PHYSICAL AND RHEOLOGICAL CHARACTERIZATION OF A SUBSTITUTED DOUGH SYSTEM USING A YEAST PROTEIN ISOLATE

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ABSTRACT

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by

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The effects of single cell yeast protein (SCP) substitution were observed in a hard red spring wheat dough system. Oxidant, surface-active agent, salts and heated protein were used to determine potentials of each as individual and combination treatments. Farinograph, extensigraph and baking studies implementing volume and sensory evaluation were used as indices for the functionality measure of the SCP substituted dough. Additional evaluations of the dough mixing characteristics following treatment with various chemical reagents were made. The dough structure with and without the SCP and surface active agent (SSL) was studied by scanning electron microscopy.

Low levels of SCP substitution (0 and 3%) yielded similar results for the farinograph, extensigraph and baking study with all additives. The higher levels (6 and 12%) caused considerable decreases in dough characteristic's quality with the 12% SCP dough being generally very poor in

performance. For the 6% and 12% SCP dough the farinograph absorptions were particularly high, the stabilities tended to be shortened, the extensigraph extensibilities decreased considerably as did the resistance to extension. Final bread quality was generally only fair for the 6% SCP system and was poor for the 12% SCP product. When oxidant and surface active agent were included in the treatment, the 6% SCP system's overall performance tended to be improved, combinations of these or with the salt or heat treatment yielded improved results.

Testing of dough mixing characteristics under treatment with various chemical reagents showed weakening of the dough by blocking amide groups with succinic anhydride. Urea at higher concentrations had a weakening effect on both doughs but interference of the H-bonds at low levels seemed to strengthen the unsubstituted dough. Higher percentages of total sulfhydryl were involved in mixing tolerance than were total disulfides. Very low percentages (approximately 2%) of the disulfides were actively involved in dough development.

Scanning electron microscopy of the dough showed the surface active agent (SSL) to yield a very fine transluscent gluten texture. Extensibility and sheeting of the gluten seemed finer than in doughs without the SSL. It appeared that SCP was carried by the gluten proteins. Doughs with 6% SCP were thick and spongy; the gluten seemed to lose its extensibility with the SCP addition. Use of the surface active agent with the SCP seemed to improve the extensibility

and sheeting character which had been lost. However, the fluid draping thin gluten was not recovered.

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INTRODUCTION

In the United States bread is frequently supplemented with proteins that will improve the nutritional quality of the product. The protein of wheat tends to be low in lysine, methionine and tryptophane. The most popular protein added to bread has been non-fat dry milk. However, with increasing cost as well as difficulty in obtaining this material, manufacturers have been turning to alternate protein sources. Among the proteins investigated have been whey, soy, favabean, cottonseed, fish protein and many others. Prior to use as a supplement, the protein must be shown to function in the bread system without hindering either the mechanical production or the final quality of the product.

In this research a commercially produced baker's yeast single cell protein was characterized in bread dough. As a control system hard red spring wheat was supplemented at 0, 3, 6 and 12% levels with baker's yeast protein (hereafter referred to as SCP). The untreated control and four treatment systems were observed: a conditioner, sodium stearoyl-2-lactylate; an oxidant, $KBrO_3$:KIO_3 (3:1); a salt, NaCl; and a micro-wave treatment of the SCP. All treatment variables were tested by farinograph, extensigraph and bread baking to determine effects on rheological properties and product quality.

The objective was to determine the maximum levels of substitution that resulted in the least alteration of production and final product. Factorial study was designed to determine the effects of the interaction of all additive treatments at the 6% level of SCP substitution.

All bread products were evaluated by a descriptive method for quality and the volume and textural characteristics were measured.

Protein bonding interactions were measured with the farinograph in 0% and the 6% SCP substituted system using dithiotreitol, n-ethylmalimide, urea, succinic anhydride and sodium dodecyl-sulfate. In this manner reactive sulfhydryls (both -SH and -S-S-) and the effects of hydrogen and electrostatic bonding were determined.

REVIEW OF LITERATURE

Flour Proteins

The endosperm of the wheat kernel contains about 70% of the total protein of the grain (77). Characteristics of bread doughs made from wheat flour result fundamentally from the properties of gluten, a complex formed during mixing and accounts for about 85% of the endosperm proteins.

Gluten was first described by Beccari in 1728 (8). Osborne (71) separated gluten (by alcohol extraction) into two roughly equal fractions (8). The fraction soluble in alcohol was called gliadin and the name glutenin was proposed for the insoluble portion. Gluten can also be separated into numerous other fractions on the basis of solubility in various solvents. However, it is the difference in solubility between the gliadin and glutenin fractions that is considered most distinctive.

The amino acid composition of gliadin and glutenin are relatively similar. Both have unusally high glutamic acid (present largely as glutamine) and proline contents. Relatively large numbers of non-polar amino acids contribute to apolar bonding. Few of the carboxyl groups of glutamic and aspartic acid are free to ionize and the low content of lysine, histidine and arginine result in a low ionic character for the

gluten proteins (105).

The gluten proteins have strong aggregation tendencies resulting from the hydrogen-bonding potential of the unusually large number of glutamine side chains (44), the potential for apolar bonding of the many nonpolar side chains, and their low ionic character. Thus, they are essentially insoluble near their isoelectric point (IEP). The IEP of the gluten proteins fall in the pH range of 6 to 9 (104). At pH values lower than 4 or 5 they are moderately soluble, presumably because they acquire an excess positive charge.

Gliadin and glutenin differ in physical properties, most notably in their viscoelasticity. Gliadin is cohesive, but not elastic, whereas glutenin is both cohesive and elastic (29). Gliadin is composed of proteins of relatively low molecular weight compared to the high molecular weights of the glutenins (24).

Protein Bonding

Protein quality among wheat varieties and the action of improving additives during mixing are related to their fundamental differences. Knowledge of the molecular organization of gluten proteins and their physical behavior permits greater manipulation of dough systems to achieve desired end products (101). Wheat flours that contain the same quantity of protein may present far different physical properties (74). To explain differences in the molecular and physical behavior

of wheat proteins an understanding of the chemical bonds is essential (102).

Proteins are generally considered to have at least three levels of structure and some may have a fourth. The primary structure or sequence of amino acids in the polypeptide chain governs the order of the latter two structures. Additionally it determines the distances and bonds between individual amino acids and segments of the protein chain and therefore bond reactivity (102). Thus far, amino acid (compositional) studies have been disappointing due to their failure to explain differences in physical characteristics of wheat varieties (76). Research has shown that both ionic and non-ionic bonds are involved in governing the viscoelastic properties of hydrated gluten (100). These forces include: covalent bonds, ionic, hydrogen, van der Walls and hydrophobic; these along with nonprotein component interactions determine gluten protein functioning (75).

Hydrogen bonds result from the affinity of hydroxyl, amide and carboxyl hydrogen for carbonyl or carboxyl oxygen (102). In aqueous media the strength of the interpeptide hydrogen bond is relatively small; however, in the interior regions of gluten where a low dielectric constant exists due to high concentrations of hydrocarbon-like amino acids, interpeptide hydrogen bonding is strengthened. Thus, while hydrogen bonds are not the dominant non-covalent dough stabilizing force their gluten strengthening properties should not be overlooked (102). The glutamine amide groups are primary

sites of association through hydrogen bonding in wheat proteins (44). The cohesive nature of hydrated wheat gluten has been ascribed to the hydrogen bonding of glutamine amide groups because this property was quickly lost in methylated-ester amide interchange reactions (10). Differences in molecular heterogeneity and intramolecular attraction resulting from amide group hydrogen bonding are responsible for the variations in flours characterized as having strong or weak gluten (47).

Deuterium bonds in most cases have higher bond energies than hydrogen bonds (102). When doughs have been mixed with deuterium oxide (D_2O) they are more stable and elastic (48). Subsequent exchange of the heavy water (D_2O) for H_2O restored normal dough physical properties in 30 minutes (102).

Adding 3M urea to dough destroyed structure immediately (48) but was reversed by addition of magnesium sulfate. Gliadin is solubilized with 2-3M concentrations of urea by masking reacting sites to inter-molecular crosslinking. The mechanism may be that the bonding sites are of a hydrogen-type (44); however, although urea has been used for many years in protein studies it is not certain whether its disruptive effect is from breakage of hydrogen or hydrophobic bonds (84).

Many of the amino acids in proteins have non-polar side chains. The term "hydrophobic bond" was proposed to account for the tendency of the non-polar residues to draw together and avoid contact wtih aqueous surroundings (50). Due to the unique physical properties of water, particularly its tendency

to exist as H-bonded clusters, the indirect effect of hydrophobic interactions may be significant in wheat dough (102). Without free water hydrophobic bonds can not be produced, and in dough about 50% of the water is not bound. Consequently, hydrophobic bonds are possible (44).

Doughs mixed with various hydrocarbons of the n-alkane family yielded glutens displaying varying degrees of decreased extensibility. The greatest decreases occurred with C_6 and C_8 alkanes; shorter or longer carbon chains had lesser effects (79). Bread prepared with organic solvents had varying degrees of acceptability but only one, a hexanol loaf, was significantly unacceptable (79). In this testing the gluten contained higher levels of protein aggregates; it was less extensible and the films were stable to expansion. Thus, organic solvents apparently favor hydrophobic associative forces of gluten components (79). The theoretical hydrophobicity of gluten proteins calculated from amino acid composition is sufficiently high at 1016 for glutenin and 1109 for gliadin to allow for both intra- and inter-molecular hydrophobic bonds (102).

The importance of ionic bonds in gluten proteins is demonstrated by the addition of salt to doughs. Theoretically, the ions influence their environment in several ways, the most important being the complexing with ionic amino acid side chains of the gluten (102). The ions enhance association and dissociation of dough components. In practice the former prevails, since upon salt addition dough rigidity

increases and extensibility reduces (13).

About 1.4 percent by weight of the amino acids of gluten are either cysteine or cystine (100). The disulfide of cystine can link together portions of the same or different polypeptide chains and contribute to dough firmness. A network with permanent crosslinks cannot show viscous flow; this requires opening of rigid crosslinks. And if these crosslinks do open, avoiding loss of cohesion demands reformation of crosslinks at a similar rate at other sites (15). By reacting with available sulfhydryl groups disulfides can interchange and provide mobility (100). The effects of oxidizing agents on the rheological properties of dough may be qualitatively explained by breaking disulfides and concurrent reformation by exchange reactions with other sulfhydryl groups (15). In practice, these effects are complicated by variations in sulfhydryl-disulfide reactivity. The reactivity depends on: the size of the molecule of which the groups are a part, stearic availability of the groups, and interactions with protein and non-protein components (99).

Baking quality has been compared to both protein content and total disulfide-sulfhydryl ratio (SS/SH). For a given protein level, optimum baking results are obtained with a total SS/SH ratio around 15 (11). The SS/SH ratio increases with flour storage (102). During dough mixing only 1-2 percent of gluten disulfide bonds are exchanged with free sulfhydryl groups (64), indicating that only a few reactive disulfide bonds are critical to rheological properties (54).

Total and reactive sulfhydryls and their ratio decrease with increasing flour strength. Total disulfide content decreases only slightly, and reactive disulfide groups also decrease with increasing mixing strength. Thus, mixing strength appears to be inversely related to reactive sulfhydryl and disulfide contents (95).

Chemical and Physical Aspects of Breadmaking

Three major phases of breadmaking are in one way or another present in the production of every loaf of bread. They include mixing, fermentation or proofing and baking. The mixing will vary by type of mixer, time mixed and speed of mixing; in some methods fermentation may be very brief or completely absent and there may be variations in the temperature, the time or the relative humidity. Conditions of baking can also vary. But for all three basic principles still remain the same.

Mixing is the process of converting flour and water into a dough by blending and distributing the ingredients with development of the gluten into a continuous phase.

When water is added the flour particles are wetted and slowly hydrate. As the water penetrates it weakens starch-protein bonds and upon mixing, several changes occur. The flour-water mass gradually becomes cohesive, loses its wet, sticky appearance and becomes a smooth dough. The dough's resistance to extension increases during mixing until a maximum

is reached. At the peak of resistance, mixing time is considered optimal. In addition to the visual and physical changes, reactions are also occurring at the molecular level. Hydration, a rather slow process, is accelerated by mixing. The proteins and other dough constituents are hydrating and free water is decreasing. As this proceeds, the dough actually feels drier, resistance to extension increases, consistency increases and dough mobility decreases. The water is penetrating the flour particles and the mixing action promotes removal of hydrated layers of protein and starch from the flour particles. The hydrated mass continues to lose more of its remaining free water to yet unhydrated flour particles. The outer layer of these particles hydrate and the process is repeated, until all of the particles are thoroughly wetted and form a homogeneous-appearing dough.

When the dough has become a coherent-mass, continued mixing begins to alter the protein structure, stretching it and developing it into a continuous phase capable of retaining gas (1,4,14,46,54,63,77). Mixing causes a uni-directional orientation of long chain molecules and a concommitant decrease in random chain entanglements. In a properly developed dough, the gluten forms an orderly continuous, three-dimensional network in which are embedded the starch granules. These structural flour proteins combine into minute elongated platelets which are partially laminated with flour lipids forming slip-planes. When stress is applied, slippage can occur along these fat planes between protein platelets (92). Good

handling properties may be defined by the ability of this structure to hold together against subsequent physical strains imposed in further processing.

The most evident change in the dough during fermentation is its progressive increase in volume. As fermentation proceeds, the dough rises to about five times its original bulk and assumes a light, spongy character (47). Causing this change are yeast cells uniformly dispersed throughout the dough, acting upon the available sugars and transforming them into the principal end products: carbon dioxide and alcohol. For proper aeration of dough, about .035 lbs of fermented sugar must be available per pound of flour; any sugar not consumed in fermentation will show in the product as residual (80). Yeast is a living organism requiring certain foods and environmental conditions before it can function properly. Environmental factors such as moisture, temperature, pH and ionic strength are the basic requisites for a proper fermentation. The yeast in the course of active functioning alters conditions by consumption of necessary fermentable substances and production of wastes in the form of CO_2, alcohols, esters and acids. These result in a highly complex system whose study is extremely difficult.

Yeast does not ferment all available sugars at the same rate or time; it displays distinct preferences for less complex sugars. Early fermentation is supported by utilization of free glucose and sucrose furnished by the flour and added ingredients. The third sugar utilized, maltose, results

mainly from B-amylase hydrolysis of starch and its fermentation is slower than that of glucose or sucrose so its content rises initially (53, 72). Conversion of maltose to glucose prior to fermentation is not detected and therefore, it has been concluded that maltose is acted directly upon by yeast (72).

Doughs are mixed so they may enter fermentation at 25° to 27°C. During a 3-4 hr fermentation the temperature usually increases by 3-4°C. Proof box temperatures of 35-37°C are usual, but temperatures up to 50°C have been successfully tested (81). Initially, the gassing power (ml CO_2/10 min.) of yeast is quite high during the initial period of fermentation of free sugars. This is followed by a decided decline in fermentation as the free sugar supply is exhausted. Subsequently, gassing increases as it adapts to fermentation of maltose which can be depleted in about three hours. The rate of these reactions becomes more rapid with increases in temperature from 27.5° to 35.0°C (38). Gas production rate increases with higher temperatures up to 38°C, above which it starts to decline (34). Gassing rate is not solely effected by temperature; sugar content, pH, alcohol concentration and other variables are constantly changing and no one condition has been individually assessed.

Research has shown that maximal gassing activity is attained between pH values of 4 and 6 (86) with a sharp decline in production occuring around pH 3.0 (33). This, however, can be effected by substrate, molasses displaying a

lower pH than cane sugar at which gas production declines. In the alkaline range, fermentation activity drops off gradually, beginning above pH 6. Generally, the fermentation activity of yeast is fairly constant over a range representing a 100-fold change in hydrogen-ion concentration, pH 4 to 6. This is the pH range encountered in most straight dough, sponge dough and other baking processes (81). Again, the effect of pH is not unidimensional, time and temperature of exposure also have an effect on gassing power with decreases occuring after longer or higher temperatures of exposure at extremes in pH (18).

Ethanol, a yeast waste product, reduces fermentation rate by about 10 to 20% toward the end of fermentation when the alcohol concentration is the highest (33). In continuous fermentation by S. cerevisiae, ethanol was not inhibitory to gassing until a concentration higher than 7% by volume was reached; concentrations below this had little or no effect (36). The alcohol content of baked bread is usually about 2.33 g ethanol/1 lb loaf or 0.8% based on flour weight (107). Alcohol concentrations in baked bread depend on baking temperature, size of loaf, cooling cycles, etc.

While yeast growth and gas development are the primary reactions of fermentation there are concurrent alterations in the rheological properties of the dough. Some factors responsible for this such as starch and gluten hydration are not dependent upon the yeast. Others, however, such as decreasing pH, alcohol's effect on gluten, physical working of the dough

due to CO_2 expansion, and slackening of the dough by enzyme catalyzed reactions are a function of yeast fermentation (81).

One enzyme catalyzed slackening effect is due to yeast reductase acting through substances such as thioctic acid in the flour. These reducing compounds, are involved in a cleavage of the flour's internal disulfides producing a slack dough (26). Additional weakening may result from catalytic disruption by yeast glutathione reducase of internal dough disulfides and reaction of the newly freed SH groups with a naturally occuring reduced glutathione equivalent of the flour (55). These and other secondary reactions appear to be responsible for changes in the visco-elastic properties of the dough but their full extent is as yet undetermined due to the difficulty in controlling research conditions.

When dough is placed in the oven a thin, but expandable surface film forms. For the first few minutes of oven time the dough continues its volume increase or "oven rise" (42). The next important reaction is "oven spring" or expansion of dough volume by about one-third of its original size. Under the influences of oven heat, gases expand and liquids such as CO_2 and alcohol volatilize to produce further pressure (42). Since crust formation has already been initiated, one result of oven spring is the characteristic "break and shred" on the side of the loaf in pan bread.

As oven spring proceeds there is a marked drop in internal pressure and at this point starch swelling begins and volume increase ceases (9). While oven spring is occuring

gluten softening also sets in but this is offset by starch swelling; the extent of which is restricted by limited water (85). The individual granules remain intact but are quite flexible due to gelatinization (83). At this time some amylose diffuses out of the granules and into the surrounding aqueous areas where it becomes concentrated as granule expansion continues. During cooling this linear fraction sets up into a gel and appears to play a significant role in bread staling (85). In addition to control by available moisture, gelatinization is also effected by temperature. Within a loaf, the starch in the outer areas which was subjected to higher temperatures for a longer time was gelatinized to a greater degree than that in the loaf core (106). In the immediate crust area, gelatinization is restricted due to highly insufficient moisture (106).

