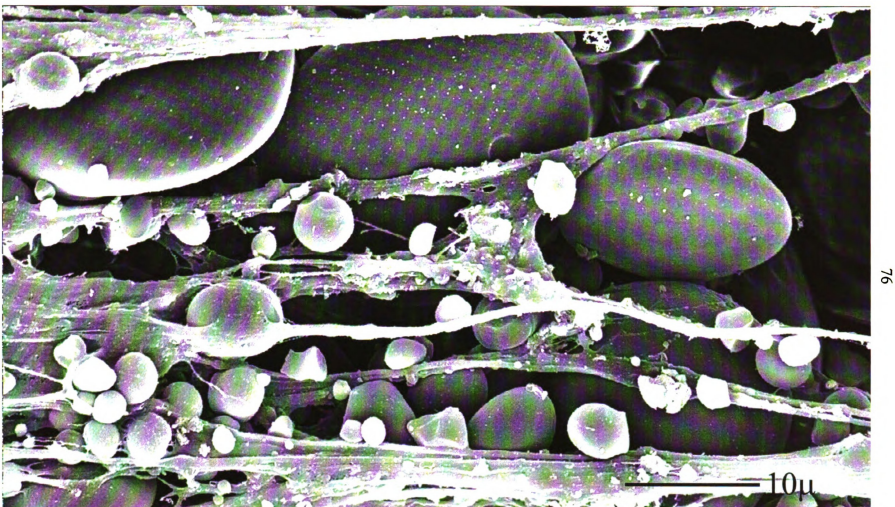

Figure A.9. Soft white winter wheat dough subjected to shear deformation.

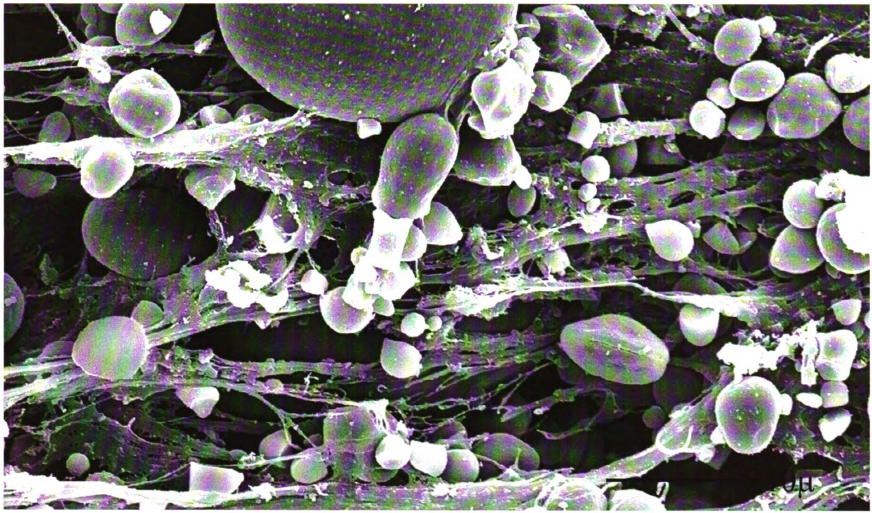

Figure A.10. Soft white winter wheat dough subjected to shear deformation.

