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MICROSTRUCTURE AND FUNCTIONALITY OF SOURDOUGH DOUGHS AND BREADS

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ABSTRACT

MICROSTRUCTURE AND FUNCTIONALITY OF
SOURDOUGH DOUGHS AND BREADS

By Katherine P. Niven

Microstructure and rheology of bread dough and bread crumb samples with, and without, the addition of salt and/or organic acids in sourdough yeast culture were examined. Scanning electron micrographs indicated that salt had a strengthening effect and organic acids had a weakening effect in dough and bread samples. When used in combination, salt counteracted the weakening effect of organic acids. Two samples ,though, standard yeast culture bread crumb without salt, and sourdough yeast culture bread crumb with salt were similar in microstructure. Rheological tests were conducted on unfermented model dough systems, standard yeast culture, and sourdough fermented doughs using the farinograph. Bread crumb was tested on the universal testing machine. The results were parallel to trends shown in the micrographs; however, differences in textural data were more pronounced.

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Table of Contents

CHAPTER PAGE

List of Tables

List of Figures

CHAPTER I

Introduction

San Francisco sourdough french bread is a trademark of the San Francisco Bay Area. It has been estimated that 15 to 20 percent of the bread consumed in the Bay Area is sourdough (Kline, Sugihara, & McCready, 1970). The unique properties of sourdough french bread have been under investigation for years. Despite much research on the subject, little work has addressed the microstructure of sourdough and how it relates to rheological behavior. Scanning electron microscopy (SEM) provides information on the microstructural characteristics of bread dough and bread crumb. An examination of microstructure as it relates to textural changes could contribute to a better understanding of the functional behavior of sourdough bread.

The separate effects of acid and salt on doughs consisting of wheat flour and water have been studied extensively. Nevertheless, the combined effects of acid and salt on model dough systems have been investigated by only a handful of investigators (Bennet & Ewart, 1962; Tanaka, Furukawa, & Matsumoto, 1967; Galal, Varriano-Marston, & Johnson, 1978; Bakhoum & Ponte, 1982). Galal and researchers (1978) isolated the organic acids found in a San Francisco style sourdough starter culture and then observed the effect these acids had on the functional behavior of wheat proteins.

Farinographs were evaluated for unfermented model dough systems which were comprised of wheat bread flour, water and added acid and/or salt. The addition of salt was found to modify or counteract the influence of acid on wheat proteins. Their study, however, did not evaluate the effect of acid and/or salt in intact fermented sourdough doughs.

Changes in microstructure in sourdough doughs and breads which could be expected to accompany textural changes have not been assessed. In addition, neither the rheology of intact fermented sourdough doughs, or sourdough baked bread has been investigated. An understanding of the microstructure and rheology of sourdough dough and bread can contribute to effective quality control and product development efforts. The examination of microstructure as it relates to textural changes will provide additional useful information on the functionality of intact sourdough dough and bread. This will, in turn, expand the knowledge of the American baking industry.

Objectives

The present research was designed to investigate two research aspects of sourdough bread. Specifically, the rheological behavior of model dough systems, intact sourdough doughs and baked breads, and the microstructure of sourdough doughs and breads were evaluated.

Before carrying out the rheological analysis, lactic acid levels were analysed in sourdough starter sponge and sourdough bread doughs during the fermentation process. The resulting levels of lactic acid were then compared to literature values for sourdough bread. This comparison was deemed necessary to verify the authenticity of the San Francisco style sourdough culture used in this study. The lactic acid analysis was also used to obtain an idea of the proper total levels of organic acids to add to the unfermented model doughs.

The rheological behavior of the model dough systems, and the sourdough doughs and breads was evaluated by using two instrumental approaches: the farinograph (C.W. Brabender, El Cerrito, California), and the Instron universal testing machine (Model T.M., San Ramon, California). The farinograph measured dough development time, dough tolerance and stability during mixing in unfermented and fermented doughs. The Instron evaluated the firmness of bread measured as resistance to compression and amount of recovery from compression.

The microstructure of unfermented doughs, fermented doughs, and baked bread crumb was examined using a scanning electron microscope. Visual observation of starch granule size and spatial orientation, as well as external characteristics of the gluten matrix, was carried out to evaluate the dough and bread microstructure.

CHAPTER 2

Literature Review

Introduction

Bread, a staple since pre-historic times, has fascinated researchers for centuries. In 1745, Beccari, an Italian chemist, kneaded a flour dough under running water. The result, an elastic cohesive mass, he named gluten (Khan & Bushuk, 1979). The vast majority of scientific research on gluten has taken place since the 1960's. Chemical solubility studies have identified two major protein components: gliadin and glutenin. Specific amino acid sequence, and molecular size and shape have been determined for both glutenin and gliadin. Extensive gel-filtration, objective textural methods, and optical rotation studies have enlightened the scientist as to the unique chemical and rheological functionality of gluten. The advent of the electron microscope has given the scientist an enhanced visual perspective from which gluten and its properties can be observed (Taranto, 1983).

Wheat Proteins

Gliadin

Gliadin, a 35-40% component of the protein in wheat flour, is soluble in 70% aqueous ethanol. Glutamic acid is the predominant amino acid in gliadin and is believed to contribute to the high degree of hydrogen bonding in the

gluten complex (Khan & Bushuk, 1979; Wehrili & Pomeranz, 1969). Gel electrophoresis suggests that gliadin is composed of intra-polypeptide disulfide linkages in single chain structures. Gliadin is thought to influence the resulting viscosity of the gluten complex (Khan & Bushuk, 1979). Scanning electron microscopic analysis of gliadin has shown the average diameter of the strandlike fibrils were 2 μm intermixed with small spherical particles that were 2-4 μm in diameter (Paredes-Lopez & Bushuk, 1983).

Glutenin

Glutenin is soluble in dilute acid or alkali and makes up 35-40% of the wheat protein. Glutenin is believed to contribute to the elastic qualities of wheat protein (Khan & Bushuk, 1979). Gel electrophoresis of glutenin isolates show a large asymetrical molecule. Sub units of glutenin are either long strands with interpolypeptide disulfide linkages, or micelles comprised of both hydrophobic and hydrogen bonds (Wehrili & Pomeranz, 1969). Scanning electron micrographs by Orth, Dronzek, and Bushuk (1973) of purified, hydrated glutenin from Manitou wheat showed intertwined fibrils 1 μm and 10 μm in diameter. Cross sections indicated the fibers were circular in shape with filmy material between strands. This fibrous network was reacted with ß-mercaptoehanol and 6 M urea. The resulting glutenin lost its fibrous appearance, indicating that the reduction of disulfide bonds had

occurred. Orth et al. (1973) concluded that disulfide bonds were essential in maintaining its structure.

Interactions in Wheat Proteins

The unique amino acid structure of glutenin and gliadin exhibit four major types of bonds found in bread dough: disulfide bonds, ionic bonds, hydrophobic bonds, and hydrogen bonds.

Wall & Huebner (1981) stated that the mechanical kneading of bread doughs breaks intra disulfide bonds into highly cross-linked protein aggregates so that smaller aggregates can associate. Tsen (1973) reviewed research on cysteine use in chemical dough development. Added cysteine reduced dough mixing time by cleaving disulfide linkages between larger protein aggregates so that smaller units were freed to realign in a functional protein matrix. Cysteine used in larger amounts had detrimental effects on bread dough by weakening gluten structure (Belitz, Kieffer, Seilmeier & Weiser, 1986). Bromate, an oxidizing agent, had the opposite effect. When added to dough, disulfide bonds were broken and reformed by exchanges with neighboring sulfhydrl groups (Wehrli & Pomeranz, 1969).

Ionic bonds are extremely important in the role of dough development. Ionic attractions between carboxylic and amide groups on neighboring amino acids are thought to strengthen the gluten complex (Belitz et al., 1986). The addition of salt to bread dough was shown to increase dough stability and

decrease extensibility (Galal, Varriano-Marston, & Johnson, 1978).

Gliadin contains a high percentage of leucine, which has extremely hydrophobic properties. When flour is hydrated, the non-polar groups of leucine interact with one another. This interaction is believed to stabilize the glutenin complex (Khan & Bushuk, 1979). Belitz et al. (1986) described gliadin as a "hydrophobic" solvent for the relatively insoluble glutenin. Hydrophobic interactions are felt to contribute significantly to the visco-elastic properties of the gliadin-glutenin complex.

Lipids, which make up 5-10% of the dry weight of gluten solids, are believed to complex to glutenin (by hydrophobic bonds) and gliadin (by hydrogen bonds). Khan and Bushuk (1979) suggest that these lipids aid in the gas holding capabilities of the gluten complex.

One third of the amino acids in gluten is glutamine. Glutamine exhibits inter- and intra-hydrogen bonding. Indirect evidence, such as the addition of ascorbic acid (Tsen, 1973) or urea (Khan & Bushuk, 1979) is thought to break hydrogen bonds, affecting the physical properties of bread dough. Thus, it is concluded that hydrogen bonding plays an important role in breadmaking.

Glycine, which is a major component of collagen, is also present in glutenin. The physical structure of glycine contributes to the elasticity of glutenin. A larger

proportion of glycine was found to be present in "stronger" wheat flours which are superior for breadmaking (Belitz et al., 1986).

Salt and Acid Effects on Dough Rheology

A number of studies have evaluated the effects of added acid or salt on bread doughs and model dough systems, but the results have not always agreed.

When acid was added to dough in the pH range of 2 to 12, Watanabe, Watanabe, & Uemura (1955) farinograph data indicated a dough weakening as pH was decreased. This effect was also observed by Bennett & Ewart (1962) and Hoseney & Brown (1983), using other rheological tests, such as an extensometer and a mixograph. However, Bayfield & Young (1964) also evaluated farinographs of bread doughs at various pH levels and concluded that the doughs didn't show weakening, though one dough "appeared" sticky at pH 4.6. Tanaka, Furukawa, & Matsumoto (1967) theorized that the variation in results between these groups of researchers was due to the variety of acids utilized and their differing ionic strength. Their data indicated that organic acids had a tendency to increase dough consistency with decreasing pH. Added organic acids also made doughs weaker and more unstable than did the addition of inorganic acids.

The addition of salt (NaCl) to model system doughs consisting of water and flour has shown conflicting results. On one hand, Tanaka et al. (1967) found that salt decreased

the consistency of dough in a farinograph when compared to a control without salt. Hlynka (1962) also had these results. However, Galal, Varriano-Marston, & Johnson (1978) indicated that salt had a strengthening effect with increased dough development time in a farinograph. Bennett and Ewart (1965) paralleled Galal's findings, showing salt decreased extension and increased resistance in an extensometer.

However, few studies have evaluated the rheology of model system doughs when both salt and acid were added. Bennett and Ewart (1962) used an extensometer to observe the effects of inorganic and organic acids in various concentrations ranging from 0-135 m moles added to doughs made from bread flour and a 2.5% NaCl solution. Yeasted bread dough was also made up with lactic acid added in various concentrations (0-135 m moles) and then baked off to record loaf volume. Their findings indicated that extensibility was decreased as acid levels increased. Weaker acids, such as acetic and propionic, had the least effect on reducing extensibility due to less hydrogen ion dissociation. The authors hypothesized that acid added to doughs donated hydrogen ions, which unfolded the protein due to the cleavage of salt bonds and the repulsion of positively charged groups in the gluten complex. The resulting protein had reduced elasticity due to the uncoiling of the gluten complex. This, in turn, decreased the extensibility of the dough. Also, baked bread had decreased loaf volume as lactic acid levels increased.

Bennett and Ewart (1962) suggested that increased levels of lactic acid reduced extension of bread dough, caused gas cells to rupture, and thus reduced the volume of baked bread.

Tanaka et al. (1967) observed physical dough properties with varying levels of acetic acid and NaCl combinations. Water and flour dough was adjusted to pH levels of 4.2, 4.8, 5.1, and 5.8 with 1 N acetic acid. Farinographs were recorded for these doughs and for another similar set of doughs with 3.0% (flour basis) NaCl added. The results indicated that unsalted dough consistency increased as pH was lowered. The opposite was true for the salted doughs: consistency decreased with lower pH values. They postulated that acid breaks the inter- and intra-molecular bonding of NaCl as seen by the lower consistency farinograph curves of the doughs at pH 4.2. Tanaka et al. (1967) also used an extensograph to measure extensibility of 0.0%, 1.0%, 3.0% salted doughs at pH values of 4.2, 4.8, 5.1, and 5.8. Findings showed that at a low pH (4.2), reduced extensibility occurred with or without the presence of salt. Also, as pH decreased, the resistance increased. Tanaka et al. (1967) discussed the contradictory nature of their results, i.e., as pH decreased, farinograph consistency increased in unsalted doughs and resistance values were decreased for unsalted doughs in extensographs. They suggested that consistency (in farinographs) and resistance (in extensographs) were not synonymous physical dough properties. However, the authors did not explain the

increased resistance of salted doughs at these same pH values in extensograph tests.

Galal, Varriano-Marston, & Johnson (1978) isolated organic acids from commercial sourdough bread doughs. Gas liquid chromatography indicated that lactic and acetic acids dominated the total titratable acidity (TTA) with six minor acids (propionic, iso-butyric, alpha-methyl-n-butyric, iso-valeric, and valeric acids) making up the rest of the TTA. In a later study, Galal et al. (1978) observed the rheological properties of sourdough bread doughs with and without the addition of salt. A series of farinographs were made of control dough, consisting of water and bread flour with additives such as organic acids, approximating fully fermented sourdough, 1.5% NaCl, and then combinations of organic acids with either 1.0% or 1.5% NaCl. The results indicated the addition of organic acids alone decreased mixing time, and generally weakened the dough structure. The control bread, with 1.5% NaCl, increased the mixing time and yielded a more viscous bread dough. When both acid and salt were added, mixing time was again increased, and the dough structure was significantly stronger. These findings were contradictory to Tanaka et al. (1967).

Galal et al. (1978) theorized that the addition of salt decreased the amount of water the protein could bind by occupying those binding sites. Hence, protein-protein interactions increased. Organic acids had the opposite

effect by lowering the pH of the system dramatically from 5.5 to 3.8-3.9. The protein unfolded due to an increase in positive net charge which lowered protein-protein interactions. The result was an augmented water holding capacity and weakened dough structure. The combination of 1.5% NaCl and organic acid worked synergistically together. The acid uncoiled the now more soluble protein with a positive (+) net charge. The salt counteracted this effect by charge shielding the positive charge, and decreasing the solubility of the protein. Galal et al. (1978) suggested that the acid/salt combination impeded the electrostatic repulsion by charge shielding, which allowed more hydrophobic groups to participate in intermolecular bonding. Therefore, the insoluble proteins had more of a tendency to associate and thus strengthen the bread dough.

Bakhoum and Ponte (1982) observed the combined effects of various levels of hydrochloric acid (HCl) and sodium chloride (NaCl) on bread doughs in a mixograph and then on the loaf volume of baked bread. Salt was added at levels 0.5% incremental levels, and HCl was added in 5 to 10 m moles increments from 5 m moles to 40 m moles. A standard bread formula was adapted with yeast (2.5%), sugar (6.0%), shortening (3.0%), commercial milk replacer (2.0%), and 0.25% dough strengthener (sodium stearoyl lactylate) based on 100% wheat flour. Results indicated that any combination of acid and salt increased dough mixing time and stability. At the

higher levels of acid and salt, increased stability and longer mixing times were noted. When salt levels were held constant at 2.0%, and acid was incrementally increased, mixing time and stability paralleled this increase. These findings were found to be in agreement with Galal et al. (1978).

In the second half of the study, loaf volume was found to increase at various levels of salt except at 2.5%, and acid levels up to 15 m moles. After 15 m moles of HCl, loaf volume decreased no matter what level of salt was used. The poorest overall quality in a baked loaf came with 2.5% salt and 25 m moles of HCl. Bakhoum and Ponte (1982) concluded that acid and salt react synergistically to increase dough stability during mixing and improve overall quality of baked bread. However, it should be pointed out in this study that the "commercial milk replacer" was not identified and possibly could have reacted with either the added salt or acid. Also, the use of hydrochloric acid in the bread dough is not equivalent to the organic acids found in sourdough fermented bread dough. Another criticism of this study is that the addition of sodium stearoyl lactylate as a dough strengthener was not included in the NaCl percentages, nor was its possible interactions with the differing salt and acid levels discussed.

The data on dough rheology as affected by the addition of acid and salt is not always in agreement nor is it always

adequately explained. Tanaka et al. (1967) concluded that salt appeared to lower the consistency of acidified doughs. Galal et al. (1978) and Bakhoum and Ponte (1982) results indicated that the combination of acid and salt increased the stability and tolerance of bread dough. The use and possible effects of commercial milk replacer, and sodium stearoyl lactylate added to doughs in Bakhoum and Ponte's study (1982) were not addressed. In the research of Galal et al. (1978), only model dough systems were used to "approximate" fully fermented sourdough doughs in farinograph analysis. Galal et al. (1978) did not attempt to use intact sourdough doughs, or fermented sourdough doughs, in rheological testing.

Rheology Methodology in Breadmaking

The rheology of bread dough and bread crumb is tested by a variety of methods. Basically, three instruments evaluate bread doughs: the farinograph, the extensometer, and the mixograph. Baked bread can be measured by tests such as the examination of loaf volume and the penetrometer. The universal testing machine is also used to give more detailed rheological information on baked bread.

The farinograph has a small mixing bowl attached to a chart recorder. Flour, water, and other additional ingredients are mixed in the farinograph bowl and a curve is drawn. The maximum height of the curve represents the consistency of the dough, the starting curve up to maximum height is recorded as dough development, and the width of the

band is defined as elasticity. The height of the band eventually drops as the dough weakens. The difference in Brabender units (BU's) from the center of the maximum height of the curve minus the height of the center of the curve measured 20 minutes later is considered dough stability (Stafford, 1970). Two methods are commonly used: Constant Flour Weight Procedure and Constant Dough Weight Procedure (AACC Methods 54-21 **A** and **B**, 1982). In the **A** method, 50 grams of flour is put in the farinograph bowl and water is added until a curve is achieved with a maximum dough development centered on the 500 BU line. In the **B** method, a pre-determined flour and water weight is added to the farinograph bowl. Again, the maximum consistency of the dough is centered on the 500 BU line. Another rheological instrument, the mixograph, is similar to a farinograph but only measures mixing time and dough stability (Hoseney & Brown, 1983).

The extensometer records extension, resistence, and dough strength. A piece of dough is attached to two pegs and pulled at a steady speed before breaking. The resistance is measured as the peak of a curve drawn on recording paper. The extension is measured as the base length of the curve and the strength of the dough is the area under the curve. Breaking stress can also be measured as the product of resistance times the length of extension (Bennett & Ewart, 1962).

Two methods are used to test baked bread. The first technique involves measuring the volume of a baked bread loaf. Conclusions are drawn about the extensibility, strength, and stiffness of the dough when evaluating loaf volume and height (Bakhoum & Ponte, 1982). The second technique, the universal testing machine (brand name: Instron or Saytec) tests the firmness, crumbliness, and elasticity of bread crumb by measuring deformation, recovery, and compression. Numerical values of resistance against time are recorded as a plunging device slowly compresses an uniformly sliced piece of bread (Redlinger, Setser, & Dayton, 1985; Short & Roberts, 1971).

Rheological testing is very useful to the food scientist as it provides numerical data by which analytical conclusions can be drawn. When used in conjunction with SEM research, rheology of dough and breads is invaluable in supporting hypotheses generated from visual analysis of SEM photographs.

Scanning Electron Microscopy of Bread Doughs and Bread Crumb

Sandstedt, Schaumburg, and Fleming (1954) observed bread dough and bread crumb under the light microscope. Samples from freshly kneaded dough showed a random arrangement of starch granules embedded in a protein matrix. Little or no gas cells were present in these samples. Baked samples showed gelatinized starch granules uniformly oriented in a continuous protein membrane. Sandstedt et al. (1954)

observed that the starch granules did not touch each other but were surrounded by a protein film. These landmark findings became the basis for research done on breadmaking for the next thirty years.

Khoo, Christianson, and Inglett (1975) did SEM analysis of bread dough at various stages in the mixing and proofing process. Bread crumb was also analyzed. Unhydrated flour was revealed as sharp pieces mixed between starch granules. As hydration occurred during the kneading of the dough, the protein thinly coated the starch granules. A fractured surface revealed starch granules surrounded by a protein network with microscopic holes. Fully proofed dough showed larger air cells which had forced the thin protein matrix into long, aggregated fibrils. Scanning electron microscopic analysis of bread crumb showed larger air cells surrounded by an even thinner protein film. The protein had a less stranded appearance than in the bread dough, and was fused with itself as well as with the gelatinized starch granules.

Paredes-Lopez and Bushuk (1983) used different types of flour and three mixing procedures in preparing bread dough (underdeveloped, optimally developed, and overmixed). They observed microstructural changes in the gluten protein. Optimally developed bread dough showed a continuous protein network enmeshed with starch granules. Underdeveloped dough had a discontinuous gluten membrane that did not adequately surround the starch granules. Overmixed dough exhibited a

marked breakdown of continuous gluten structures, which, in a previous study (Paredes-Lopez & Bushuk, 1981), produced a decrease in loaf volume. The "weakest" of the flour types showed discontinuous gluten development with all three mixing procedures. The gluten matrix appeared to be "torn" in areas and unable to adequately surround starch granules. On the other hand, the "strongest" flour type showed more uniformity throughout the three mixing procedures, though the underdeveloped and overmixed doughs still exhibited a discontinuous gluten matrix. The underdeveloped dough was remixed and it almost approximated the continuous gluten formation of optimally mixed dough. Paredes-Lopez and Bushuk (1983) postulated that gliadin and glutenin aggregated and began to form a matrix in underdeveloped dough. As the dough became optimally fixed, a continuous gluten membrane formed. Upon overmixing of the dough, the protein matrix was ruptured.

Only one study to date has examined sourdough doughs and sourdough breads with SEM techniques. The researchers Pomeranz, Meyer, and Seibel (1984) prepared three bread doughs with different flours: 100% wheat flour, 60% wheat-40% rye flour, and 90% rye meal-10% rye flour. The wheat bread formula had sugar, fat, commercial baking aid, in addition to the standard yeast culture, salt, water, and flour. The wheat/rye flour, and rye meal/rye flour breads consisted of a combination of standard yeast culture and

sourdough yeast culture, salt, water, and flour (and/or rye meal depending on formula). Pomeranz et al. (1984) did SEM analysis of bread dough and bread crumb at various stages of mixing, fermentation, and baking.

The 100% wheat bread dough exhibited starch granules embedded in a protein matrix. Fermented dough revealed much the same picture, but the starch granules were more swollen. The interaction of the large starch granules in the protein matrix were retained in the baked bread samples. In the wheat/rye sourdough bread dough, the starch granules became distorted due to organic acid interaction. Baked bread samples exhibited a weakened structure with damaged starch granules in the protein matrix. The rye meal/rye flour bread appeared to have small starch granules "glued" together with a gum-like substance or adhering to larger starch granules. The baked bread crumb had larger vacuoles with a "rugged" appearing crust. Small starch granules remained completely intact in some of the baked bread samples. Pomeranz et al. (1984) concluded that the dough structure of wheat bread was due to the interaction between the starch and protein matrix. To a lesser extent this was also true in the rye/wheat sourdough bread, though starch granules appeared to be distorted by the production of organic acids. The rye flour/rye meal sourdough bread structure consisted of modified starch granules interacting with one another to yield a continuous dough network.

This study compared two different sourdough formulations with a standard wheat bread formula. Unfortunately, the wheat bread formula had additives such as sugar, fat, and commercial dough improvers which the other two formulations did not include. These additives altered the protein matrix and hydration of starch granules in such a way that a SEM comparison between this type of bread and sourdough breads would be extremely difficult. This study should have used a wheat formulation more similar to the wheat/rye and rye/rye meal doughs and excluded the additives in order to have a valid SEM comparison. Also, the addition of baker's yeast to both the sourdough formulations decreased the fermentation times drastically, hence organic acid production had a lesser effect on the resulting dough structure. It would have been more effective to utilize a true sourdough formulation excluding baker's yeast in this study. The organic acid content of the bread would have been increased and produced some interesting data for SEM analysis.

Scanning Electron Microscopy Methodology

It is generally recognized in the field of SEM that meticulous preparation of the sample must be followed so artefacts and distortions of the sample are not produced. Varriano-Marston (1978) compared several SEM preparation techniques for bread doughs. It was concluded that dough samples frozen in liquid nitrogen, cryofractured, and then freeze-dried at -65° C for 48 hours produced the best

photographs. Good micrographs were also achieved by drying frozen (via liquid nitrogen) dough samples in a vacuum dessicator over silica gel for 24 hours at room temperature. Other researchers (Khoo et al., 1975; Paredes-Lopez & Bushuk, 1983) also froze bread dough samples in liquid nitrogen, followed by freeze drying, which produced excellent results. The worst SEM photographs occurred when unfrozen dough samples were fixed with either osmonium tetraoxide or glutaraldehyde and then critical point dried in acetone. The resulting micrographs showed structural distortion of starch granules, separation of the starch granules from the gluten matrix, and an overall dry and brittle appearance. It is well documented in the field of SEM (Hayat, 1970; Chabot, Hood, & Liboff, 1979; Postek, Howard, Johnson, & McMichale, 1980) that chemical fixation and solvent dehydration can alter the morphology or chemical structure of the sample.

After the sample was dehydrated, and before it was viewed under the microscope, a general procedure was followed. The specimen was attached to a stub, and gold (or gold alloy) coated in a vacuum chamber (Varriano-Marston, 1978; Khoo et al., 1975; and Paredes-Lopez & Bushuk, 1983).

Sample preparation of bread crumb was less tedious. Khoo et al. (1975) froze freshly baked bread in liquid nitrogen before attaching it to stubs. Davis (personal communication, 1987) simply put bread crumb samples in a vacuum dessicator over silica gel and dried the specimens for 48 hours at room

temperature before attaching them to stubs. Scanning electron micrographs using either technique produced acceptable results.

CHAPTER 3

Materials and Methods

Determination of Lactic Acid

The first objective was to determine the lactic acid content in sourdough sponge and sourdough bread dough samples during the fermentation process. The lactic acid analysis technique was based on the principle that L-lactate dehydrogenase (L-LDH) catalyzes the oxidation of L-lactate by nicotinamide adenine dinucleotide (NAD) to pyruvate. The amount of NADH formed in the reaction is equivalent to the amount of L-lactate. The increase in NADH is determined on the basis of its absorption at 340 nm (Holz & Bergmeyer, 1970).