Gluten coagulation is initiated at about 74°C and continues until the end of baking. During this process the gluten matrix surrounding the gas cells is transformed into a semi-rigid film. As the gas cells expanded, the starch granules embedded in the protein elongated and the gluten film became thinner; rupture of this film may occur without collapse (83). The major phenomena that does occur during gluten coagulation is a loss of its water to the starch phase, thereby facilitating gelatinization (21).

Throughout the baking period other reactions such as enzyme inactivation, crust formation, carmelization and Maillard reaction are occuring; all of these interact to

yield a product with the familiar and desirable organoleptic properties of baked bread (80).

Physical Testing of Protein Substituted Doughs

The farinograph and extensigraph are the most commonly used instruments in measuring and recording the physical properties of dough. They are used in testing flour for quality and performance in baking. The farniograph measures dough viscoelasticity as a function of continuous mixing. It outlines the mixing characteristics of a flour with respect to absorption, dough development and the ability of the flour to produce a maximum consistency in mixing and stability to prolonged mixing (43, 22, 17, 18).

The extensigraph demonstrates the extensibility and elasticity characteristics of a dough; the effects of ingreients, particularly oxidants are studied. Retention of strength after experiencing mechanical stress as in continuous mixing, kneading and rest periods is important in machinability of doughs (17, 22, 43).

Farinograms and extensigrams measure functional characteristics and baking quality of flour gluten proteins. With increases in protein content there are increases in farinograph absorptions, mixing requirements, tolerance to mix and extensigram dimensions (2, 35, 60).

Yeast protein isolates like most protein supplements for

bread contain neither gliadin nor glutenin-like proteins of the wheat gluten type. Therefore, they do not contribute to the visco-elastic breadmaking properties of flour. Farinogram and extensigram patterns reveal marked changes upon addition of yeast protein supplement to wheat flour (17). Generally, water absorption increased, mixing requirements for optimum development decreased and mixing tolerance was reduced (2, 35, 60). The degree of these changes was determined by the type of supplement, processing of the supplement, level of replacement of wheat flour and the particle size of the supplement (7, 14, 52, 62, 82). Heat treatment of the supplement can significantly effect mixing tolerance and dough stability. Apparently, a factor present, probably a protein that adversely interferes with gluten development is denatured and rendered less harmful by the heat. Additionally, control of supplement particle size, strength of the wheat flour, baking procedure and use of dough stabilizing compounds are significant effectors of final product quality (52, 82).

Resistance to deformation and extensibility in extensigrams decreases in dough supplemented with concentrations of chickpea flour from 2-20% (87). Extensigrams of doughs with twelve percent defatted soy flour showed increased resistance to extension and lower extensibilities (19).

Cottonseed protein supplements that had been wet processed and dried at 6.8 pH showed large increases in dough water absorption (27). Farinograph water absorption increases significantly with replacement of wheat by four oilseed flours

at levels of 17.5 and 20%; heat treatment of the oilseed flour increases the water absorption even further. Although the type of oilseed does not influence water absorption, evaluation of the mixing curves reveals differences in the effects on dough properties. Sunflower and peanut weaken dough structure while cottonseed destroys dough stability while sesame shows only slight weakening of mixing strength (82). In baking tests sunflower and cottonseed dramatically reduced loaf volume; peanut and sesame flours were more compatable for bread volume and interior properties (82).

Comparison of NFDM solids, fish protein concentrate, soy flour and spray-dried yeast cell contents shows that rheologically the yeast performed the poorest in peak time and stability in farinograms and in extensibility and resistance in extensigrams. However, in baking tests the performance of yeast was second only to that of NFDM solids (57). When soluble yeast protein extracts were tested their functionality in bread decreased compared to the dried whole cells (56).

Baker's yeast protein disperses in water rather than dissolves. Solubility through the pH 3 to 7 range is limited to an extractable nitrogen level of approximately 5%. A water:protein absorption ratio of 3:1 and a fat:protein absorption ratio of 1:1 is displayed by yeast protein. The yeast protein may form stable emulsions or gels of varying firmness; it may be extruded or spun. These characteristics point out the functionality of this creamy-colored, nutty-flavored powder in food product systems (88). The protein powder has

shown good results when used in crackers, cookies and other baked goods (88).

Additives Used in Bread Making

A food additive may be defined as a substance added to food either directly and intentionally for a functional purpose, or indirectly during some phase of production, processing, storage or packaging without intending that it remain in or serve a purpose in the final product (5). The additives in the following discussion are of the former group. They are ingredients used in relatively small quantities in baked foods. However, it is only in the sense of quantity that these may be considered "minor" components. In terms of effects on the sensory qualities and physical characteristics of the products, they are important, even indispensible ingredients (68). Among the additives that may be studied in relation to baked products are malts, enzyme preparations, mold inhibitors, yeast foods, minerals and buffers, gelatin, salt, oxidizing agents and dough conditioners. This section reviews the last three.

Salt is a basic additive of dough systems under the Standards of Identity. The 2% salt in most dough systems functions to improve taste but also plays an important role in dough rheology. It aids in: stabilizing dough fermentation, toughening wheat gluten, retarding enzymatic activity, which can effect gluten (68).

Low levels of sodium chloride stiffen doughs and makes it less sticky. The increased stiffness has been measured with the extensigraph; curves of doughs treated with salt show higher resistance (32, 37). However, farinograph studies usually fail to show this trend and in fact decreases in consistency (viscosity) have been reported (37, 41, 43). Variation of temperature, pH and other conditions have displayed increased farinograph consistencies in tests with increasing salt concentration but these are usually exceptions (59). The decreased consistency is generally explained as decreased stickiness rather than stiffness (32, 37).

Not only do extensigrams of salted doughs show increased resistance but they also indicate increased extensibility. This differs from oxidizing agents which usually decrease the dough extensibility while increasing resistance. The explanations of salt effects are incomplete. Some of the effects of salts in dilute acid solutions can be explained in terms of electrostatic effects (25, 59). In doughs the concentration of salt is usually higher than in solutions and therefore electrostatic effects are an insufficient explanation.

Oxidizing agents exert a two-fold effect on flour. They are: 1) flour improvers; and 2) bleaching agents. The bromates and iodates function only in the former role which may be defined as changing the rheological properties of the dough.

Oxidation shows minimal effects in mixing or farinograph studies but clearly demonstrates its role in extensigraph

tests (58, 69). In extensigrams oxidants increase resistance and decrease extensibility depending on the type and amount of oxidant used and reaction time. A corresponding decrease of thiol and increase of disulfide occurs (39, 40, 107) with the use of oxidants. Bromates form reactive sites at the disulfide linkages that interchange during structural activation such as mixing; in a resting dough these groups are too far removed from one another and will react only when they are brought into a closer position (77). The reaction of bromate is slow except at elevated temperatures (28) while the rate of reaction of iodate is more rapid (16, 20). Doughs treated with bromate tend to show increased relaxation constants with time as oxidant is still available to react. Doughs dreated with iodate have higher relaxation constants in the initial testing which tend to decrease with time as not much oxidant is left to react (27).

The most recently introduced surfactants are used primarily for their dough strengthening capabilities and to some extent their usage levels are determined by the strength of the flour. Historically surfactants were used for their antifirming effect in bread but the development of new compounds has changed this aim.

The dough strengtheners or conditioners have been found to provide increased tolerance of the dough to mechanical abuse during processing. This tolerance is particularly important when conditioners are used in conjunction with weaker flours or with protein supplements that have a weakening

effect (96). Calcium and sodium stearoyl-2-lactylate have an improving effect on loaf volume, increase dough absorption, improve mixing tolerance and machinability of the dough, accelerate proofing, improve grain and texture, tenderize the crust and extend shelf life (61). They significantly strengthen soy, cottonseed and fish protein supplemented bread doughs (96).

The mechanism of the stearoyl-2-lactylates is only partially understood. Evidence indicates that it reacts with only the flour protein to alter gluten structure of the dough similar to the effect of the flour lipids. The stearoyl-2-lactylates act to retard the swelling of starch during baking. This has the effect of decreasing the association of the starch granules in the continuous protein film that makes up the crumb structure, thus yielding a less firm initial consistency to the crumb (1).

EXPERIMENTAL PROCEDURE

Apparatus and Equipment

For bread production dough was mixed in a Hobart Kitchenaid K5-A mixer and proofed in a Cres-Cor Model 120-1828 fermentation cabinet. The dough was sheeted and molded with a National Manufacturing Company sheeter and molder. Bread was baked in a General Electric deck oven, Model #CN16, equipped with a Honeywell Versa-Tronik Indicating and controlling potentiometer, Model #R761B. Bread volume was measured using a National Manufacturing Company loaf volumeter. Bread was sliced on a Hobart-Model 410 slicer and stored in a Puffer-Hubbard Sa-F-15-1-SC freezer.

Farinographic studies were completed using a C. W. Brabender Instruments, Inc. Farinograph equipped with a Type Pl-2H Dynamometer and a Type 3-S-300 Measuring Head. A Brabender Extensigraph Type E-1 was used in visco-elastic studies. Temperature of these instruments was controlled by a Heat-Transfer Circulator Type T-60-B (C. W. Brabender Instruments, Inc.).

Lab Con-Co digestion rack #21621 was used for micro-Kjeldahl sample preparation. A Scientific Glass Associates distillation apparatus equipped with 100 ml non-transfer

Kjeldahl flasks was used for nitrogen determinations.

For ashing, a Temco Muffle furnace Model #F1740 manufactured by ThermoElectric Manufacturing Company was used. Temperature was controlled by a Thermolyne Corporation Barber-Colman Model CPS-4032P thermostat.

A Precision Scientific Model 18 Thelco drying oven and a Hotpack #633 vacuum drying oven were used for sample and glassware drying and moisture determinations.

In the Ellman's sulfhydryl determinations a Corning PC-351 Hot Plate Stirrer was used for heating. Following color development samples were centrifuged in a Sorvall GLC-1 centrifuge equipped with a Type GSA rotor. Spectrophotometric measurements were made with a Beckman DB-G Grating spectrophotometer equipped with visible and ultraviolet light sources. Samples were analyzed in 1-cm pathlength quartz cuvettes.

All pH measurements were made on a Beckman Expandomadic pH meter.

Chemicals and Ingredients

The principal ingredients and chemicals used in this study are listed below. All chemicals were reagent grade unless otherwise stated. The water used was distilled for baking and rheological studies and distilled and deionized for all chemical studies.

Ingredients used in dough tests: the flour was Balancer, Code 2831, a hard red spring wheat flour donated by the

Pillsbury Company, Minneapolis, Minn. Red Star Active Dry Yeast was supplied by Universal Foods, Milwaukee, Wisc. Sugar and salt were purchased from Michigan Sugar Company, Saginaw, Mich. and Mallinckrodt Chemical Works, St. Louis, Mo., respectively. The protein supplement (SCP) was Baker's Yeast Protine (BYP) donated by Anheuser-Busch, Inc., St. Louis, Mo.

Patco, a division of C. J. Patterson Company, Kansas City, Mo. donated sodium stearoyl-2-lactylate (Emplex). Analytical reagent potassium bromate and potassium iodate were purchased from Mallinckrodt Chemical Works.

Chemicals used in nitrogen (micro-Kjeldahl) determinations included: tryptophane for recovery studies purchased from Sigma Chemical Company, St. Louis, Mo. Potassium sulfate was obtained from J. T. Baker Chemical Company, Phillipsburg, N.J. Mercuric sulfate was supplied by Fisher Scientific Company, Fairlawn, N.J. Potassium biiodate used for titration was purchased from Sigma Chemical Company.

Chemicals used in the sulfhydryl groups determinations: Ellman's reagent, 5, 5'-dithiobis (2-nitrobenzoic acid), also known as DTNB, was purchased from Aldrich Chemical Co., Inc., Milwaukee, Wisc. The disodium salt of ethylenediamine tetraacetic acid, di-NaEDTA, was supplied by Mallinckrodt Chemical Works. Sodium lauryl sulfate was brought from Fisher Scientific Company.

Chemicals used in the sulfhydryl groups plus disulfude determinations were: DTNB and di-NaEDTA came from the same sources as above. Urea was purchased from Aldrich Chemical

Company, Inc., and the sodium borohydride was manufactured by Fisher Scientific Company. L-cysteic acid monohydride was purchased from Sigma Chemical Company.

In the reactive sulfhydryl determinations the N-ethyl-maleimide was purchased from Aldrich Chemical Company, Inc. The dithiothreitol for the reactive disulfide test was supplied by Aldrich Chemical Company, Inc. In the bonding interaction studies on the farinograph the succinic anhydride was bought from Eastman Kodak Company, Rochester, N.Y. while the urea and sodium lauryl sulfate were obtained from the above noted sources.

EXPERIMENTAL DESIGN

This research project was divided into four separate studies and for continuity will be discussed as such.

The initial section included an overall physical and rheological characterization of a hard red spring wheat substituted at 0, 3, 6 and 12% of flour weight with a commercial yeast protein isolate (SCP). Five treatments were investigated at each level: no additive; 50 ppm Potassium Bromate ($KBrO_3$)-Potassium Iodate (KIO_3) (3:1); 0.5% sodium stearoyl-2-lactylate (SSL); 2% sodium chloride (NaCl); and a 1.50 min microwave heat treatment. Testing in this section included farinograph, extensigraph, baking, sensory evaluation, pH of dough, and proximate analysis.

The second section was an investigation of the potential for interaction among the four additives of the treatments on the 0 and 6% SCP substituted systems. Testing included farinograms, extensigrams of doughs and volume and sensory evaluation of baked bread.

The third sequence of testing was a farinograph investigation of the effects of different chemicals on flour bonding during dough mixing. Among the reagents tested were sodium dodecyl-sulfate, succinic anhydride, urea, N-ethylmaleimide and dithiothreitol.

The final test was a scanning electron microscopic examination of four different dough systems. The 0 and 6% SCP untreated doughs and the 0 and 6% SCP sodium stearoyl-2-lactylate treated doughs were mixed to peak dough development and then evaluated.

Farinograph Testing

Farinograph measurements were made according to the AACC Constant Dough Weight Procedure 54-21B (3).

In this test variable weights of flour and water, determined by the absorption, were selected by estimate from AACC Farinograph Table 54-28A (3). The flour was premixed in the farinograph 300 g bowl for 1 min at 63 RPM and then it was titrated with the water in 20 sec. Bowl sides were scraped and the cover was replaced. The dough was allowed to mix for 15 min. If the curve did not center on the 500 B.U. (Brabender Unit) line adjustments were made on the basis of 20 B.U. = 0.6% absorption as read from Table 54-28A. The curve was then repeated with corrected absorptions until a curve centering on the 500 B.U. line was achieved. The apparatus temperature was maintained at 30°C. Triplicate curves of each sample were made for the variable testing and the interaction study. The curves provided values for absorption (%), arrival time, development time, stability and departure time.

Extensigraph Testing

AACC Extensigraph Method 54-10 (3) was the basis for extensigraph procedures. A dough for testing was prepared in the farinograph 300 g bowl. Three hundred grams of flour sample plus 6 g NaCl were premixed at 63 RPM and titrated with a sufficient amount of water to yield a peak centered on the 500 B.U. line; the dough was mixed for 1 min at which time the machine was shut down and the dough allowed to rest for 5 min. Mixing was continued and then stopped at the predetermined peak time. The dough was scaled into two pieces of 150 g. It was rounded on the extensigraph rounder at a rate of 20 revolutions per piece and formed into a cylinder on the shaping unit. Cylinders of dough were clamped into the lightly greased dough holders. The dough was proofed in the humidified chamber for 45 min. Next it was placed in the sample balance arm and the chart pen adjusted to 0 B.U. The stretching arm was started on the down stroke and continued until the test piece broke. Following testing, the same dough was reshaped and replaced in the humidity chamber. Testing was repeated at 90 and 135 min. Each variable was tested in triplicate. Test curves were analyzed for extensibility (mm) and resistance to extension (B.U.).

Baking Study

Bread was baked using the above basic formula (Table 1) with conditions of mixing fermentation, bench rest, proofing and baking optimized for the 0% formula according to AACC Method 10-10 (3).

Table 1. Formulation for Test Pan Bread.

Ingredient		Level of SCP (%)			
		0	3	6	12
	%	g	g	g	g
Flour	100	200	194	188	176
BYP	0,3,6,12	-	6	12	24
Yeast	3	6	6	6	6
Salt	1.5	3	3	3	3
Sugar	5	10	10	10	10
Water (24°C)	-	variable	variable	variable	variable

The yeast was hydrated in the mixing bowl for 5 min after which the sugar, salt and flour were added. The ingredients were blended at low speed for 1 min and then the dough was mixed at speed 10 for 9 min. Mixed doughs were fermented for 60 min at 30°C and 85% R.H. Doughs were degassed with a dough sheeter, scaled to 150 g and given a 10 min bench rest at room temperature. The loaf was molded and then panned; the loaf

was proofed for 35 min at 30°C and 85% R.H. Proofed dough was baked at 218°C for 20 min.

Baked bread was wrapped in plastic wrap after cooling. Loaf volume was measured approximately 10 hr after baking by rapeseed displacement on a loaf volumeter. Loaf volume (cc) represented the average of three replications of two loaves per treatment baked on three different days. An interval of six days occurred between replications.

pH of Dough

The pH electrode was inserted directly into the dough and 0.5 min was allowed for equilibration before pH of the dough was measured at 0 time and at 10 min intervals during fermentation and at 0, 15 and 35 min of the proofing.

Sensory Evaluation

The quality of the bread was evaluated according to a descriptive system designed by Solle (90). In this procedure loaf characteristics of primary interest were listed and given a qualitative range of short descriptive terms. Each term was assigned a value from 1 to 5. The resulting table constituted a descriptive matrix (Appendix I).

With all characteristics described they were grouped into broad classifications (i.e. shape, grain, etc.). The classifications were given weighing points dependent on their inherent importance. The total value of the points equaled 100.

Within each classification another 100 points was divided among the characteristics depending on their relative importance. The characteristic weighings were determined by multiplying characteristic values times classification values and dividing by 100.

A realistic target loaf was outlined by selecting the appropriate descriptive level for each characteristic on the matrix. All test loaves were evaluated according to this theoretical reference and quality assessment was determined by difference from the profile of the target loaf.

The score obtained was a deviation from the target by the test loaves. A perfect match would be a score of zero while departure would result in penalty. All departures are positive numbers which are multiplied times the characteristic weighing to give the penalty. The penalties of all characteristics were summed to give a final value, "The nonconformity Index" (NCI), a total of the products deviation from target or desired quality. The larger the NCI the greater the deviation from target quality.