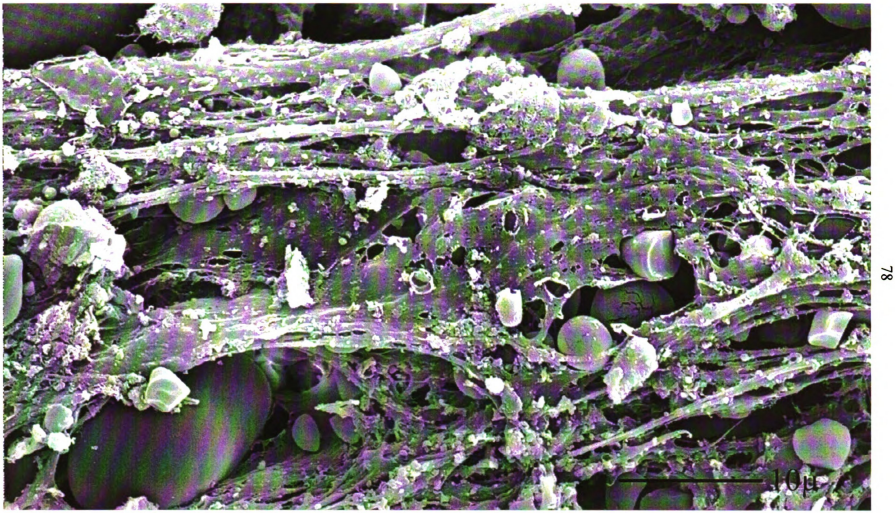

Figure A.11. Hard red winter wheat dough subjected to shear deformation.

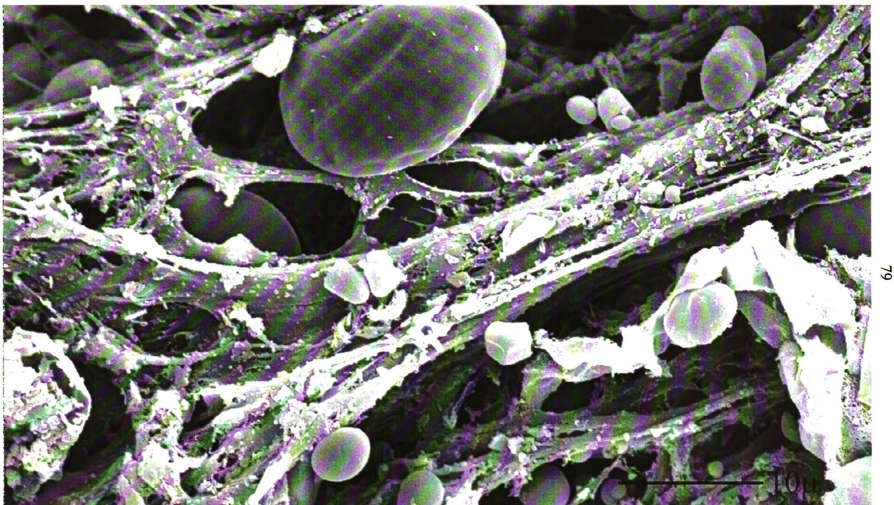

Figure A.12. Hard red winter wheat dough subjected to shear deformation.

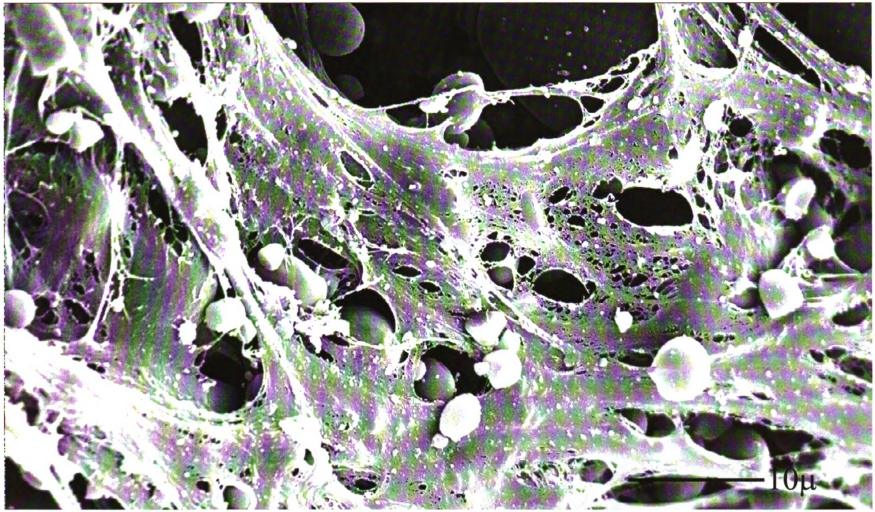

Figure A.13. Soft white winter wheat dough subjected to extensional deformation.