Materials

The formulas used for sourdough sponge and sourdough bread dough were adapted from research done by Kline and associates (1970) (Table 1).

For the sponge, the sourdough culture was donated by Acme Bakery in Berkeley, California, which is reputed to have an authentic San Francisco style sourdough bread. Acme Bakery had maintained the 10 hour sourdough sponge culture at 40° F in a covered plastic bucket. The ingredient proportions of the sponge were similar to the formulation outlined in Table 1, although Berkeley city water was used instead of distilled

water. The high gluten flour for the sponge was donated by Con Agra Milling, Oakland, CA. (Isis brand).

The ingredients of the starter sponge were mixed together and kneaded 5 minutes at medium speed with a dough hook in a 5 quart mixer bowl. The resulting sponge was allowed to ferment at 80° F, covered with cellophane for 10 hours in a small proof box. Samples of approximately 15 grams each were analyzed for L-lactate at intervals of 3, 5, and 10 hours during the fermentation process . They were wrapped in cellophane, put in zip-lock bags and frozen at 30° F immediately. The samples remained frozen until they were analyzed.

The sourdough bread was made from the 10 hour sponge, bread flour (Judith Mello brand donated from Con Agra Milling Company, Oakland, CA.) distilled water, and iodized salt (Table 1). The dough was mixed and kneaded for 8 minutes at medium speed in a 5 quart Hobart mixer with a dough hook. The dough was fermented at 80° F covered in a small proof box. Then the bread dough samples were taken at 0, 3, 6, and 8 hour fermentation times, and frozen immediately, wrapped in cellophane and in zip-lock bags. The samples were maintained at 30° F until they were analyzed.

Lactic Acid Measurement Procedure

1. A Glycine/Hydrazine buffer solution was prepared by dissolving 5.7 g glycine (0.5 **m**) in 100 ml deionized water. 12.5 ml of hydrazine hydrate (o.4 **m**) was added

and this mixture was diluted to 150 ml with deionized water. The pH was checked (pH = 9.0) and adjusted.

2. A nicotinamide adenine dinucleotide solution was prepared by dissolving 900 mg NAD in 30 ml deionized water (40 **m M** NAD).
3. An L-lactate dehydrogenase suspension (5 mg protein/ml) was used undiluted.
4. An 1 N L-lactate standard solution was diluted 1:2000 with 0.01 N NaOH. The L-lactate standard was prepared by mixing 0.9 g lactate standard solution in 10 ml deionized water. 0.05 ml of this mixture was pipetted into a 100 ml volumetric flask and diluted to volume with 0.01 N NaOH (0.5 **m**N L-lactate).
5. The samples were prepared for the actual analysis by taking the frozen bread dough or starter sponge of 5.0 grams a piece, mixing immediately with distilled water and grinding with mortar and pestle. The dough and water mixture were transferred to centrifuge tubes and centrifuged at low speed (5000 rpm) for 10 minutes. The subsequent supernatant was decanted from the centrifuge tube into a 100 ml volumetric flask and diluted to volume with deionized water.
6. The amounts of prepared solutions used for the actual L-lactate determination are summarized in Table 2. Samples or the standard were measured against a blank. The standard or samples were mixed in the cuvette without the

L-LDH. The L-LDH was pipetted in last, mixed, and time 0 optical density was recorded immediately and then recorded after a 3 minute interval. All readings were taken at 340 nm.

7. Lactic acid concentration in micro equivalents/wet basis was then calculated as follows:

$$c = \frac{(A2 - A1) \times 0.045 \times 500}{(S2 - S1) \times 1000 \times 9 \times 10^{*-5} \times \text{sample wt.(g)}} \quad (1)$$

where:

A1 = absorbancy of sample at time 0
A2 = absorbancy of sample at time 3 minutes
S1 = absorbancy of standard at time
S2 = absorbancy of standard at time 3 minutes
c = concentration µeq/wet basis
* = to the exponent
DF = dilution factor = 100/0.2 = 500 (sample dilution)
90.1 g = molecular weight of lactic acid

To determine µeq/dry basis:

$$\frac{\text{µeq/wet basis}}{100-\% \text{ moisture}} \times 100 \quad (2)$$

Table 1

Formulation of Starter Sponge and Bread Dough*

Proportion	Ounces	Grams	Ingredient
		Starter Sponge	
100 parts	2	56.7	sourdough sponge
100 parts	2	56.7	high-gluten flour
50 parts	1	28.4	distilled water
		Bread Dough	
20 parts	2.0	56.7	sponge
100 parts	10.0	283.5	bread flour
60 parts	6.0	170.1	distilled water
2 parts	0.2	5.7	iodized salt

Note: *Formula was adapted from "Nature of the San Francisco sourdough french bread process" by Kline et al., 1970, Baker's Digest, 44, p.48-50.

Table 2

L-lactate Assay

Reagent	Blank (ml)	Sample (ml)	Concentration
Buffer	2.50	2.50	0.43 M glycine 0.34 hydrazine
NAD	0.20	0.20	--
Sample or Standard	--	0.20	2.75 mM
Water	0.20	--	up to 75 µM
L-LDH	0.02	0.02	--
Total	2.92	2.92	34.2 µg/mg

Note: Adapted from Verlag Chemie (p.1486) by Holz & Bergmeyer, 1970, Germany: Weinheim.

Rheological Analysis

The second objective was to evaluate the influence of salt and acid on the rheological behavior of unfermented and fermented doughs and baked breads. Two measurement techniques were used. First, farinograph measurements were determined for model dough systems and fermented bread doughs. Then, an Instron TM model universal testing machine was used to compress baked bread samples to ascertain the

resistance of baked bread crumb and relative amount of recovery.

Farinograph Measurements Method

Farinographs using a C. W. Brabender model were determined for unfermented and fermented doughs.

Unfermented Doughs

The model dough systems consisted of flour, water, and added salt and/or acid, depending on the treatment condition (Table 3). Based on research by Galal et al.(1978), an assumption was made that the three major organic acids found in fully fermented sourdough doughs constituted 100% of the acids as 73.14% lactic acid, 26.25% acetic acid and 0.61% propionic acid. The formulation of the acid mixture used in the farinograph trials is outlined in Table 4.

Table 3

Unfermented Dough Formulation

17.71 g	high-gluten flour
155.93 g	bread flour
95.70 g	distilled water
2.34 g	iodized salt
1.36 g	acid (Table 4)

Table 4

Acid Formulation of Treatment 2 and 4 in Unfermented Dough Farinographs

74.00 µeq acid∂	156.47 µeq acidƒ	Ingredient
4.89 g	10.31 g	lactic acid
1.17 g	2.47 g	acetic acid
0.02 g	0.04 g	proprionic acid
10.00 g	10.00 g	distilled water

Note: ∂ Adapted from "Lactic and volatile (C2-C5) organic acids of San Francisco sourdough french bread" by Galal et al., 1978, Cereal Chemistry, 55, p. 466..

ƒ Adapted from lactic acid levels found in bread dough at 8 hour fermentation mark determined in this research (Table 7).

Two sets of farinographs were obtained. The first set of farinographs replicated the studies done by Galal et al.(1978) at the levels of 74.00 µeq of organic acid/gram dough. The second set of farinographs had added acid based on the lactic acid levels determined in this study (Table 4 and Table 7). The lactic acid value of 114.44 µeq at the 8 hour fermentation mark was taken (Table 7) and assumed that it was 73.14% of the total organic acid mixture--a value derived from the research done by Galal et al. (1978). An

additional 41.07 µeq acetic acid and 0.96 µeq propionic acid was added to the dough; these amounts were also determined from this same study by Galal et al.(1978). The added salt was 0.86% the dough weight (or 1.5% the flour weight). The flour was a combination of high-gluten flour and bread flour. The high-gluten flour was included to approximate the addition of the sponge in sourdough bread which is made with this particular flour. The treatment conditions for the unfermented doughs all included the same proportions of high-gluten flour, bread flour, and distilled water. Four treatment conditions were prepared as follows:

1. No added salt and no added acid
2. Acid only
3. Salt only
4. Added salt and added acid

Unfermented Dough Farinograph Procedure

A constant dough weight procedure was followed for the unfermented doughs (AACC Method 54-21 B, 1982). The method was somewhat modified in that the doughs were pre-mixed in a small 5 quart table top Hobart mixer (El Cerrito, CA.) for 30 seconds at low speed with a paddle attachment instead of in the farinograph bowl. The modification was necessary to fully disperse the acid mixture and to entirely dissolve the salt in the water before adding the flour. An eighty gram piece of dough at ambient temperature was measured and put into a 50 g farinograph bowl. The farinograph was maintained

at 30° C and the dough was mixed for 20 minutes. Dough development time, dough tolerance to mixing, dough stability, and drop-off during mixing were measured by chart recorder.

Fermented Doughs Procedure

The fermented doughs consisted of high-gluten flour, bread flour, distilled water, yeast starter culture or sourdough starter culture and added salt (0.86% the dough weight), depending on the treatment condition (Table 5 and Table 6). Four treatment conditions were prepared as follows:

1. No added salt in dough fermented with yeast starter culture which did not produce acid
2. Yeast fermented dough containing salt
3. No salt added to dough fermented with sourdough culture
4. Sourdough fermented dough containing salt

The yeast doughs were mixed and then kneaded 10 minutes in a 5 quart table top Hobart mixer with a dough hook at medium speed. The doughs were allowed to rise, covered with cellophane, for 1 and 1/2 hour in a small proof box at 80° F. Doughs were punched down and allowed to rise a second time at 80° F, covered with cellophane, for one hour. They were then shaped into round loaves. After 30 minutes rest time at 80° F covered with cellophane, 80 grams of each dough load was evaluated in the farinograph. Farinograph conditions were the same as for the unfermented doughs.

The sourdough bread dough was first prepared by making a sourdough sponge. The sourdough sponge was mixed for 3 minutes in a 5 quart table top Hobart mixer with a dough hook. The sponge fermented 8 hours at 80° F and then was used in the sourdough bread dough. The sourdough bread dough was mixed and then kneaded for 10 minutes at medium speed in a 5 quart Hobart mixer with a dough hook. The dough was allowed to rest, covered, for 30 minutes at ambient temperature. It was shaped into a round loaf and put in a round, canvas-lined bread mold that had been dusted with rice flour. The dough was covered with cellophane and proofed at 80°F for 8 hours.

After the 8 hour fermentation time, 80 gram samples were evaluated in the farinograph under the same conditions as the yeast doughs.

Table 5

Formulation of Bread Dough with Yeast Culture

Treatment 1		Treatment 2		Ingredient
Ounces	Grams	Ounces	Grams	
1.25	35.4	1.25	35.4	high-gluten flour
10.00	283.5	10.00	283.5	bread flour
--	--	0.20	5.7	iodized salt
0.18	5.2	0.18	5.2	dry yeast
6.75	191.4	6.75	191.2	distilled water

Table 6

Formulation of Bread Dough with Sourdough Yeast Culture

Treatment 3		Treament 4		Ingredient
Ounces	Grams	Ounces	Grams	
2.00	56.7	2.00	56.7	sourdough sponge (Table 1)
10.00	283.5	10.00	283.5	bread flour
--	--	0.20	5.7	iodized salt
6.00	170.1	6.00	170.1	distilled water

Farinograph Analysis of Data

Figure 1 illustrates a typical faringraph. Normally the doughs are made to a standard consistency so that the peak of the curve (point of maximum resistance) is centered on the 500 Brabender Unit (BU) line. Most of the farinographs in this study were not centered on the 500 BU line, and therefore a new line was drawn at the maximum resistance of the curve parallel to the 500 BU line. Various physical dough characteristics are determined from the farinograph curve and they are as follows (Figure 1):

A: **Peak Time** is the time in minutes (to the nearest 0.5 minutes) from time O for the curve to reach the maximum resistance BU line (MR BU line).

B: **Stability** is the time difference in minutes (closest to 0.5 minutes) between the point where the top of the curve

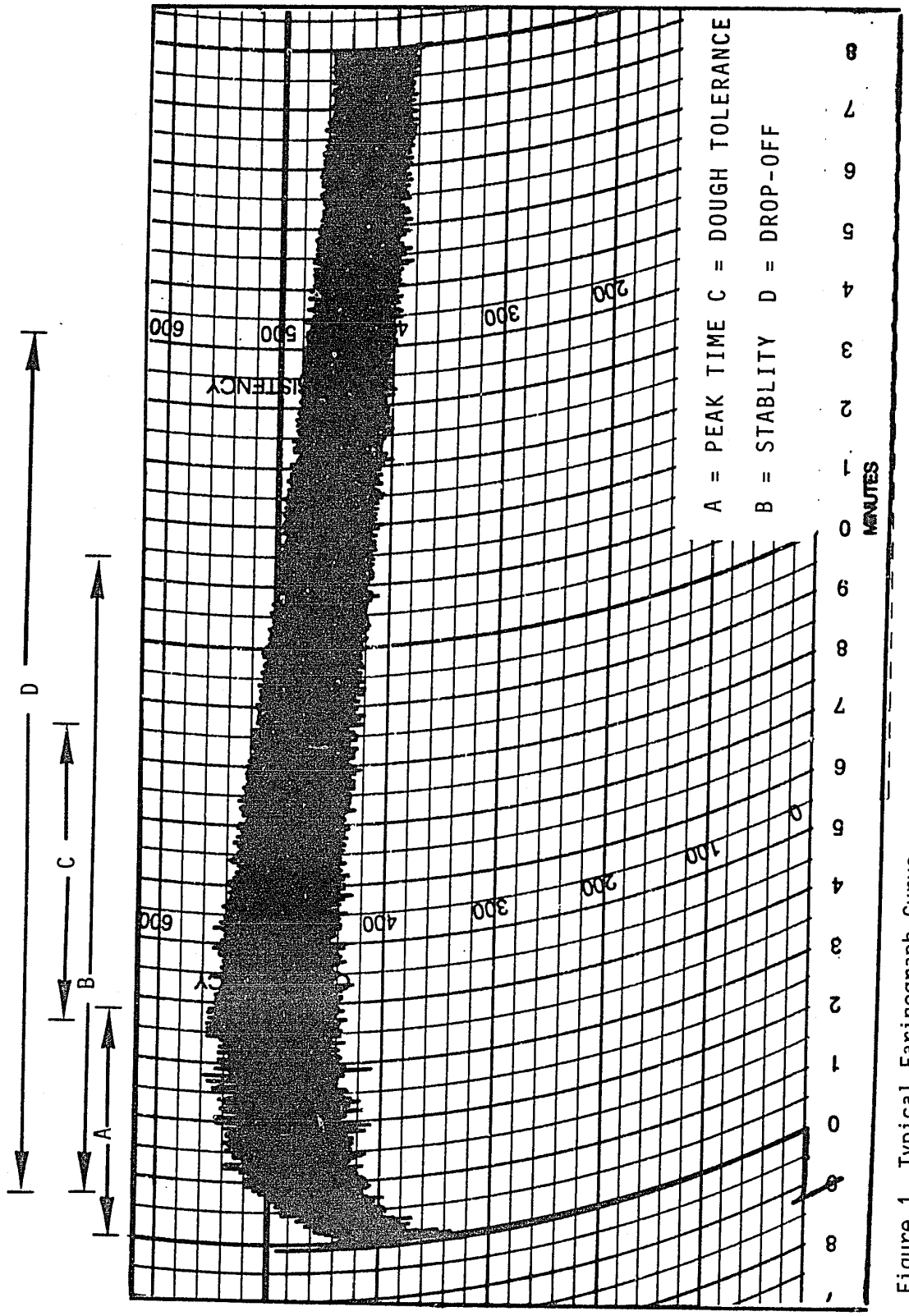

Figure 1. Typical Farinograph Curve

first intersects the MR BU line and the point where the top of the curve first leaves the MR BU line.

C: **Dough Tolerance** is the difference in BU's from the top of the curve at the peak to the top of the curve measured at 5 minutes after the peak is reached. This is an inverse relationship--the larger the value, the weaker the dough.

D: **Drop-Off** is the difference in BU's from the center of the MR BU line to the center of the curve measured 20 minutes later.

BU's are arbitrary units.

Instron Measurement Method

Compressibility, deformation, and recovery of baked bread samples were determined from the four treatments of fermented doughs outlined in Table 5 and Table 6.

Materials

The baked bread samples tested by the Instron (Model TM) were made from doughs described in the farinograph fermented dough section. Doughs were shaped into loaves and baked in standard 3" x 5" x 9" loaf pans at 375° F (191° C). Yeast fermented doughs were shaped into loaves after 2 1/2 hours total rising time. The loaves were proofed at 80° F (27° C) for 30 minutes before being baked. During the first half of the baking process, the bread was sprayed with water (to simulate a steam injection oven). The total baking time was 40 minutes.

Sourdough yeast fermented bread was allowed to rise in the loaf pan for 8 hours at 80° F (27° C). Baking was similar to the yeast fermented doughs: the oven was 375° F (191° C) and water was sprayed on the bread during the first half of the baking cycle.

After baking, the loaves were cooled, wrapped in cellophane, put in zip-lock bags, and frozen immediately at 23° F (-5° C). Before testing, the loaves were thawed out and uniformly sliced 1/2" thick (1.27 cm) by a bread slicer.

Method

Each bread slice was placed on the bottom plate of the Instron (Model TM). The 2 1/4" diameter anvil was positioned directly up against the center of the bread slice before the commencement of the test. The following settings were used for the test:

	U.S.	Metric
Drive speed	2"/minute	5.08 cm/minute
Chart speed	10"/minute	25.40 cm/minute
Compression Depth	1/4"	0.64 cm
Force Load	0 - 20 lb.	0 - 9.07 kg

The test for each sample slice was 1 minute in duration and compressed the slice by 50% of its thickness (or 1/4"). Six slices of each treatment condition 1 through 4 (outlined in Table 5 and Table 6) were tested by the Instron.

The curve drawn by the Instron was analyzed for compressibility, deformation, and recovery (Figure 2). The

bread crumb characteristics are determined for the Instron curve as follows (Figure 2):

a: Deformation is the amount in inches or mm from the start of the curve to the peak of the curve.

b: Recovery is calculated in inches or mm from the peak of the curve to the end of the curve.

c: Compression is derived by the peak of the rising portion of the curve in units of pounds/inch or kg/mm.

Scanning Electron Microscopy Analysis

The third objective was to observe the influence of acid and salt on the microstructure of unfermented and fermented bread doughs and baked bread crumb.

Materials

The four bread dough formulations are outlined in Table 5 and Table 6. Bread doughs were prepared as discussed in the farinograph materials section. Fermented bread doughs were baked as free form loaves in a 375° F oven. Yeast fermented bread dough was shaped into a round loaf after 2 1/2 hours total rising time. The loaf proofed at 80° F for 30 minutes before being baked. The dough was slashed with a sharp knife in 3 parallel lines across the loaf at a depth of 1/2 inch. During the first half of the baking process, the bread was sprayed with water (to simulate a steam injection oven). The total baking time was 40 minutes.

Sourdough yeast fermented bread was allowed to rise in a canvas-lined, rice flour dusted basket for 8 hours at 80° F.

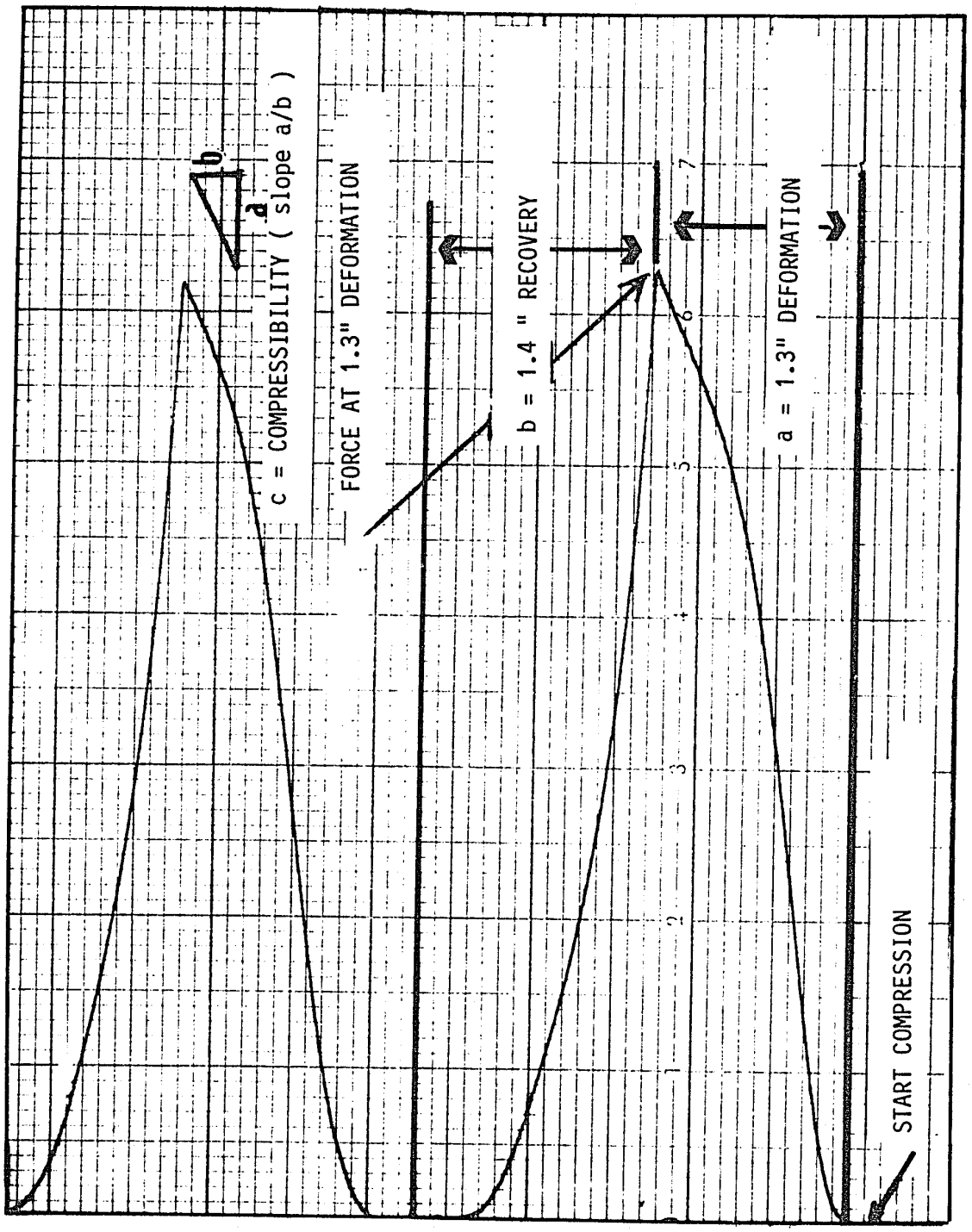

Figure 2. Typical Instron Curve

Prior to baking, the loaf was unmolded from the basket, slashed (described in the previous section), and baked at 375° F for 40 minutes. Again, it was sprayed with water during the first half of the baking cycle.

Method

Unfermented Doughs

Unfermented dough samples were taken from dough immediately after the kneading process. Samples were put in zip-lock bags and frozen at 23° F (-5° C) to halt yeast growth. Frozen samples were cut with scissors into 1/4" (5 mm) square pieces and frozen in liquid nitrogen (-196 °C) for 10 minutes. The samples were then removed and immediately fractured with metal forceps, quickly transferred to petri dishes, and put in a vacuum dessicator over drierite for 24 hours at ambient temperature (20° C). Samples were then placed on stubs and coated to a depth of 200 Å with a standard target gold:palladium mixture (60:40). An ISI Super 3 scanning electron microscope (San Jose, CA.) at 10 kV accelerating voltage was used to examine samples at 1100X to 1300X magnification. Micrographs were obtained of representative areas after scanning the specimens in at least 10 different areas.

Fermented Doughs

Yeast culture fermented dough samples were taken from dough that had risen a total of 3 hours. Sourdough yeast culture dough samples were taken from dough that had risen a

total of 8 hours. The same procedure for sample preparation was followed as for unfermented doughs in the previous section.

Baked Bread

After the fermented dough samples were taken, the dough was rounded up and baked off as described in the materials section. Baked bread samples were taken from the middle of the cooled loaves, placed in zip-lock bags, and frozen at 23° F (-5° C) until the next step. The frozen samples were placed in petri dishes and put in a vacuum dessicator over drierite for 48 hours at ambient temperature (68° F/20° C). Samples were placed on stubs and coated with 200 Å of a gold:palladium (60:40) mixture. A Super ISI 300 scanning electron microscope was used at 10 kV to view the samples. At least ten areas were examined of all specimens before representative micrographs were obtained.

CHAPTER 4

Results and Discussion

Lactic Acid Determination

The results for lactic acid levels in sourdough sponge and sourdough doughs during the fermentation process are shown in Table 7 and Appendix A. The values for lactic acid in sourdough sponge and sourdough bread dough research done by Galal et al. (1978) are summarized in Table 8. The results indicated that lactic acid formation in sourdough sponge increased from 45.50 µeq to 85.74 µeq/wet basis in 10 hours, and sourdough bread dough increased from 18.16 µeq to 114.44 µeq/wet basis in 8 hours. Generally, the lactic acid increased five to six fold in the sourdough sponge and bread dough by the end of the fermentation process. The baked bread from this experiment produced excellent loaf volume and texture, indicating the sourdough yeast was not affected by the high acid concentrations. The deviation between runs varied from ±2.10 µeq to ±12.93 µeq per wet basis.

The lactic acid levels in the sponge were similar to those levels produced by the research of Galal et al.(1978) at the 3 (Table 7) and 4 hour (Table 8) fermentation mark. Levels in the sponge from research by Galal et al. (1978) after 8 hours of fermentation (65.64 µeq) were decreased slightly from the levels presented here (85.74 µeq). In the

bread dough section, the 0 time result in Table 7 (18.16 µeq) and the 0.5 time result in Table 8 (28.68 µeq) were very similar. However, by the end of the fermentation process,

Table 7

Microequivalents of Lactic Acid in Sourdough Samples

Sample	Proof Time (hr.)	µeq/ wet basis	µeq/ dry basis	rate constant*
sponge	3	45.50 ±2.10	77.71 ±3.56	-
sponge	5	64.74 ±2.30	110.57 ±3.95	-
sponge	10	85.74 ±12.23	146.44 ±20.89	9.31
bread dough	0	18.16 ±6.58	33.04 ±11.98	-
bread dough	3	65.18 ±6.74	118.50 ±12.26	-
bread dough	6	101.84 ±12.93	185.30 ±23.53	-
bread dough	8	114.44 ±4.83	208.22 ±8.79	22.22

Note: Average moisture was 41.45% for sponge and 45.05% for bread dough samples.