Under scoring procedure, each panelist was presented with one slice of bread and a whole loaf on which they could make their judgments. The variable testing bread was scored for four replicate bakes while the bread from the interaction study was scored for duplicate bakes.

Chemical Analyses

Moisture

Moisture content of the bread and ingredients were determined in triplicate by AACC Method 44-40 (3). A 2 g sample was weighed to the tenth mg into an aluminum sample dish that had been previously dried at about 168°C for 30 min, cooled in a dessicator and weighed after attaining room temperature. The sample was dried at 90°C for about six hours under vacuum at pressure equivalent to approximately 25-30 mm Hg.

The samples were reweighed after cooling to room temperature in a dessicator. Percent moisture was computed as grams of weight lost divided by total sample weight time 100.

Kjeldahl-Total Nitrogen

Total nitrogen was determined using the Kjeldahl method described by McKenzie (65). This consisted of digesting a dry sample of approximately 30 mg with 1.5 g of powdered potassium sulfate, 1.5 ml of sulfuric acid and 0.5 ml of mercuric sulfate solution. The mercuric sulfate solution was prepared by dissolving 13.7 g mercuric sulfate in a total volume of 100 ml of 2 M sulfuric acid. After digestion the flasks were allowed to cool and each digest was diluted with approximately 20 ml of ammonia-free water. The flasks were transferred to a micro-kjeldahl distillation apparatus, 10 ml

of sodium hydroxide-sodium thiosulfate solution were added and the mixture steam distilled into 5 ml of boric acid indicator solution contained in a 50 ml beaker. The sodium hydroxide-sodium thiosulfate solution was prepared by dissolving 200 g of sodium hydroxide and 12.5 g sodium thiosulfate in 400 ml water. The boric acid indicator solution was a mixture of 20 g boric acid in 800 ml water; 6.67 mg methylene blue dissolved in 50 ml water; and 13.3 mg methyl red dissolved in 10 ml ethyl alcohol; all of these were combined and brought to a liter.

Distillation was continued until about 40 ml had been distilled. The beaker was lowered from the condenser tip which was rinsed with a few ml of water and about 5 ml more of distillate was collected. Distillation was then halted. The distillate was titrated to a grey-lilac end-point with potassium biiodate which was prepared by dissolving in 1 liter of water 3.8994 g of potassium biiodate that had been dried over dessicant.

Recoveries of nitrogen were affirmed with dl-tryptophane which had been dried over dessicant for one month. Throughout testing a blank was run to correct for nitrogen contamination in the system. The mg of nitrogen were calculated by multiplying ml of potassium biiodate titrant by .1401.

Lipid

Lipid material was determined as crude fat according to AACC Method 30-10 (3). After moistening 2 g of sample in a beaker with 95% ethyl alcohol it was mixed with 10 ml of HCl. The HCl was a mixture of 25 parts concentrated HCl to 11 parts of water. The mixture was held in a water bath at 70-80°C for 30-40 min and was stirred frequently. Following heating, the mixture was cooled in an ice bath and 10 ml of 95% ethyl alcohol was added to it. The mixture was transferred to a Mojonnier fat extraction flask with 25 ml of ethyl ether divided into three portions. The stoppered flask was shaken vigorously for 1 min. After adding 25 ml of petroleum ether (b.p. below 60°C) the flask was again shaken for 1 min. Following centrifuging of the mixture for 20 min at approximately 600 RPM, the upper fat-ether layer was filtered through a glass wool plug into a dried preweighed 125 ml flask.

The material remaining in the flask was reextracted twice more according to the above procedure modified in using only 15 ml of each ether in extraction. The ether-fat solution in the flask was dried slowly on a steam bath and then the flask was dried in a drying oven at 100°C to constant weight (about 90 min). The flasks were air cooled for 30 min and then reweighed. A blank determination was run to correct for reagent residue. The percent crude fat was calculated as the weight of extract remaining divided by sample weight times 100.

Ash

Determination of ash was made according to AACC Method 08-01 (3). Approximately 3 g of dry sample were weighed into porcelain or vicor ashing dishes that had been dried and cooled in a dessicator prior to preweighing. The samples were ignited before being placed into a muffle furnace preheated to 575°C. Incineration was continued for 25 hr. The samples were cooled in a dessicator until reaching room temperature at which time they were reweighed. Ash was calculated as weight after ashing divided by sample weight times 100.

Sulfhydryl Groups

Sulfhydryl groups were determined by a modification of the procedure developed by Ellman (30). To approximately 10 mg dry sample were added 5 ml of a 0.01 M sodium phosphate buffer, pH 8.0, containing 1.0% sodium lauryl sulfate and 0.4% diNa-EDTA. This solution was boiled for 30 min, cooled and then 0.2 ml of a 5,5'dithiobis-(2-nitrobenzoic acid) solution, DTNB, was added. The DTNB solution was prepared by dissolving 40 mg DTNB in 10 ml 0.1 M sodium phosphate buffer, pH 7.0. The color was allowed 45 min to develop, and the sample was centrifuged for 10 min at 1000 RPM. Absorbency at 412 nm and 600 nm was read in a spectrophotometer. Concentration of the sulfhydryl groups was determined using an extinction coefficient of 12,000. A blank was run along with the protein samples.

Total Sulfhydryl

In this test disulfides were first reduced with sodium borohydride to form sulfhydryl groups. The reducing agent was destroyed with acid and acetone and the concentration of sulfhydryl groups was determined. The method is a modification of one developed by Cavallini et al. (23).

About 3 mg dry sample was placed in 1 ml 0.05 M sodium phosphate buffer, pH 7.4 containing 10 ml 0.02 M di-Na-EDTA per 200 ml of buffer. To this solution was added 2 ml 1-octanol, an anti-foaming agent, and 1 ml of a urea-sodium borohydride solution. This solution contained 10 g urea, 0.25 g sodium borohydride, and 10 ml of water. The protein, urea, borohydride mixture was shaken and incubated in a 40°C water bath for 30 min. After cooling to room temperature 0.5 ml of a low pH buffer was added. It consisted of 13.6 g KH_2PO_4 plus 1.66 ml concentrated HCl brought to a final volume of 100 ml with water. This solution was introduced dropwise to prevent excessive foaming. The walls of the reaction vessel were wetted by the low pH buffer.

A reaction time of five min was allowed and 1 ml acetone was added to complete borohydride destruction. Again the mixture was shaken to wet the walls of the reaction container. Finally 0.02 ml DTNB (as used in the sulfhydryl determination) was added and absorbency at 412 nm and 600 nm was measured after 45 min. The extinction coefficient of 12,000 was used.

A blank determination was run parallel to the test

solution to check for complete destruction of sodium borohydride. Undestroyed borohydride would reduce DTNB causing the blank to yellow and absorb at 412 nm.

Chemical Modification of Doughs During Mixing

In this test 300 g of flour was titrated with water to develop a farinograph curve of 500 B.U. The percent absorption of the dough was maintained constant throughout testing. Table 2 lists all chemicals and quantities used to determine effects of bonding systems on the mixing characteristics. Solid reagents were added dry to the systems unless otherwise noted while liquids were pipetted into the dough mass at the time of water titration. The volume of liquid reagents was used on a substitution basis for the water.

All curves were evaluated for overall effect on mixing and points of particular effect by the chemical modifying agent, among which may be included arrival time, departure time, stability or 30-minute drop.

Estimation of Reactive Sulfhydryl and Disulfides

In the determination of rheologically active sulfhydryls and disulfides the procedure of Jones et al. (49) was followed. All experiments were carried out at 30°C in the large bowl of the farinograph for sulfhydryl and the small bowl of the farinograph for disulfides, using 300 g and 50 g of flour or

Table 2. Reagents and Quantities Used for Evaluation of Bonding System Functionality in Flour and 6% SCP Substituted Dough Systems

Reagent	Quantity	Comments
Acetic anhydride	0.5, 1.0, 1.5 2.0, 2.5, 5.0 10.0 ml	liquid
Sodium-dodecyl sulfate	1.0, 1.5, 2.0 5.0 g	dry powder
Succinic anhydride	0.1, 0.5, 1.0 5.0, 10.0 g	dry powder pH 9.4
Urea	0.1, 0.5, 5.0 g 1.0M, 5.0M	liquid
N-ethylmaleimide	see reactive sulfhydryl	
Dithiothreitol	see reactive sulfhydryl	

6% SCP substituted flour for each mix. The percent absorption of the control systems was determined. This volume of water was maintained constant for all testing. The speed of the mixing blades was set at 63 RPM.

The duration of the mixing required to bring the dough to a maximum resistance was reported as development time. The loss of B.U. at the end of 30 min mixing was recorded as loss of resistance to mixing.

Reactive sulfhydryls in the dough were determined by N-ethylmaleimide (0-400 µmoles), which was added in approximately 0.1 - 1.0 ml of ethanol to the total volume of H_2O titrated at zero time. The dough was mixed for 30 minutes at which time the resistance to mixing was measured.

Reactive dough disulfide was measured by dithiotreitol (0-300 µmol) added in 0.1 ml of ethanol to the total volume of H_2O titrated at zero time. The dough was mixed for 30 minutes at which time the resistance to mixing was measured.

Scanning Electron Microscopy Studies

Doughs were prepared with hard red spring wheat flour with and without yeast single cell protein (SCP) and with and without the surfactant, sodium stearoyl-2-lactylate (SSL). Supplementation levels were at 6.0% for SCP and 0.5% for SSL, both based on the weight of the flour (100 grams). Dough samples were prepared by mixing components in a fork-type mixer to optimum development.

Immediately after mixing, dough samples were prepared for scanning electron microscopy (SEM) using methods developed by Hooper (45). Thin strips of dough were excised with a pair of scissors from a smooth freshly exposed surface. Each strip was divided into shorter segments (about 3 mm long), with care being taken to not disturb the surface of the dough, and immersed in .1 M phosphate buffered glutaraldehyde (5%). Fixation time was 24 hours at 10°C followed by stepwise dehydration in graded ethanol and critical point drying with CO_2 as the ambient liquid. Specimens were mounted on aluminum stubs with television Tube Coat (liquid carbon compound), coated with gold, and viewed at 10 KeV in an ISI-Super-Mini scanning electron microscope. All sample preparations for SEM were duplicated and the areas representative of each dough sample were selected for photography after careful study by the microscopist.

RESULTS AND DISCUSSION

Increasing SCP Levels and Single Treatments

Farinograph Studies

Absorption is defined as the amount of water required to center the farinograph curve on the 500 B.U. line for a flour-water dough. Research has shown that when the 580 B.U. line is used for the optimum consistency, the absorption obtained from the farinograph agrees within 1% of those determined in the bake shop (70). The height of the farinograph curve at the maximum development time increased with the percent protein of the flour; that is, employing optimum absorptions from baking tests, the plasticities of the farinograph increased with an increase in protein content (66). In studies with defatted heat treated soy flour, farinograph absorptions increased with raising levels up to 5% supplementation (73).

In the single cell yeast protein system increasing absorption (Table 3) is a function of the high water-binding capacity of yeast protein, three grams of water per one gram of SCP.

A measure of the rate at which water is taken up by the flour is the arrival time. Generally, as protein content

Table 3. Farinograph Data[1] for Doughs Prepared with 0, 3, 6 and 12% Single Cell Yeast Protein Supplemented Flour Under Varied Treatments[2]

Farinograph Measure	Treatment	Level of SCP Substitution			
		0%	3%	6%	12%
Arrival time (min)	None (control)	1.9	1.5	1.5	2.3
	Oxidant	5.1	2.0	1.5	1.6
	Salt	3.1	2.1	1.8	2.5
	Conditioner	1.6	1.8	1.8	2.0
	Heat	-	3.0	3.0	3.1
Peak time (min)	None (control)	6.1	3.0	2.5	2.9
	Oxidant	9.1	7.1	2.0	2.1
	Salt	15.0	15.0	4.0	5.3
	Conditioner	3.0	3.3	3.3	2.8
	Heat	-	5.6	5.1	5.3
Stability (min)	None (control)	>13.0	12.5	3.0	1.0
	Oxidant	9.1	12.5	2.0	0.8
	Salt	15.0	15.0	>13.1	8.5
	Conditioner	>13.3	10.3	2.0	2.1
	Heat	-	7.0	5.6	4.5
Absorption (%)	None (control)	65.6	69.7	75.8	92.0
	Oxidant	66.6	69.4	74.3	83.9
	Salt	63.9	68.3	74.2	82.3
	Conditioner	63.4	68.5	73.4	84.3
	Heat	-	69.3	72.5	73.3

[1] Average of three replications.

[2] Treatments: None (control)
Oxidant - $KBrO_3$:KIO_3 (3:1); 50 ppm
Salt - NaCL; 2%
Conditioner - Sodium stearoyl-2-lactylate; 0.5%
Heat - Microwave heat on SCP; 1.25 min

increases, the arrival time increases. Single cell protein showed varied effects on arrival depending on treatments (Table 3). With the control (no additives) system, the arrival decreased from 1.9 min for 0% SCP dough to 1.5 min for the 3% and 6% SCP levels but increased to 2.3 min for the 12% SCP dough. In the oxidant and salt treated systems the arrivals decreased as the SCP increased to 6%. However, the time increased slightly for the highest level. The SSL treated system increased in arrival time from 1.6 to 2.0 min as the supplement increased from 0 to 12%. The arrival times of the heat-treated system were not effected by supplement level.

The peak or dough development time is the time from first addition of water to the development of the dough's maximum consistency or minimum mobility. A variety of effects resulted depending on treatment and substitution level (Table 3). In the control system as the SCP increased from 0 to 6% the peak time decreased. A slight increase was observed for the 12% SCP substituted system. This also occurred in the oxidant, salt and heated systems. However, in the SSL system, the peak time increased as the supplement increased from 0 to 6% and decreased at the 12% level.

One study found only a 0.27 correlation between the farinograph peak and the baking mixing time on 186 samples containing eight different varieties of winter wheat (67). Another pointed out that the time just before the peak of maximum plasticity on the farinograph curves appeared to be

the state of optimum development of a dough as far as baking quality is concerned when the dough mixed in the farinograph contained the "baking formula" (91).

The stability of a dough which indicates the flour mixing tolerance is the difference between arrival and departure times. Except for the oxidant system, as the percentage of SCP rose the stability of the dough decreased with very small differences between the 0% and 3% levels but with considerable drops after that point (Table 3). Under oxidant treatment the stability of the 3% SCP dough increased considerably over the 0% but declined progressively at 6% and 12% SCP.

The mixing properties of the dough are effected only slightly by substitution to the 3% SCP level but 6% and 12% substitution showed rather negative effects. It may be that the lower levels of SCP acted as an enhancer to some of the chemical reactions of dough development while the higher levels of SCP acted as a diluent of the flour gluten and thus resulted in a weakened dough and inferior final product.

The farinograms of the control and SSL systems at 0 and 3% SCP had curves with short development time and long stability characteristics. The salt treated systems showed short to medium development with long stability at the 0, 3 and 6% SCP levels and medium stability at the 12% SCP level. Heat treatment resulted in medium peak time and medium stability for 3% SCP substitution but short stability at 6% and 12% SCP levels. The oxidant treated system had long development and stability at the 0% level but short development and long

stability at the 3% SCP level. The 6 and 12% SCP levels displayed short development and stability.

Most farinograph mixing curves are of seven general types, which can indicate to the baker their overall mixing characters. These types of curves include:

1) short development; short stability
2) short development; long stability
3) medium development; short stability
4) medium development; long stability
5) long development; short stability
6) long development; long stability
7) double peak, sway back, dip in early curve.

Generally, if a curve indicates a higher absorption, longer peak time and longer stability, the flour will be strong and tolerant. It will require more mixing and will withstand more mechanical abuse. It is with this knowledge that bakers may make appropriate flour or ingredient system selections depending on the final product desired.

Extensigraph Studies

The length (mm) of the extensigram indicates extensibility of the dough and the height (B.U.), its resistance to extension. The extensibility of a dough is an expression of the ease of stretching or dislocating structure; the resistance to extension is the inverse of the energy required to stretch or dislocate that structure. A soft, flowy

dough will yield a long and low extensigram, while a tight dough will result in a high narrow curve.

As indicated in Table 4, in all treatments except oxidant, the dough's extensibility decreased as substitution increased. In the oxidant system however, the extensibility for the 3% SCP dough increased above that of the 0% system for all three test times (45, 90, 135 min). Except for the 0% control and the 0, 3 and 12% SSL variables the extensibility decreased with time at all levels and treatments.

The resistance to extension consistently decreased as the level of substitution increased for all treatments (Table 5). Except for the 3% and 12% SCP control, 0% SCP with oxidant, 0% SCP with salt and 6% SCP with SSL variables, the resistance to extension increased with time.

The decrease in dough extensibility with increased substitution and time may mean that at the molecular level the dough became increasingly tighter or less flowy due to bonding forces. The SCP inhibits the smooth unfolding and stretching of the proteinaceous sheets in the gluten structure so that easy tearing of the sheets occurred. This was accompanied by decreasing dough resistance as SCP level increased and indicated mounting interference at the molecular level of bonds that were less strong than in the all flour dough. With time, this interference or bonding increased quantitatively such that while tightening was occuring the forces responsible for this were of a less energetic nature.

Table 4. Means[1] of Extensibility Measures (mm) for Dough Prepared with 0, 3, 6 and 12% Yeast Single Cell Protein Supplemented Flour Under Varied Treatments[2]

Treatment	Time of Measurement (min)	Level of SCP Substituted			
		0% (mm)	3% (mm)	6% (mm)	12% (mm)
None	45	237	235	211	137
	90	251	225	201	115
	135	256	210	193	103
Oxidant	45	240	245	207	132
	90	195	217	181	121
	135	171	200	170	108
Salt	45	295	278	230	170
	90	275	257	216	142
	135	233	217	203	141
Conditioner	45	316	258	231	106
	90	295	263	207	126
	135	305	245	187	146
Heat	45		267	230	155
	90		247	208	142
	135		243	196	131

[1] Average of four replications.

[2] Treatments: None (control)
Oxidant - $KBrO_3$:KIO_3 (3:1); 50 ppm
Salt - NaCL; 2%
Conditioner - Sodium stearoyl-2-lactylate; 0.5%
Heat - Microwave heat on SCP; 1.25 min

Table 5. Means[1] of Extensigraph Resistance to Extension Measurements (BU)[2] for Dough Prepared with 0, 3, 6 and 12% Single Cell Yeast Protein Supplemented Flour Under Varied Treatments[3]

Treatment	Time of Measurement (min)	Level of SCP Substitution 0% (BU)	3% (BU)	6% (BU)	12% (BU)
None	45	570	426	316	307
	90	601	484	384	325
	135	700	465	416	315
Oxidant	45	846	616	516	490
	90	1001	787	660	524
	135	995	907	752	524
Salt	45	762	654	495	315
	90	937	724	579	341
	135	925	791	604	355
Conditioner	45	640	449	399	261
	90	667	490	421	270
	135	750	512	416	270
Heat	45		494	286	266
	90		504	336	285
	135		526	375	289

[1] Average of four replications.