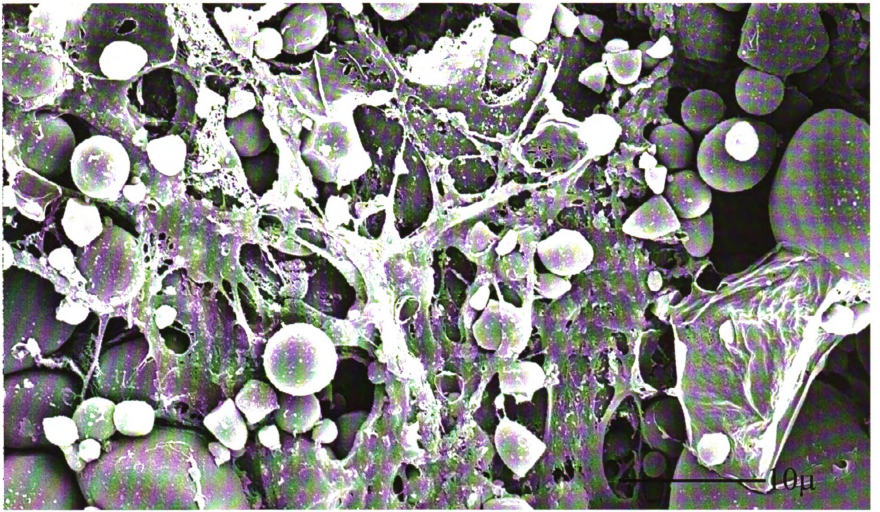

Figure A.14. Soft white winter wheat dough subjected to extensional deformation.

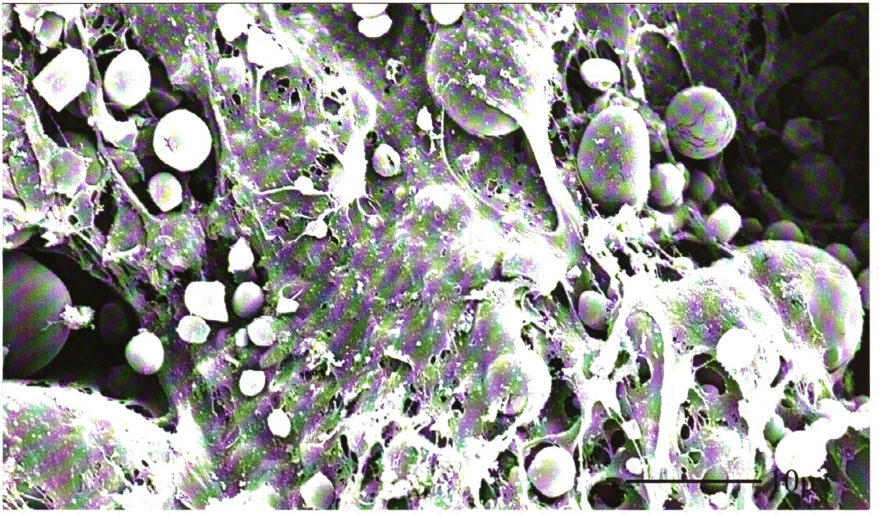

Figure A.15. Hard red winter wheat dough subjected to extensional deformation.