* The rate constant was determined on a dry basis (µeq/hr.).

Table 8

Literature values of Microequivalents of Lactic Acid in Dough Samples

Sample	Fermentation Time (hr.)	µeq/ wet basis	µeq/ dry basis	rate constant*
sponge	0	28.68	48.98	-
sponge	4	46.41	79.27	-
sponge	8	65.64	112.11	7.89
bread dough	0.5	16.69	30.37	-
bread dough	3	42.38	77.11	-
bread dough	5	54.32	98.84	15.36

Note: The data in column 3 are from "Lactic and volatile (C2-C5) organic acids of San Francisco sourdough french bread" by Galal et al., 1978, Cereal Chemistry, 55, p.466.
*The rate constant was determined on a dry basis (µeq/hr.).

the levels of lactic acid in the bread dough were over two times higher (114.44 µeq as opposed to 54.32 µeq) than those of Galal et al. (1978). Many reasons can account for these differences. For example, Galal et al. (1978) used 15 parts in the sourdough sponge in the bread dough as opposed to 20 parts in the formulation presented here. Also, Galal et al. (1978) proofed the dough at a higher temperature (105° F), for a shorter length of time (5 hr.) as opposed to 8 hours at

80° F in the research presented here. The ratio of lactic to acetic acid formed in the sourdough process is dependant on the dough yield, fermentation time, and temperature (Pomeranz et al., 1984). Therefore, there was a higher amount of lactobacillus in the bread dough at time 0 which was given a longer time to reproduce at a lower temperature. This might account for the the higher microequivalents of lactic acid found in the bread dough at time 3, 6, and 8 hour intervals. Also, it is possible that the sourdough culture used in this experiment produced more lactic acid due to the fact that sourdough cultures vary widely within the San Francisco area (Sugihara, Kline, & McCready, 1970).

Rheology

Farinograph Measurements

Model Dough Systems

The farinograph results are outlined in tables 9 and 10 and in appendices B, C, and D. The model dough systems' (Table 9) results show that with the doughs with added salt, 5.3 ml acid was the most stable and tolerant, followed by 2.7 ml acid dough. The salt-only dough followed, having a stronger tolerance index and drop-off values than the doughs with no added salt. The two doughs with added acid and no salt had the lowest stability and tolerance values. In general, the three doughs with added salt were more stable and had increased tolerance to mixing over a period of time. Salt had a strengthening affect on the physical characteristics of

Table 9

Effects of Organic Acids, and Salt on Flour Farinograph Values*

Condition	Peak (min.)[a]	Stability (min.)[b]	Tolerance (BU's)[c]	Drop-off (BU'S)[d]
ø salt, ø acid	1.1 ±0.5	5.8 ±2.0	90 ±21	159 ±31
salt only	2.9 ±1.3	11.2 ±2.4	46 ±13	67 ±20
5.3 ml acid, ø salt	1.3 ±0.4	3.0 ±0.4	275 ±50	420 ±14
2.7 ml acid, ø salt[e]	3.5	3.0	170	340
5.3 ml acid, + salt	1.0 ±0.0	20.0 ±0.0	55 ±7	10 ±0
2.7 ml acid, + salt[e]	1.0	15.0	110	70

Note: *:In most farinographs the curve was not centered on the 500 BU line and therefore a new line was drawn at the maximum resistance parallel to the 500 BU line.

a: Time to reach maximum resistance BU line (MR BU line).

b: Time difference between point where top of curve first intersects MR BU line and point where top of curve first leaves MR BU line.

c: Difference in BU's from top of curve at peak to top of curve measured at 5 minutes after peak is reached. This is an inverse relationship--the larger the value, the weaker the dough.

d: Difference in BU's from the center of the MR BU line to the center of the curve, measured 20 minutes after the addition of water.

e: These farinographs were run once to verify work by Galal et al. (1978).

the dough even in combination with acid. However, the doughs made without salt were considerably weakened with reduced dough stability and tolerance to mixing over a period of time. This was especially evident in the acidified doughs. When these doughs were actually retrieved from the farinograph after 20 minutes of mixing, they were exceedingly sticky and runny. These findings are consistent with Galal et al. (1978) and Bakhoum and Ponte (1982). The standard deviation of the farinographs varied tremendously. Since the farinograph research was completed over a period of a year and a half, several conditions such as no acid/no salt and no acid/added salt had several runs done over that time period. The deviation was most likely due to the varying moisture in the flour used (even though it was stored in tightly closed heavy plastic bags). Also, if two farinograph runs looked similar, then a third run was not completed; and this accounts for the two run trials of the 5.3 ml with and without salt. The 2.7 ml runs were done only to verify the data of Galal et al. (1978) and were not repeated since the results were similar to his research.

Fermented Doughs

The fermented doughs (Table 10) showed a large variation in the tolerance and drop-off values. After 20 minutes of mixing, the sourdough without salt dropped off 440 BU's as opposed to 85 BU's for the yeasted dough with salt. These findings follow the same trend as shown by the model dough

systems, indicating that the organic acids in sourdough considerably weaken the bread dough. On the other hand, salt has a strengthening effect when used in combination with the sourdough or when added to yeast dough. The yeasted dough with added salt was definitely the strongest dough, followed by the sourdough dough with added salt, and than the yeast with no salt. The sourdough with no added salt was still the weakest dough. Also, the peak time and stability values show dough development during mixing. Since the fermented doughs were already kneaded, these parameters were not used as part

Table 10

Effects of Salt on Fermented Sourdough and Yeast Culture Farinograms

Yeast Type/ condition	Proof Time (hr.)	Tolerance Index (BU's)	Drop-off (BU's)
Sourdough ø salt	8	405 ±78	440 ±28
Sourdough + salt	8	150 ±58	163 ±53
Yeast ø salt	3	160 ±28	160 ±28
Yeast + salt	3	70 ±28	85 ±7

of the farinograph data. The standard deviations of the fermented doughs were fairly similar except for the sourdough with added salt runs. Since these trials were run on two separate days, perhaps humidity or temperature was a factor during the fermentation process. It is interesting to note that the model dough systems (Table 9) and the fermented dough farinographs (Table 10) show few similarities in values but show much the same trends, though the 2.7 ml acid level with salt is more analagous to the sourdough with added salt than the 5.3 ml with salt test runs. However, the 5.3 ml acid without salt and the sourdough without salt are closer in tolerance and drop-off values than the 2.7 ml acid without salt farinograph. It is difficult to conclude which model system more closely approximates the level of organic acids in fully fermented sourdough bread dough.

There are two possible explanations for these results. Bushuk and Hlynka (1964) indicated that the hydration of gluten was decreased in the presence of salt. They postulated that the salt occupied the molecularly bound water sites and increased the amount of free water in the dough. Farinographic evidence supported this hypothesis as less water was required to obtain the same consistency rate when salt was added to a flour and water dough. Galal et al. (1978) explained their results similarly. However, when acid is added, the pH of the dough is lowered to 3.8-3.9. The gluten proteins, which normally have an iso-electric point

between pH 6 and pH 9 (Wrigley, 1968), become protonated and positively charged. Intra- and inter-molecular repulsions cause the protein to unfold, and the water holding capacity of the gluten protein is increased. This is shown in the reduced extensibility of doughs with added acids (Bennett and Ewart, 1962; Tanaka et al., 1967) and the decrease of stability and tolerance in farinographs (Galal et al., 1978). Although Tanaka et al.(1967) did not compare stability or tolerance values in their farinographs, the curves drawn of lactic acid and acetic acid at pH 4.2 appeared to be less stable and tolerant than the other farinographs using other acids. It should be noted here that the majority of acid in sourdough bread is made up of lactic and acetic acid (Galal et al., 1978).

When acid and salt are added to bread dough, a complex interplay of bonding most likely occurs. Galal et al.(1978) suggested that the addition of salt to acidified doughs counteracts the unfolding of the protein by shielding intermolecular repulsions and allowing more hydrophobic bonding to occur. Since hydrophobic interactions contribute significantly to the visco-elastic properites of gluten by stabilizing ionic bonds, a more compact protein aggregate results (Belitz et al., 1986). This would explain the strengthening effect salt had in the acidified dough farinographs. Tanaka et al.(1967) had a different explanation, shared by Bennett and Ewart (1962): the

addition of acid cleaves both inter- and intra-molecular ionic bonds to protein molecules. Thus, extensibility was reduced and an actual dough "breakdown" occurred. Salt appeared to cancel this effect by occupying binding sites where acid bonds existed, in addition to decreasing bound water. This explains why salt decreased the consistency of the farinographs in the Tanaka et al. (1967) study and is much the same hypothesis Galal et al.(1978) used for explanation of decreased water absorption in the farinograms with combined salt and organic acids.

Instron Measurements

The results of the compression testing of bread crumb (Table 11 and Appendix E). The sourdough without salt was the least compressible and the yeasted bread with salt was the most compressible. The sourdough with salt and the yeasted bread without salt had similar recoveries and compressibilities. As noted in Table 11, when the highest and lowest values were not Table 11averaged into the test runs,the yeasted bread without salt was less compressible than the sourdough with salt. When all values were averaged, the opposite was true by a small margin: the sourdough with salt was less compressible than the yeasted bread without salt. The opposite was true of the recovery of the bread crumb. The sourdough bread without salt recovered the least and the yeasted bread with salt recovered the most. The deformation of all four conditions of bread crumb samples

Table 11

The Effects of Salt on Sourdough and Yeast Cultured Bread Using the Instron

Yeast Type/ condition	Deformation (inch)	Recovery (inch)	Compressibility (lb/inch)
Sourdough ø salt	1.21 ±0.02	0.79 ±0.05	23.99 ±1.78
Sourdough + salt	1.23 ±0.01	0.86 ±0.03	16.17 ±1.66 15.90*
Yeast ø salt	1.25 ±0.05	0.90 ±0.04	15.92 ±4.04 16.30*
Yeast + salt	1.23 ±0.02	0.95 ±0.02	10.63 ±1.96

Note: *Numerical values were averaged, minus the highest and lowest values.

were similar. The standard deviation results were fairly consistent within each condition. The runs varied from ±0.01 to ±4.04.

Though standardized loaf volume tests were not conducted in the research presented here, it was observed that the sourdough bread without salt was a more compact, denser loaf than the other three test condition breads. Bayfield, Lannuir, and Young (1963) confirmed this finding where the highest loaf volume in their research was recorded in the 4.0-5.0 pH range and reduced loaf volumes were obtained in

the 3.6-3.8 pH range. The mechanism by which the sourdough bread without salt had a reduced loaf volume, was the hardest to compress, and the least likely to recover, can be explained by the effect organic acids had on the gluten proteins and starch hydration.

The degree to which a slice of bread can be compressed and spring back after the plunger is removed has to do with the elasticity of the bread crumb (Taranto, 1983). Already, in several studies discussed (Bennett & Ewart, 1962; Tanaka et al., 1967; Galal et al., 1978; Bakhoum & Ponte, 1983), the extensibility of doughs was decreased by the addition of acid due to increased intra- and inter-molecular replusions which caused the gluten protein to uncoil. An increased water holding capacity of the now unfolded protein also contributed to the weakening of the dough structure. Hence, the dough's ability to expand during fermentation was hampered. Gas cells ruptured, which caused a loss of aeration in the dough, and a denser loaf of bread was the result. This hypothesis explains how sourdough bread without salt had increased resistance to compression. The loss of elasticity in the dough also caused the subsequent bread to recover less when the Instron plunger was released. When salt was added to the sourdough dough, increased hydrophobic bonding occurred, which in turn, contributed to the elasticity of the dough structure (Galal et al.,1978). The gluten protein formed a more compact aggregate and was able to expand more readily

with improved gas retention during fermentation. However, acid still interferes with the dough elasticity to a point, as the yeasted bread with salt was much more compressible and recovered the most. This finding was also confirmed in the fermented dough farinographs (Table 10): yeasted bread with added salt was the strongest of all the doughs. When the bread dough had no added salt or acid, it had approximately the same degree of elasticity as the sourdough bread with salt. This result fits in with the theory that salt counteracts and actually cancels out the effect of acid. In addition, the drop-off and tolerance index in the farinographs of fermented sourdough dough with salt and the yeasted bread without salt had fairly close values when compared to the other two bread doughs.

Besides the effect of acid on protein conformation, the presence of acid could also impede the hydration of starch granules. As discussed previously, the acidified doughs have increased water holding capacity due to the uncoiling of the gluten protein. Therefore, the protein bonds with more water, decreasing the amount of "free" water in the bread dough system. During fermentation, then, there is less water available for starch hydration. The result is a more compact, denser bread loaf as seen in the compression testing of the unsalted sourdough bread. Salt appears to increase the amount of free water in the dough and hence the yeasted dough with salt had more water available for starch

hydration. This accounts for the higher loaf volume of this test bread, easier compressibility, and higher recovery values. The sourdough bread with added salt and the yeasted bread without salt had similar protein configurations, and one would assume the water available for starch hydration was approximately the same. Galal et al. (1978) did not agree with this latter hypothesis. He suggested that the salt and acid combination in doughs formed the most compact protein aggregate of all test conditions. The suppressed electrostatic repulsion and exposed hydrophobic group interaction caused the proteins to become very insoluble.

Scanning Electron Micrograph Analysis

The micrographs of the unfermented doughs (Figures 3A, 3B, 4A, 4B) exhibited small and large starch granules embedded in a protein matrix. This has been shown previously by many researchers (Khoo et al. 1975; Varriano-Marston, 1977; Betchel et al., 1978; Fretzdorff et al., 1982; Pomeranz et al., 1984). In the doughs containing salt (both yeasted and sourdough culture: Figures 3A, 4A), the protein matrix was so thin and stretched that the starch granules were prominent and easily identified. The two doughs without salt (Figures 3B, 4B), however, showed a more relaxed matrix with patches of proteinaceous material, and larger spacing between starch granules.

The fermented doughs (Figures 5A, 5B, 6A, 6B) showed

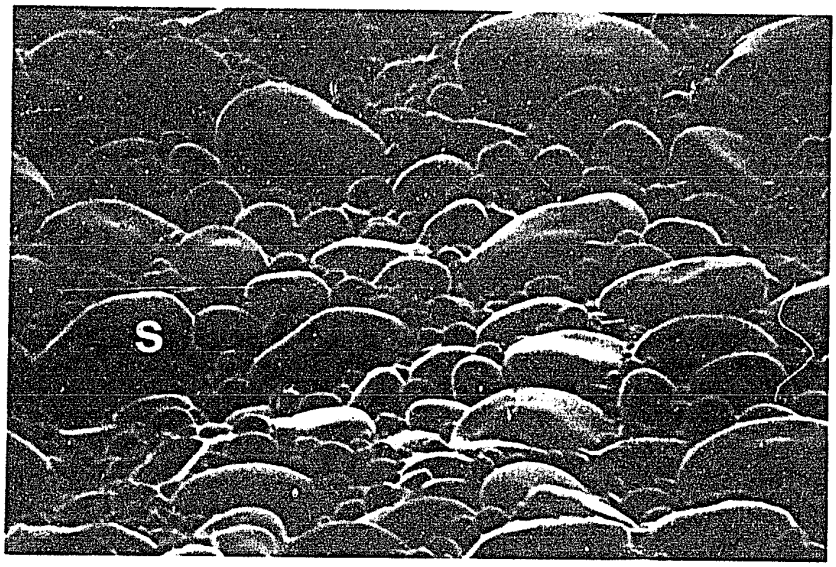

Figure 3A. Unfermented Yeast Culture Bread Dough + Salt

p = protein; s = starch X1120

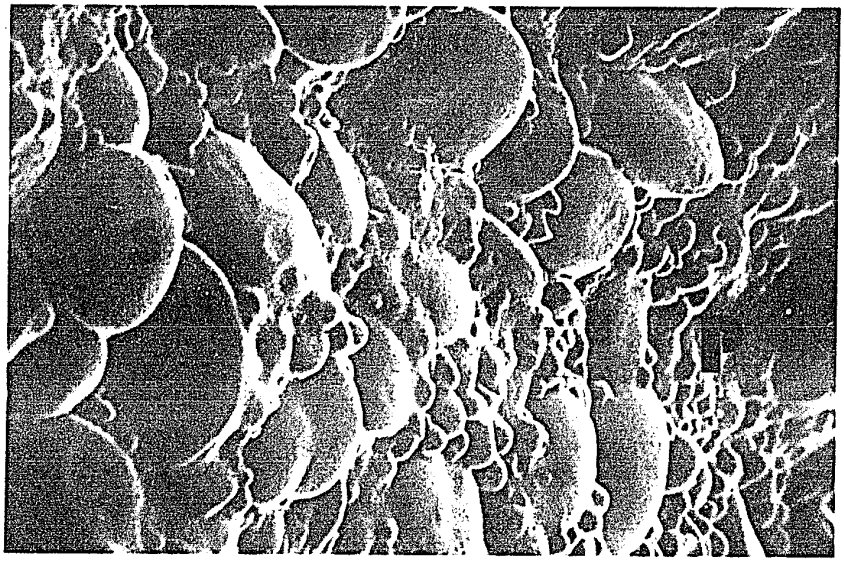

Figure 3B. Unfermented Yeast Culture Bread Dough Ø Salt

p = protein; s = starch X1320

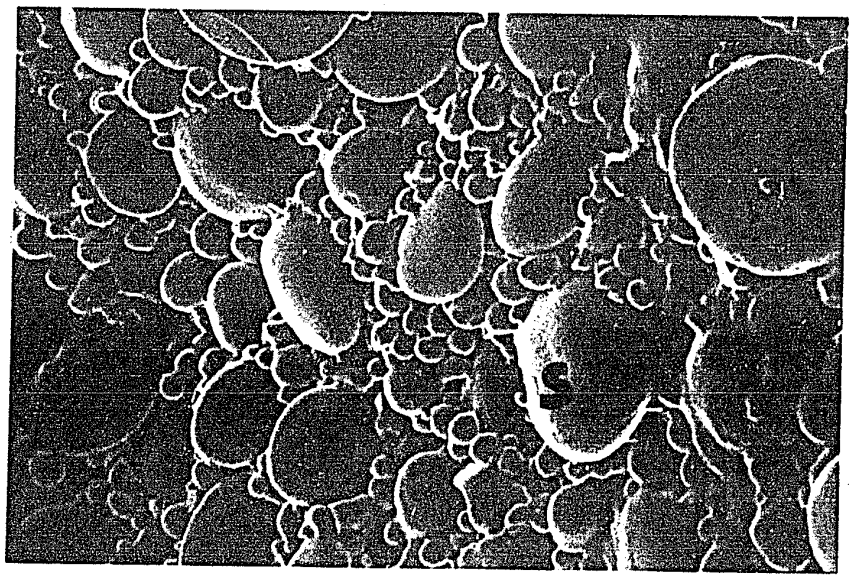

Figure 4A. Unfermented Sourdough Bread Dough + Salt

p = protein; s = starch X1120

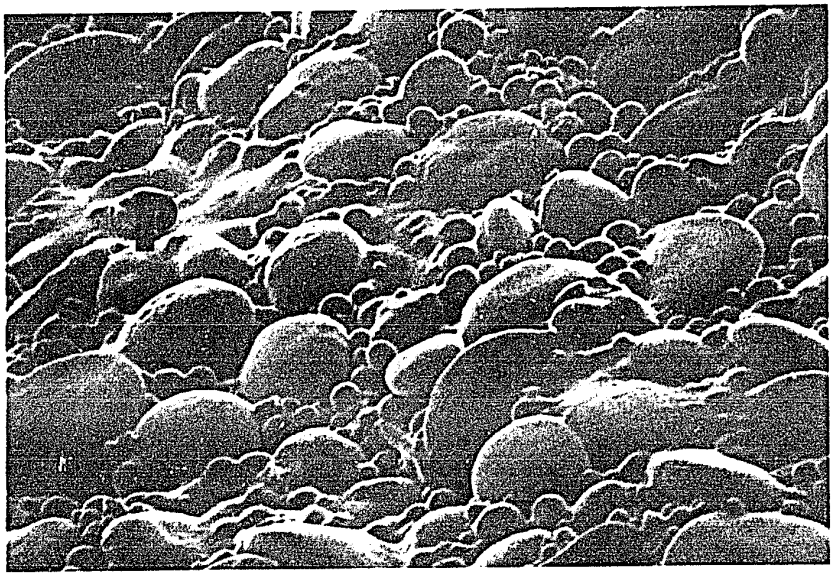

Figure 4B. Unfermented Bread Dough Ø Salt (sourdough)