[2] Brabender Units.

[3] Treatments: None (control)
Oxidant - $KBrO_3$:KIO_3 (3:1); 50 ppm
Salt - NaCL; 2%
Conditioner - Sodium stearoyl-2-lactylate; 0.5%
Heat - Microwave heat on SCP; 1.25 min

Baking Study

The water absorptions used in the baking tests (Table 6) were higher by about 3% than those determined by farinograph measure. In the dough fermentation and proofing it was found that the SCP had a buffering effect on the dough's pH. The data graphed in Figures 1 A-E indicate that with increasing levels of SCP the final pH of the dough increased. The rate of pH decrease was similar but there were instances in the control variables where the rate of pH drop of a higher supplemented dough exceeded that of the immediately preceeding lower level. In the case of the initial pH of the doughs there was only about 0.05 pH unit difference among all levels for the control, salt and SSL treated systems. The oxidant

Table 6. Water Absorption (ml/%) Used in Preparation of Bread from Flour Supplemented with 0, 3, 6 and 12% Single Cell Yeast Protein Under Varied Treatments[1]

Treatment	Level of SCP Substitution			
	0%	3%	6%	12%
None	137/68.6%	145/72.7%	158/86.9%	180/95.0%
Oxidant	139/69.6%	145/72.4%	155/77.3%	174/78.8%
Salt	134/66.9%	143/71.3%	154/77.2%	171/85.3%
Conditioner	133/66.4%	143/71.5%	153/76.4%	175/87.3%
Heat		145/72.3%	151/75.5%	153/76.3%

[1] Treatments: None (control)
Oxidant - $KBrO_3$:KIO_3 (3:1); 50 ppm
Salt - NaCL; 2%
Conditioner - Sodium stearoyl-2-lactylate; 0.5%
Heat - Microwave heat on SCP; 1.25 min

Figure 1. pH of 0, 3, 6 and 12% SCP Substituted Doughs as a Function of Time Through Fermentation

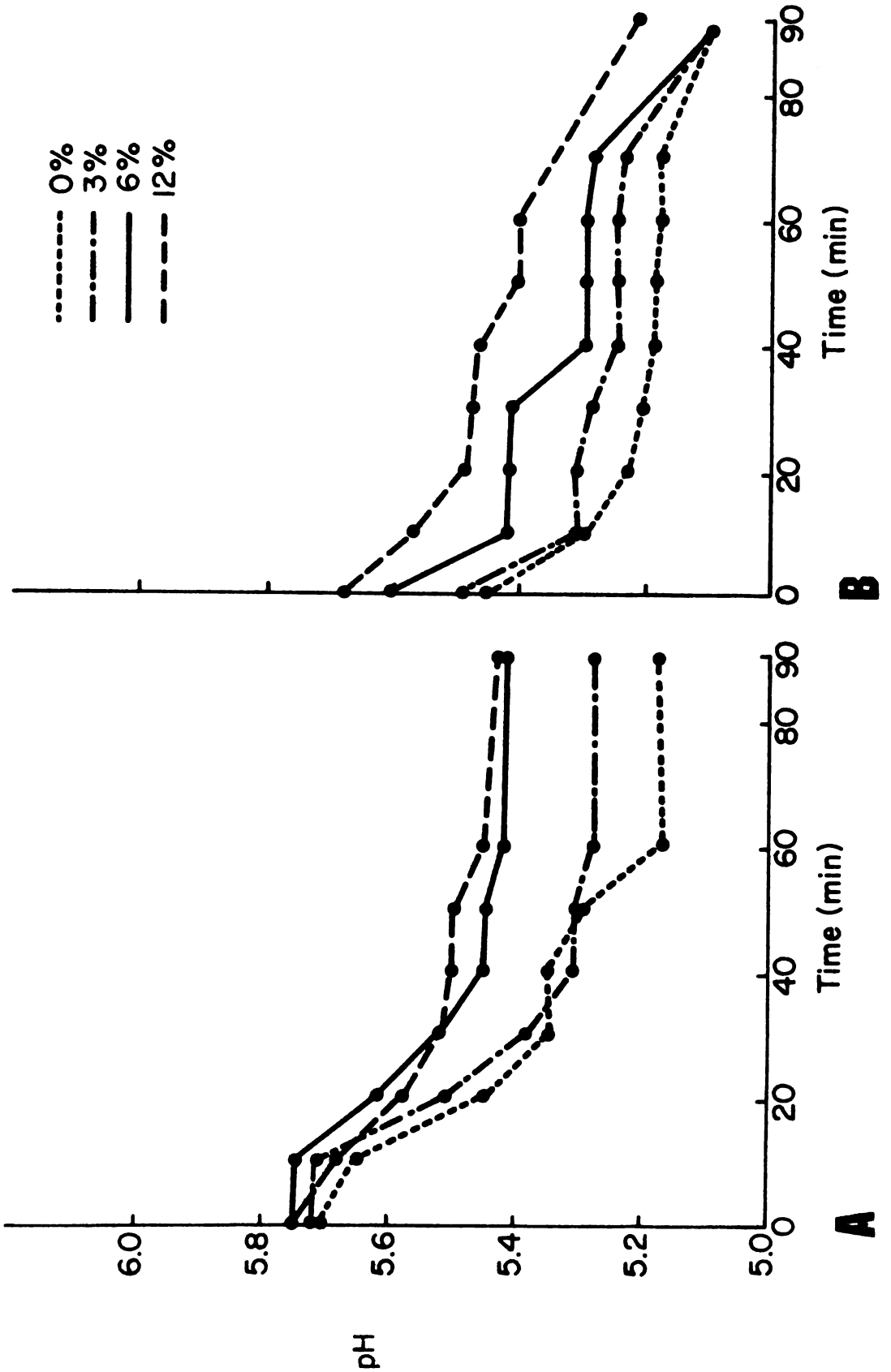
a) Control (untreated) System

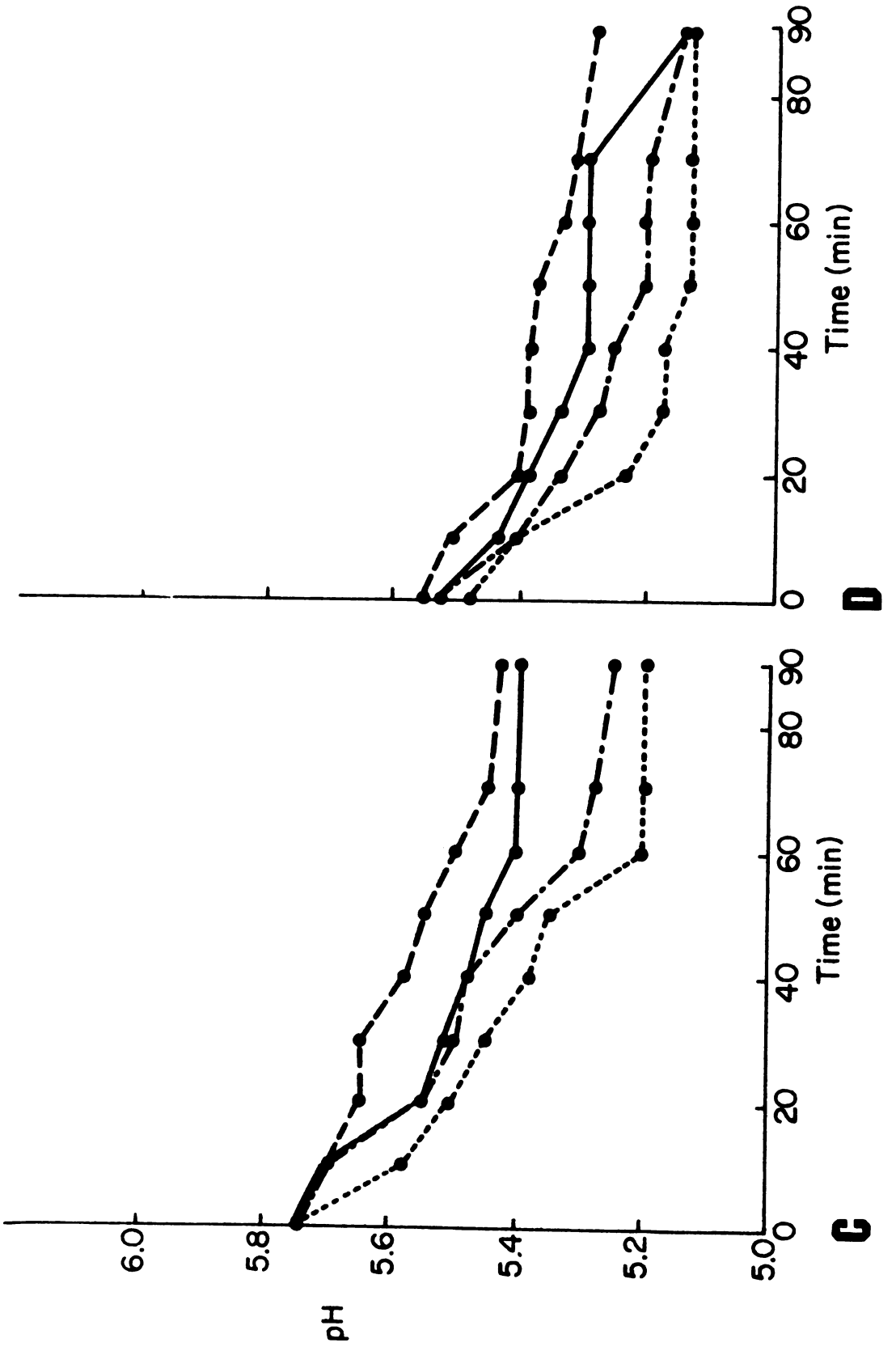
b) Oxidant System

c) Sodium Chloride System

d) Sodium Stearoyl-2-lactylate System

e) Heat System


0%
3%
6%
12%
pH
6.0
5.8
5.6
5.4
5.2
5.0
0
20
40
60
80
90
Time (min)
A
B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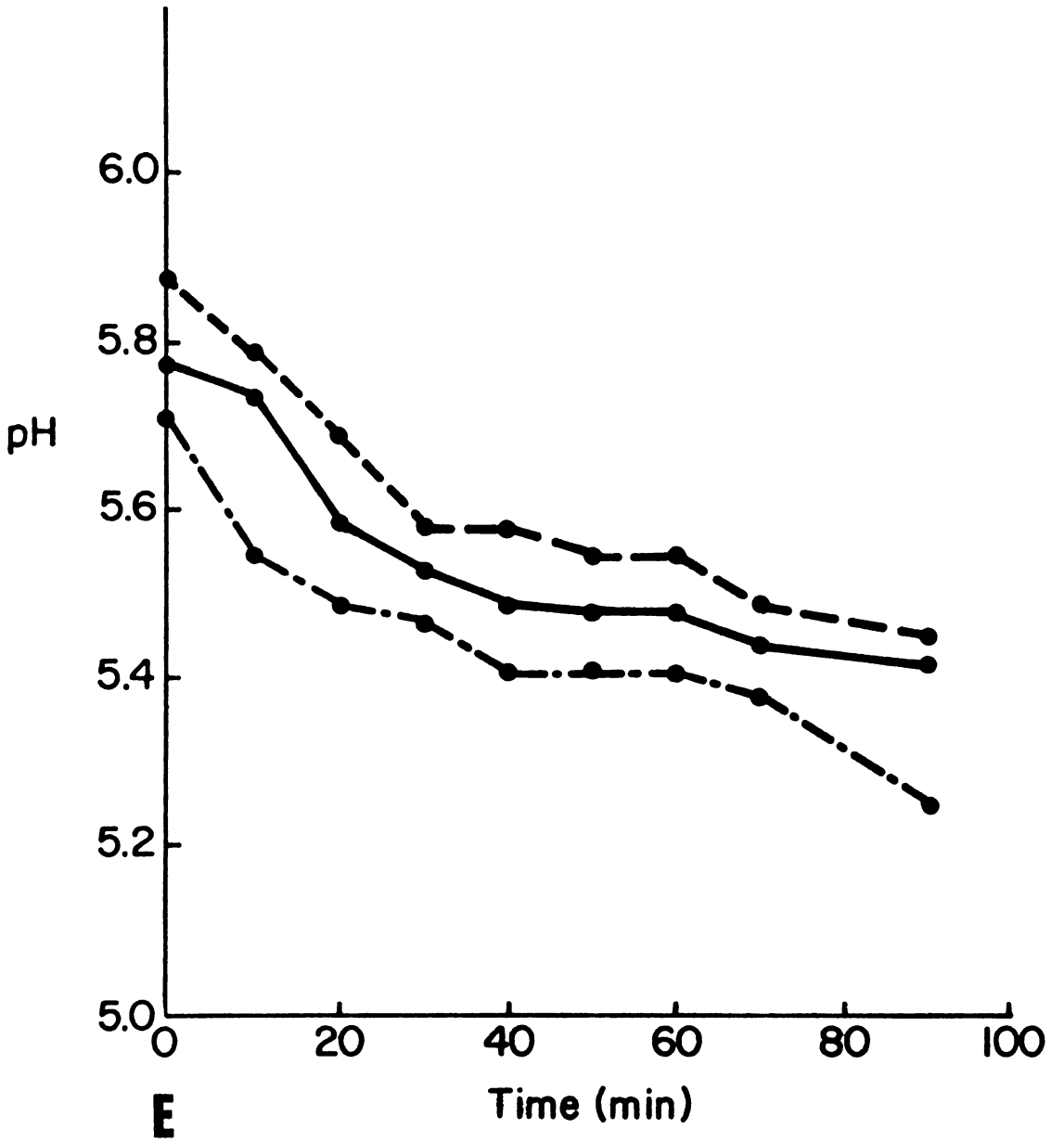
20
40
60
80
90
Time (min)
C
D


6.0
5.8
5.6
5.4
5.2
5.0
pH
0
20
40
60
80
100
Time (min)
E

and heat treatments showed higher differences at initiation of fermentation. In all instances the final pH of the 0 and 3% SCP doughs fell below 5.3, while the 6 and 12% SCP variables did so only after oxidant and SSL treatment. It is noteable that despite final pH differences which were within 0.01 pH units, the SSL treated doughs at 0, 3 and 6% SCP had a difference of about 90 cc in loaf volume.

The loaf volumes of breads containing 0, 3, 6 and 12% SCP are shown in Table 7. Bread with 3% SCP had a similar loaf volume to the 0% control, oxidant and salt treatments. The higher SCP levels of these treatments yielded loaves of reduced volumes. However, the 6% SCP oxidant treated loaf had a volume higher than those of the 0 and 3% levels with salt. The breads treated with SSL had acceptable volumes. With 0 and 3% SCP the volumes were quite different at 495$\pm$ 28.21 and 451.25$\pm$7.49 cc. The volume of 6% SCP SSL treated bread improved compared to the control 6% SCP loaf with respective volume of 406.25$\pm$51.23 and 348.75$\pm$38.43 cc. Heat treatment resulted in a volume decrease for 3% SCP bread as compared to the control 0% SCP loaf. In two cases, the salt treated and oxidant treated breads with 30% SCP, there were improvements over the 0% SCP salt and oxidant variables. In all treatments use of 12% SCP resulted in bread of highly reduced volumes, ranging from 228.12 cc to 296.8 cc.

Early research had shown that the use of full fay soy decreased loaf volume (19) but later this effect was found to be overcome by increasing bromate concentrations in the

Table 7. Mean[1] and Standard Error of Volume (cc) of Bread Prepared with 0, 3, 6 and 12% Single Cell Yeast Protein Supplemented Flour with Varied Treatments[2]

Treatment	Level of SCP Substitution			
	0%	3%	6%	12%
	cc	cc	cc	cc
None	473.12 ±37.49	463.12 ±29.95	348.75 ±38.43	265.00 ±20.51
Oxidant	498.12 ±21.73	498.75 ±33.00	435.00 ±50.04	252.50 ±12.41
Salt	410.62 ±18.07	421.20 ±29.75	380.60 ±10.68	296.80 ±25.76
Conditioner	495.00 ±28.21	451.25 ± 7.49	406.25 ±51.53	272.50 ±56.08
Heat		447.50 ±22.45	355.60 ±22.02	228.12 ± 8.50

[1] Average of 2 loaves of each of four replications.

[2] Treatments: None (control)
Oxidant - $KBrO_3$:KIO_3 (3:1); 50 ppm
Salt - NaCL; 2%
Conditioner - Sodium stearoyl-2-lactylate; 0.5%
Heat - Microwave heat on SCP; 1.25 min

bread formulas (31). Pollack and Geddes reported that 1% unheated soy improved loaf volume but higher levels were deleterious (73). With the SCP substitute, oxidant performed as it does with soy to improve loaf volume. The salt treatment appears to have decreased loaf volume in the 0% and 3% SCP samples over that of the control 0% and 3% SCP bread possibly by tightening of the gluten structure. This is verified by data in Table 6 which indicates increased resistance to extension for the salt variables.

A proximate analysis of the bread crumb shows the the protein content of the bread increased as the SCP increased (Table 8). The 3% SCP bread contained 17.61% protein (as is moisture) which would permit its acceptance as high protein bread. In turn, the higher two SCP levels were more than within the commonly accepted 15% protein level for high protein bread status.

Table 8. Proximate Analysis[1] of Bread Prepared with 0, 3, 6 and 12% Yeast Single Cell Protein Supplemented Flour

Component	Level of SCP Substitution			
	0%	3%	6%	12%
Moisture	39.26	40.60	42.36	45.49
Protein	14.35	17.61	20.31	23.49
Fat	2.30	2.57	2.85	3.63
Ash	1.94	2.13	2.13	2.22
Carbohydrate (by difference)	42.15	37.09	32.35	25.17

[1] Average of triple replications.

The moisture levels of all the breads were above the federal standard of 38%. This would require some adjustment by formula manipulation but lower moisture for adequate dough handling could probably be achieved.

Data on sensory evaluation (Table 9) shows that the most acceptable product is the 3% SCP bread treated with SSL. Despite its failure to improve loaf volume the SSL effected crumb color and grain positively, yielding a very acceptable product (Figure 2). Generally, most of the 3% SCP bread scored either better or nearly as good as the 0% SCP in the individual treatment series.