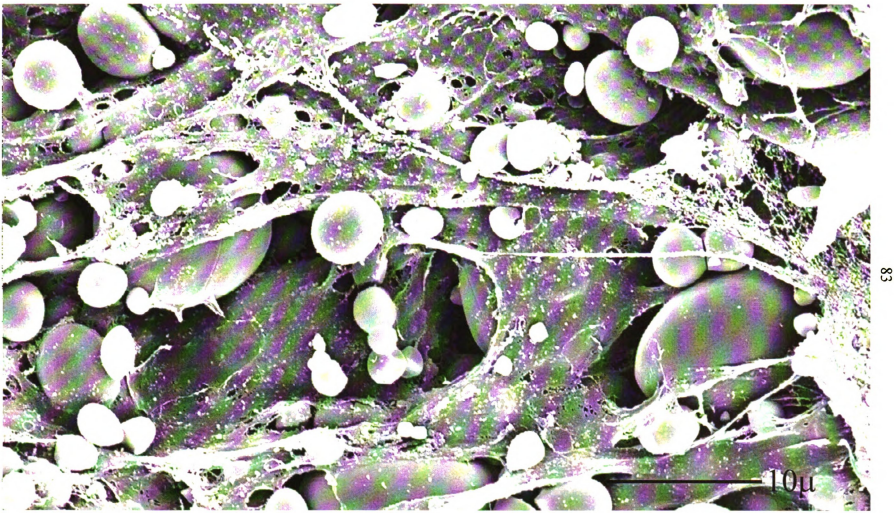

Figure A.16. Hard red winter wheat dough subjected to extensional deformation.

APPENDIX B

Table B.1 Protein texture values from numerical digital image analysis of developed soft white winter wheat dough.

sample	total pixels	PTV pixels	PTV%
1	403456	132258	32.78
2	403456	139869	34.67
3	403456	121153	30.03
4	403456	117627	29.15
5	403456	112164	27.80
6	403456	167579	41.54
7	403456	144660	35.86
8	403456	165976	41.14
9	403456	107596	26.67
10	403456	124453	30.85
11	403456	172144	42.67
12	403456	156510	38.79
13	403456	126792	31.43
14	403456	125437	31.09
15	403456	135288	33.53
16	403456	118356	29.34
17	403456	108023	26.77
18	403456	128830	31.93
19	403456	133216	33.02
20	403456	147153	36.47
21	403456	115473	28.62
22	403456	142027	35.20
23	403456	156032	38.67
24	403456	119680	29.66
25	403456	145929	36.17
26	403456	144392	35.79
27	403456	134054	33.23
28	403456	135320	33.54
29	403456	185970	46.09
30	403456	162505	40.28
31	403456	139832	34.66
32	403456	163388	40.50
33	403456	152965	37.91
34	403456	131659	32.63
35	403456	168386	41.74
36	403456	168426	41.75
37	403456	143637	35.60
38	403456	131901	32.69
39	403456	166743	41.33
40	403456	139052	34.47
41	403456	135552	33.60
42	403456	120322	29.82
43	403456	134285	33.28
44	403456	124451	30.85
45	403456	127474	31.60
46	403456	181670	45.03
47	403456	155532	38.55
48	403456	131972	32.71
49	403456	228302	56.59
50	403456	158001	39.16
		AVERAGE	***35.34***
		STAND. DEV.	***5.68***

Table B.2 Protein texture values from numerical digital image analysis of soft white winter wheat dough subjected to shear deformation.

total pixels	PTV pixels	PTV%
403456	161529	40.04
403456	142062	35.21
403456	121699	30.16
403456	156292	38.74
403456	132406	32.82
403456	141616	35.10
403456	126182	31.28
403456	158224	39.22
403456	131468	32.59
403456	99076	24.56
403456	133236	33.02
403456	146851	36.40
403456	143733	35.63
403456	126369	31.32
403456	127848	31.69
403456	108493	26.89
403456	158228	39.22
403456	152979	37.92
403456	147694	36.61
403456	136136	33.74
403456	165908	41.12
403456	113063	28.02
403456	174353	43.21
403456	179822	44.57
403456	151628	37.58
403456	134296	33.29
403456	139031	34.46
403456	111265	27.58
403456	113180	28.05
403456	124862	30.95
403456	146825	36.39
403456	151936	37.66
403456	121865	30.21
403456	105036	26.03
403456	117936	29.23
403456	134951	33.45
403456	140120	34.73
403456	138151	34.24
403456	135406	33.56
403456	104153	25.82
41	403456	123801	30.69
42	403456	128195	31.77
43	403456	109415	27.12
44	403456	113107	28.03
45	403456	111095	27.54
46	403456	132852	32.93
47	403456	128374	31.82
48	403456	141002	34.95
49	403456	100956	25.02
50	403456	124345	30.82
51	403456	112214	27.81
52	403456	122807	30.44
53	403456	123339	30.57
54	403456	143740	35.63
55	403456	134957	33.45
56	403456	83553	20.71
57	403456	110707	27.44
58	403456	133187	33.01
59	403456	107335	26.60
60	403456	124674	30.90
61	403456	98177	24.33
62	403456	115558	28.64
63	403456	113194	28.06
64	403456	130242	32.28
65	403456	148405	36.78
66	403456	138217	34.26
67	403456	165220	40.95
68	403456	160395	39.76
69	403456	113041	28.02
70	403456	127012	31.48
71	403456	133697	33.14
72	403456	115358	28.59
73	403456	132843	32.93
74	403456	148900	36.91
75	403456	112534	27.89
76	403456	129654	32.14
77	403456	142976	35.44
78	403456	119359	29.58
79	403456	118655	29.41
80	403456	124348	30.82
81	403456	135142	33.50
82	403456	128441	31.84