p = protein; s = starch X1120

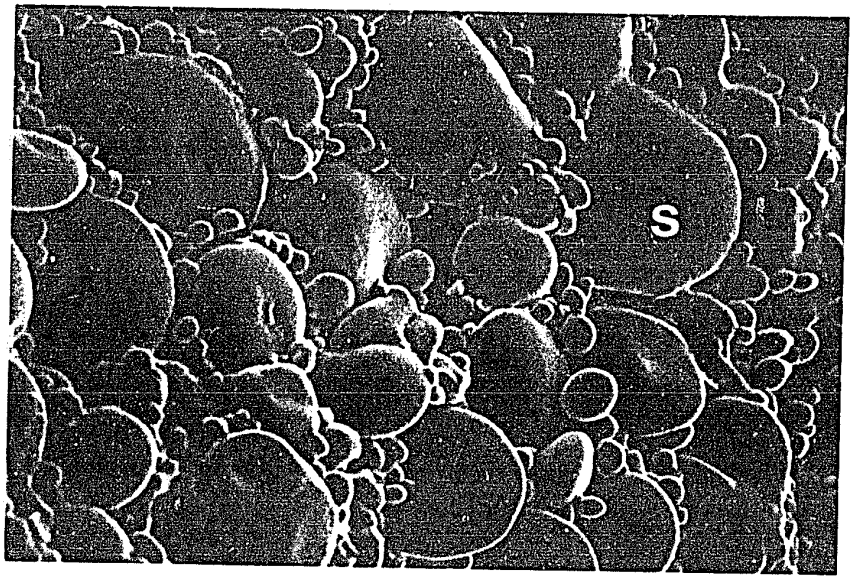

Figure 5A. Fermented Yeast Culture Bread Dough + Salt

p = protein; s = starch X1280

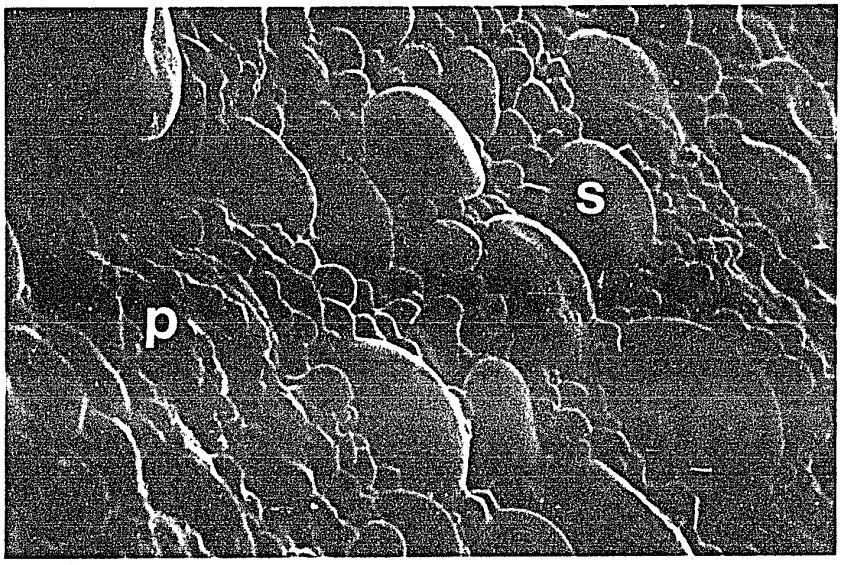

Figure 5B. Fermented Yeast Culture Bread Dough Ø Salt

p = protein; s = starch X1280

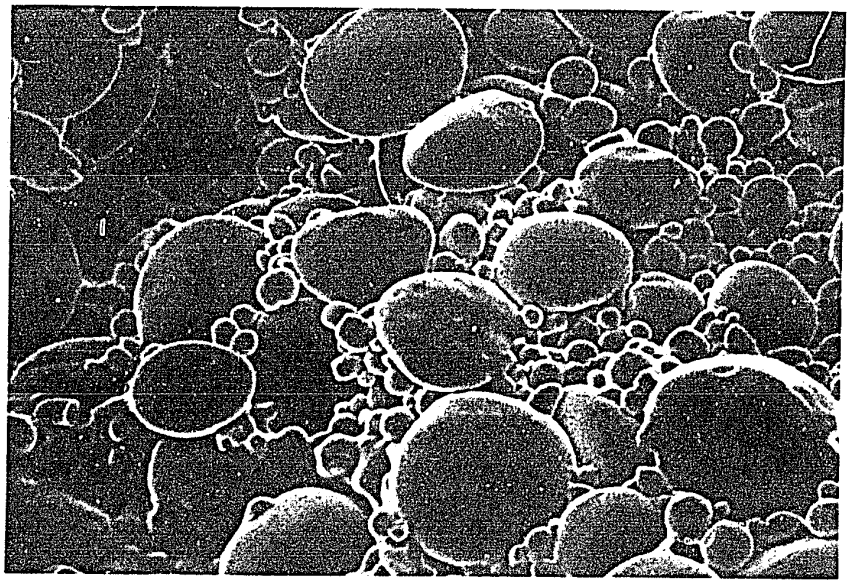

Figure 6A. Fermented Sourdough Bread Dough + Salt

p = protein; s = starch X1120

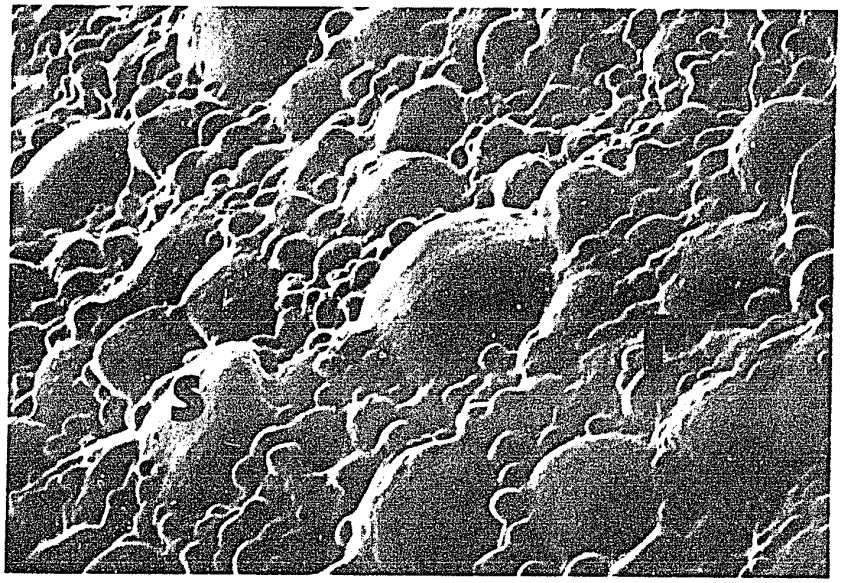

Figure 6B. Fermented Sourdough Bread Dough Ø Salt

p = protein; s = starch X1120

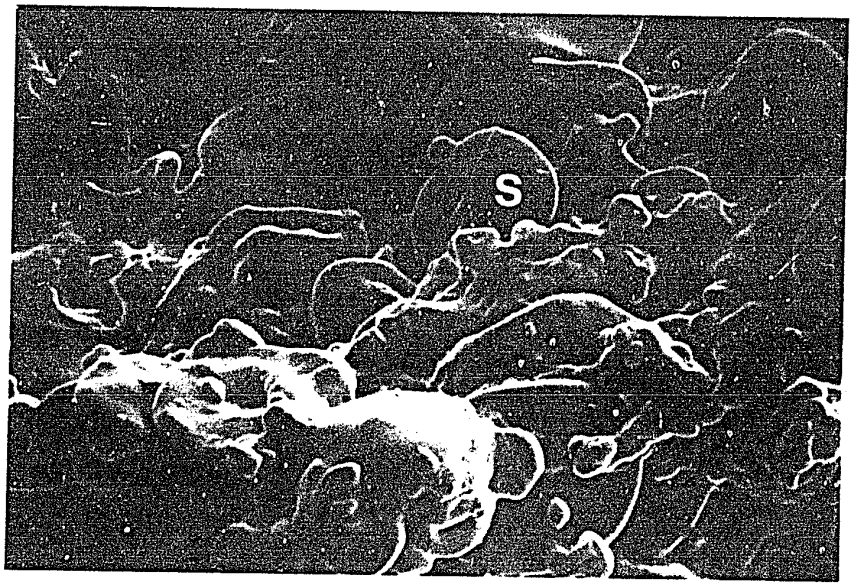

Figure 7A. Yeast Bread Crumb + Salt

p = protein; s = starch X1190

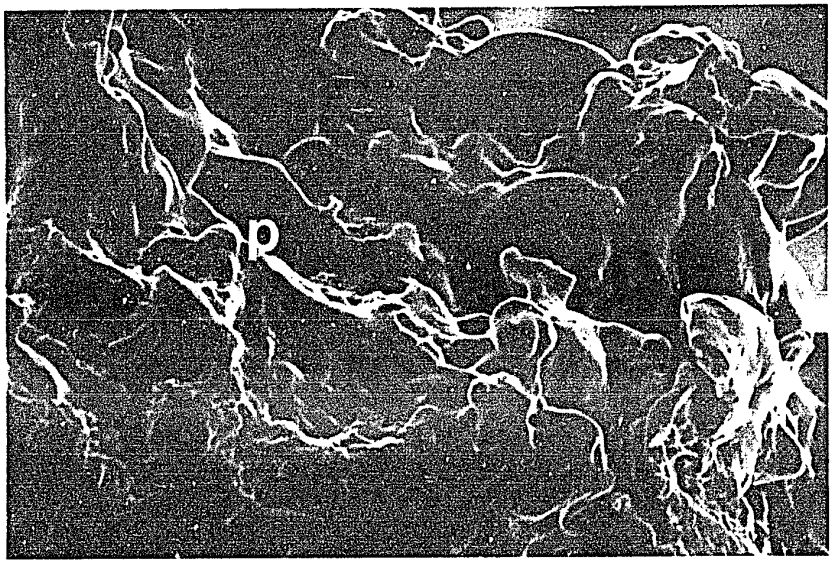

Figure 7B. Yeast Bread Crumb Ø Salt

p = protein; s = starch X1190

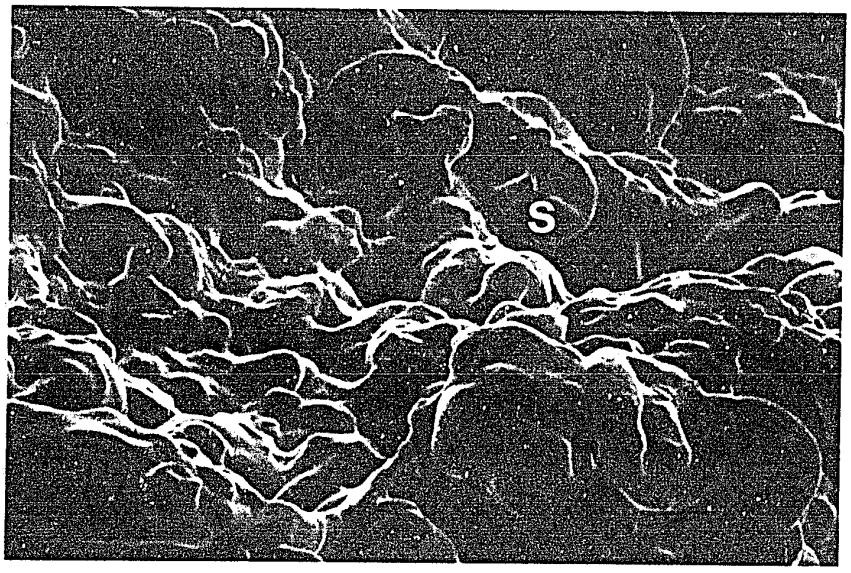

Figure 8A. Sourdough Bread Crumb + Salt

p = protein; s = starch X1190

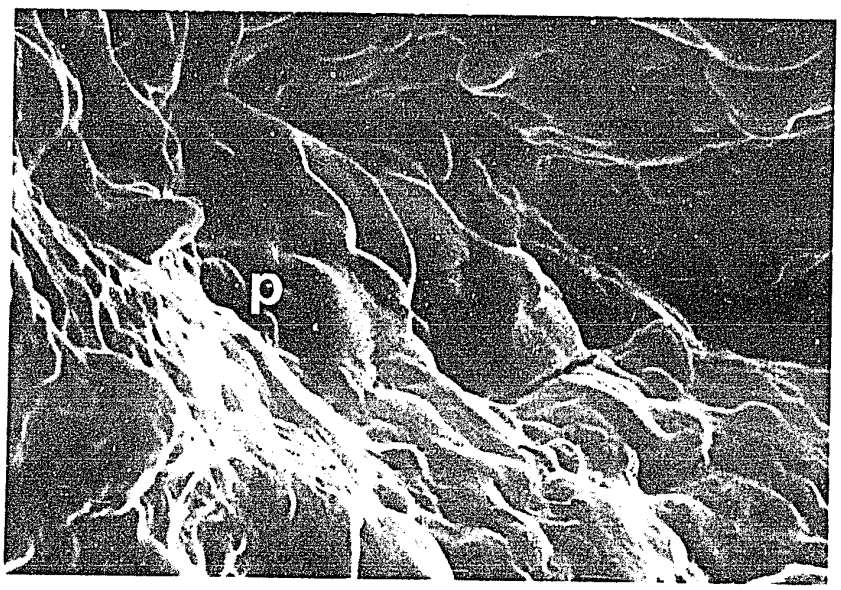

Figure 8B. Sourdough Bread Crumb Ø Salt

p = protein; s = starch X1190

hydrated small and large starch granules spaced further apart from one another than in the unfermented doughs. Fretzdorff et al.(1982) noted this in his comparison of unfermented and fermented doughs. The sourdough without salt (Figure 6B) had an extremely relaxed appearance, with the starch granules looking partly buried in a veil-like protein network. Pomeranz et al. (1984) indicated that SEM of sour wheat/rye bread fermented dough showed considerable weakening of the protein-starch structure. The yeasted dough without salt (Figure 5B) was similar to the sourdough without salt, yet the starch granules appeared to be more defined. The two bread dough samples with added salt (Figures 5A,6A) had distinct starch granules crowded together and enrobed in a very thin protein matrix.

The bread crumb micrographs (Figures 7A, 7B, 8A, 8B) showed outlines of starch granules totally enveloped by the gluten structure. This phenomena is well documented (Khoo et al., 1975; Fretzdorff et al., 1982; and Pomeranz et al., 1984). The yeast fermented bread with salt (Figure 7A) had the most dense structure with distinct starch granules compacted together. The sourdough bread without added salt (Figure 8B) had no discreet starch granules in view: the proteinaceous material totally masked any starch definition. The appearance of the yeast fermented dough with no salt (Figure 7B) and the sourdough fermented bread with salt had very similar ultrastructure. There was some relaxation of

background components, though some enmeshed starch granules were seen.

In general, the scanning electron micrographs of bread doughs and bread crumb confirm the results of the rheological studies. In the farinograph research, the model dough systems with added salt or fermented yeast bread doughs with added salt were the strongest doughs. In addition, the model dough systems with added acid and no added salt, or fermented sourdough dough without salt, were the weakest doughs. Since micrographs were not taken of the model dough systems, it is difficult to ascertain why the 5.3 ml acid with added salt dough was the strongest of all the tested model doughs. The SEM photographs do, however, parallel the trends of the fermented yeast and sourdough culture farinographs. For instance, Micrograph 5A (yeast culture with added salt) showed a tight gluten matrix with uniformly hydrated starch granules: an indicator of a strong dough. However, micrograph 6B (sourdough culture no added salt) exhibited patches of gluten network, less starch crowding, and an altogether more relaxed appearance. The similar ultrastructure of yeasted bread dough without salt (Figure 5B) to sourdough culture with added salt (Figure 6A) in the micrographs is a consistent finding with the similar tolerance and drop-off values of these doughs in the farinograph trials (Table 10).

The scanning electron micrographs also support the Instron data. For instance, the large patches of proteinaceous material in Micrograph 8B (sourdough breadcrumb without added salt) indicated unfolded protein structures with less starch/starch interaction. This contributed to the weakening of the bread structure, which made it a denser loaf: hence, the larger compressibility values and smaller recovery values. Also, the similarity in microstructure of yeast fermented bread crumb with no salt (Figure 7B), and the sourdough fermented bread crumb with salt (Figure 8A), corresponds to the similar compression values of these two conditions in the Instron data. The ultrastructure of yeast culture bread with salt (Micrograph 7A) had the most starch/starch interaction which contributed to its elasticity. This would account for the ease of compression and the larger recovery values in the Instron testing.

Only one group of researchers, Pomeranz et al. (1984), did SEM analysis of a sourdough system, wheat/rye sourdough and 100 % rye meal sourdough, and compared it to a standard wheat bread dough and bread crumb. Some general findings were similar between this study and the research presented here. In fermented wheat bread dough, starch granules appeared to be uniformly hydrated, with larger granules interacting with the protein-starch interface. Scanning electron micrographs of wheat bread crumb had uniformly distributed starch granules interacting with the surrounding

protein matrix. In the sourdough samples, distortion of starch granules was apparent, as well as a weakening of the dough structure. Pomeranz et al. theorized that this condition affected oven-spring and the elasticity of the dough during baking. The comparison ends at this point, as the wheat bread dough formulation is entirely different and the sourdough doughs were prepared from flours with high bran and aleurone layer particles.

Conclusion and Implications

The unique properties of sourdough bread have been demonstrated by the research presented here. Lactic acid levels in sourdough dough increased six fold over an eight hour fermentation period. Rheological testing using a farinograph and universal testing machine, determined dough and bread functionality. Model systems comprised of flour, water, and varying levels of acid and/or salt showed that dough consisting of acid only had less stability, tolerance, and more drop-off than the other test conditions. Salted doughs were generally stronger. When used in combination with acid, salt cancelled out the weakening effect of acid. One model system, 5.3 ml acid with added salt, was the strongest of all the doughs tested. Fermented dough farinographs indicated that sourdough without salt was the weakest dough, with lower tolerance and drop-off values, followed by two conditions which had very similar results: the unsalted yeast culture dough and the salted sourdough

dough. The strongest dough was the yeast culture dough with added salt.

The compression testing of baked bread determined that sourdough bread without salt was the hardest to compress and least likely to recover. On the other hand, the yeasted bread with added salt was the easiest to compress and had the highest recovery rate. Again, as in the fermented dough farinographs, the yeast dough without salt and the sourdough with salt had similar compressibilities and recoveries.

Scanning electron microscopy reinforced both the fermented farinograph results and Instron data. Micrographs indicated that the sourdough doughs and breads without salt had the weakest structure, with large areas of proteinaceous material and little definition of starch granules. The yeasted doughs and breads with salt showed uniformly hydrated small and large starch granules tightly held in a gluten matrix. The yeasted doughs and breads without salt and the sourdough with salt had similar structure, with starch granules placed further apart and a little more relaxation of the protein matrix.

It is quite evident that organic acids and salt have a profound influence on sourdough bread. Organic acids alone, have a disruptive quality on sourdough, which can be explained by increased protein unfolding which causes augmented water holding capacity, and reduced starch hydration. This produces a weakened dough structure, and

poor gas retention. The resulting baked bread has a reduced loaf volume and dense crumb texture. Optimum levels of salt added to sourdough cancel out the deleterious effects of the organic acids. Salt enhances the protein hydrophobic bonding, and therefore the elasticity of the dough is increased. Also, since the protein has more inter- and intra- molecular bonding participation, less water is bound and freed up to hydrate starch granules. Gas retention is improved and the baked bread has an increased loaf volume and lighter texture. Still, though, standard yeast bread with salt has a springier and lighter texture than sourdough bread. However, these textural qualities are just part of the uniqueness that consumers equate with sourdough bread.

Limitations and Suggestions for Further Research

This study could have been improved in a number of ways. First, four laboratories were used for the different testing conducted in this research. For this reason, a source of error was introduced into the results. The pH should have been taken for all doughs used in the research: sourdough culture, standard yeast culture, and the model dough systems. Since it is not known why the 5.3 ml with added salt model dough was stronger than other doughs, a pH would have been valuable in determining an explanation for this result. If time would have permitted, SEM analysis of the model dough systems would have also added to the theoretical framework of the research presented here. Since sourdough cultures do

differ in the Bay area, a gas chromatography analysis of the organic acids in the sourdough culture used in this research would have specifically identified the levels of lactic, acetic, and propionic acids. Thus, the acid levels used in the farinographs of the model dough systems could have been more exact. Despite limitations inherent in this study and the old adage "more research needs to be done," the examination of the microstructure as it relates to textural changes has given insight into the functional behavior of sourdough bread.

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Appendixes

Appendix A

Microequivalents of Lactic Acid in Sourdough Samples

Sample	Proof time (hr.)	µeq/ wet basis	µeq/ dry basis
Sponge	3	43.40	74.17
		45.50	77.71
		47.60	81.30
Mean		45.50	77.71
STD.DEV. ±		2.10	3.56
Sponge	5	64.95	110.93
		66.93	114.31
		62.35	106.43
Mean		64.74	110.57
STD.DEV. ±		2.30	3.95
Sponge	10	72.33	123.53
		96.28	164.44
		88.62	151.35
Mean		85.74	146.44
STD.DEV. ±		12.23	20.89
bread dough	0	25.33	46.09
		12.39	22.54
		16.77	30.51
Mean		18.16	33.04
STD.DEV. ±		6.58	11.98
bread dough	3	64.23	116.87
		72.34	131.62
		58.97	107.30
Mean		65.18	118.50
STD.DEV. ±		6.74	12.26

Appendix A (cont.)

Microequivalents of Lactic Acid in Sourdough Samples

Sample	Proof time (hr.)	µeq/ wet basis	µeq/ dry basis
bread	6	98.34	178.93
dough		116.16	211.35
		91.01	165.59
Mean		101.84	185.30
STD.DEV. ±		12.93	23.53
bread	8	119.89	218.14
dough		112.73	205.11
		110.69	201.40
Mean		114.44	208.22
STD.DEV. ±		4.83	8.79

Appendix B

The Effect of Acid and Salt on Farinogram Values of Model Dough Systems

Condition	Figure #	Peak (min.)	Stability (min.)	Tolerance (BU's)	Drop-Off (BU's)
ø acid	9	1.8	6.8	100	170
ø salt	10	0.5	7.0	70	110
	11	0.5	7.0	60	130
	12	1.5	2.5	120	200
	13	1.8	4.0	80	170
	14	1.0	5.5	100	180
	15	1.0	8.0	100	150
Mean		1.1	5.8	90	159
STD. DEV. ±		0.5	2.0	21	31
ø acid	16	2.0	11.0	40	70
+ salt	17	1.0	8.3	40	80
	18	4.5	15.0	30	30
	19	3.0	10.0	50	80
	20	4.0	9.5	50	80
	21	3.5	10.8	40	80
	22	2.0	14.0	70	50
Mean		2.9	11.2	46	67
STD. DEV. ±		1.3	2.4	13	20

Appendix C

The Effect of Acid and Salt on Farinogram Values

Condition	Figure #	Peak (min.)	Stability (min.)	Tolerance (BU's)	Drop-Off (BU's)
+ acid (2.7 ml) ø salt	23	3.5	3.5	170	340
+ acid (5.3 ml) ø salt	24	1.0	3.0	310	430
	25	1.5	3.5	240	410
Mean		1.3	3.3	275	420
STD. DEV. ±		0.4	0.4	50	14
+ acid (2.7 ml) + salt	26	1.0	15.0	110	70
+ acid (5.3 ml) + salt	27	1.0	20.0	50	10
	28	1.0	20.0	60	10
Mean		1.0	20.0	55	10
STD. DEV. ±		0.0	0.0	7	0

Appendix D

The Effects of Salt on Sourdough and Standard Yeast

Culture Bread Dough on Farinograph Values

Condition	Figure #	Tolerance (BU's)	Drop-off (BU's)
Sourdough	29	100	210
+ Salt	30	100	90
	31	200	190
	32	200	160
Mean		150	163
STD. DEV. ±		58	53
Sourdough	33	460	460
ø salt	34	350	420
Mean		405	440
STD. DEV. ±		78	28
Yeast	35	50	80
+ Salt	36	90	90
Mean		70	85
STD. DEV. ±		28	7
Yeast	37	140	140
ø Salt	38	180	180
Mean		160	160
STD. DEV. ±		28	28

Appendix E

The Effects of Salt on Sourdough and Yeast Culture Bread Using the Instron

Sample	Figure #	Deformation (inch)	Recovery (inch)	Compression (lb./inch)
ø salt	39	1.13	0.90	14.86
ø acid	40	1.25	0.95	20.00
	41	1.20	0.90	10.68
	42	1.25	0.90	14.05
	43	1.25	0.85	20.00
Mean		1.22	0.90	15.92
STD. DEV. ±		0.05	0.04	4.04
+ salt	44	1.22	0.95	13.67
	45	1.25	1.00	10.43
	46	1.22	0.95	8.71
	47	1.25	0.95	8.34
	48	1.25	0.95	11.02
	49	1.22	0.95	11.59
Mean		1.24	0.96	10.63
STD. DEV. ±		0.02	0.02	1.96

Appendix E (cont.)

Sample	Figure #	Deformation (inch)	Recovery (inch)	Compression (lb./inch)
+ acid	50	1.25	0.75	21.28
ø salt	51	1.20	0.80	22.80
	52	1.22	0.88	23.66
	53	1.20	0.80	26.24
	54	1.22	0.78	24.98
	55	1.20	0.77	24.98
Mean		1.22	0.80	23.99
STD. DEV. ±		0.02	0.05	1.78
+ acid	56	1.23	0.88	14.46
+ salt	57	1.23	0.88	14.52
	58	1.25	0.88	16.15
	59	1.22	0.80	18.97
	60	1.23	0.85	16.15
	61	1.25	0.85	16.77
Mean		1.24	0.86	16.17
STD. DEV. ±		0.01	0.03	1.66