Most of the 12% SCP bread scored higher than 100 in the sensory evaluation, this was the cut off for a minimally acceptable product. A loaf with a score of 90 could be described as fairly acceptable. The 6% SCP control and heat-treated variables scored minimally acceptable while the 6% SCP SSL, salt and oxidant breads scored fairly acceptable.

Table 9. Mean[1] and Standard Deviation of Sensory Evaluation[2] of Bread Prepared with 0, 3, 6 and 12% Single Cell Yeast Protein Supplemented Flour Under Varied Treatments[3]

Treatment	Level of SCP Substitution			
	0%	3%	6%	12%
None	68.21 ±16.35	68.15 ±11.79	99.25 ±13.69	112.24 ±18.08
Oxidant	73.68 ±15.97	72.31 ±15.86	89.31 ±22.91	117.93 ±10.65
Salt	71.46 ±17.09	66.62 ±11.07	86.49 ±10.91	124.56 ±15.62
Conditioner	72.96 ±15.62	63.24 ±15.40	89.03 ±12.23	119.01 ±13.72
Heat		70.28 ±18.49	96.90 ±16.67	126.49 ±20.15

[1] Average of 2 loaves of each of four replications.

[2] See Appendix I for components of score. The number reflects the degree of deviation from a standard bread.

[3] Treatments: None (control)
Oxidant - $KBrO_3$:KIO_3 (3:1); 50 ppm
Salt - NaCL; 2%
Conditioner - Sodium stearoyl-2-lactylate; 0.5%
Heat - Microwave heat on SCP; 1.25 min

Figure 2. Bread Prepared with 0, 3, 6 and 12% SCP Substituted Flour

a) Control (untreated) System

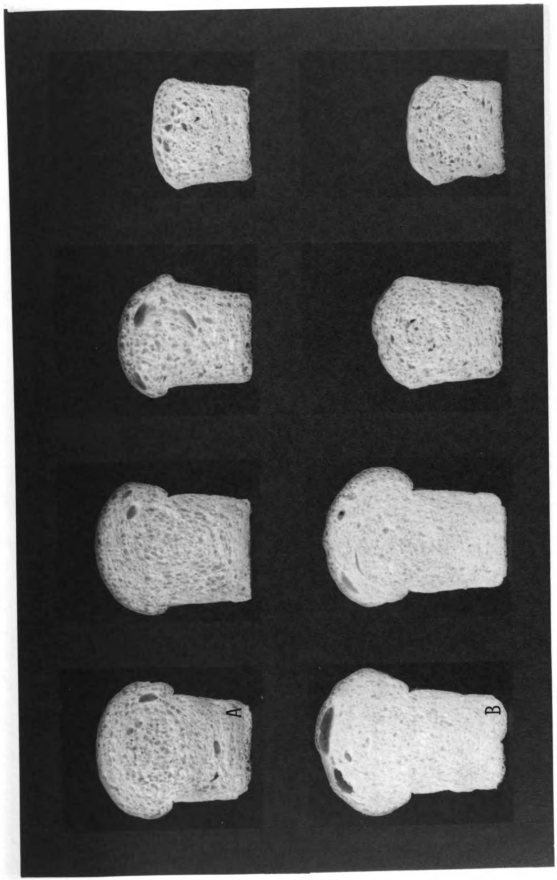
b) Oxidant System

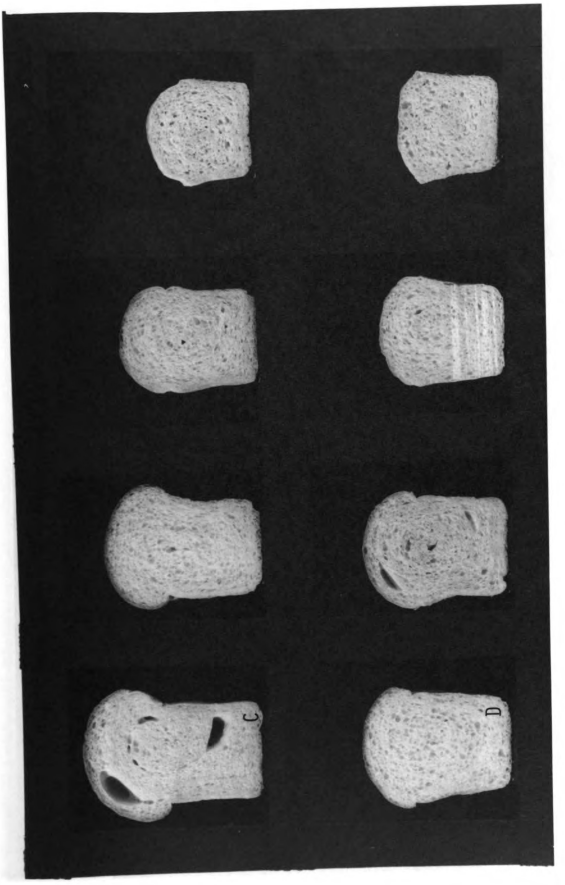
c) Sodium Stearoyl-2-lactylate System

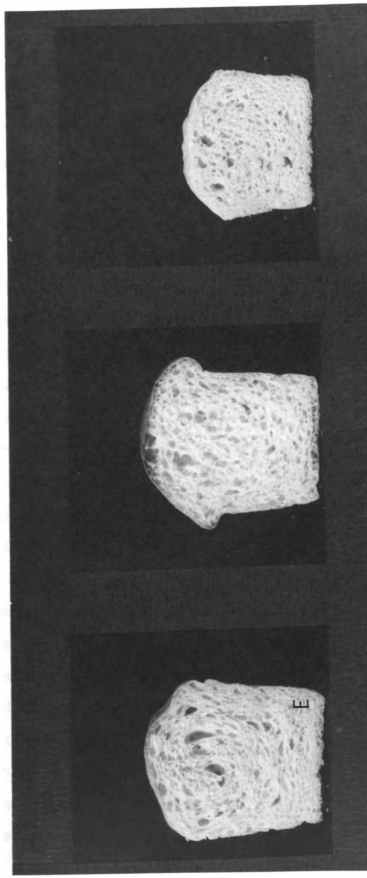
d) Sodium Chloride System

e) Heat System

59 A-B




C
D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Interaction of Additives

Farinograph Studies

A combination of two additives in the 6% SCP doughs had a variety of effects on mixing characteristics. Outlined in Table 10, the arrival times for doughs containing combinations of two additives show several noticeable changes compared to doughs in which the additives were individually tested (Table 3). The arrivals for doughs with a double combination of additives ranged from 1.0 - 1.8 minutes. In single additive doughs the arrivals ranged from 1.5 - 3.0 minutes with the 3.0 minute arrival time being in the heat treated system. In the testing of doughs with double additive combinations, however, the arrivals of the variables which included a heat treatment had at least halved in time ($p \leq 0.001$). Thus, the other additives have an apparent effect of facilitating hydration. In practical terms, a baker would expend less time and energy on hydration of his mix thereby conserving mix time. Doughs with double combinations including salt as one of the additives exhibited the longest arrival times ($p \leq 0.001$)(Table 10). The longest being 1.8 min or equal to the time needed to develop the 6% SCP supplemented dough with salt alone. The salt heat pairing had the longest arrival for all combinations with a heat treatment. The salt may have had a dehydration effect on the flour protein bonding sites or it may have acted as a blocking agent.

Table 10. Farinograph Data[1] for Doughs Prepared at the 6% Single Cell Yeast Protein Substituted Flour Testing for the Effect of Interaction of Oxidant, Salt, Conditioner and Heat

Treatments[2]	Arrival Time	Peak Time	Stability	Absorption
	min	min	min	min
DOUBLE ADDITIVES				
Oxid-cond	1.4	3.4	8.0	71.8
Heat-oxid	1.4	4.0	6.8	72.5
Heat-cond	1.0	2.0	4.0	73.8
Salt-cond	1.5	2.5	5.0	69.7
Salt-oxid	1.8	5.5	9.8	71.0
Salt-heat	1.5	2.5	3.6	74.0
TRIPLE ADDITIVES				
Salt-oxid-cond	1.8	3.5	6.5	72.8
Salt-heat-cond	2.0	3.8	5.5	73.7
Salt-heat-oxid	1.4	4.5	9.1	71.5
Heat-oxid-cond	1.0	2.5	8.0	72.5

[1] Average of

[2] Treatments: Oxidant - $KBrO_3$:KIO_3 (3:1); 50 ppm
Salt - NaCL; 2%
Conditioner - Sodium stearoyl-2-lactylate; 0.5%
Heat - Microwave heat on SCP; 1.25 min

The peak times display an interesting trend with decreases to 2.0 and 2.5 min respectively for the heat lactylate and heat salt treated doughs (Table 10). This compares with a peak time of 5.1 min for the heat treated dough. The least decrease in peak time was for the oxidant heat treated dough. An examination of the other oxidant pairings showed that peak times of doughs with oxidant lactylate and salt oxidant combinations were not decreased at all; in fact, they showed slight increases ($p \leq 0.001$). This could be predicted because the bromates have little effect during early mixing times.

Doughs which contained heat treated SCP and any other additives had lower stabilities ($p \leq 0.001$) (Table 10). The salt oxidant treatment yielded the most stable curve at 9.8 minutes, the salt lactylate was less stable with a measure of 5.0 minutes. This leads to a search of the other treatments for improving effect. And while the lactylates do not seem to be involved in any trends, the oxidant combinations with all the other treatments yielded the longest stabilities ($p \leq 0.001$). This indicated that sometime during the first 5 to 6 minutes the oxidants became reactive and after that they promoted sulfhydryl-disulfide interchange. In turn, this resulted in a stronger more durable dough.

The absorption trends for dough with the double additive combinations (Table 10) showed that the combinations which include heat treatment have slightly higher percent absorptions. In general, the percent absorptions ranged

from 69.7% to 74.0% which was similar to individual applications of the treatments where the range was 72.5% to 74.3% (Table 4).

For the series of doughs containing triple combinations of treatments, the arrival times do not seem to follow any set pattern (Table 10) except that those doughs containing a salt treatment displayed longer arrival times than those without. The range of arrival times was 1.0 to 2.0 min. The heat oxidant lactylate treated dough exhibited a short arrival time, 1.0 min. When salt was added to the heat treatment in combination with either lactylate or oxidant, a slow down in hydration occured with arrival times of 2.0 and 1.4 min respectively, the lactylates seemed to have less ability ($p \leq 0.001$) to overcome the time lengthening effect of the salt than did the oxidant.

Triple combinations of additives that contained salt showed the longest peak times (Table 10). Treatments with salt and heat along with either oxidant or lactylate resulted in peak times of 4.5 and 3.8 min, respectively. The lactylate seemed to be more effective than the oxidant in decreasing peak development. The heat oxidant lactylate combination resulted in a dough with a peak time of 2.5 min compared to a peak time of 3.8 min for the doughs containing the salt heat lactylates ($p \leq 0.001$). Lactylate reduced the peak time when compared with doughs containing oxidant. In the double additive combinations the heat oxidant peak time was 4.0 min compared with 2.0 min for the heat lactylate doughs.

In stability doughs with combinations including salt or oxidant were more stable ($p \leq 0.001$), however, combination of these two did not result in the greatest stability (Table 10). The salt oxidant heat treated dough was the most stable (9.1 min) while the lactylate oxidant heat treated dough ranked second in stability (8.0 min) and the lactylate salt oxidant treated dough was third in stability. Oxidant treatment again showed strengthening of the dough in later mixing.

The percent absorption data for the triple combinations showed a range from 71.5% to 73.7% with no significant trends (Table 10).

Extensigraph Studies

Double combinations of additives in doughs had little effect on extensibility (mm) after fermentation (Table 11). The range of dough extensibilities after individual treatment by the additives at the 45 min test time was 207 to 231 mm (Table 5) while the range of extensibilities in doughs with double combinations of treatments was 210 to 230 mm. There were no extensive changes in extensibility at 90 minutes. However, at 135 min the extensibility for the doughs with double combination of additives decreased. Treatment of dough with oxidant and lactylate decreased the extensibility to 150 mm at the 135 min test time ($p \leq 0.001$). Doughs with the other possible combinations including oxidant also showed lower extensibilities, neither of which were significant. This supports the oxidant treatment data of 170 mm,

Table 11. Means[1] of Extensibility Measures (mm) for Doughs Prepared with the 6% Single Cell Yeast Protein Substituted Flour Testing for the Effect of Interaction of Oxidant, Salt, Conditioner and Heat

Treatment[2]	Time of Extensibility Measure		
	45 min	90 min	135 min
DOUBLE ADDITIVES			
Oxid-cond	210	185	150
Heat-oxid	230	200	190
Heat-cond	210	205	200
Salt-cond	220	195	185
Salt-oxid	215	190	170
Salt-heat	225	215	205
TRIPLE ADDITIVES			
Salt-oxid-cond	220	210	200
Salt-heat-cond	225	210	200
Salt-heat-oxid	230	205	200
Heat-oxid-cond	235	225	215

[1] Average of three replications.

[2] Treatments: Oxidant - $KBrO_3$:KIO_3 (3:1); 50 ppm
Salt - NaCL; 2%
Conditioner - Sodium stearoyl-2-lactylate; 0.5%
Heat - Microwave heat on SCP; 1.25 min

the lowest value, for the individual additive testing.

Data from the triple additives testing did not affirm this observation (Table 11). Over the 135 min the range (235 - 200 mm) of decrease in extensibility amounted to only 35 mm.

Data in Table 12 indicates that in the double combinations of additives, dough systems that included a heat treatment had the lowest resistance to extension at the 45 min testing. The heat lactylate treatment dough had the lowest resistance, 425 BU ($p \leq 0.001$) while the heat oxidant and heat salt variables followed with respective values of 525 ($p \leq 0.001$) and 540 BU. The lactylate oxidant followed with a reading of 690 BU ($p \leq 0.001$) while the lactylate salt and oxidant salt ($p \leq 0.001$) followed with respective values of 780 and 900 BU. In order of degree of effect in tightening the molecular structure and resulting in increased resistance to extension the additives ranked: heat < lactylate < oxidant < salt. With increasing fermentation the oxidant and salt treatment showed some interchangeableness with respect to degree of effect on the tightening of the gluten.

These observations were supported by the triple combinations of treatments in doughs (Table 12). The salt oxidant lactylate treatment displayed the most resistance to extension at all times. The second most resistant system at all times was the salt oxidant heat treated dough. The other two combinations, salt lactylate heat and oxidant lactylate heat, ranked third and fourth in degree of effect on resistance to

Table 12. Means[1] of Resistance to Extension Measure (BU) for Doughs Prepared with the 6% Single Cell Yeast Protein Substituted Flour Testing for the Effect of Interaction of Oxidant, Salt, Conditioner and Heat

Treatment[2]	Time of Measure of Resistance		
	45 min	90 min	135 min
DOUBLE ADDITIVES			
Oxid-cond	690	785	895
Heat-oxid	525	650	720
Heat-cond	425	445	475
Salt-cond	780	835	860
Salt-oxid	900	1065	1150
Salt-heat	540	610	655
TRIPLE ADDITIVES			
Salt-oxid-cond	865	1075	1100
Salt-heat-cond	590	650	700
Salt-heat-oxid	675	870	975
Heat-oxid-cond	405	620	780

[1] Average of three replications.

[2] Treatments: Oxidant - $KBrO_3$:KIO_3; 50 ppm
Salt - NaCL; 2%
Conditioner - Sodium stearoyl-2-lactylate; 0.5%
Heat - Microwave heat on SCP; 1.25 min

extension at 45 and 90 min of fermentation but they reversed order at 135 min.

Thus, it seemed that the doughs were tightened more by salt and oxidant while the degree of effect by the lactylate and heat was considerably less.

Bread Volume

The effect of the combination of additives can be seen in the bread volume data (Table 13). All double combinations resulted in loaf volumes considerably higher than the individual treatment systems for 6% SCP breads (Table 7). Volumes of bread with double combinations of additives were higher than any of the 0 and 3% SCP substituted breads (Table 7).

Heat in combination with the other three dough treatments yielded loaf volumes greater than 500 cc in each case. Use of the heat treated SCP in combination with oxidant, salt and lactylate in doughs yielded loaves with respective volumes of 570, 555 and 545 cc. Two of these systems yielded the highest extensibility measures for doughs at 45 min, the heat oxidant and heat salt. Support for the idea that extensibility may be a measure of possible volume was found in that the lowest extensibility was for the oxidant lactylate treated dough which also had the lowest bread volume.

In the triple combinations of treatments the heat treatment along with all possible combinations of the other additives ranked first, second and third in loaf volume measurement (Table 13). The heat oxidant lactylate loaf had the

Table 13. Means[1] of Volumes (cc) and Sensory Evaluations[2] of Bread Prepared with the 6% Single Cell Yeast Protein Substituted Flour Testing for the Effect of Interaction of Oxidant, Salt, Conditioner and Heat

Treatment[3]	Volume	Sensory Score
	cc	
DOUBLE ADDITIVES		
Oxid-cond	455	71.65
Heat-oxid	570	56.35
Heat-cond	545	49.05
Salt-cond	460	70.95
Salt-oxid	455	63.25
Salt-heat	555	51.05
TRIPLE ADDITIVES		
Salt-oxid-cond	475	62.50
Salt-heat-cond	540	60.00
Salt-heat-oxid	555	56.45
Heat-oxid-cond	591	60.20

[1] Average of three replications.

[2] Score is deviation from norm.

[3] Treatments: Oxidant - $KBrO_3$:KIO_3 (3:1); 50 ppm
Salt - NaCL; 2%
Conditioner - Sodium stearoyl-2-lactylate; 0.5%
Heat - microwave heat on SCP; 1.25 min

highest volumes of all double and triple combinations with a value of 591 cc. The salt heat oxidant and salt heat lactylate loaves ranked second and third with respective volumes of 555 and 540 cc ($p \leq 0.001$).

The improvement in volumes for all of the loaves with combinations including oxidant was between 20 and 160 cc over the 435 cc volume for the oxidant treated 6% SCP substituted loaf.

Also it seemed that the heat treatment effected some unidentified factor, possibly a protein in the SCP, and in conjunction with the other additives resulted in bread of considerably higher volume.

Sensory Evaluation

Sensory evaluation data (Table 13) for the 6% SCP bread with combinations of treatment resulted in scores that were considerably lower (better) than in any individual treatment products (Figure 3). Following the trends of the volume data, the bread prepared with heat treated SCP plus other additives scored first, second and third. The heat lactylate ($p \leq 0.05$), heat salt ($p \leq 0.001$) and heat oxidant ($p \leq 0.001$) loaves scored respectively 49.05, 51.02 and 56.35. The salt oxidant ($p \leq 0.01$) and salt lactylate ($p \leq 0.001$) bread ranked fourth and fifth with scores of 63.25 and 70.95. The poorest scoring product was the bread with oxidant and lactylate ($p \leq 0.001$).