Table B.2 (Cont'd.)

83	403456	136630	33.86
84	403456	120828	29.95
85	403456	100920	25.01
86	403456	116799	28.95
87	403456	105841	26.23
88	403456	115169	28.55
89	403456	138948	34.44
90	403456	145195	35.99
91	403456	118412	29.35
92	403456	130242	32.28
93	403456	100816	24.99
94	403456	152583	37.82
95	403456	173769	43.07
96	403456	151287	37.50
97	403456	136642	33.87
98	403456	125167	31.02
		AVERAGE	***32.34***
		STAND. DEV.	***4.64***

Table B.3 Protein texture values from numerical digital image analysis of soft white winter wheat dough subjected to extensional deformation.

sample	total pixels	PTV pixels	PTV%
1	403456	96553	23.93
2	403456	65665	16.28
3	403456	48314	11.98
4	403456	81605	20.23
5	403456	97521	24.17
6	403456	125722	31.16
7	403456	111174	27.56
8	403456	134882	33.43
9	403456	118930	29.48
10	403456	145726	36.12
11	403456	133704	33.14
12	403456	143437	35.55
13	403456	125338	31.07
14	403456	155491	38.54
15	403456	90495	22.43
16	403456	86928	21.55
17	403456	78860	19.55
18	403456	100418	24.89
19	403456	148069	36.70
20	403456	95615	23.70
21	403456	84639	20.98
22	403456	71556	17.74
23	403456	97519	24.17
24	403456	110551	27.40
25	403456	93746	23.24
26	403456	129277	32.04
27	403456	86022	21.32
28	403456	122670	30.40
29	403456	109449	27.13
30	403456	128166	31.77
31	403456	116615	28.90
32	403456	109736	27.20
33	403456	110907	27.49
34	403456	117495	29.12
35	403456	97815	24.24
36	403456	108739	26.95
37	403456	129087	32.00
38	403456	134380	33.31
39	403456	129369	32.07
40	403456	134500	33.34
41	403456	99584	24.68
42	403456	120772	29.93
43	403456	98890	24.51
44	403456	91123	22.59
45	403456	87410	21.67
46	403456	150898	37.40
47	403456	108570	26.91
48	403456	132682	32.89
49	403456	223384	55.37
50	403456	127492	31.60
		AVERAGE	***28.00***
		STAND. DEV.	***7.00***

Table B.4 Protein texture values from numerical digital image analysis of undeveloped soft white winter wheat dough.