Appendix F

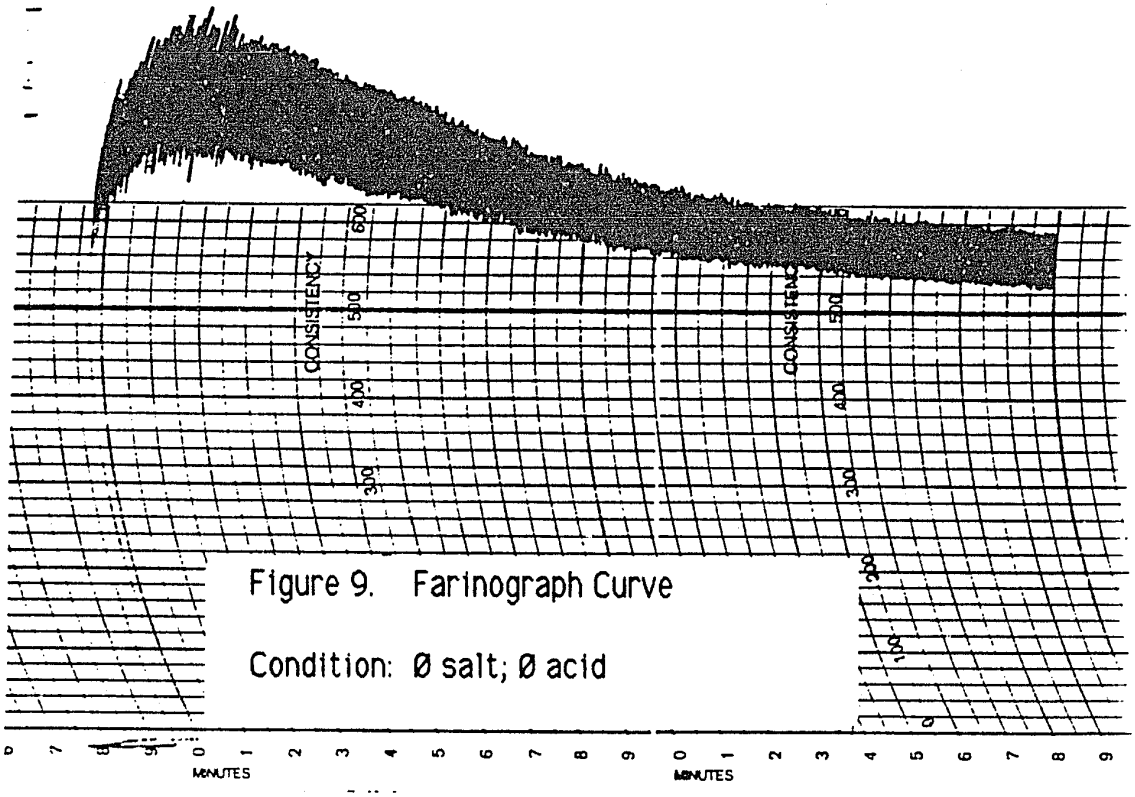


Figure 9. Farinograph Curve

Condition: Ø salt; Ø acid

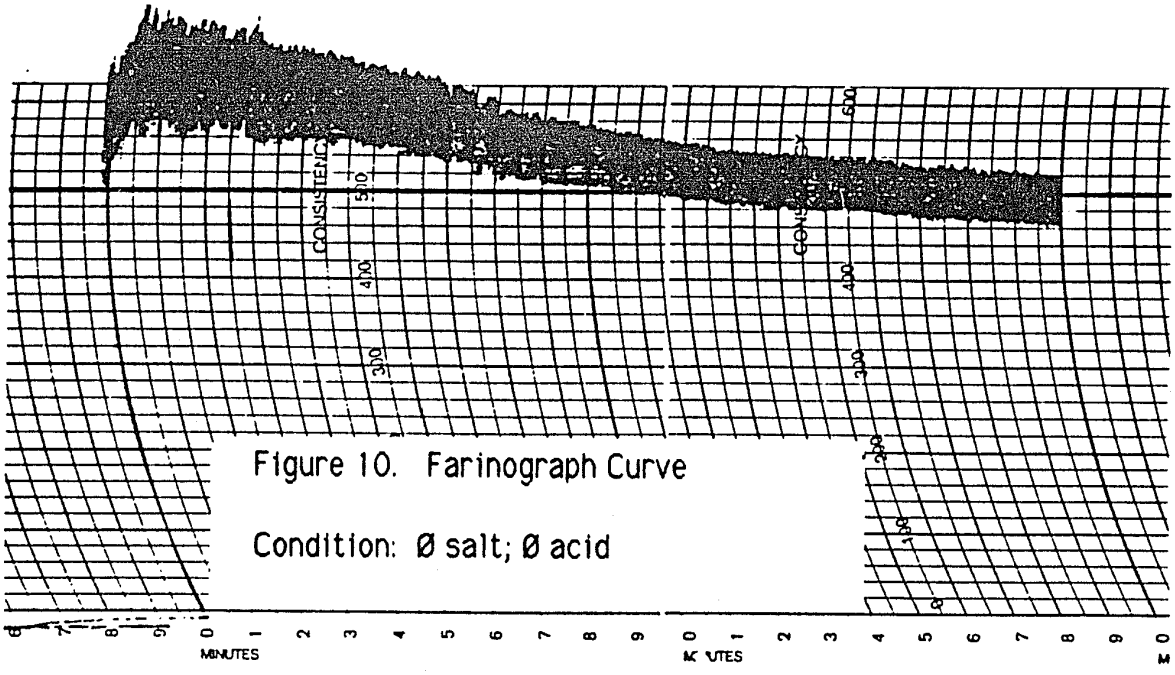


Figure 10. Farinograph Curve

Condition: Ø salt; Ø acid

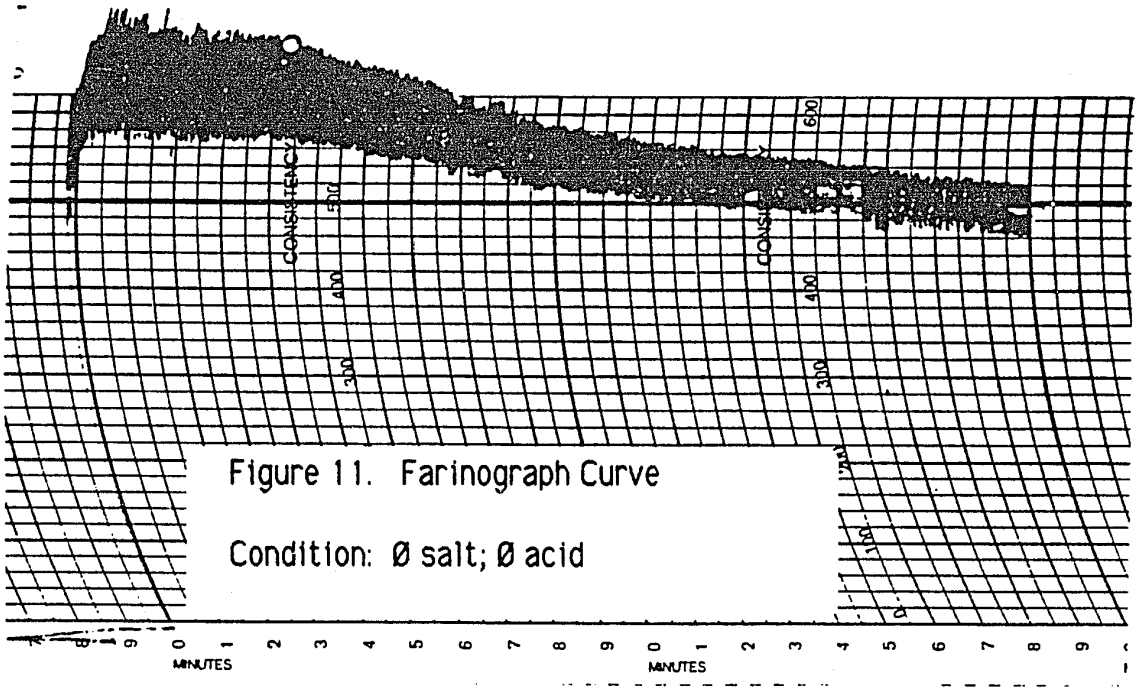


Figure 11. Farinograph Curve

Condition: Ø salt; Ø acid

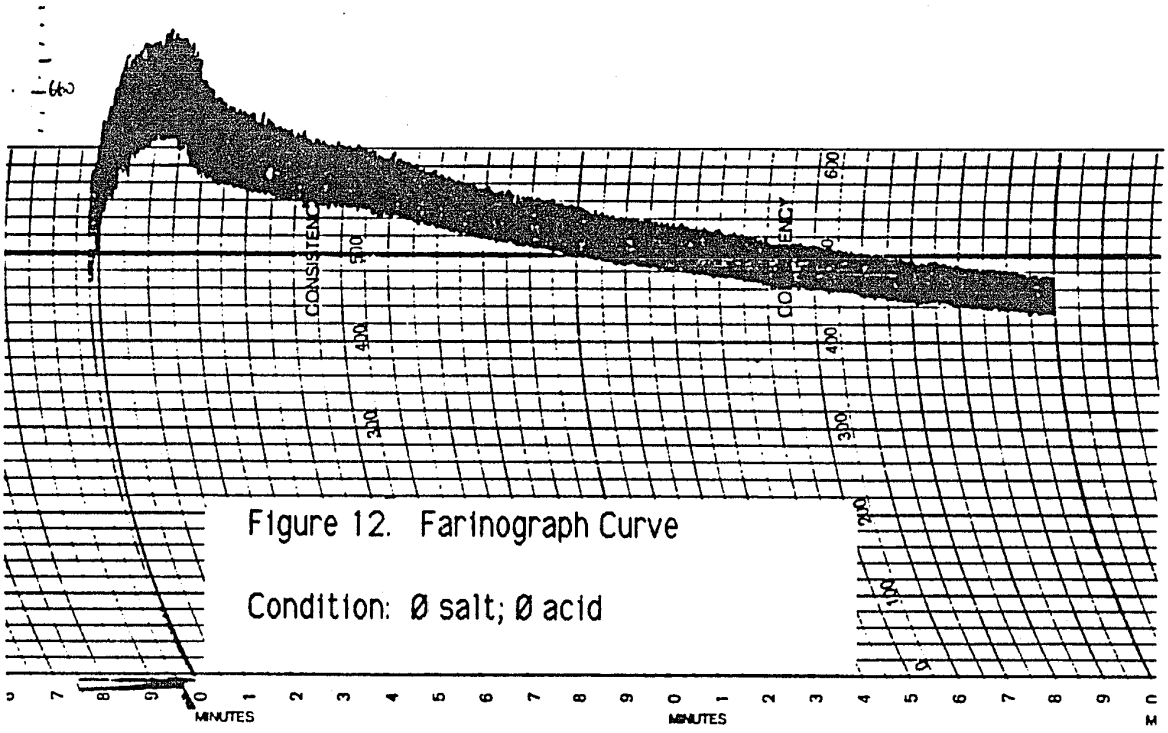


Figure 12. Farinograph Curve

Condition: Ø salt; Ø acid

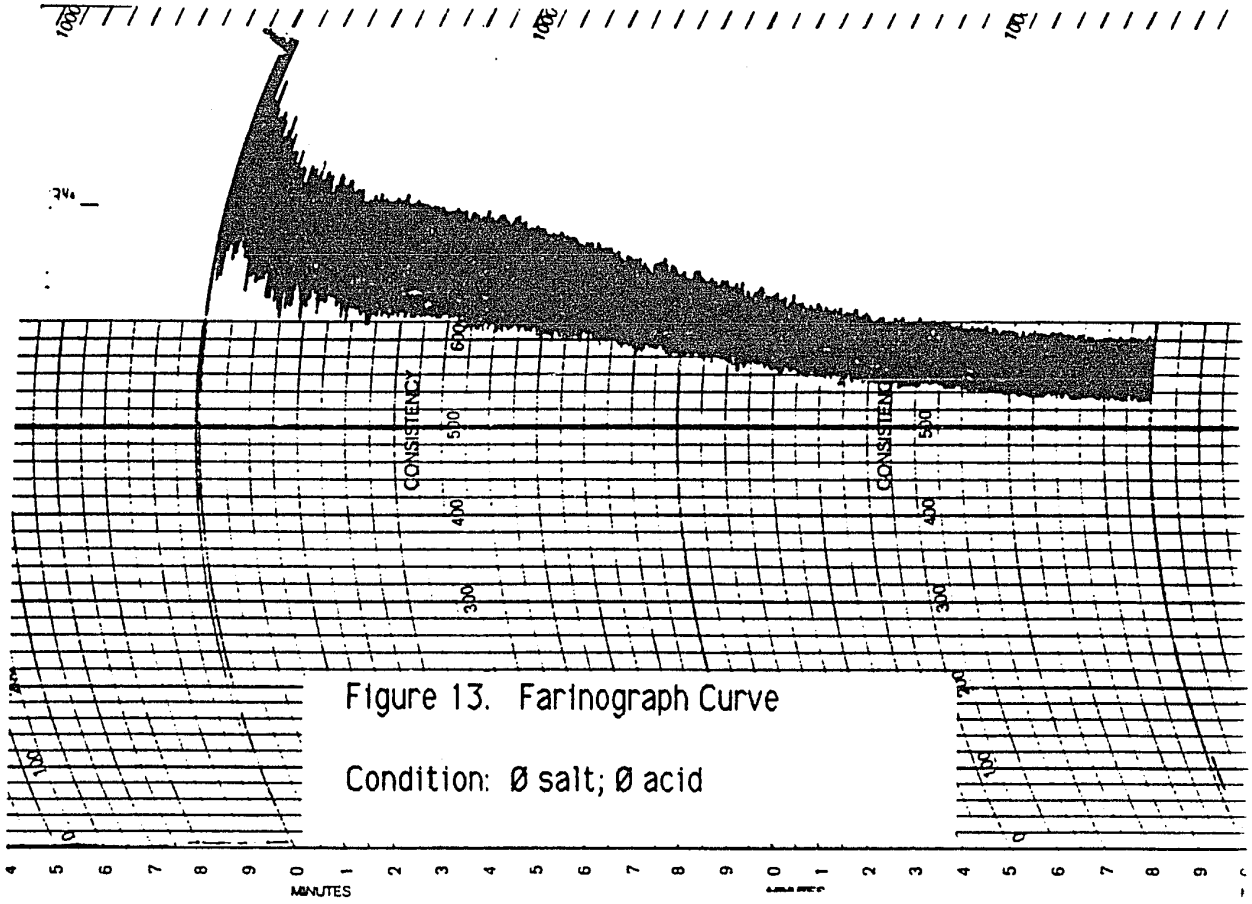


Figure 13. Farinograph Curve

Condition: Ø salt; Ø acid

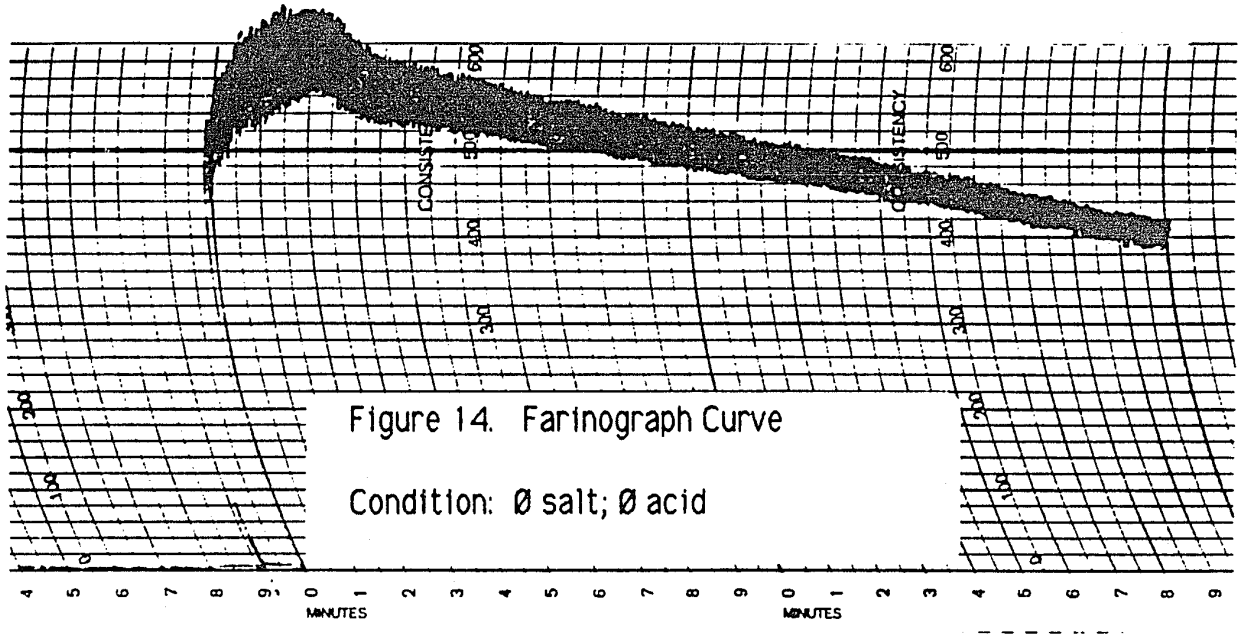


Figure 14. Farinograph Curve

Condition: Ø salt; Ø acid

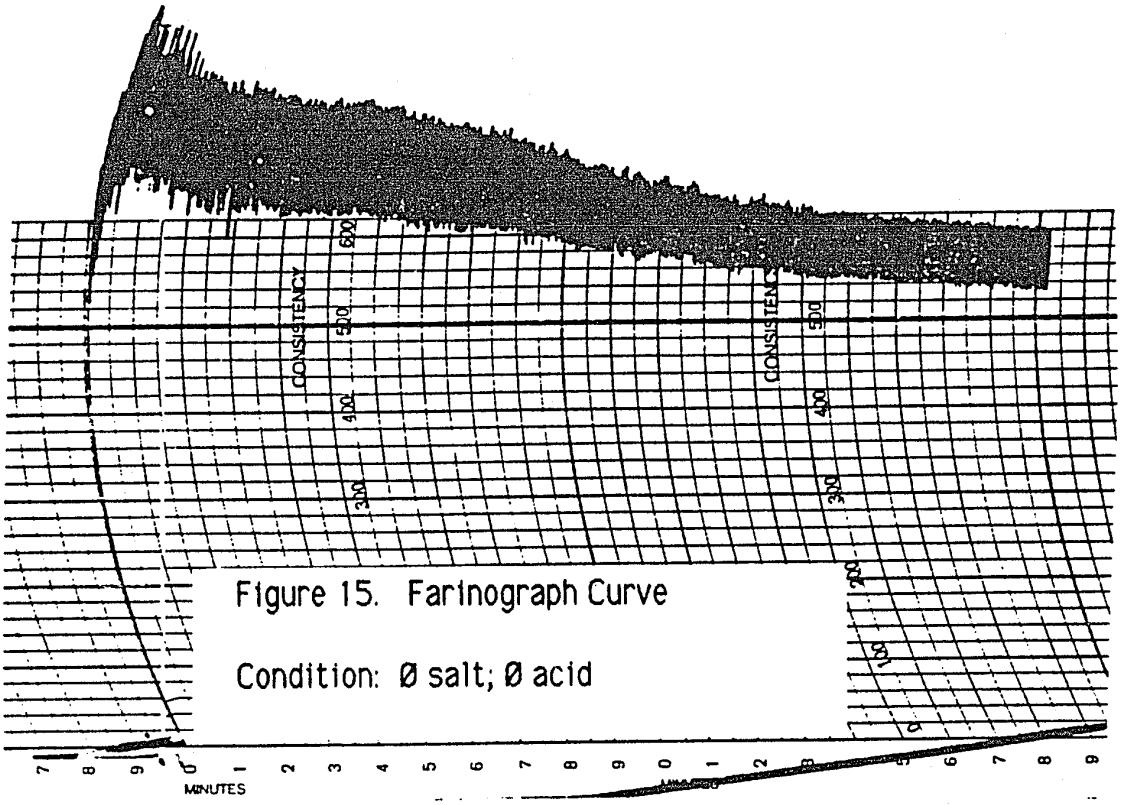


Figure 15. Farinograph Curve

Condition: Ø salt; Ø acid

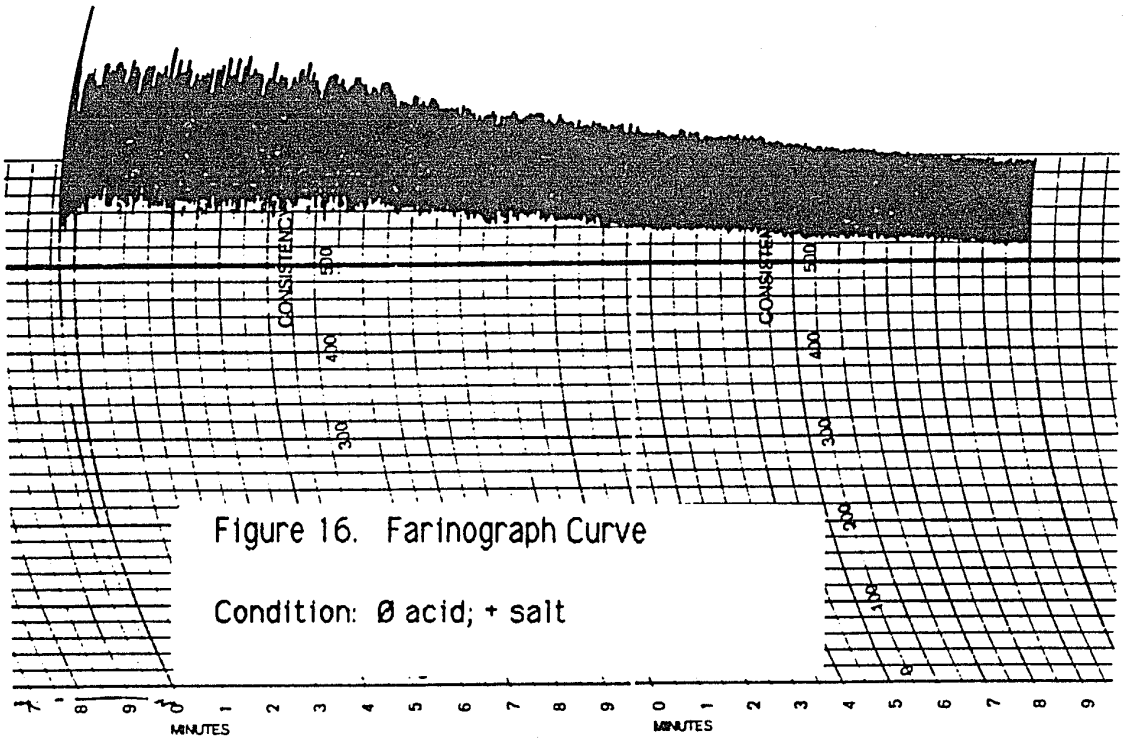


Figure 16. Farinograph Curve

Condition: Ø acid; + salt

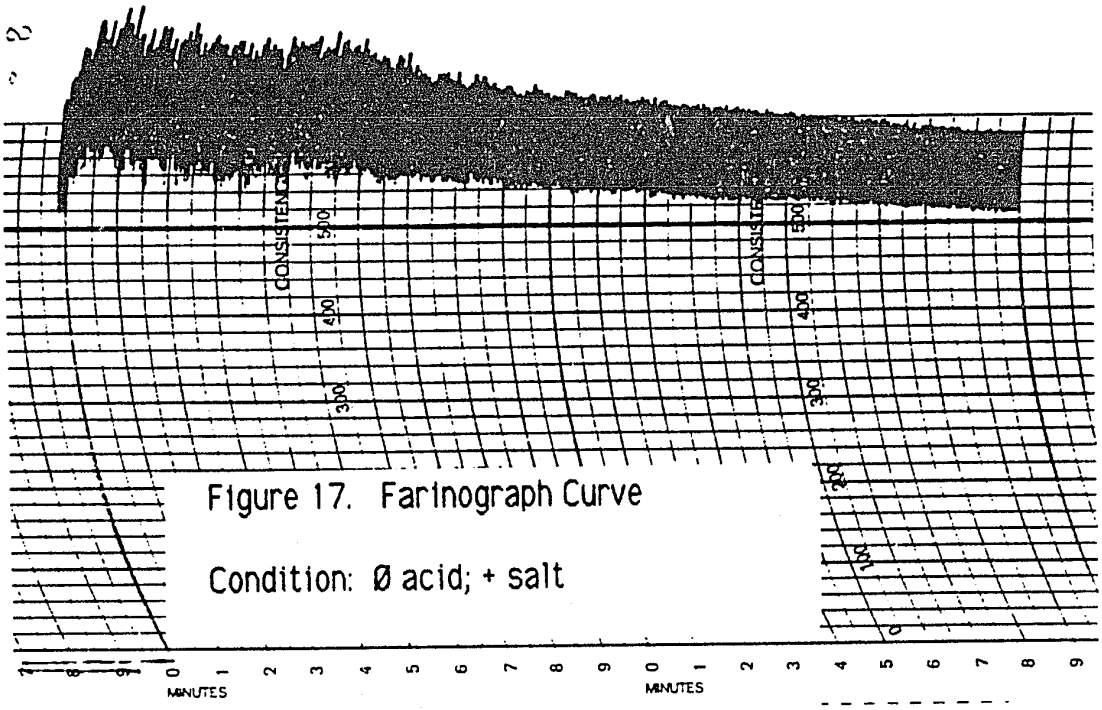


Figure 17. Farinograph Curve

Condition: Ø acid; + salt

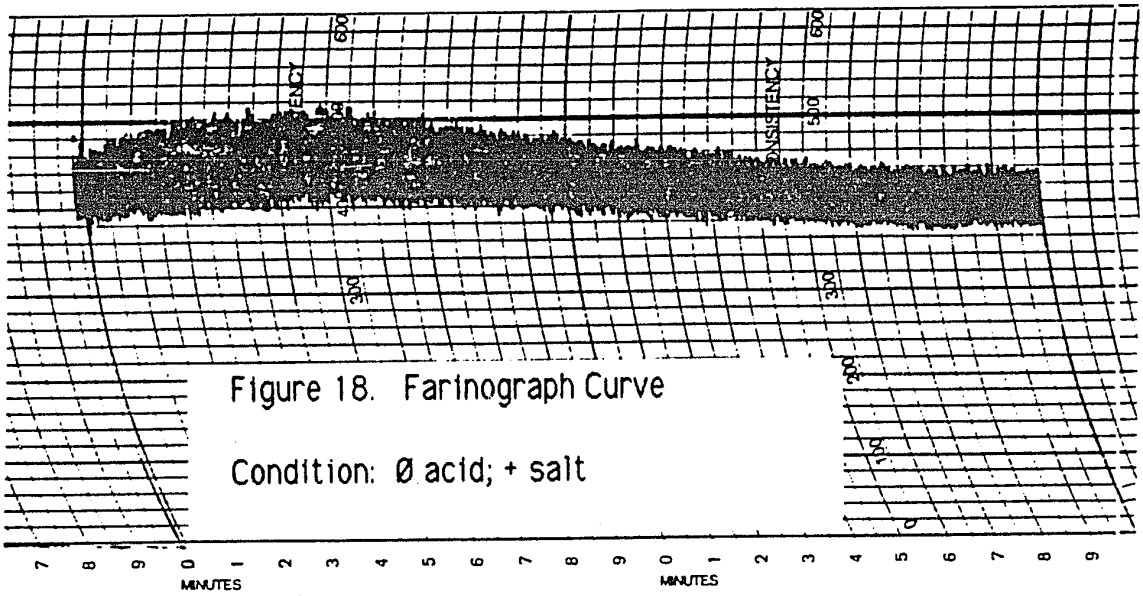


Figure 18. Farinograph Curve

Condition: Ø acid; + salt

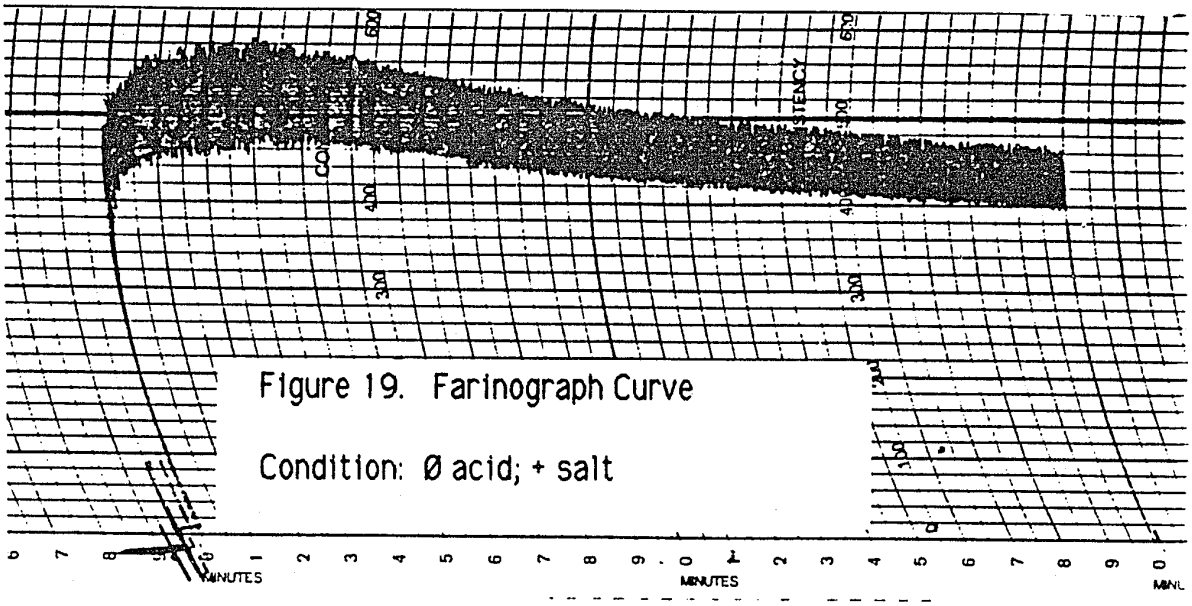


