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STUDIES OF WATER BINDING IN DOUGH AND BREAD BY DIFFERENTIAL THERMAL ANALYSIS

by

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ABSTRACT

Water binding in dough and bread was studied using boiling and melting curve modes of differential thermal analysis (DTA). The boiling curve results showed that water binding of dough depends on the mixing strength of the wheat flour (wheat variety). Stronger mixing flours appear to bind water more tightly compared to weaker mixing flours. Increase in the water absorption of dough produced a curvilinear increase in water binding. The binding energy decreased with increase in protein content and increased linearly with the increasing starch damage and water soluble pentosan content. During dough mixing, water binding increased to a maximum and then decreased at about the same rate as farinograph consistency in case of the two stronger varieties but in case of the weak variety investigated the binding energy mixing-time curve was anomalous. Addition of salt, N-ethylmaleimide, cysteine and iodate decreased the energy of water binding.

Melting curve DTA results showed that moisture in dough to about 25% was completely bound. With the increase in bound moisture above this limit only one third of the added moisture was bound. However, doughs of different mixing strength did not show any significant difference in bound water content. Flour protein content, starch damage, mixing time and added chemicals did not affect the amount of bound water determined by the DTA melting curve method.

DTA melting curve results on bread showed a definite decrease in bound water content of bread crumb with increasing storage time. The rate

of decrease with time was slowed down considerably by including fat in the bread formula. The decrease in bound water with storage time was strongly dependent on storage temperature; it was much slower at lower temperatures. Baking absorption had a definite influence on bound water content of bread crumb; it increased with increasing baking absorption and the rate of decrease with storage time was slower in loaves baked at higher absorption. Flour protein content and starch damage did not directly effect the change in bound water content. It is concluded that the change in bound water content of bread crumb during post-baking storage can be used as an index of bread staling.

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INTRODUCTION

Water is a critical constituent of wheat flour dough as it contributes significantly to the dough's functional or processing properties in the conversion of flour into bread. Even after baking, water remains as a major constituent of bread. Accordingly, it plays an important role in the economics of the bread industry by controlling the production yield and by affecting consumer acceptance of the bread.

Bakers have always been interested in increasing the amount of water that could be added to flour to obtain a higher yield of bread at little extra cost. Moreover, a considerable fraction of the total bread produced is discarded daily as stale bread thus increasing the cost of bread and generally decreasing the availability of food. To increase bread yield and to solve the problem of bread staling by extending the shelf-life of bread, the baker must have detailed information on the factors that affect the uptake, distribution and binding of water by flour when it is processed into dough and bread. A study of some of these factors forms the basis of this thesis.

It is now well established, as will be seen from the review of the literature that follows, that, in dough and in bread, water (moisture) exists in two distinctly different physical states. A small proportion of the water is "free" water; this has all of the normal properties of liquid water. The remainder is "bound" water, so-called because it is attracted to various flour constituents and dough ingredients; this water does not behave like ordinary liquid water and is generally unavailable in the complex dough system as a

solvent or lubricant. It is the bound water that is of special interest to cereal chemists because of its presumed lesser effect on the physical (rheological) properties of dough than that of free water even though the bound water forms the major portion of the moisture in dough.

There are many techniques for measuring bound water in biological materials. The more commonly used techniques are dilatometry, cryoscopy, refractometry, calorimetry, nuclear magnetic resonance spectrometry, differential scanning calorimetry, thermogravimetric analysis, and differential thermal analysis. For the present study on water binding in dough and bread the differential thermal analysis technique was selected because it is rapid, easy to use, and extremely sensitive.

For the studies of water binding in dough, flours of three wheat cultivars (varieties) with widely different dough mixing characteristics (at constant protein content) were selected. The factors investigated in this part of the study were: (1) the mixing strength of wheat flour, (2) the amount of water used to make the dough, (3) the flour protein content, (4) the degree of starch damage in the flour, (5) flour pentosan content, (6) the length of dough mixing time, and (7) the effects of added sodium chloride, N-ethylmaleimide, cysteine, potassium iodate and ascorbic acid.

For studies of bread, flours from two cultivars of Canadian hard red spring wheat (Pembina and Manitou) were used. The variables investigated in this part of the study were: (1) post-baking storage time, (2) the amount of fat in the bread formula, (3) post-baking storage temperature, (4) the amount of water used to make the dough, (5) flour protein content, and (6)

degree of starch damage in the flour.

The results obtained in these studies on dough and bread are described in this thesis.