In triple combinations the heat combined with the other

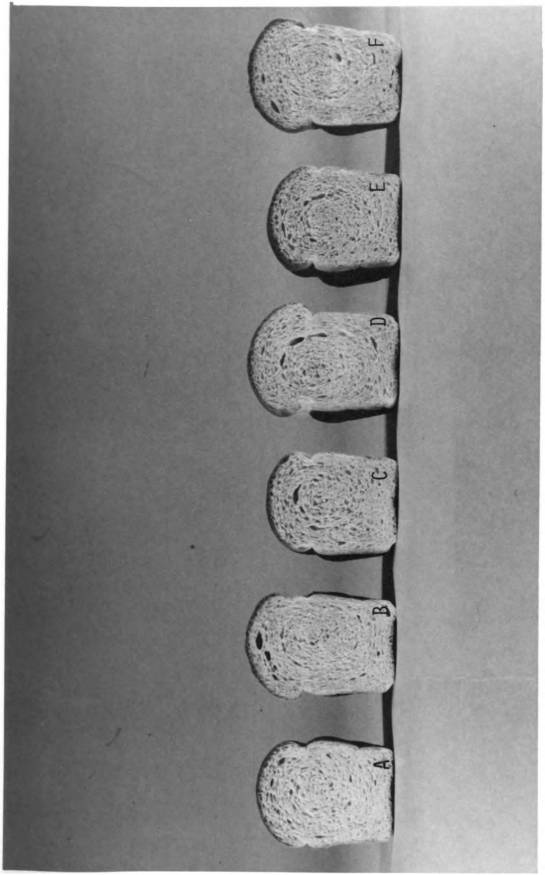
Figure 3. Bread Prepared with 6% Single Cell Yeast Protein Substituted Flour Testing for the Effect of Interaction of Oxidant, Salt, Conditioner and Heat

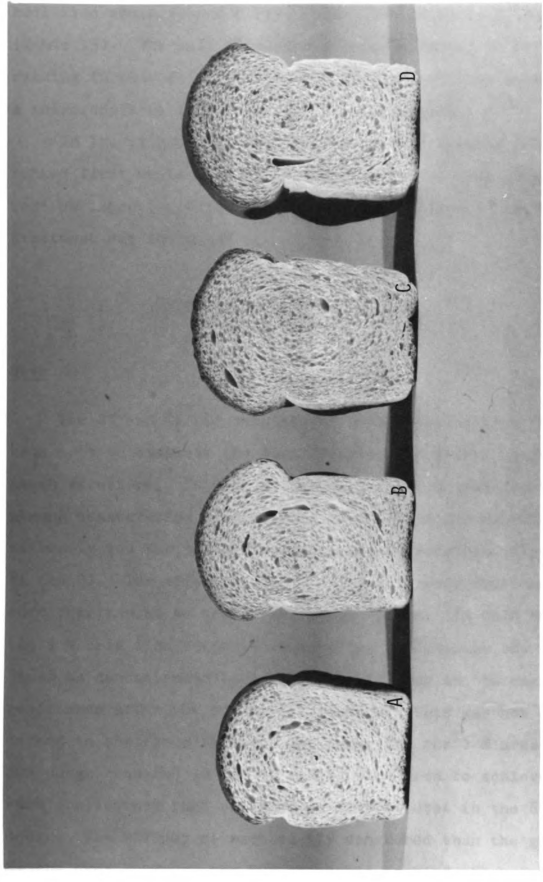
Double Combinations

a) Salt-Conditioner
b) Heat-Conditioner
c) Oxidant-Conditioner
d) Heat-Salt
e) Salt-Oxidant
f) Heat-Oxidant

Triple Combinations

a) Salt-Oxidant-Conditioner
b) Salt-Heat-Conditioner
c) Salt-Heat-Oxidant
d) Heat-Oxidant-Conditioner


A
B
C
D
E
F


A
B
C
D

additives again scored first, second and third ($p \leq 0.001$) (Table 13). The salt also seemed to be a factor in high ranking in this factorial level. It was heat plus salt plus a third additive which ranked first and second.

In the single treatment systems, a salt treated loaf ranked first while heat treated loaves ranked last (Table 9). Thus an improving effect by the other additives on the heat treatment was indicated.

Chemical Modification of Bonding Systems

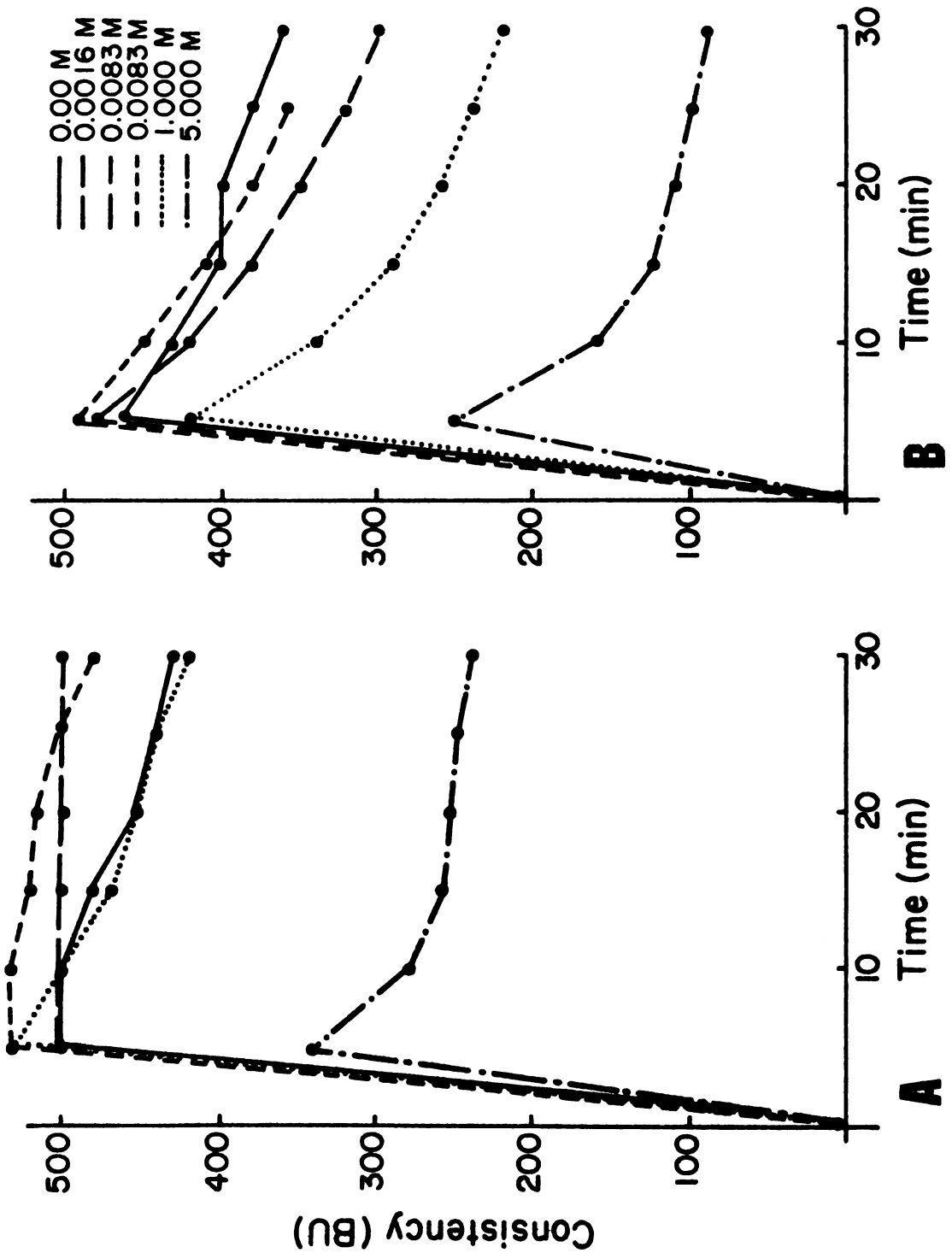
Urea

The 0% and 6% SCP substituted dough systems were treated with urea to evaluate the significance of hydrogen bonding on dough structure. In both the doughs, the 5 M urea showed strong denaturation of the wheat proteins as demonstrated by extremely low farinograph resistance (BU) readings (Figures 4A and B). The effect of the urea at all concentrations was more detrimental to the 6% SCP dough system. In this dough the 1 M urea also tended to solubilize or denature the protines as demonstrated by a rather rapid drop in the mixing resistance after the first five minutes. This was not evidenced in the flour system. The curve for the 1 M urea 0% SCP dough required 30 min of mixing with urea to achieve the same consistency that occurred at five minutes in the 6% SCP dough. The SCP may be more easily denatured than the gluten by the urea . Thus, the SCP is unable to bind the water

Figure 4. Effect of Various Concentrations of Urea on Consistency (BU) of Dough as a Function of Time (min)

a) Control

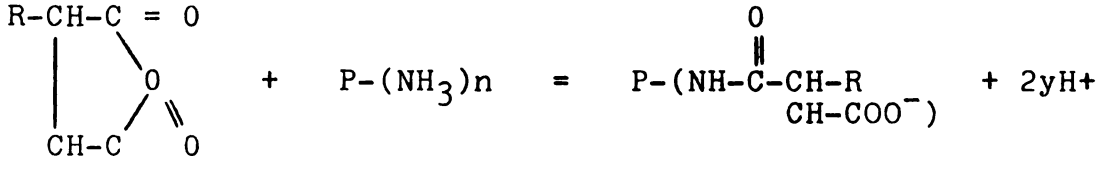
b) 6% SCP Substituted Dough


Consistency (BU)
500
400
300
200
100
10
20
30
Time (min)
A
B
0.00 M
0.00016 M
0.0083 M
0.0083 M
1.000 M
5.000 M

necessary to maintain a higher early peak resistance. At the lower concentrations of urea (0.0016, 0.0083, 0.083 M) in the flour system (Figure 4A) the urea either had no effect at earlier times (five and ten min at 0.0016 M and 0.0083 M) or it displayed a strengthening (0.083 M) compared to the control (0.0 M). A similar trend was observable in the SCP dough when at early times the 0.0083 and 0.083 M urea treatments showed an initial strengthening of the dough but the viscosity dropped rather quickly in this system (10 min). In both the 0% and 6% SCP doughs, the 0.083 M urea had a greater strengthening effect than the 0.0083 M urea. It may be that the gluten was partially denatured, unfolding the chains and making available bonding sites that had associative and thus strengthening effects. The 6% SCP dough (Figure 4B) was effected to a greater degree through loss of resistance than was the 0% SCP dough at the constant 10 minute measurement.

Succinic Anhydride

In testing the effect of the high concentrations of amide ($-NH_3$) groups in the wheat protein, succinic anhydride was applied at a variety of concentrations to the dough. Succinic anhydride reacted with protein as shown:

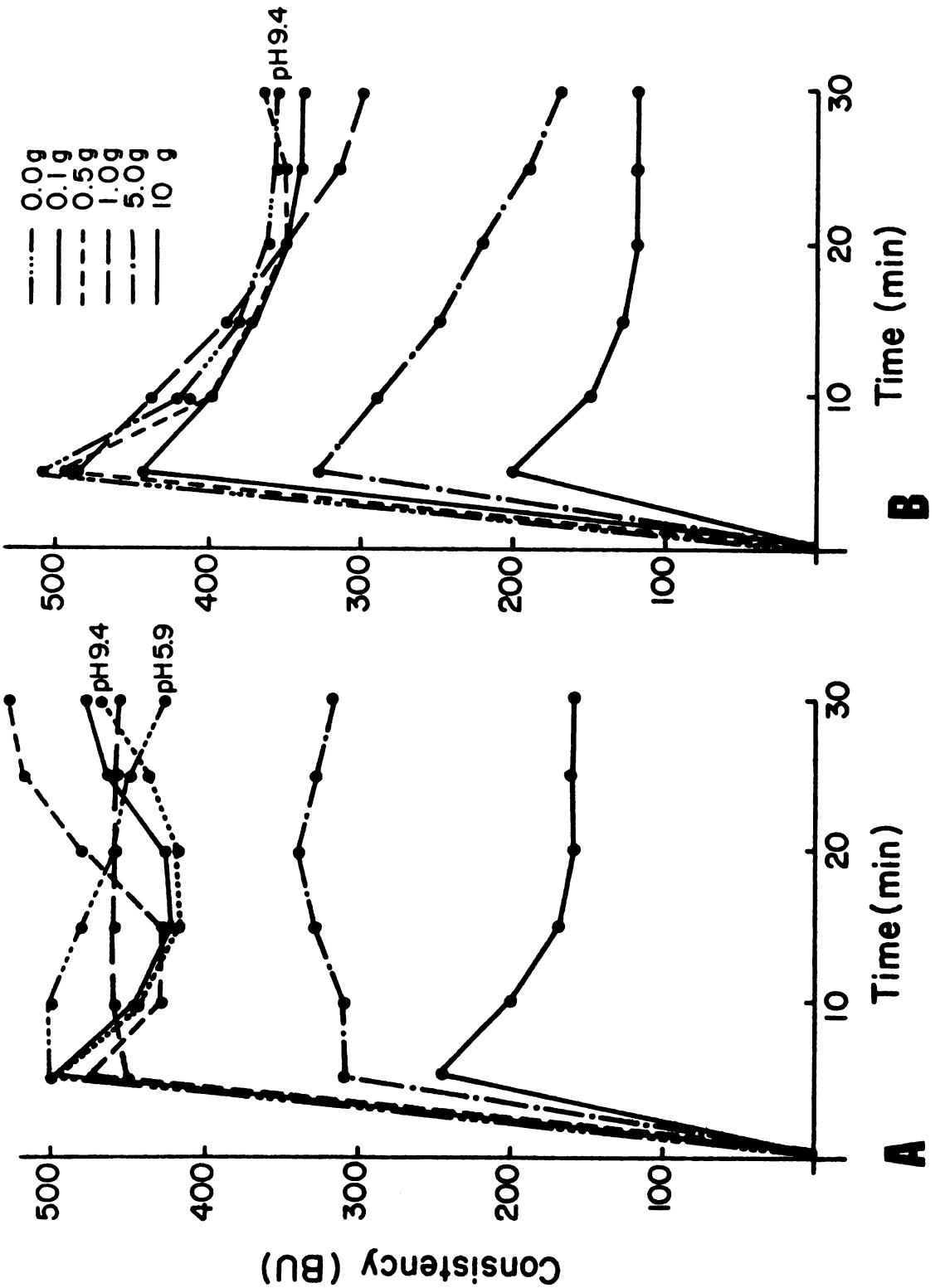


In this testing it was necessary to mix at a higher pH than normal. Usual farinograph dough pH was approximately 5.9, but in this testing the dough was titrated with N NaOH to a consistent dough pH of 9.4. Degradation effects may have been a problem thus controls at 5.9 and 9.4 pH are displayed (Figure 5A). Some early degradation of protein may have occured in the alkaline system but after 20 minutes of mixing this was equilibrated. As evidenced in Figures 5A and 5B the higher concentrations of succinic anhydride (5.0 and 10.0 g) tended to have extremely detrimental effects on the farinograph mixing pattern of the 0% and 6% SCP substituted doughs. The 0.1 g treatment with succinic anhydride had virtually no effect; in the 6% SCP dough the 0.5 g treatment had no effect. But the 0% SCP dough with 0.5 g succinic anhydride showed a strengthening in the mixing pattern at 20 min and had a final resistance, 60 BU greater than the untreated system. After treatment with 1.0 g succinic anhydride, the 0% SCP dough was virtually uneffected; however, the 6% SCP dough displayed a delayed weakening with a final resistance 50 BU less than the untreated system. The succinic anhydride at all concentrations (Figures 5A and B) except one (5 g) showed a more detrimental effect on resistance of the 6% SCP dough than on the 0% SCP dough at the 10 minute time measurement.

Figure 5. Effect of Various Concentrations of Succinic Anhydride at pH 9.4 on Consistency (BU) of Flour Dough as a Function of Time (min)

a) Control

b) 6% SCP Substituted Dough


Consistency (BU)
500
400
300
200
100
pH9.4
pH5.9
10
20
30
Time (min)
A
B
0.0g
0.1g
0.5g
1.0g
5.0g
10 g

Sodium-dodecyl-sulfate

The effect of sodium dodecyl sulfate (SDS) was observed on two characteristics of each dough system: mixing resistance and arrival time. Concentrations from 1.0 - 2.0 g SDS in the 0% SCP dough showed that as the concentration increased the resistance increased (Figure 6A). The rate of resistance increase at 1.0 and 1.5 g was parallel but decreased at 2.0 g. This was further substantiated by checking the rate of increase with 5.0 g SDS which demonstrated a much slower development to maximum resistance than at lower treatment levels. The 1.0, 1.5 and 2.0 g SDS tests in the 0% SCP dough seemed to plateau in resistance after 10 to 15 min of mixing.