Figure 19. Farinograph Curve

Condition: Ø acid; + salt

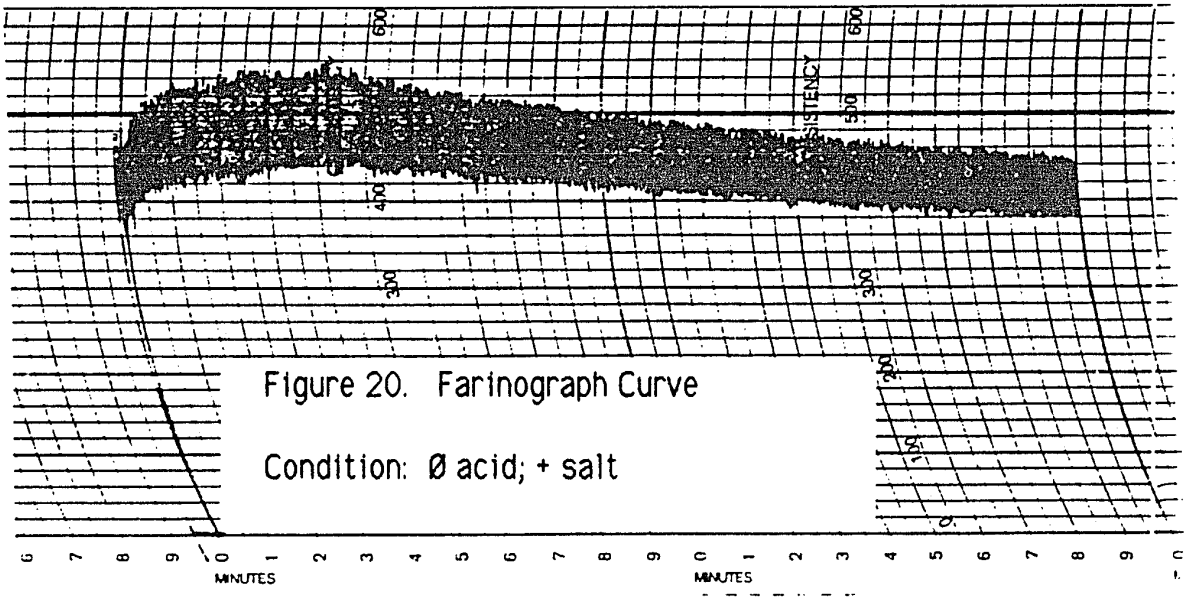


Figure 20. Farinograph Curve

Condition: Ø acid; + salt

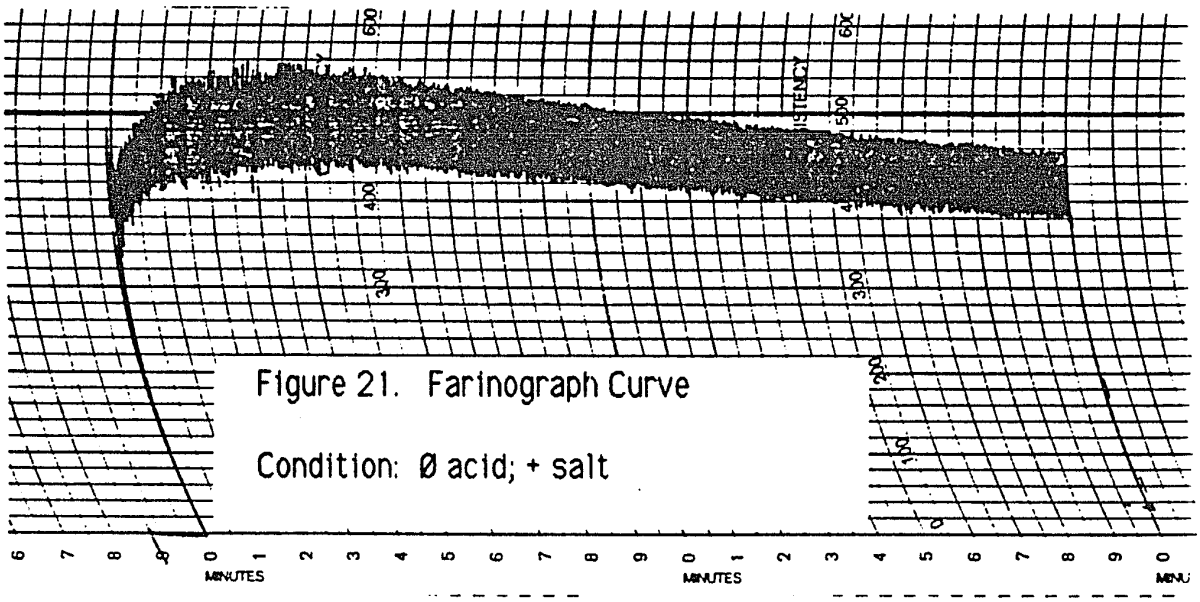


Figure 21. Farinograph Curve

Condition: Ø acid; + salt

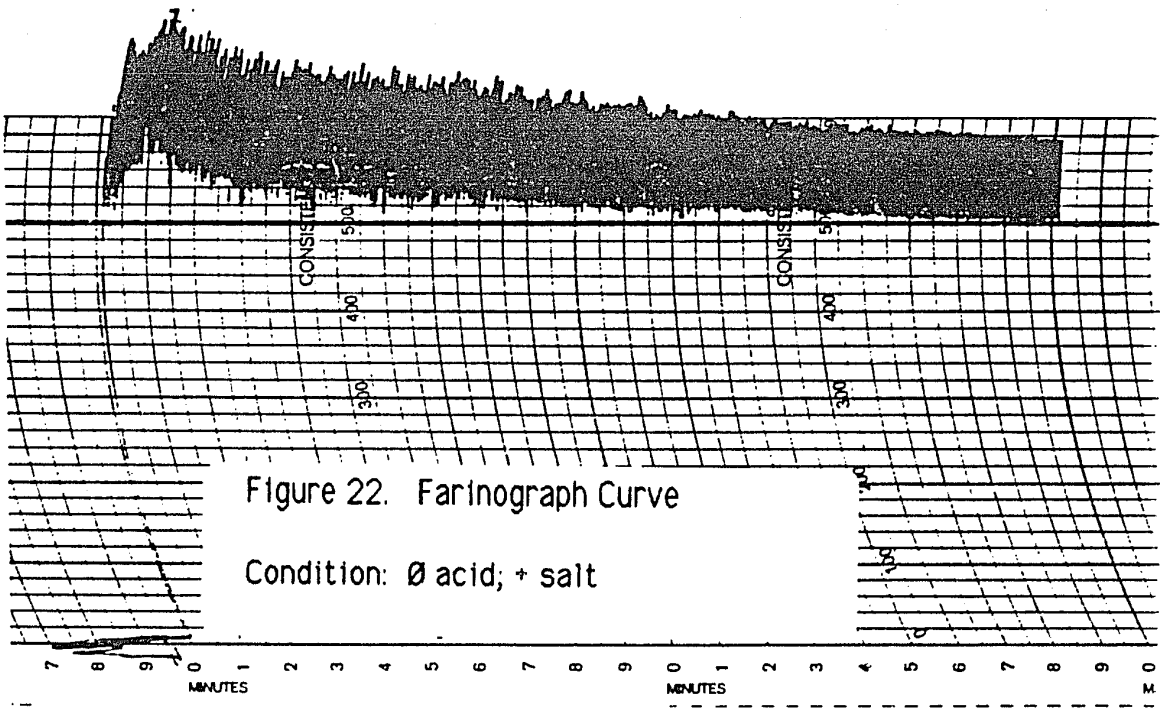


Figure 22. Farinograph Curve

Condition: Ø acid; + salt

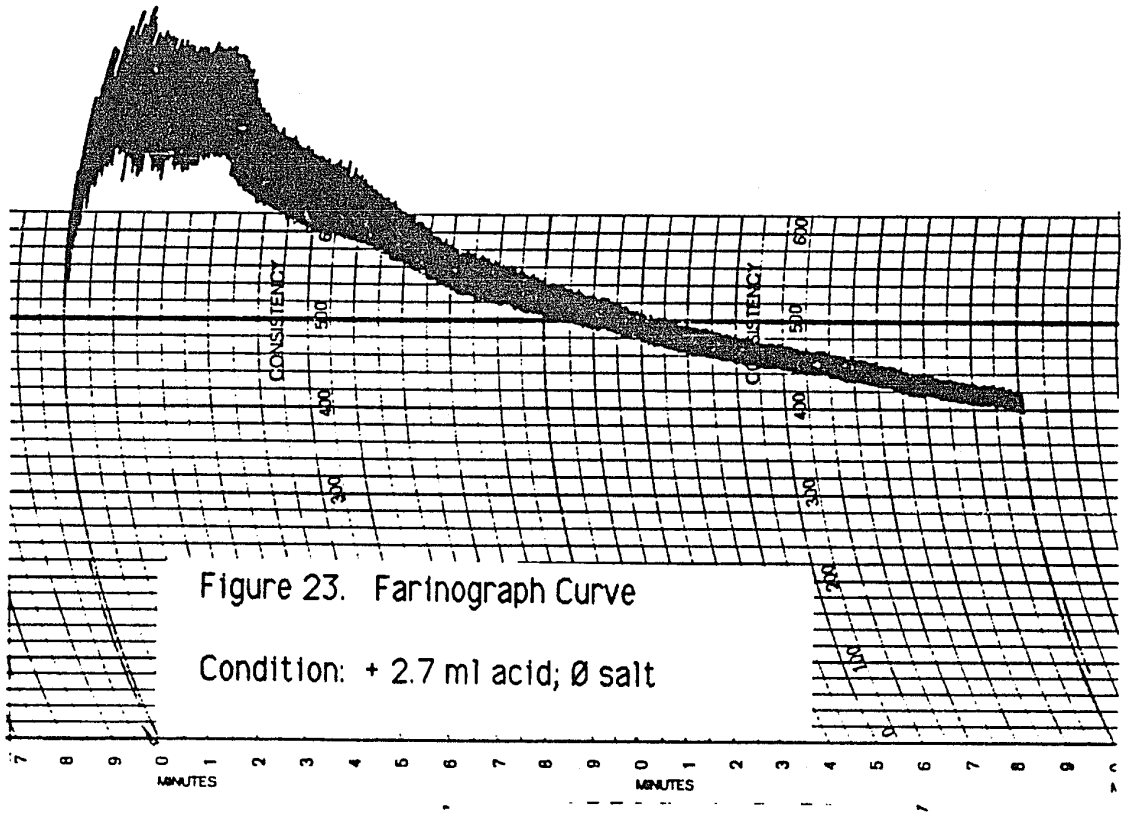


Figure 23. Farinograph Curve

Condition: + 2.7 ml acid; Ø salt

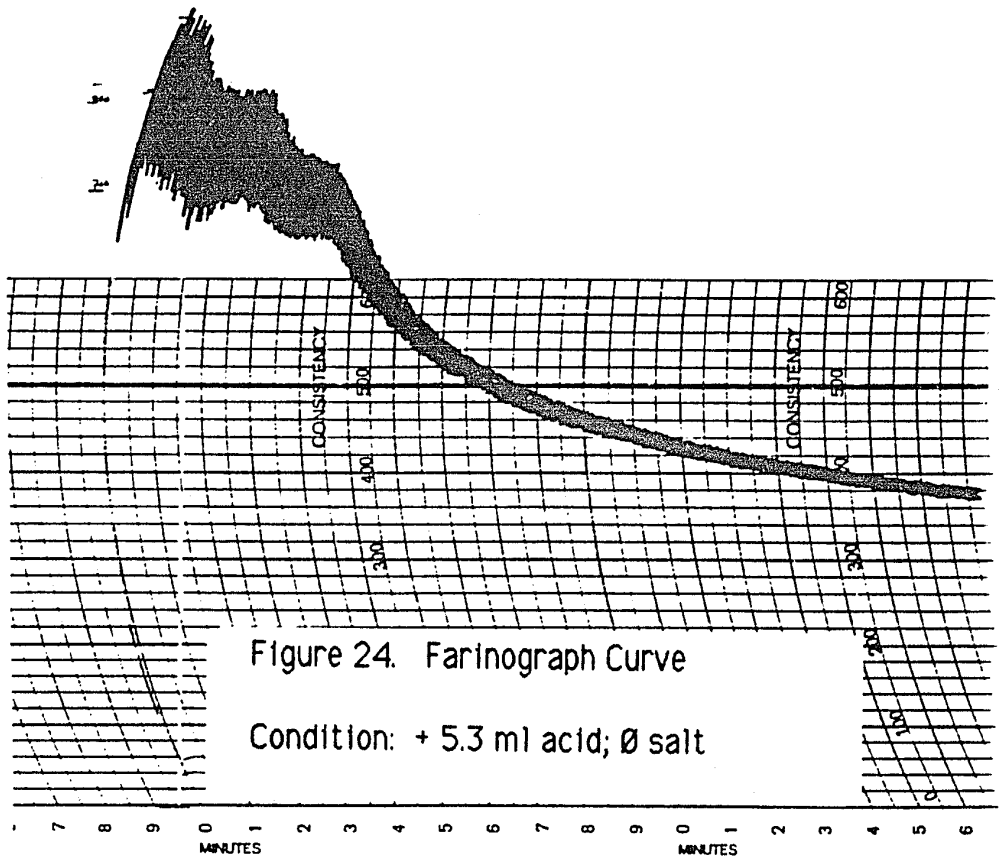


Figure 24. Farinograph Curve

Condition: + 5.3 ml acid; Ø salt

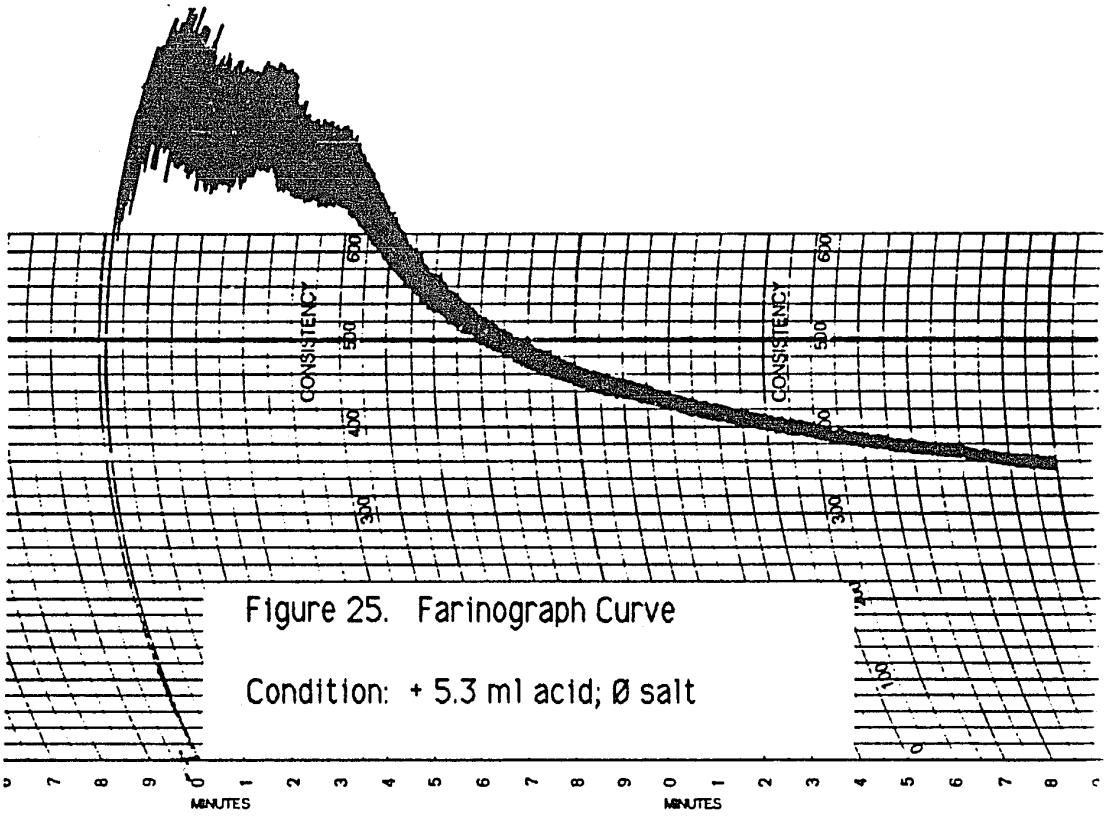


Figure 25. Farinograph Curve

Condition: + 5.3 ml acid; Ø salt

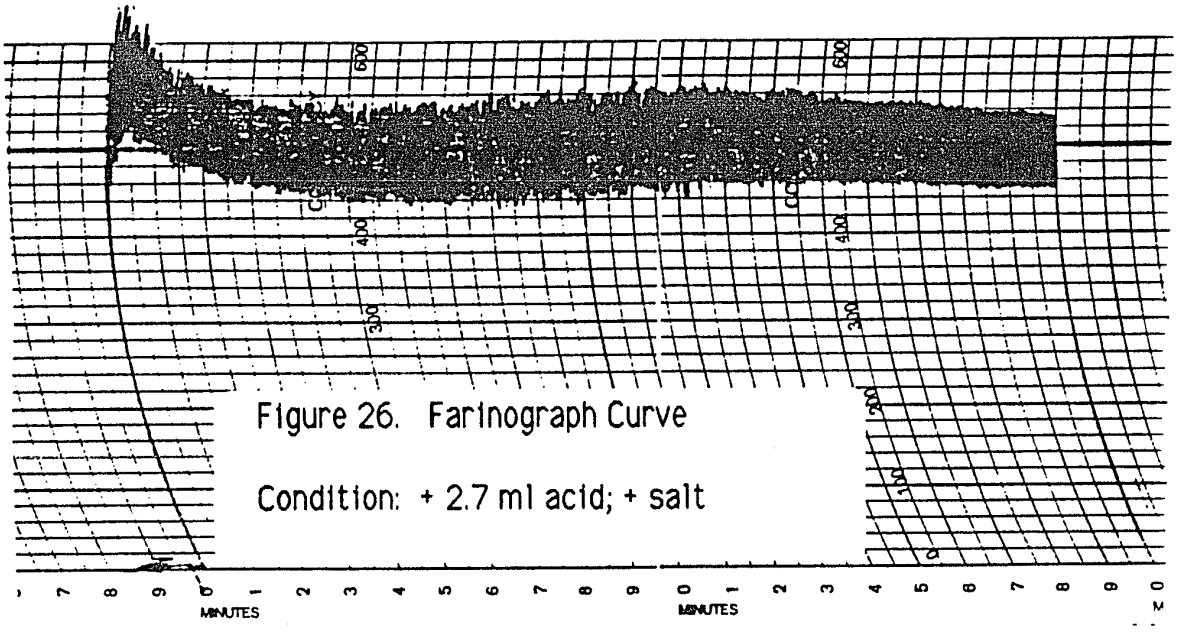


Figure 26. Farinograph Curve

Condition: + 2.7 ml acid; + salt

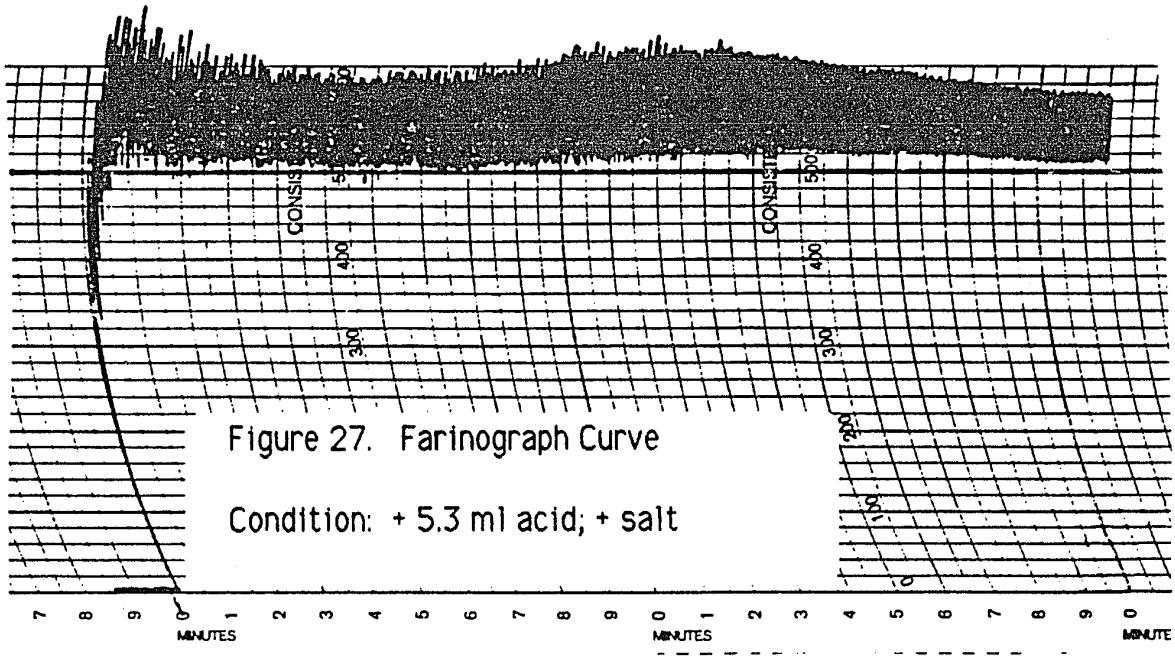


Figure 27. Farinograph Curve

Condition: + 5.3 ml acid; + salt

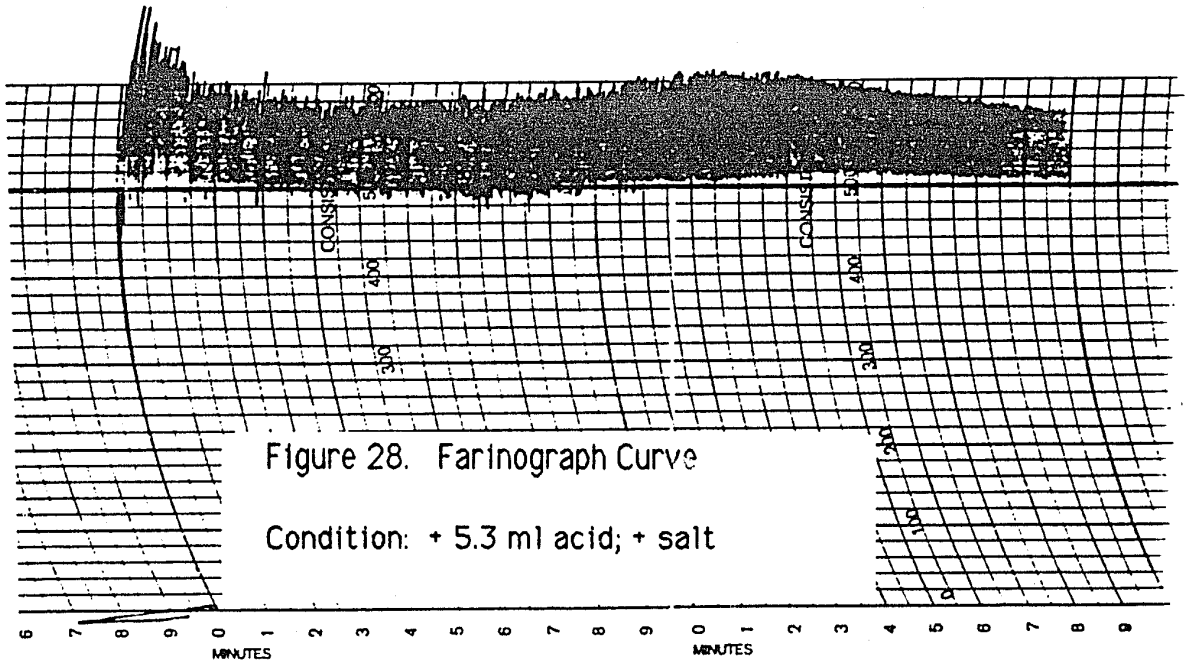


Figure 28. Farinograph Curve

Condition: + 5.3 ml acid; + salt

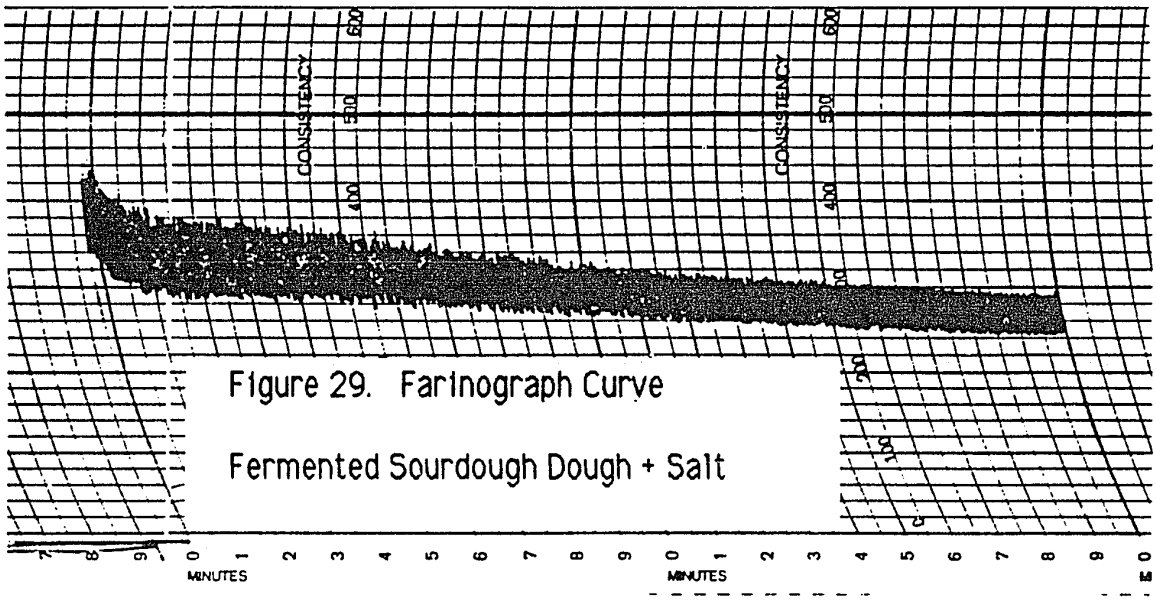