LITERATURE REVIEW

A. Water in Flour Dough and Bread

Water is a normal constituent of flour and is essential for the transformation of flour to dough which is ultimately baked into bread. No other liquid can transform flour into a dough with just the right viscoelastic properties to enable the dough to leaven into a structure that forms the basic structure of a baked loaf of bread (Bushuk and Hlynka 1964; Bushuk 1966; Pyler 1973). Water also plays a major role in the consumer acceptance of the bread as it remains one of the major constituents of bread after baking and is critically involved in the post-baking changes commonly referred to as staling (Bechtel et al. 1953; Bice et al. 1954; Herz 1965; McIver et al. 1968; Willhoft 1971A, 1971B, 1973; Zobel 1973). Although the importance of water in controlling dough and bread characteristics has been known for a long time, it is only in recent years that detailed studies have been undertaken on the physical status of the water in dough and bread. Only the literature that is pertinent to the subject of this thesis will be reviewed in this section.

Bloksma and Hlynka (1964) were first to emphasize that water is unique among known liquids in that it forms a viscoelastic dough with wheat flour and suggested that water probably is directly involved in the structure of dough by forming crosslinks among the flour constituents. A possible type of crosslink is one in which a water molecule is hydrogen

bonded between two polar groups on protein or starch as suggested by Bushuk (1956). It was subsequently shown by Kretovich et al. (1964) and Vakar et al. (1965) by experiments on doughs made with heavy water (D_2O), that the hydrogen bond plays an important role in gluten structure. Their elongation tests of lyophilized samples of gluten hydrated with D_2O, indicated that the mechanical strength of the D_2O-gluten was significantly greater than that of H_2O-gluten. Tkachuk and Hlynka (1968) extended the work with D_2O of the Soviet workers and showed that a much stronger dough and gluten (on the basis of the farinogram) was formed with D_2O than with H_2O. This was explained by the fact that the deuterium bond is stronger than the hydrogen bond. It was concluded from these studies with D_2O that hydrogen bonds play a very significant role in determining the physical structure and processing behaviour of bread doughs.

Relative to the mechanism of water binding in dough, Webb et al. (1970) suggested that since water constitutes about 42% of the total weight of bread dough, it is unlikely that it is present simply as a diluent. They proposed that the water in dough was attracted to polar groups with varying degrees of strength and that the distribution of water depended on the mechanical work input used to develop the dough. Hlynka (1959) and Larsen (1964) introduced the concept of "free" and "bound" water in considering the relationship between water and the individual components of flour: bound water being an integral part of the structure of dough and free water being responsible for the fluidity or mobility of the dough. Larsen (1964) suggested that only a small fraction of the moisture of dough is lost during baking [a bread dough formula contains 42% water and bread normally contains

37% moisture, (Bushuk et al. 1964). This is further evidence that essentially all of the water in bread is of the bound type. Work of Wagenaar and Dack (1954), which showed that Botulinum bacteria could not grow in canned bread having a moisture content of 36% or less, even though other environmental conditions were favourable, indicates that the water in bread is firmly attached to hydrophilic groups of the solid constituents of bread and thus is unavailable to support growth of the microorganism. In summary, it is now well established that the moisture in dough and bread exists in two quite distinct physical states - bound and free.

1. Concept of Bound Water

It is now an established fact that the total water in dough and bread comprises bound and free water (Skohvolt et al. 1935; Vail and Bailey 1940; Hlynka 1959; Larsen 1964; Toledo et al. 1968; Davies et al. 1969; Shanbhag et al. 1970; Webb et al. 1970). However there is no sharp distinction between the two states except that if one can be measured the other can be determined by difference (Lee 1970).

Although distinction between free and bound water has been made quite frequently (Vail and Bailey 1940; Hlynka 1959; Larsen 1964; Toledo et al. 1968; Davies et al. 1969; Webb et al. 1970), there is no complete agreement on the specific definition of each because each depends entirely on the method of determination (Kuprianoff 1958; Fennema et al. 1964; Davies et al. 1969)

Bound water has been defined as that fraction of the total moisture of the system which does not freeze (Meryman 1966; Davies et al. 1969), or does not crystallize (Luyet 1961), or does not contribute to hydrolytic

enzyme action (Lee 1970), or is unavailable as a solvent (Bull 1943) or generally differs in physical properties from those of normal water (Kuprianoff 1958, GurArieh et al. 1967). For the purpose of this study, the definition according to which bound water is that portion of the water (moisture) in a biological system that does not freeze at the normal freezing temperature of water was adopted.