sample	total pixels	PTV pixels	PTV%
1	403456	93904	23.27
2	403456	95453	23.66
3	403456	91517	22.68
4	403456	80765	20.02
5	403456	73658	18.26
6	403456	106772	26.46
7	403456	90049	22.32
8	403456	95025	23.55
9	403456	94308	23.38
10	403456	95942	23.78
11	403456	113000	28.01
12	403456	104627	25.93
13	403456	98809	24.49
14	403456	89976	22.30
15	403456	95574	23.69
16	403456	95838	23.75
17	403456	98640	24.45
18	403456	85733	21.25
19	403456	64807	16.06
20	403456	86147	21.35
21	403456	92070	22.82
22	403456	80840	20.04
23	403456	78393	19.43
24	403456	107395	26.62
25	403456	87433	21.67
26	403456	76041	18.85
27	403456	95217	23.60
28	403456	104788	25.97
29	403456	128852	31.94
30	403456	92532	22.93
31	403456	86989	21.56
32	403456	103796	25.73
33	403456	80407	19.93
34	403456	105044	26.04
35	403456	71191	17.65
36	403456	136535	33.84
37	403456	131620	32.62
38	403456	108568	26.91
39	403456	82930	20.55
40	403456	125763	31.17
41	403456	82301	20.40
42	403456	89935	22.29
43	403456	85644	21.23
44	403456	74064	18.36
45	403456	69967	17.34
46	403456	97593	24.19
47	403456	102667	25.45
48	403456	107446	26.63
49	403456	94357	23.39
50	403456	83510	20.70
		AVERAGE	***23.37***
		STAND. DEV.	***3.83***

Table B.5 Protein texture values from numerical digital image analysis of developed hard red winter wheat dough.

sample	total pixels	PTV pixels	PTV%
1	403456	138132	34.24
2	403456	143215	35.50
3	403456	152999	37.92
4	403456	141388	35.04
5	403456	157483	39.03
6	403456	118539	29.38
7	403456	128313	31.80
8	403456	130482	32.34
9	403456	151147	37.46
10	403456	171128	42.42
11	403456	128592	31.87
12	403456	97980	24.29
13	403456	119763	29.68
14	403456	101348	25.12
15	403456	114997	28.50
16	403456	136236	33.77
17	403456	110278	27.33
18	403456	118435	29.36
19	403456	128019	31.73
20	403456	105884	26.24
21	403456	96898	24.02
22	403456	92944	23.04
23	403456	124935	30.97
24	403456	114629	28.41
25	403456	122002	30.24
26	403456	91277	22.62
27	403456	83344	20.66
28	403456	116794	28.95
29	403456	129992	32.22
30	403456	156137	38.70
31	403456	139989	34.70
32	403456	109067	27.03
33	403456	108492	26.89
34	403456	151064	37.44
35	403456	111161	27.55
36	403456	109814	27.22
37	403456	93750	23.24
38	403456	152104	37.70
39	403456	137811	34.16
40	403456	137730	34.14
41	403456	116675	28.92
42	403456	143129	35.48
43	403456	136372	33.80
44	403456	149426	37.04
45	403456	148541	36.82
46	403456	122821	30.44
47	403456	166149	41.18
48	403456	189729	47.03
49	403456	157922	39.14
		AVERAGE	***31.89***
		STAND. DEV.	***5.71***

Table B.6 Protein texture values from numerical digital image analysis of hard red winter wheat dough subjected to shear deformation.

sample	total pixels	PTV pixels	PTV%
1	403456	148524	36.81
2	403456	110260	27.33
3	403456	154156	38.21
4	403456	154108	38.20
5	403456	154842	38.38
6	403456	147242	36.50
7	403456	129641	32.13
8	403456	130404	32.32
9	403456	113159	28.05
10	403456	156843	38.87
11	403456	92728	22.98
12	403456	97160	24.08
13	403456	132897	32.94
14	403456	127329	31.56
15	403456	144951	35.93
16	403456	141596	35.10
17	403456	122635	30.40
18	403456	133091	32.99
19	403456	90793	22.50
20	403456	131216	32.52
21	403456	168055	41.65
22	403456	164451	40.76
23	403456	152258	37.74
24	403456	140429	34.81
25	403456	132221	32.77
26	403456	109009	27.02
27	403456	134519	33.34
28	403456	113406	28.11
29	403456	119294	29.57
30	403456	184823	45.81
31	403456	157956	39.15
32	403456	150587	37.32
33	403456	188811	46.80
34	403456	142912	35.42
35	403456	109382	27.11
36	403456	120585	29.89
37	403456	119140	29.53
38	403456	114959	28.49
39	403456	99145	24.57
40	403456	129947	32.21
41	403456	92728	22.98
42	403456	97160	24.08
43	403456	132897	32.94
44	403456	127329	31.56
45	403456	144951	35.93
46	403456	141596	35.10
47	403456	122635	30.40
48	403456	133091	32.99
49	403456	90793	22.50
50	403456	131216	32.52
		AVERAGE	***32.62***
		STAND. DEV.	***5.74***