Figure 29. Farinograph Curve

Fermented Sourdough Dough + Salt

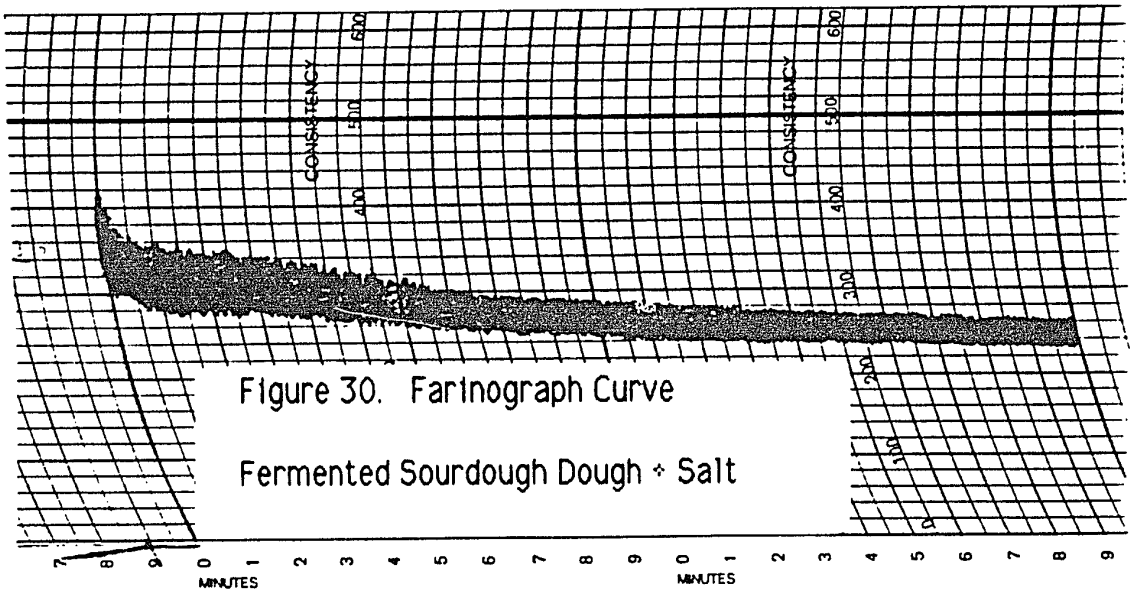


Figure 30. Farinograph Curve

Fermented Sourdough Dough + Salt

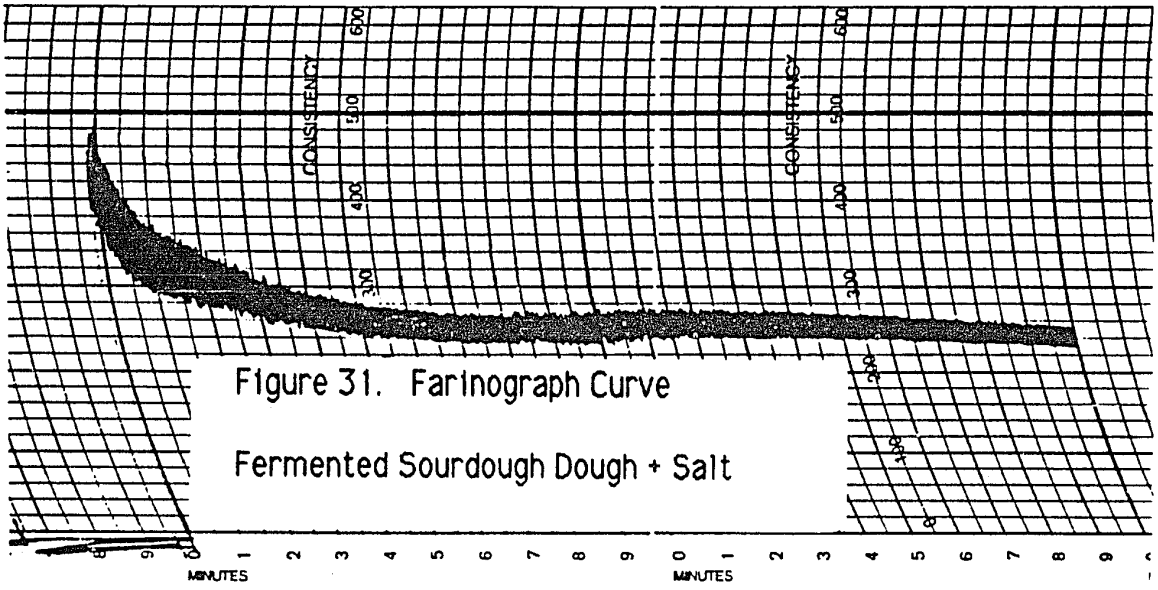


Figure 31. Farinograph Curve

Fermented Sourdough Dough + Salt

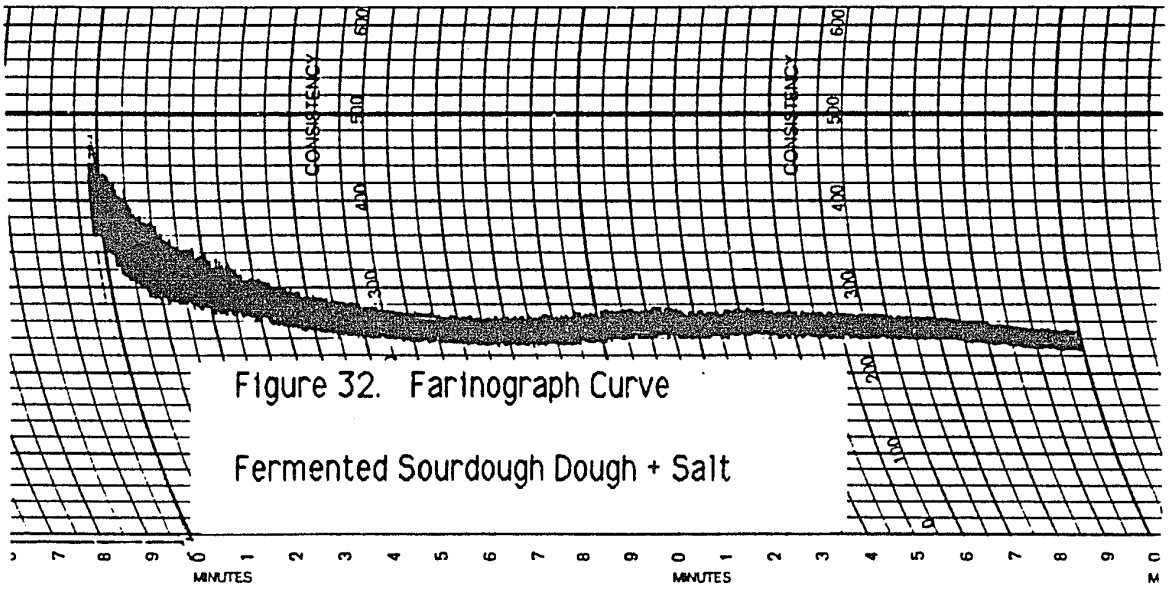


Figure 32. Farinograph Curve

Fermented Sourdough Dough + Salt

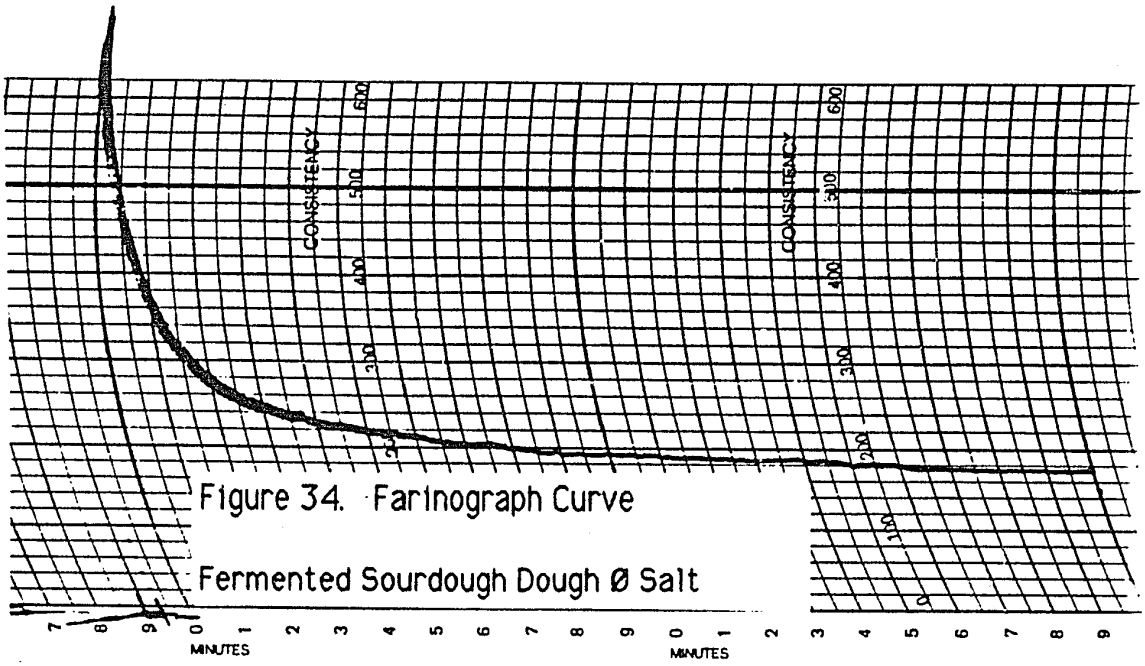


Figure 34. Farinograph Curve

Fermented Sourdough Dough Ø Salt

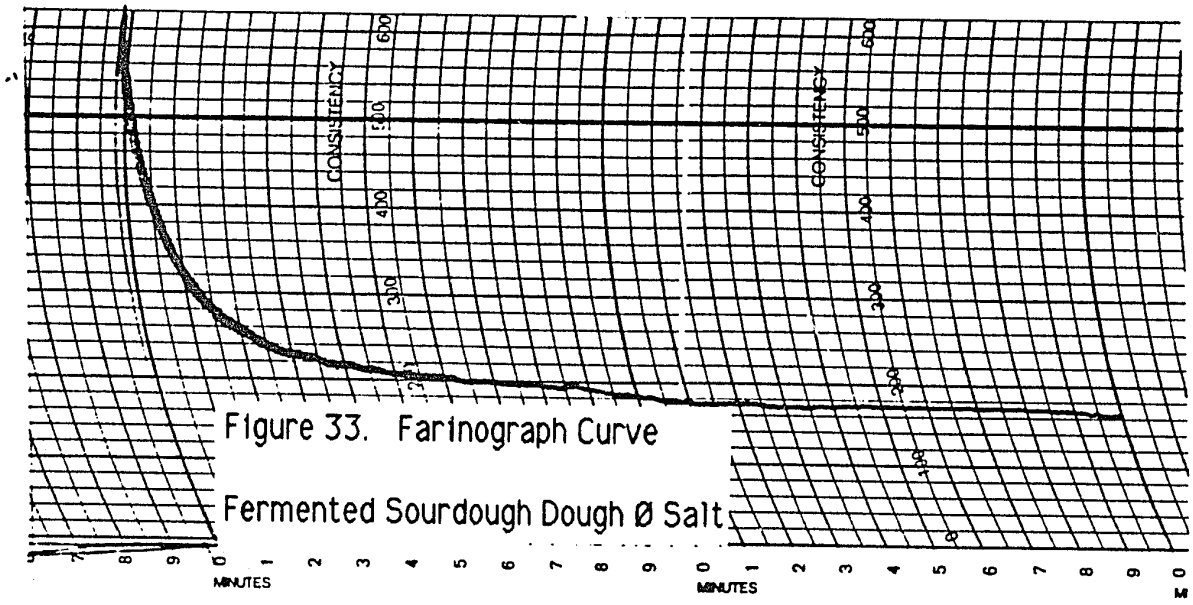


Figure 33. Farinograph Curve

Fermented Sourdough Dough Ø Salt

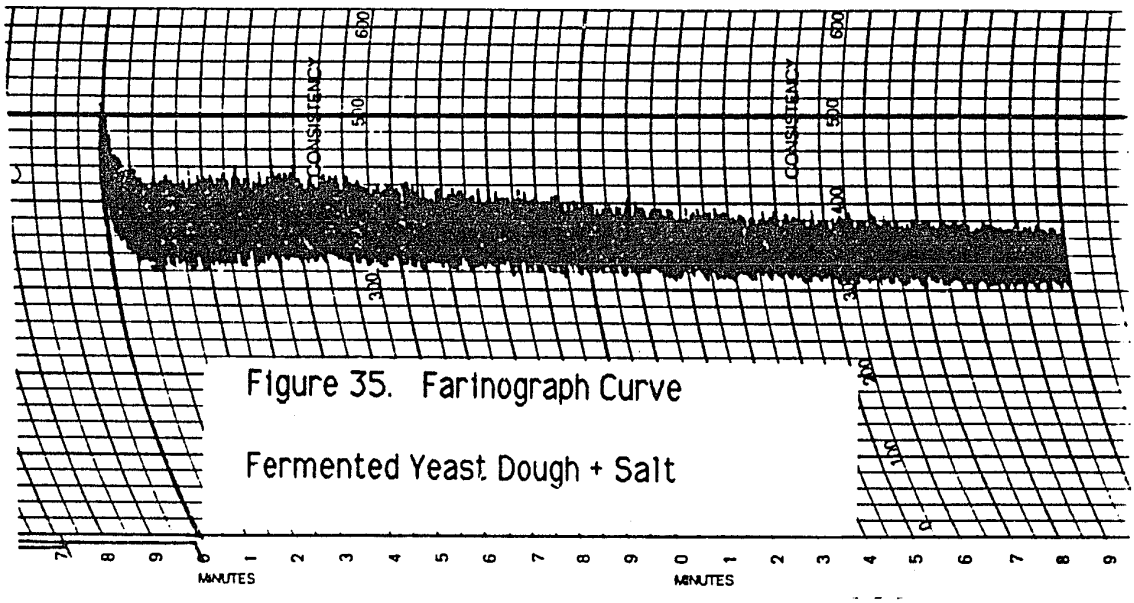


Figure 35. Farinograph Curve

Fermented Yeast Dough + Salt

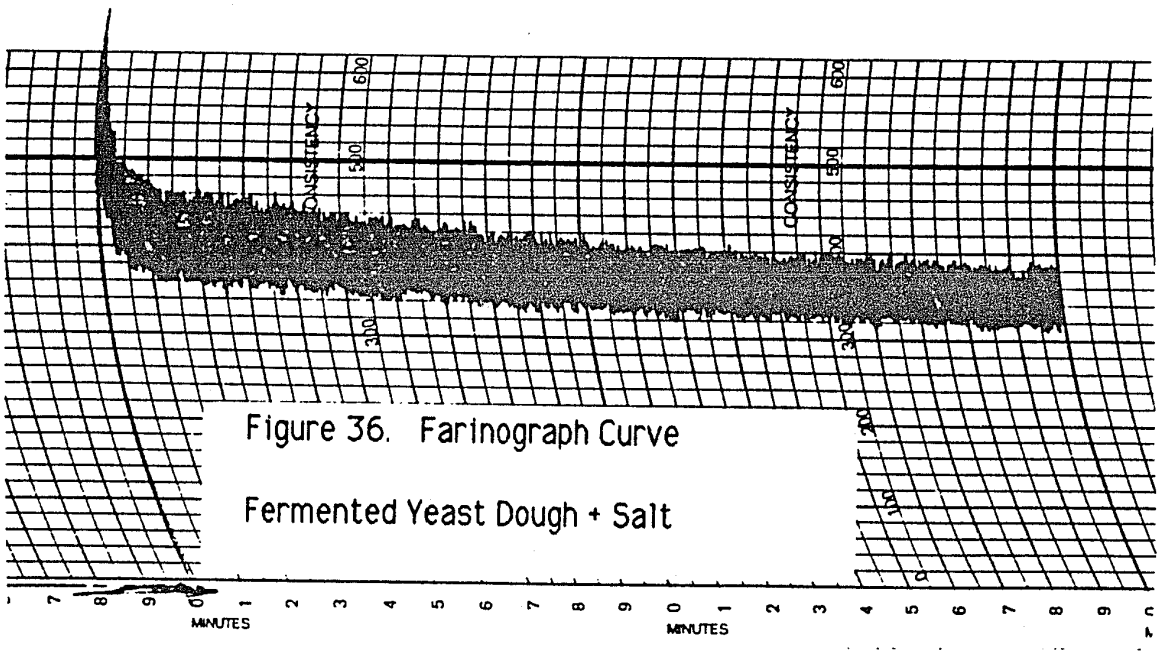


Figure 36. Farinograph Curve

Fermented Yeast Dough + Salt

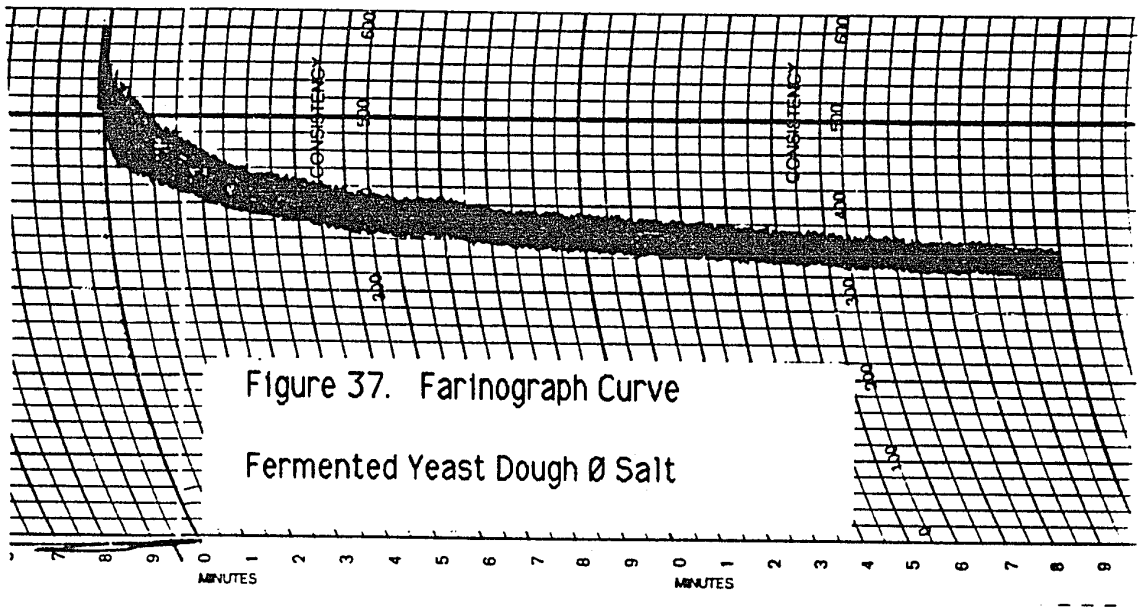


Figure 37. Farinograph Curve

Fermented Yeast Dough Ø Salt

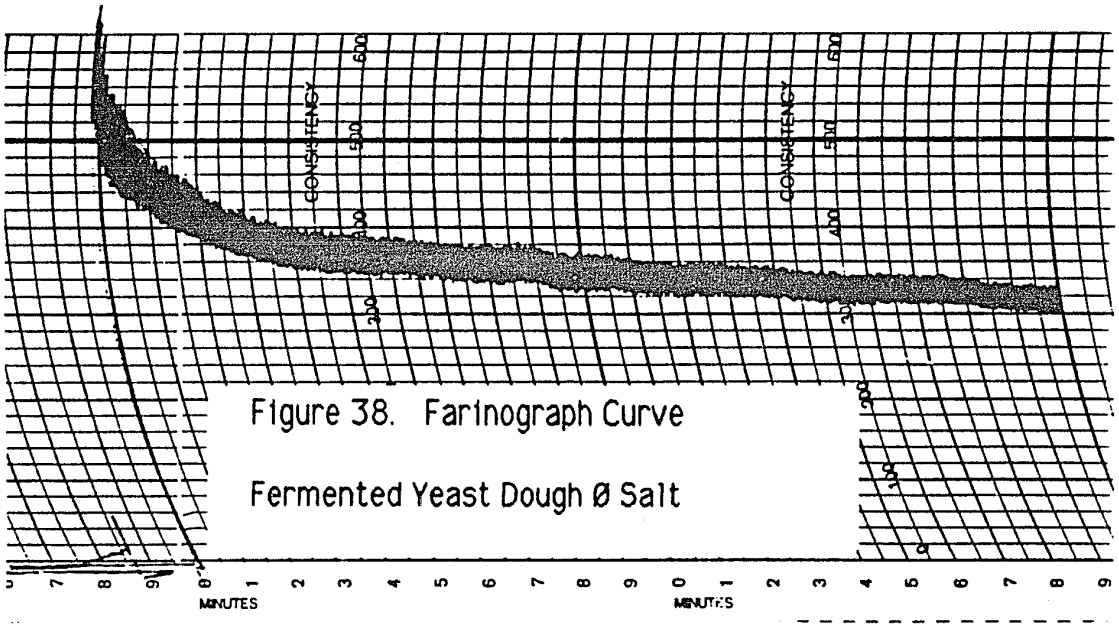
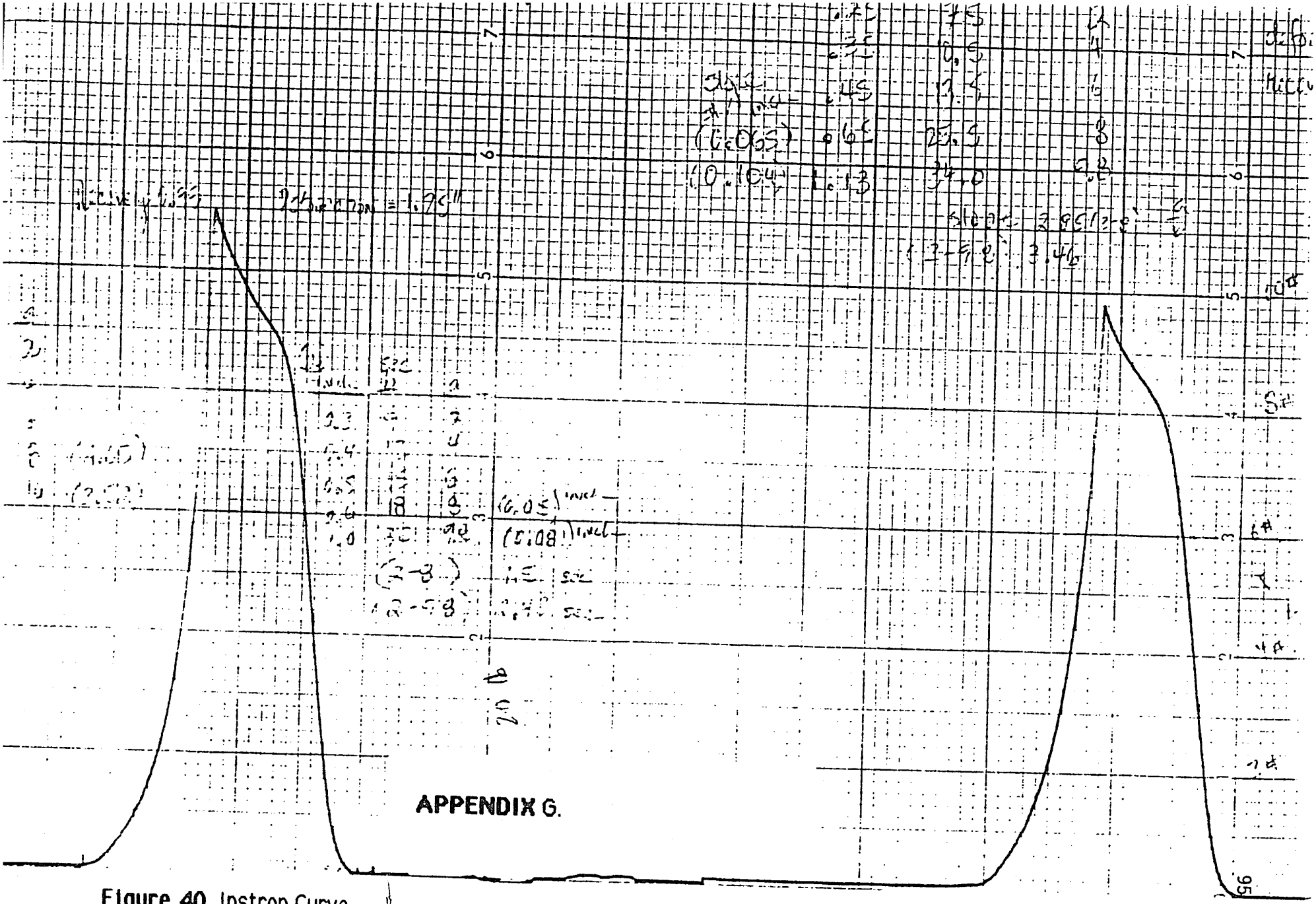


Figure 38. Farinograph Curve

Fermented Yeast Dough Ø Salt



APPENDIX G.