2. Determination of Bound Water

A number of different methods have been employed to determine the amount of bound water in a system such as dough. It can be determined directly or indirectly by first measuring the amount of free water and then determining bound water by difference.

Kuprianoff (1958) and Duckworth et al. (1963) concluded that the most reliable method for the determination of bound water is to measure the water that persists unfrozen at sub-freezing temperatures. Moran (1926,1931), in early studies, used a dilatometric method to determine the amount of ice that formed in gelatin gels on freezing. He later abandoned this method in work on egg albumin and muscle tissue (Moran 1935) in favour of a method in which ice was caused to form only at the surfaces of the test specimen. The residual non-freezable or bound water within the bulk of specimen was determined directly on the assumption that all of the free water was at the surface. Methods based on cryoscopy (Newton and Cook 1930; Skovholt et al. 1935; Vail and Bailey 1940), refractometry (Kuhlmann and Golossowa 1936), alkaline water retention capacity (Yamazaki 1953), distilled water retention capacity (Sollars 1972) and dough mobility (Hlynka 1959) have also been used

to determine free and bound water in dough. Other workers in the field have successfully employed calorimetry (Mennie 1932; Daughters and Glenn 1946; Fleming 1969). In recent years, nuclear magnetic resonance (NMR) spectroscopy (Sussman and Chin 1966; Toledo et al. 1968; Shanbhag et al. 1970), differential scanning calorimetry (DSC) (Davies and Webb 1969, Wood et al. 1972), thermogravimetric analysis (TGA) (Neher et al. 1973) and differential thermal analysis (DTA) (Duckworth 1971; Neher et al. 1973) have been added to the list of techniques that can be used to determine the amount of bound water. Since the DTA technique was adopted for this thesis research project, the literature dealing with this technique will be reviewed in some detail.

Since the development of DTA, its application has been limited almost exclusively to examination of inorganic materials. During the last twenty years, its use has spread rapidly, first to the study of relatively simple organic substances and then to more complex systems of biological origin (Smothers and Chiang 1958; Barrall and Johnson 1966; Schwenker et al. 1969; Mackenzie 1970). The technique has also been applied to flour, dough and bread. (Axford and Colwell 1967; Axford et al. 1968; Colwell et al. 1969; Duckworth 1971; Neher et al. 1973).

Low temperature DTA of aqueous solutions was used first by Rey (1961) and later by Greaves and Davies (1965) in their studies on the freeze-drying of biological materials. Similar studies were carried out by Rasmussen and co-workers (Rasmussen 1969; Luyet and Rasmussen 1969). Mazur (1963) used low temperature DTA on aqueous systems containing living micro-organisms. A considerable amount of work on DTA of frozen food materials and on the application of the method for direct determination of unfreezable or bound water in

biological materials has been published by Duckworth (1971). Neher et al. (1973) used both TGA and DTA for studying the state of the moisture in grains by scanning above freezing and sub-freezing temperatures. There have been numerous studies involving the application of DTA to starch retrogradation and bread staling in recent years (Axford and Colwell 1967; Axford et al. 1968; Greenwood et al. 1967; McIver et al. 1968; Colwell et al. 1969). These will be reviewed later in the section on bread staling.

3. Distribution of Water in Flour, Dough and Bread

Since water has different functions or roles in flour, dough and bread, a separate section will be devoted to each product.

a. Flour. Wheat is generally tempered at about 16% moisture which, on milling, yields a flour containing about 14% moisture (Wheat Flour Institute 1966). This level of moisture is also very close to the equilibrium moisture of flour at a relative humidity of 70% (Bushuk et al. 1964). This moisture level does not support microbial growth in the flour nor does it induce any change in the flour's chemical properties. Also flour with this moisture content has satisfactory storage stability (Pyler 1973). Accordingly, this level of moisture is considered to be an integral part of the composition of flour (Bushuk et al. 1964) and is not available as a solvent (Pyler 1973).

Water molecules present in flour are thought to be associated with specific chemical groups in the starch, the proteins and the pentosans (Bushuk et al. 1964; Hagenmaier 1972). For example, starch which is a

natural high polymer of glucose, contains numerous hydroxyl groups, in addition to keto groups in the pyranose ring and in the linkages between the glucose units (West et al. 1967; Lehninger 1972), all of which can interact with water through hydrogen bonds (Greenwood 1952; Bushuk et al. 1964; Wolfrom et al. 1965; Myhre 1970).