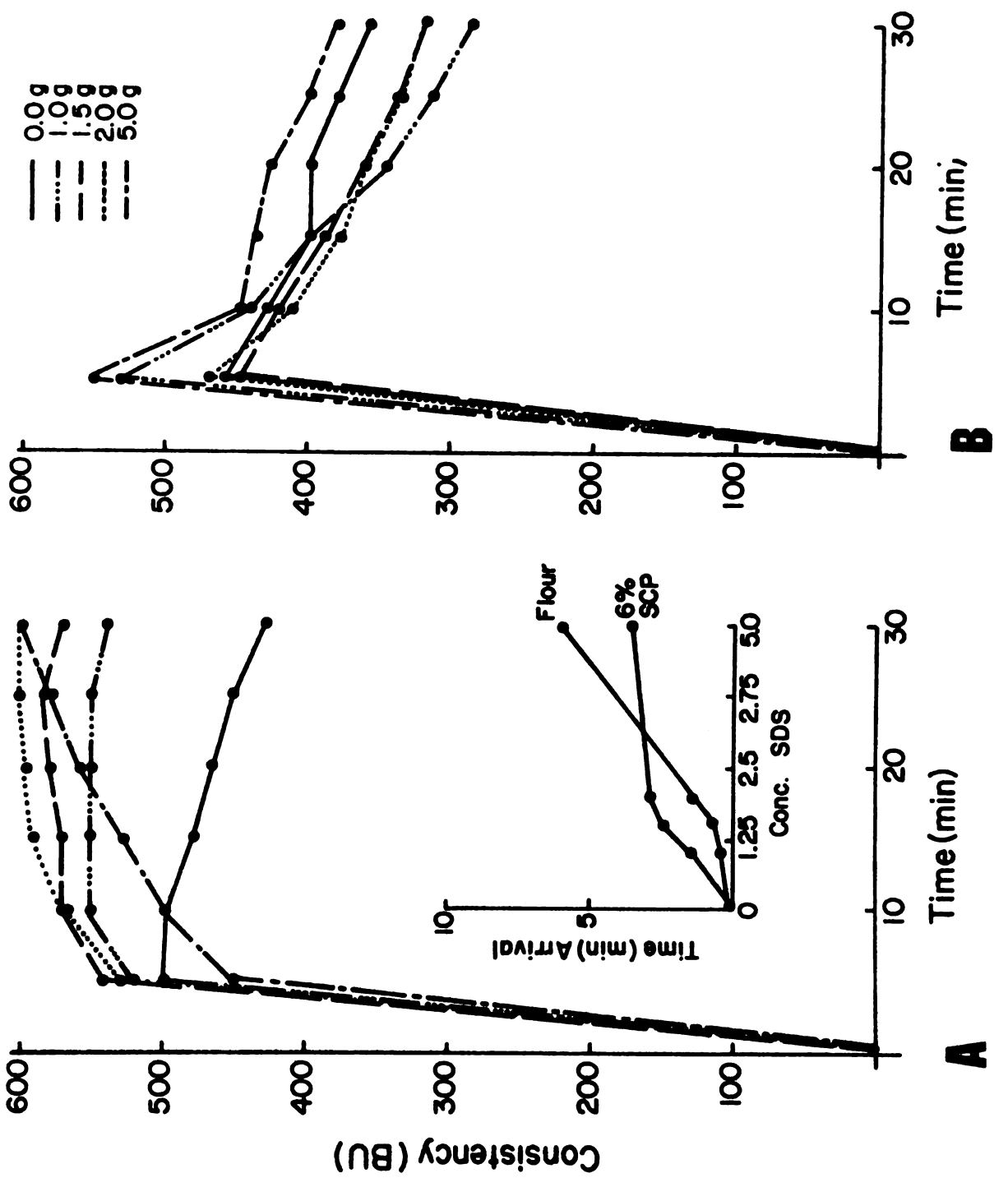
In the 6% SCP dough the mixing trends were not as clear as in the 0% SCP dough (Figure 6B). The doughs with 1.5 and 2.0 g SDS had approximately the same curve as the control (0.0 g SDS) dough up to 15 min at which time they displayed some weakening. The 6% SCP dough treated with 1.0 g SDS seemed stronger initially (5 min) with a fairly rapid and consistant drop in resistance occuring thereafter; no plateau effect was seen at any level of SDS. The 5.0 g SDS treatment of the 6% SCP dough caused high initial resistance measurements with subsequent decreases but at no time did the resistance drop below that of untreated 6% SCP dough.

If in the 0% and 6% SCP doughs the SDS was weakening the hydrophobic bonds it was making available other hidden bonding sites that may interact. This unfolding and association at previously unavailable sites was most obvious at the

Figure 6. Effect of Various Concentrations of Sodium-dodecyl Sulfate on Consistency (BU) of Flour Dough as a Function of Time (min)

a) Control

b) 6% SCP Substituted Dough


Consistency (BU)
600
500
400
300
200
100
10
20
30
Time (min)
A
Time (min) Arrival
10
5
0
1.25
2.5
2.75
5.0
Conc. SDS
Flour
6% SCP
0.0g
1.0g
1.5g
2.0g
5.0g
B
Time (min)

5.0 g concentration of SDS.

The effect of the SDS on the 6% SCP dough was more intense than on the 0% SCP dough; the SDS additions up to 2 g caused increased mixing resistance in the 0% SCP dough with a drop in the resistance at the 5.0 g level. The 6% SCP dough displays the opposite effect from SDS treatment with the mixing resistance decreasing with addition up to 2 g SDS and then increasing with the 5.0 g SDS addition.

Reactive Sulfhydryl and Disulfide

Sulfhydryl (-SH) and disulfide (-SS-) bond importance in rheological properties of doughs is a long established belief (49). It has been determined however, that not all sulfhydryls and disulfides are reactive and display an effect on the rheological parameters of a dough (49). As shown in Table 14, the 0% and 6% SCP doughs displayed total sulfhydryl counts of 123.5 and 210.4 mol/50 g respectively. Of these, 22.5 and 46.6 mol/50 g were involved in the mixing tolerance of the respective 0% and 6% SCP doughs (Figures 7A and B). Thus, only 19.2% and 22.1% of the total sulfhydryl groups were reactive in the respective systems. The remaining -SH groups were in all liklihood either buried inside of the protein molecule or unreactive due to some other chemical or physical force.

The total disulfide counts of the two systems (0% and 6% SCP dough) measured 888.0 µmols/50 g and 1057.0 µmol/50 g.

Table 14. Chemical and Physical Mixing Properties of Flour and 6% BYP Substituted Flour as a Function of Total and Reactive Thiol and Disulfide

	Flour	6% BYP
Protein (%)	14.35	19.31
Water absorption (%)	57.2	72.5
Development time (min)	6.1	2.5
Total thiol (μmol/50 g)	123.5	210.4
Thiol involved in mixing tolerance (μmol/50 g)	22.5	40.6
Total disulfide (μmol/50 g)	888.0	1057.00
Disulfide involved in development (μmol/50 g)	11.5	25.0
Disulfide involved in resistance to mixing (μmol/50 g)	82.5	92.5
Mixing SS/ mixing SH	3.66	2.28
Total SS/ SH	7.190	5.0223

Figure 7. Determination of Sulfhydryls Involved in Mixing Tolerance by Treatment of Flour Dough with N-ethylmaleimide

a) Control

b) 6% SCP Substituted Dough

Determination of µmols of (-SS-) Disulfides Effecting Development Time of Flour Dough

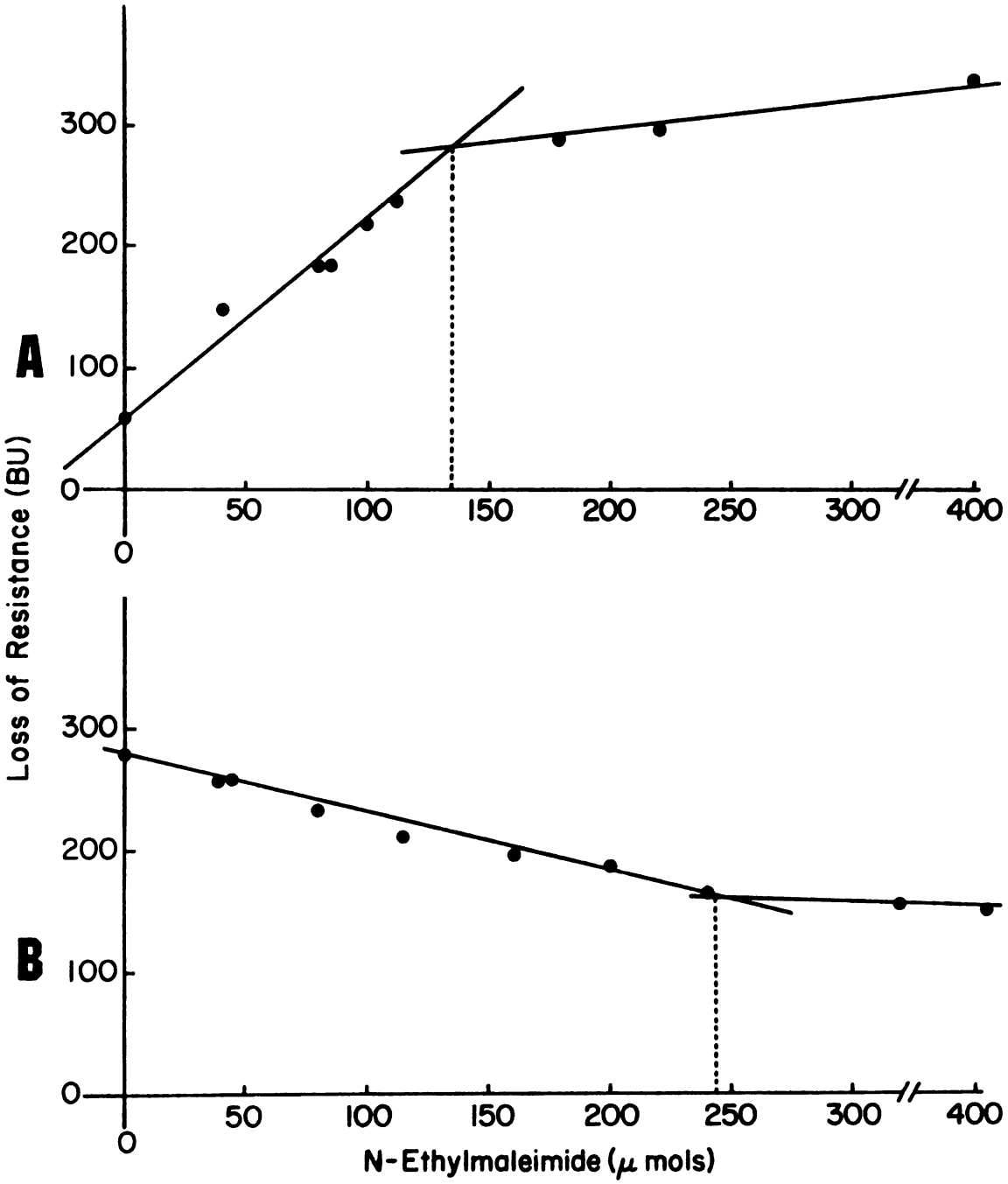
c) Control

d) 6% SCP Substituted Dough

Determination of Disulfides Involved in Mixing Tolerance by Treatment of a Flour Dough with Dithiothreitol

e) Control

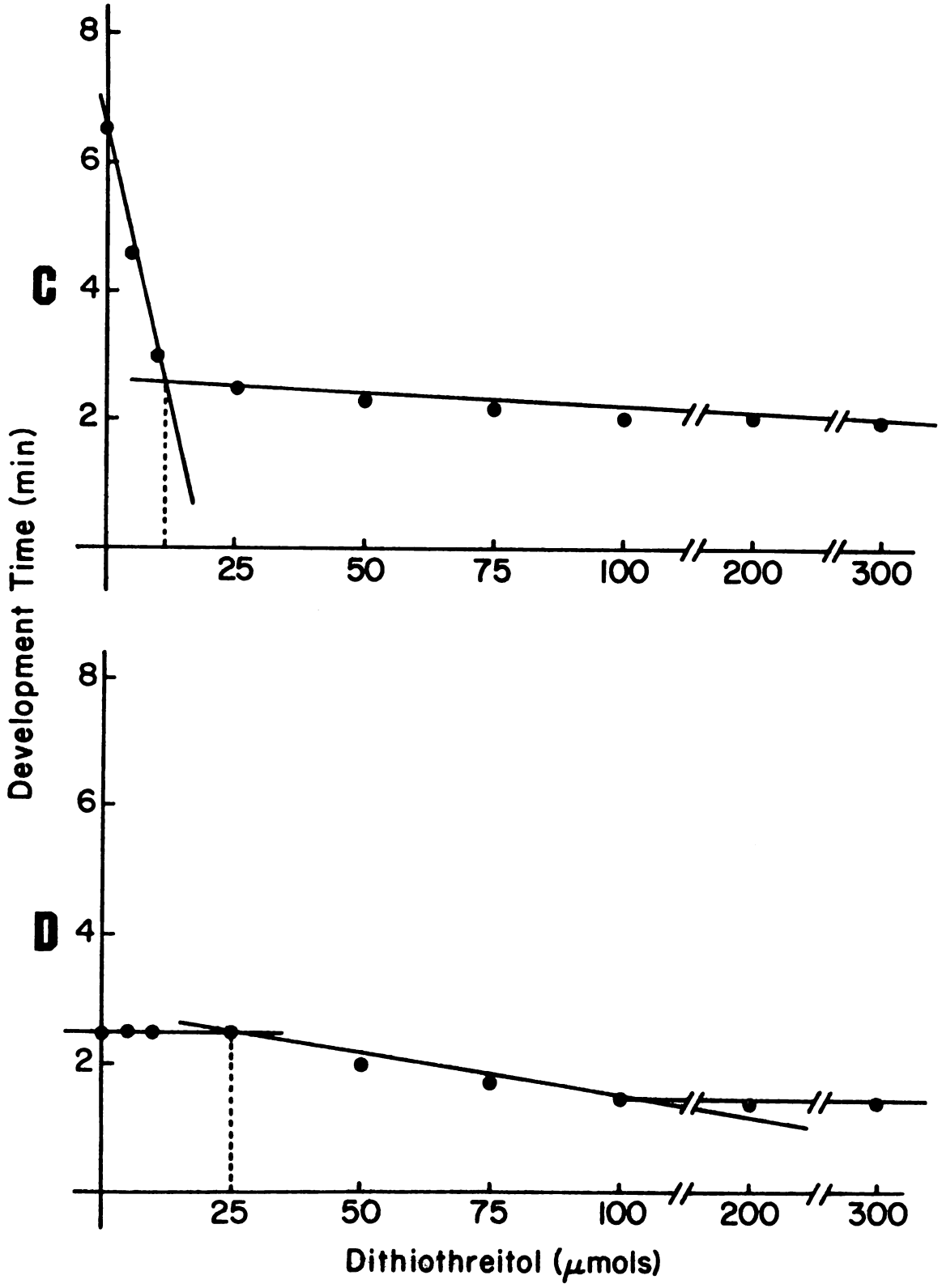
f) 6% SCP Substituted Dough

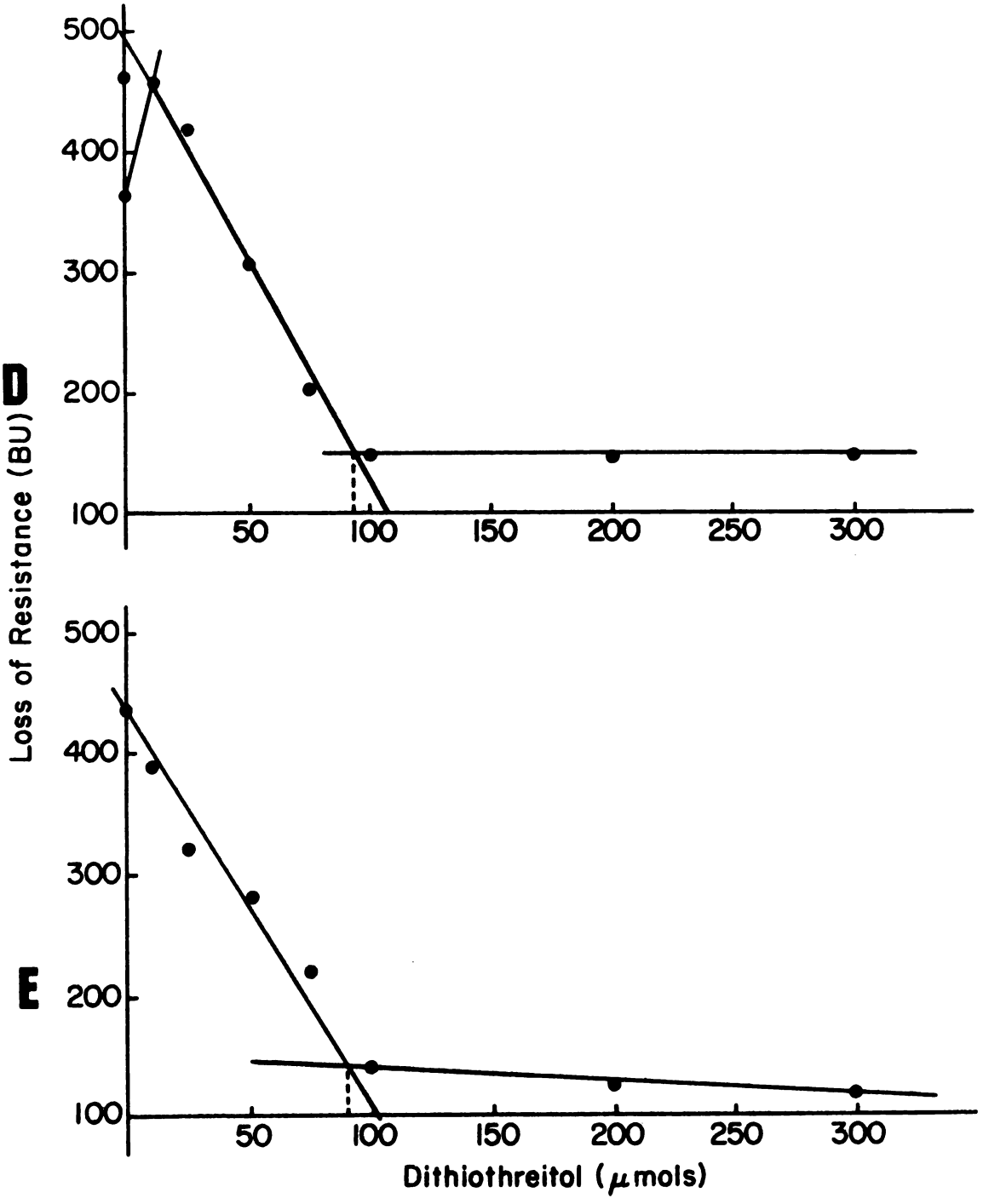

A
B
Loss of Resistance (BU)
300
200
100
0
0
50
100
150
200
250
300
400
N-Ethylmaleimide (μ mols)

Actively involved in dough development were 11.5 µmol/50 g and 25 µmol/50 g of the disulfides (Figures 7C and D). This was about 2.08% of the total groups in the 0% SCP dough and 2.56% of the total content of the 6% SCP system. The reactive disulfides involved in mixing tolerance were 82.5 µmol/50 g or 9.29% for the 0% SCP dough and 92.5 µmol/50 or 8.75% for the 6% SCP dough (Figures 7E and F). These values were slightly low as the reported range is 11-13% (49) but they are still viable.

The mixing SS/mixing SH of the 0% SCP dough was 3.66 versus 2.28 for the 6% SCP dough. In contrast, the total SS/SH ratios were somewhat higher, 7.19 and 5.02 respectively.

It had been reported that of the sulfur containing peptides endogenous to flour only gluathione was acting as a determinant of dough properties. As can be seen from Table 14, the content of total sulfhydryl and disulfide in the 6% SCP flour dough increased considerably over that of the all flour dough. Because of the non-gluten nature of the SCP and yet the arrarent reactivity of some of its -SH and -SS- groups it may be suggested that the SCP could function as does glutathione, a non-structural sulfur bearer, which may enter into the sulfhydryl-disulfide interchange. In turn because of its inability to aggregate with the gluten network other than through these -SH and -SS- groups the SCP in the dough tended to be a weakening factor in the overall mixing, extensibility and resistance to extension characteristics (Table 3 and 4).