Figure 40. Instron Curve

Bread Crumb: Ø acid; Ø salt

Figure 39. Instron Curve

Bread Crumb: Ø acid: Ø salt

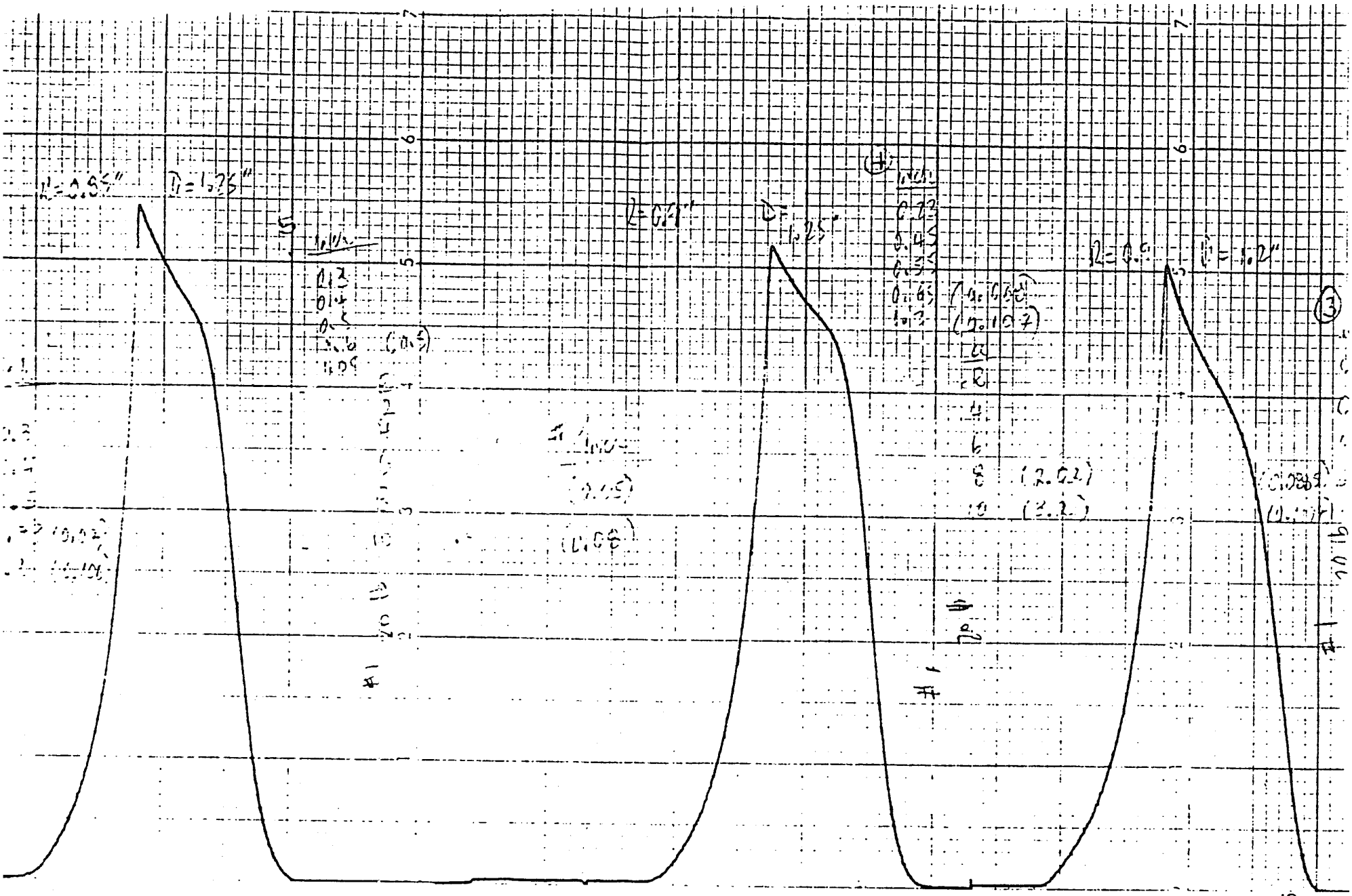


Figure 43. Instron Curve

Bread Crumb: Ø acid; Ø salt

Figure 42. Instron Curve

Bread Crumb: Ø acid; Ø salt

Figure 41. Instron Curve

Bread Crumb: Ø acid; Ø salt

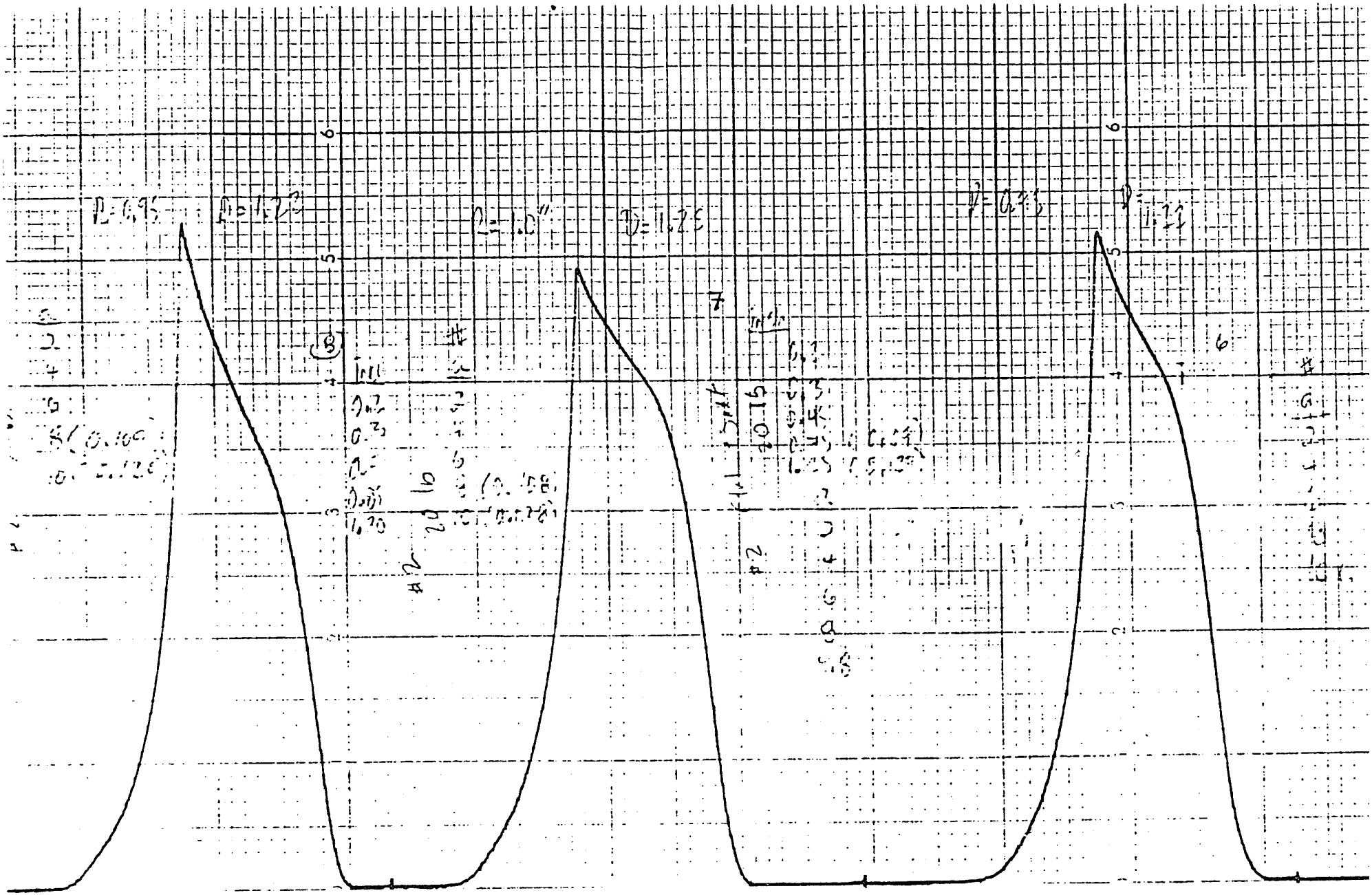


Figure 46. Instron Curve

Bread Crumb: Ø acid; + salt

Figure 45. Instron Curve

Bread Crumb: Ø acid; + salt

Figure 44. Instron Curve

Bread Crumb: Ø acid; + salt

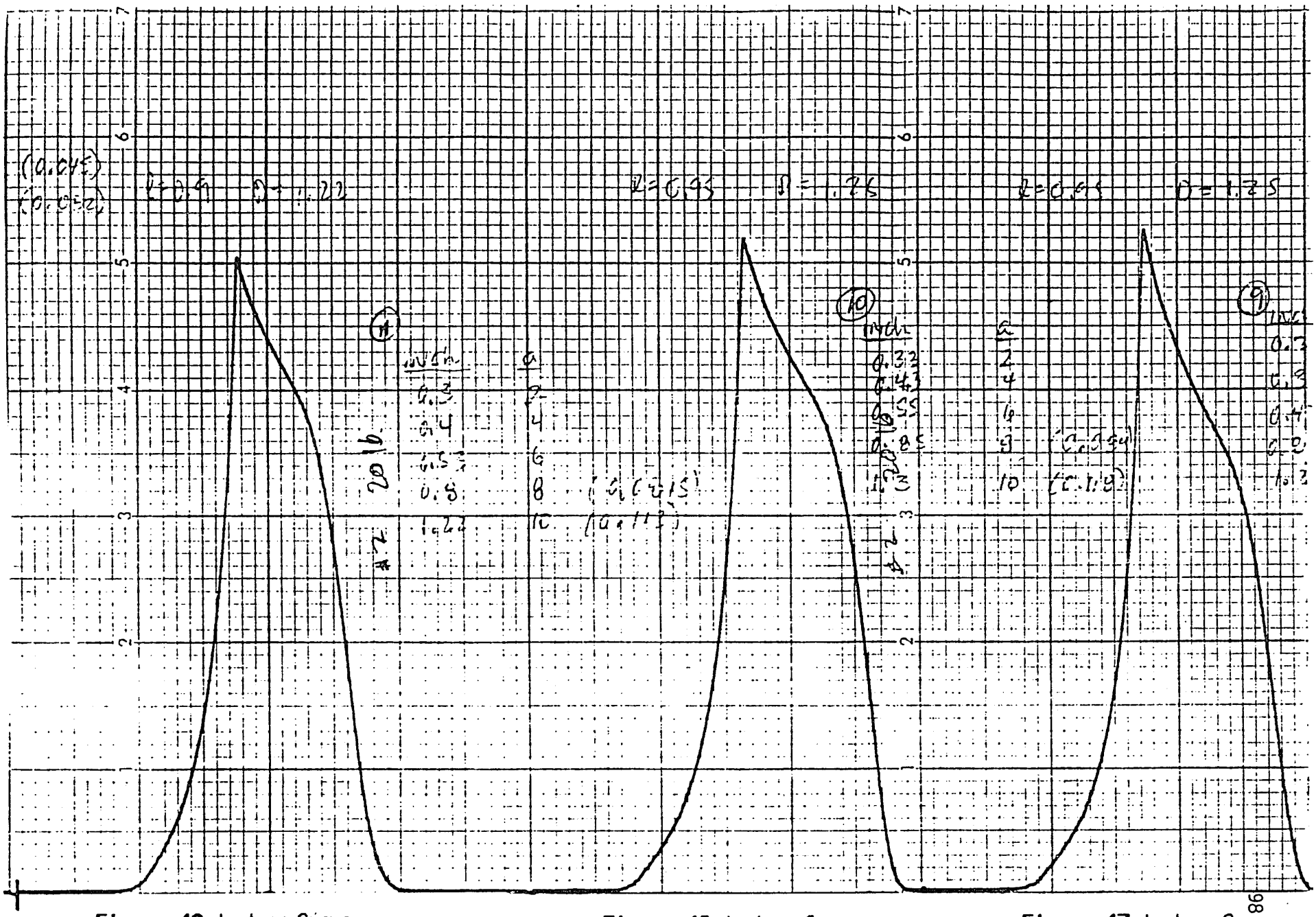


Figure 49. Instron Curve

Bread Crumb: Ø acid; + salt

Figure 48. Instron Curve

Bread Crumb: Ø acid; + salt

Figure 47. Instron Curve

Bread Crumb: Ø acid; + salt

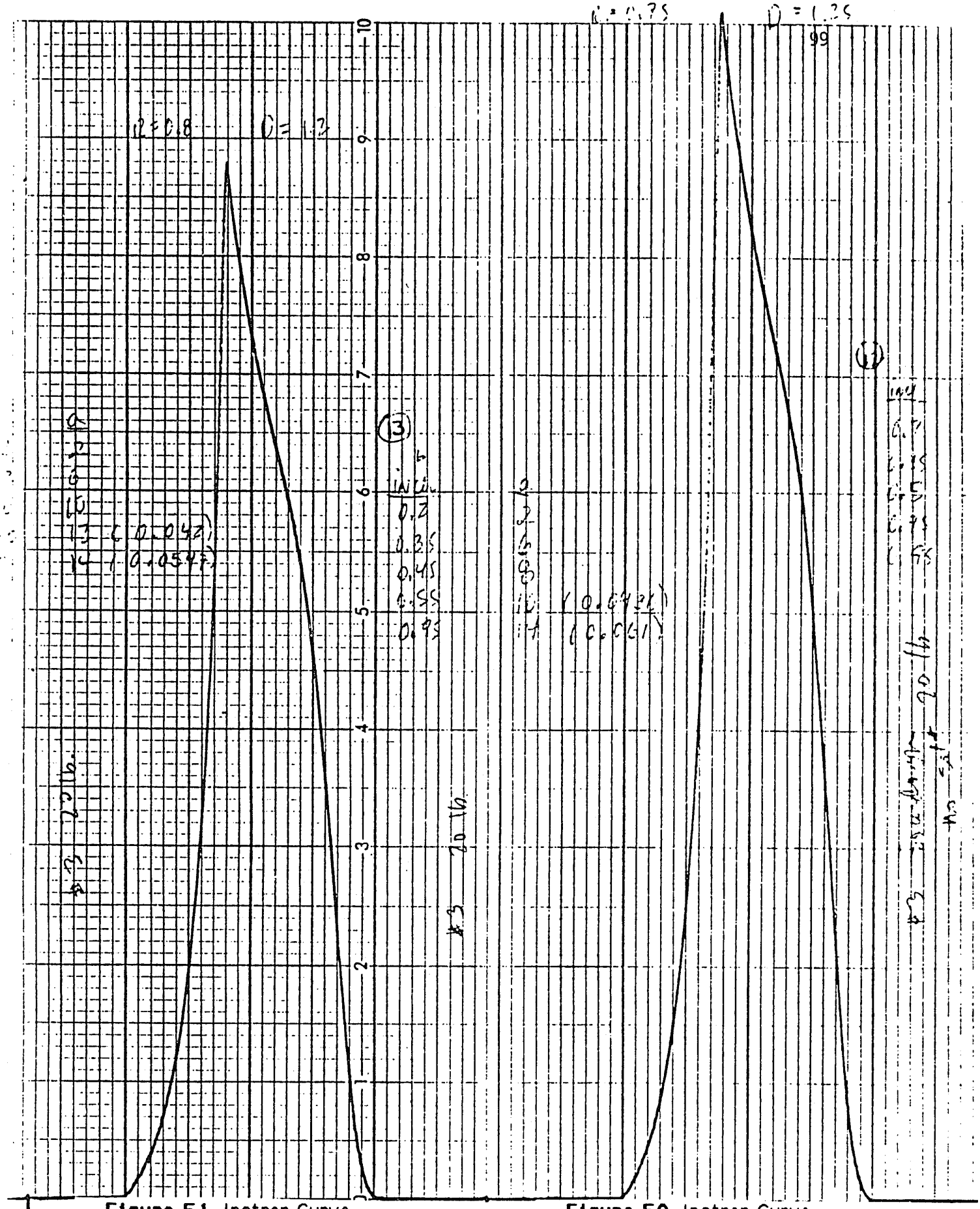


Figure 51. Instron Curve

Bread Crumb: + acid; Ø salt

Figure 50. Instron Curve

Bread Crumb: + acid; Ø salt

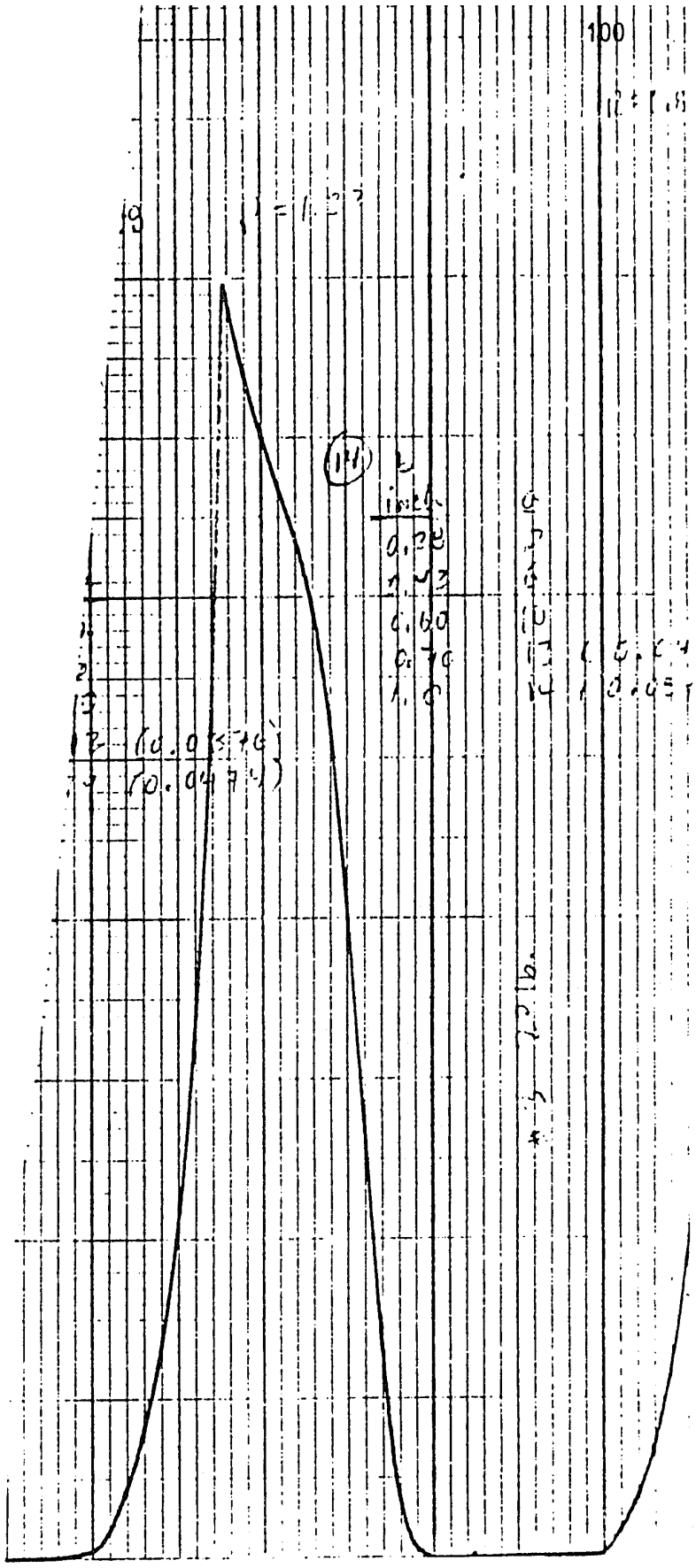


Figure 52. Instron Curve

Bread Crumb: + acid; Ø salt

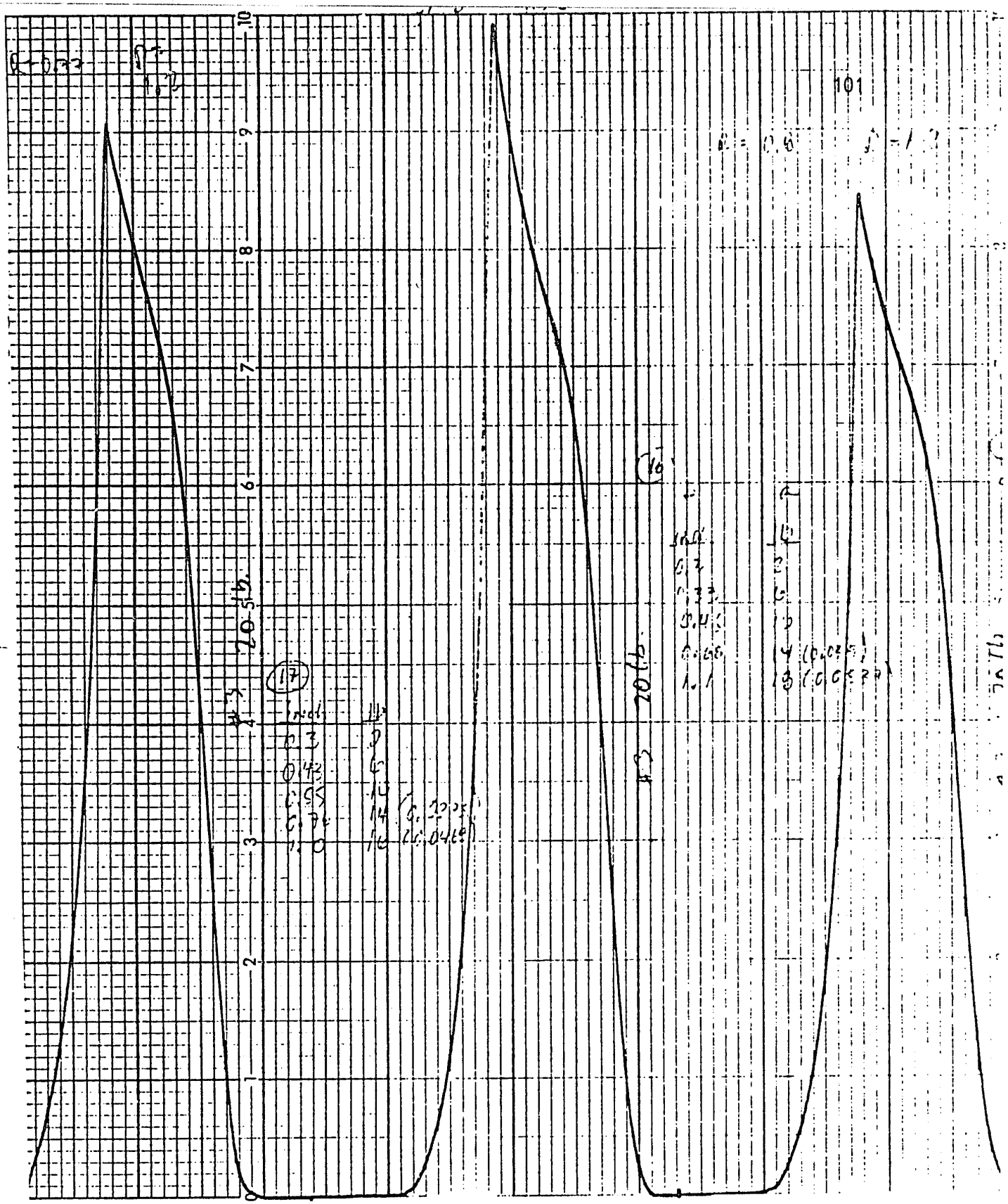


Figure 55. Instron Curve

Bread Crumb: + acid; Ø salt

Figure 54. Instron Curve

Bread Crumb: + acid; Ø salt

Figure 53. Instron Curve

Bread Crumb: + acid; Ø sa

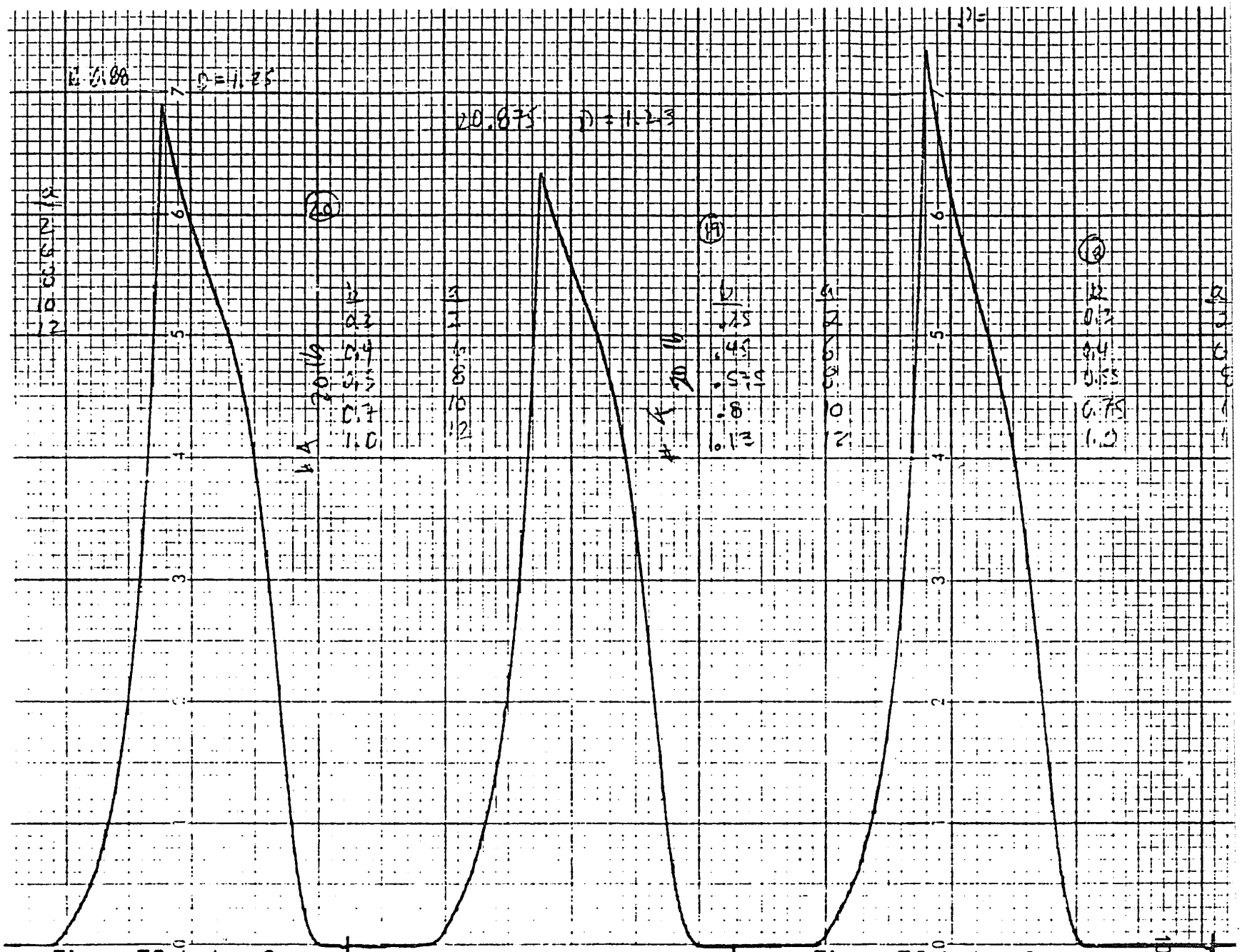


Figure 58. Instron Curve

Bread Crumb: + acid; + salt

Figure 57. Instron Curve

Bread Crumb: + acid; + salt

Figure 56. Instron Curve

Bread Crumb: + acid; + salt

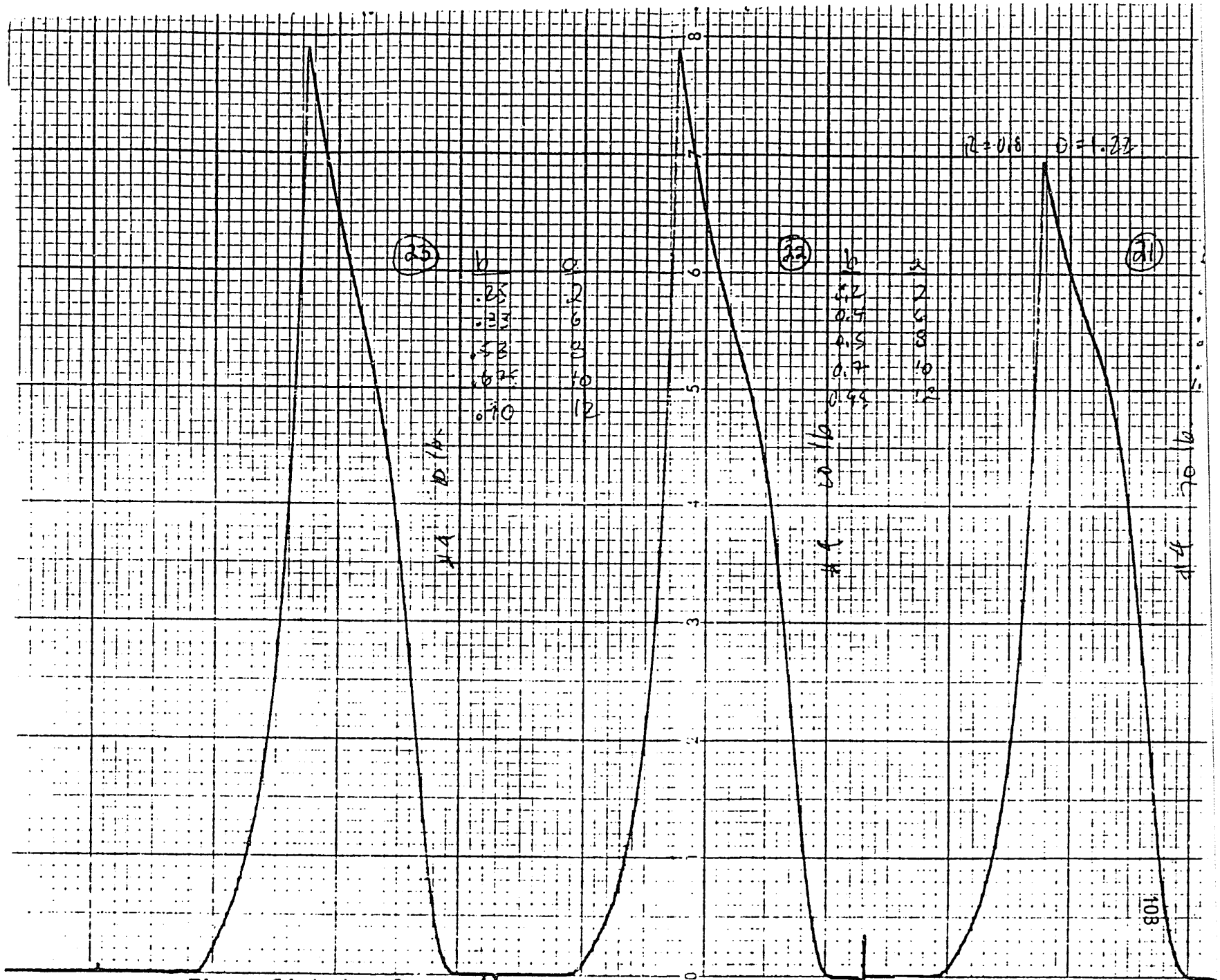


Figure 61. Instron Curve

Bread Crumb: + acid; + salt

Figure 60. Instron Curve

Bread Crumb: + acid; + salt

Figure 59. Instron Curve

Bread Crumb: + acid; + salt