Table B.7 Protein texture values from numerical digital image analysis of hard red winter wheat dough subjected to extensional deformation.

sample	total pixels	PTV pixels	PTV%
1	403456	133451	33.08
2	403456	151252	37.49
3	403456	129732	32.16
4	403456	142214	35.25
5	403456	144676	35.86
6	403456	131427	32.58
7	403456	153798	38.12
8	403456	137604	34.11
9	403456	135925	33.69
10	403456	131158	32.51
11	403456	130511	32.35
12	403456	117649	29.16
13	403456	119887	29.72
14	403456	129875	32.19
15	403456	137830	34.16
16	403456	132660	32.88
17	403456	131870	32.69
18	403456	136743	33.89
19	403456	135460	33.57
20	403456	192075	47.61
21	403456	124533	30.87
22	403456	146641	36.35
23	403456	163126	40.43
24	403456	164000	40.65
25	403456	155328	38.50
26	403456	113302	28.08
27	403456	115606	28.65
28	403456	106845	26.48
29	403456	123174	30.53
30	403456	128801	31.92
31	403456	180868	44.83
32	403456	161654	40.07
33	403456	137868	34.17
34	403456	187442	46.46
35	403456	176604	43.77
36	403456	119893	29.72
37	403456	128074	31.74
38	403456	123961	30.72
39	403456	118039	29.26
40	403456	124029	30.74
41	403456	158693	39.33
42	403456	128519	31.85
43	403456	144064	35.71
44	403456	147304	36.51
45	403456	134639	33.37
46	403456	71848	17.81
47	403456	98900	24.51
48	403456	56701	14.05
49	403456	59527	14.75
50	403456	63966	15.85
		AVERAGE	***32.82***
		STAND. DEV.	***7.04***

Table B.8 Protein texture values from numerical digital image analysis of undeveloped hard red winter wheat dough.

sample	total pixels	PTV pixels	PTV%
1	403456	46221	11.46
2	403456	67586	16.75
3	403456	114363	28.35
4	403456	64216	15.92
5	403456	76103	18.86
6	403456	119868	29.71
7	403456	122554	30.38
8	403456	84255	20.88
9	403456	101249	25.10
10	403456	99534	24.67
11	403456	166554	41.28
12	403456	124209	30.79
13	403456	101660	25.20
14	403456	92553	22.94
15	403456	56791	14.08
16	403456	73254	18.16
17	403456	24076	5.97
18	403456	99564	24.68
19	403456	51092	12.66
20	403456	73204	18.14
21	403456	56262	13.95
22	403456	79009	19.58
23	403456	53755	13.32
24	403456	60724	15.05
25	403456	54391	13.48
26	403456	60908	15.10
27	403456	59667	14.79
28	403456	56609	14.03
29	403456	56683	14.05
30	403456	64051	15.88
31	403456	63696	15.79
32	403456	113421	28.11
33	403456	60122	14.90
34	403456	74615	18.49
35	403456	58479	14.49
36	403456	95685	23.72
37	403456	56220	13.93
38	403456	78019	19.34
39	403456	106783	26.47
40	403456	90574	22.45
41	403456	45018	11.16
42	403456	59716	14.80
43	403456	43599	10.81
44	403456	44227	10.96
45	403456	63967	15.85
46	403456	77766	19.27
47	403456	82870	20.54
48	403456	84229	20.88
49	403456	61782	15.31
50	403456	62581	15.51
		AVERAGE	***18.76***
		STAND. DEV.	***6.59***

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