Similarly, proteins are also high polymers of some 20 different amino acids, joined together by peptide linkages (West et al. 1967; Lehninger 1972). The nitrogen and oxygen of the peptide linkage can interact quite strongly with water molecules (Bushuk 1956). In addition, certain amino acids, for example arginine, aspargine, aspartic acid, glutamic acid, glutamine, histidine, hydroxyproline, lysine, proline, serine, threonine, tryptophan and tyrosine have polar side groups which can attract water molecules by forming hydrogen bonds (Bushuk 1956; Kasarda et al. 1971; Pyler 1973). Moreover, glutamine, which makes up over one-third of the amino acids of flour protein (Tkachuk 1966; Wall et al. 1969), contains a second amino group which is an extremely important site of hydrogen bonding in dough (Krull et al. 1965; Krull and Wall 1966; Pyler 1973).

Pentosans, although present in small quantity in wheat flour (about 1 to 1.5%), absorb considerably more water per unit weight than starch or gluten (Yamazaki 1955; Kulp 1968; D'Appolonia et al. 1970; D'Appolonia 1971; Jelaca et al. 1971, 1972). The highly branched structure of flour pentosan has numerous hydroxyl groups ideally placed for the interaction with water molecules (Gilles 1960; D'Appolonia 1971).

Lipids, present in flour, may form contiguous and continuous layers on starch granules or protein particles and might serve to keep bound moisture present inside these substances or prevent further penetration of moisture from outside (Herz 1965).

Of the minor constituents of flour, the amylases (Pomeranz et al. 1962; Pomeranz et al. 1966; Tipples 1969) and proteases (Reed et al. 1964; McDonald 1969; Bushuk et al. 1971; Madle and Tsen 1973), could have secondary effects on water absorption if present in excessive amounts. These will be referred to in the next section.

b. Dough. Since the distribution of water in dough is an essential part of the present study, the literature dealing with dough will be reviewed in some detail. This part of the review will be divided into five subsections: (1) water absorption, (2) effect of mixing, (3) role of sulfhydryl and disulfide groups, (4) effect of other bread ingredients, and (5) chemistry of dough.

(1) Water Absorption. Water absorption of flour is defined as the amount of water required to produce a dough of pre-determined consistency (Pratt 1971; Pyler 1973). The amount of water required to give a dough consistency of 500 Brabender Units (B.U.) in the farinograph is referred to as the farinograph water absorption. On the other hand, the amount of water used by the baker for optimum dough consistency in baking is called baking absorption. The farinograph absorption, which can be easily measured, is generally used to estimate the baking absorption.

When water is added to flour, it is absorbed at varying rates by the starch, the proteins and the pentosans of the flour (Greer and Stewart 1959; Sosulski 1962; Larsen 1964). It has been estimated that starch, which represents some 68% of the flour, will absorb 45.5% of the total water in the dough; the proteins which comprise some 14% of the flour solids will bind about 13.2% of the water, while the pentosan materials, which normally constitute only 1.5% of flour, will absorb some 23.4% of the dough water (Yamazaki 1955; Tracey 1964; Bushuk et al. 1964; Bushuk 1966; Jelaca et al. 1971; Pomeranz 1971; Pyler 1973). Intact starch granules are capable of absorbing only about one-half their own weight of water whereas damaged starch granules can absorb about twice their weight (Greer and Stewart 1959). Native flour proteins can absorb twice their weight of water (Larsen 1964; Bushuk 1966). In contrast, pentosans absorb in excess of 15 times their own weight of water (Bushuk 1966; Kulp 1968). Accordingly small variations in pentosan content can be a significant factor in the total water absorption of flour.

During the process of wheat milling, a portion of the wheat starch becomes mechanically modified as a consequence of the grinding action of the mill rolls (Pomeranz 1968; Tipples 1969; Williams et al. 1969). The granules that are physically altered are referred to as damaged starch (Jones 1940). It has been shown that the level of starch damage is partially responsible for differences among flours in water absorption, handling properties of dough, and sugar production and dough slackening during fermentation (Farrand 1964; Pomeranz 1968; Tipples 1969). Different workers have reached different conclusions as to whether damaged starch or protein content

is the more important factor in flour water absorption. Mullaly and Moss (1961) considered protein content the more important factor while Greer and Stewart (1959) reported that damaged starch had the greater influence. Meredith (1966) suggested that the two factors are equally important.