C
D
8
6
4
2
25
50
75
100
200
300
Development Time (min)
Dithiothreitol (μmols)


D
E
Loss of Resistance (BU)
500
400
300
200
100
50
100
150
200
250
300
Dithiothreitol (μmols)

Scanning Electron Microscopic Investigation (SEM)

When flour and water were mixed into an optimally developed dough the most striking observation was the envelopment of the starch granules by a continuous sheet of gluten protein. The visual nature of this gluten network when prepared with and without 6% SCP and with and without 0.5% sodium stearoyl-2-lactylate will be the topic of this section.

Previously Argani and Hawrylewicz (6) described the interaction of flour proteins during dough development as forming a smooth veil-like network that stretched over the starch grains. The nature of this network was both visually and rheologically effected by the above listed treatments of the dough.

At the lowest magnification (Figures 8, 9, 10, 11-A) the doughs appeared roughly similar. There was a pebbly character with no evidence of a trend in directional placement of the granules. Both large and small starch granules were apparent in all doughs, and they could be seen both on the surface of the dough and under the gluten sheeting. All doughs showed crater-like structures that were about the same size as the large granules. In all liklihood starch granules that were not firmly positioned in the gluten network had dislodged leaving these pock-like structures. Figure 8C showed a starch granule that appeared to be weakened in its positioning in the gluten network.

At the next range of magnification (Figures 8B, C, D)

Figure 8. Scanning Electron Micrographs of Flour and Water Doughs

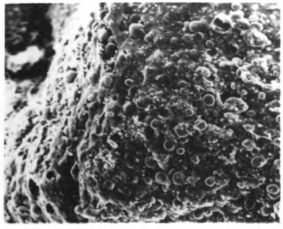
a) 200X

b) 800X

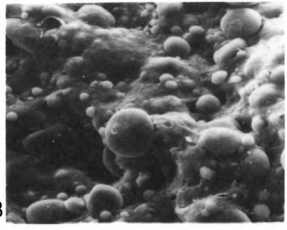
c) 1,000X

d) 1,600X

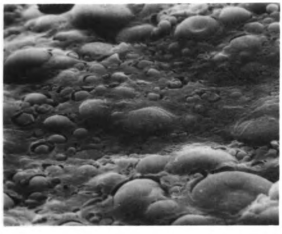
e) 2,800X



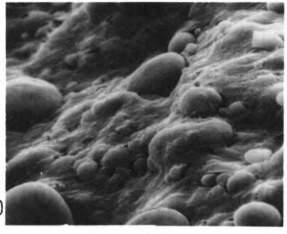
A



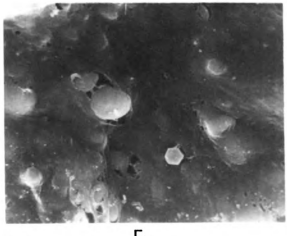
B



C



D



E

Figure 9. Scanning Electron Micrographs of Flour, Water and Na Stearoyl-2-lactylate

a) 200X

b) 800X

c) 1,000X

d) 2,800X

e) 8,000X

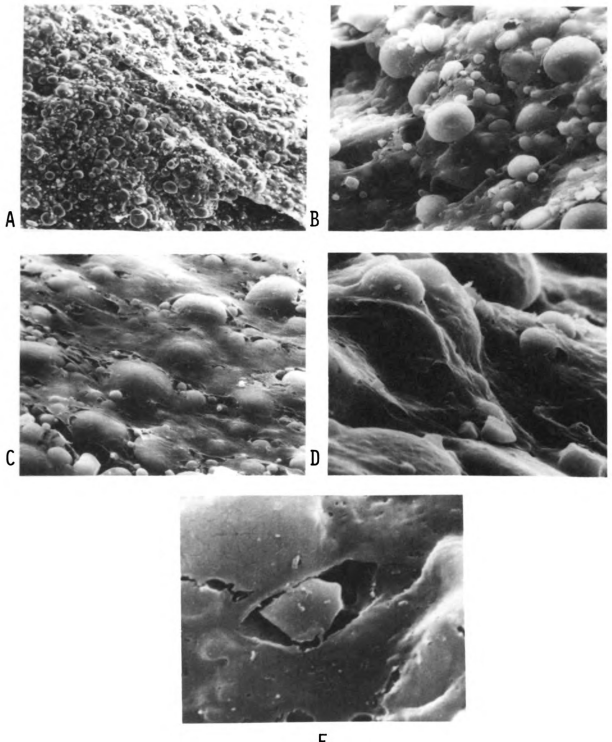

A
B
C
D
E

Figure 10. Scanning Electron Micrographs of 6% SCP, Flour and Water Dough

a) 200X

b) 800X

c) 1,000X

d) 8,000X

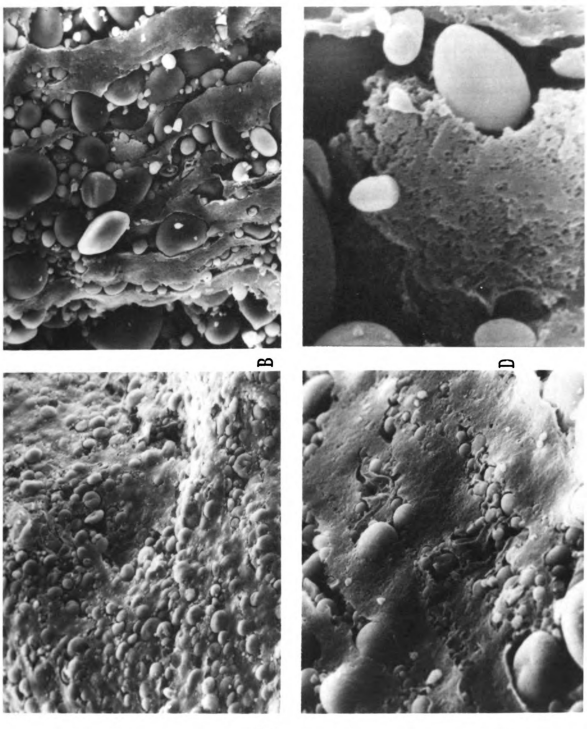

A
B
C
D

Figure 11. Scanning Electron Micrographs of 6% SCP, Flour Water and Na Stearoyl-2-lactylate

a) 200X

b) 800X

c) 1,000X

d) 7,000X

A
B
C
D

the gluten sheet looked thin with a crepe-like texture. With increasing magnification (Figure 8E) the crepy roughness in texture was diminished but the surface of the sheet was noticeably marred by very small random pockets. These pockets displayed a multiple layering phenomenon, the extent of which governed the thickness of the protein sheet. It was interesting to note that the diameters of these pockets were comparable to the sizes of osmiophilic inclusions discussed and demonstrated by Simmonds (89) and Khoo et al. (51) which they accredited to the redistribution and aggregation of membranes and organelles, a source rich in lipids.

It was also noted that the most severe rupture of the gluten sheet occurred at the starch/protein interface (Figure 8C), an area subject to great stress during dough manipulations. The mixing process developed and stretched the gluten into thin sheets flexible enough to snuggly conform to the shapes of underlying starch granules. However, stress forces during mixing sometimes exceeded the elastic strength of the gluten allowing sheet breakage at points of weakness. On a microscopic level, the breakage was most obvious at the base of protruding starch granules (Figure 8C). The torn areas were not clean breaks and many still exhibited finger-like projections spanning the gap between the two parting edges indicating the adhesive nature of the gluten. In addition, an affinity between granules and the membraneous protein that coats them was quite apparent in many cases (Figures 8B, D, D). There was, however, in other cases a

definite separating space between granules and the protein. Whether the difference in the closeness of the relationship between the granule and the gluten sheet was a function of the degree of gluten development or an artifact of sample preparation was not clear.

The addition of SSC to the 0% SCP dough resulted in alteration of the system (Figure 9). When examined it was similar to that of the control but subtle changes were evident. The gluten sheet was extremely thin, in fact, translucent in many areas; such that starch granule silhouettes were visible beneath the veiling protein. It appeared very extensible and draped fluidly over the pebbly mass of granules (Figure 2B). The sheeting character of the gluten in the emulsified dough seemed finely improved over the control; although, higher magnification (Figure 9E) showed that the smoothness of the gluten was interrupted by small pockets as observed in the control (Figure 8E).

The most obvious change in dough structure was observed in the sample containing 6% SCP substitute (Figure 10). The gluten structure was severely altered in the presence of SCP indicating that the supplement was carried by the gluten. The gluten did not develop into a continuous smooth network which could conform to the surface contours of starch granules as in the control (Figure 8) but, being less flexible, it was physically disrupted by the irregular shapes. There was no translucency to the malformed sheet and the outlines of underlying granules were masked. In contrast to the control the

gluten mass was opaque with respect to granule contours, very thick and rough textured to the point of appearing spongy and much less coherent (Figures 10B, C, D).

It appeared that the SCP gluten could not maintain or produce the sheeting typical of its nature. There was no evidence (Figures 10B, C) of the finger-like fibrils which seemed to indicate the cohesive quality of the gluten mass observed in the control (Figure 8B).

The SCP dough with the emulsifier (SSL) exhibited improved cohesion of the gluten compared to the protein substituted dough (Figure 11B compared with Figure 10B). The texture was slightly smoother, less spongy and thick and showed increasing sheeting ability. There was still discontinuity of the gluten phase though not as extensive as without emulsifier. The gluten sheet of this preparation was thinner than that of the SCP alone but still failed to completely coat starch granules as it was stretched over their surfaces. The sheet had a tendency to rupture at stress points and settle around the base of granules.

When different dough treatments had been tested for extensibility (Table 4), it was observed that SSL added to the mixes increased extensibility while substitution of 6% SCP reduced it. SSL added in conjunction with the 6% SCP somewhat restored extensibility at the initial testing time. These results were consistent with the physical SEM observations.

One point of concern in a flour/water dough containing

additives was the problem of uniform distribution of ingredients throughout the system and therefore the equal distribution and random selection of samples for SEM could lead to a misinterpretation of the results. Ample sampling of each variable, as in this work, may minimize this problem.

SUMMARY AND CONCLUSION

The primary objective of this study was to observe the effects of substitution of varying levels (0, 3, 6 and 12%) of SCP on the physical and rheological effects of doughs and bread. The SCP substituted doughs and bread systems were treated with oxidants, conditioners, salt and heat to determine additive functionality. In addition, interactions of these treatments in the 6% SCP substituted system were also observed. The effect of chemical reagents on the mixing character of the 0% and 6% SCP substituted dough was tested and scanning electron microscopic investigation of the 0% and 6% SCP substituted dough with and without conditioner was performed.

Farinograph studies of the 0, 3, 6 and 12% SCP substituted doughs showed that overall mixing character of the dough at the 0 and 3% SCP substitution level were quite similar for all treatments, displaying similar peak and stability times. However, inclusion of the SCP at the 6 and 12% levels in the dough generally resulted in severe decreases in these two mixing characteristics under all treatments. Consistently, as the level of SCP increased the dough absorption increased. The absorptions of all doughs above the 3% level was abnormally high.

Extensigraph measures showed a loss of extensibility of the doughs as the level of substitution of SCP increased. This loss of dough extensibility at the 3% SCP level was in most cases rather small but greater differences occurred at the 6 and 12% SCP levels. All of the treatment systems resulted in greater extensibility of the 3% SCP doughs than in the untreated 0% SCP dough. In general, most systems displayed a loss of extensibility with increasing time. Resistance to extension values decreased with increasing levels of SCP substitution but they concurrently showed increases with time. In most cases, even at the longest testing time, the dough at the level of SCP substitution was not able to display a resistance equal to the earliest test measure of the preceeding level's dough system.

In baking tests the bread volumes decreased as the SCP substitution level increased. The differences in volume between the 0 and 3% SCP substituted bread was negligible but the volume loss in the 6 and 12% SCP bread, was quite extensive. The oxidant and conditioner treatments seemed to be the most effective additives in controlling volume decrease.

As the level of SCP substitution increased in bread the protein, moisture, lipid and ash contents also rose.

Sensory evaluation of the breads showed a slight preference for the product prepared with 3% SCP, however, the scores of the 0 and 3% SCP bread were quite similar. Considerable decline in overall acceptability occurred with 6% SCP inclusion in the bread, making this product only fairly

acceptable. The 12% SCP bread was unacceptable under all treatments.

Considerable interaction of the additives was found for the doughs prepared at the 6% SCP level with double and triple combinations of additives. Farinograph arrival time for all doughs except one with combinations of treatments halved in value. In all instances doughs treated with combinations including salt displayed the longest arrival times.

Combinations of dough treatments which included heat resulted in peak times in all cases that were decreased from that of use of heat treatment alone. Pairing oxidant with other treatments resulted in later peak times for doughs compared to the use of oxidant alone. Doughs containing oxidant in combination with other additives were most stable while those with a heat treatment as long as it was not combined with oxidant were least stable.

The extensibility measures of the 6% SCP doughs treated with combinations of additives showed very little change as compared to use of additives individually. However, combinations of treatments resulted in doughs that were considerably higher in resistance to extension compared to use of the additives alone. The oxidant and salt treatments were most effective in increasing dough resistance to extension.

The use of combinations of treatments resulted in higher volumes for 6% SCP substituted bread than when the additives were tested alone. Most of the volumes of 6% SCP bread treated with additive combinations had volumes as high or

higher than the 0% and 3% SCP bread with the individual additives.

Sensory evaluation of the 6% SCP bread treated with combinations of additives found the acceptability to be considerably improved. With the exception of two, the 6% SCP products' with additive combinations scores were higher in acceptability than all of the 0% and 3% SCP bread with individual additives.

The chemical reagents were generally more detrimental to the mixing strength of the 6% SCP substituted doughs than in the 0% SCP dough. The urea treatment which was being evaluated for the effect on hydrogen bonds exhibited a strenthening in mixing at lower concentrations while higher concentrations showed distinct weakening for the 0% SCP dough. In the 6% SCP dough lower concentrations of urea resulted in early strengthening with subsequent weakening and higher concentrations were definitely detrimental to the mixing strength.

The succinic anhydride was generally increasinly detrimental to the mixing character as the concentration increased in both the 0% and 6% SCP doughs. In both systems the weakening effect of the succinic anhydride at the lower concentrations seemed to be overcome with longer mixing times.

In determining the reactive sulfhydryl-disulfide levels it was found that 19.2 and 22.1% of the total sulfhydryl groups were involved in mixing tolerance of the 0% and 6% SCP substituted doughs, respectively. In the 0% and 6% SCP substituted doughs, 9.29 and 8.75% of the disulfides were reactive in

mixing tolerance, respectively. About 2.08 and 2.36% of the disulfides were actively involved in dough development of the respective 0 and 6% SCP substituted doughs.

In the scanning electron microscopic investigation of the 0 and 6% SCP doughs it was found that the SCP made the gluten sheeting appear bucky and quite thick compared to that of the dough without SCP. Addition of conditioner (SSL) to both 0% and 6% SCP doughs resulted in a thinner and smoother gluten sheet.

The overall results of this study indicated that SCP may be added to bread dough up to the 3% level with individual additive treatment and it may be added up to 6% when used with combinations of the additives. The electron microscopic investigation showed that the SCP was carried by the gluten proteins but the exact nature of their interaction is uncertain.

PROPOSALS FOR FUTURE RESEARCH

Future investigation dealing with the content of this dissertation could be multiple particularly with respect to the area of increasing protein content of bread products. However, an even more timely need is seen for the technical methods of evaluating the effect of inclusion of foreign substances i.e. proteins or fiber in bread products.

Methods for evaluation of objective parameters of the bread such as volume or compressibility are available but need further standardization. The ultimate decision as to product quality as done by subjective evaluation are satisfactory but again lack consistency as to importance of parameters.

This research amply demonstrated that there are food additives i.e. conditioners, oxidants, salt, etc. that have a multiplicity of effects on a system that has a protein substitute in it. But rapid determination of which of these will alone or in combination maximize final product quality when using a range of concentrations of ingredients i.e. protein is still beyond our technological grasp. If it could be determined what effects protein ingredients actually have on the molecular level in the dough then additives that have an improving function may be more easily and rapidly selected.

Further research using model systems should be developed to study the molecular bonding of gluten and to determine which are most significant types of bonding in development and strengthening the system with respect to final product quality. If the functioning of these could be realized accurately, a tool for all stages of manipulation of production would be available.

Further development in scanning electron microscopic (SEM) examination of doughs and bread should also be undertaken. It may be that SEM will provide excellent information particularly with respect to structural faults that occur in products of newly devised formulations. Currently, the only real method for improving a faulty dough or bread is via trial and error and this takes more time usually than would several reliable technical evaluation procedures working at a micro level of protein, starch or lipid functionality in this heterogeneous system.

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A P P E N D I X

APPENDIX I

BREAD SCORE CARD

Name _____

Date _____

Use the following scales:

	CRUST COLOR	CRUST CHARACT.	GRAIN	FLAVOR	CRUMB COLOR	TEXTURE
5	Rich golden brown; very even	Soft, tender, breaks easily	Fine & distinct cells throughout; not compact	Mild, sl. sweet	Creamy white & bright	Sm. sl. elong. cells; even size & dist. w/thin cell walls
4	Rich golden brown w/some unevenness	Sl. soft, tender crust; breaks easier	Mostly fine cells with a few coarse	Sl. bland, sl. observable flavor	Creamy interior w/yellowish shadows	uneven sm. sl. elong. cells; thin walls
3	Sl. light; sl. dark; uneven thru loaf	Sl. tough, thick, rubbery or sl. soft	Coarse & fine cells; uneven dist. w/some air holes	Bland w/o distinguishable flavor	Sl. grey w/ yellowish shadows	Irr. sm. & lg. cells; thick & thin walls
2	Sl. light; sl. dark; over full loaf	Top tough, thick, rubbery & mod.-dif. to break	Mod coarse cells; uneven w/lg. holes	Somewhat off; yet not completely distasteful	Grey w/yellowish shadows	Irr. cell; thick walls but not exc. large
1	Too light; too dark	Tough, thick, rubbery, dif. to break	Coarse cell structure, uneven lg. holes	Flat or sour, distasteful	Grey, dull	Lg. irr. cell; uneven & thick walls

Sample No.	CRUST COLOR	CRUST CHARACT.	GRAIN	FLAVOR	CRUMB COLOR	TEXTURE	COMMENTS