Recent work of Farrand (1964, 1969) has been extremely useful in relating quantitatively the protein content and starch damage to maximum water absorption consistent with good bread quality. He has developed a mathematical equation which gives the water-absorption with a precision similar to that of objective measurement with the farinograph. An ancillary equation was also developed relating optimum levels of alpha-amylase activity to the level of starch damage required to produce doughs with adequate gassing power and minimum rheological instability (Farrand 1969). Tipples (1969) showed that the baking absorption is closely linked with the alpha-amylase activity of the flour. Damaged starch is more susceptible to alpha-amylase attack than granular starch. This enzyme is necessary for proper gassing power, however, if it is present in excess, it will over produce dextrins which could make the doughs too sticky. Alpha-amylase can also indirectly reduce baking absorption by making the dough too slack during fermentation since the starch degradation products have a lower water binding capacity than intact starch (Halton 1961). On the other hand, in breadmaking methods where the doughs are mechanically developed and the fermentation time is far shorter, more water can be added without the detrimental effects noted above (Chamberlain et al. 1962). Effective use of this fact has been made to increase bread yield by the use of the new Chorleywood

Bread Process (Farrand 1964; Elton 1965; Pyler 1973).

(2) Effect of Mixing. The primary function of mixing is the uniform blending of all the dough ingredients (Bushuk et al. 1968; Pomeranz 1968; Hoseney and Finney 1974). Water is one of the major dough ingredients, accordingly its distribution in dough is an important function of mixing. Maximum hydration of the flour constituents is a prerequisite for proper dough formation. Complete equilibrium hydration is important in order to realize the maximum uptake of water by the flour (Greer and Stewart 1959; Larsen 1964; Bushuk 1966; Bushuk et al. 1968).

Although it is agreed that the overall water binding capacity of flour is important, some workers have suggested that the rate of absorption during mixing may be equally important in the continuous breadmaking processes which use very short mixing times (Larsen 1964; Bushuk 1956; Meredith 1966; Pratt 1971). Gracza (1960) and Ponte et al. (1961) suggested that flour particle size is the main factor that affects the rate of water absorption. Other workers (Bushuk et al. 1957; GurArieh et al. 1967) have suggested that factors other than particle size, for example particle hardness, protein content (endosperm vitreousness) and degree of starch damage may also be important in determining the rate of hydration. Muller and Hlynka (1964) reported that flour hydrates very rapidly (in 2-3 minutes) while Gracza et al. (1965) found that 10 minutes were required for complete hydration in the farinograph mixer. The conclusion of Gracza et al. (1965) is questionable since it was based on results obtained by using a dye of much higher molecular size than water to follow the rate of hydration.

The rate of hydration thus appears to be a matter primarily of the rapidity with which the water can be brought into intimate contact with each flour particle by mixing (Bushuk 1966). The extremely large surface area of flour, (estimated by Bushuk and Winkler (1957) to be about 235 sq. m. per gram) assures the availability of sufficient surface for extremely rapid hydration.

Vigorous mixing used to achieve optimum gluten development should not significantly change the distribution of water in dough unless other side effects, such as enzymic degradation of starch or gluten, come into effect (Bushuk 1966). However, overmixing may diminish the water holding capacity of dough because of gluten breakdown (Mecham et al. 1962; Tsen 1967). This effect resulting from gluten breakdown appears to be more severe in the presence of thiol-blocking or oxidizing reagents (Mecham et al. 1963, Tanaka and Bushuk 1973A, 1973B, 1973C). Tanaka and Bushuk (1973A) reported that doughs which showed a marked decrease in farinograph consistency also showed an increase in the amount of acetic acid-soluble glutenin. This was attributed to the depolymerization of the high molecular weight glutenin through the disulfide interchange mechanism. The lower molecular weight depolymerization products of glutenin are assumed to have a lower water binding capacity than intact glutenin.

The decrease in farinograph consistency produced by excessive mixing could also be explained in terms of a change in the distribution of water in dough (Bushuk 1966; Hoseney and Finney 1974). Webb et al. (1970) supported the hypothesis that the distribution of water in dough depends on the mechanical work input during mixing. They showed that the input of mechanical

energy increased the amount of free water.

(3) Role of Sulfhydryl and Disulfide Groups. Disulfide (S-S) bonds of the flour proteins play an important role in the rheological properties of dough (Dimler 1963, 1965; Wall 1964, 1967; Sullivan 1965; Holme 1966; Pomeranz 1968; Bloksma 1972; Tanaka and Bushuk 1972). During dough mixing, S-S groups can interchange with sulfhydryl (SH) groups in gluten protein and thus relieve the strain imposed on the dough by mixing without the breakdown of other covalent bonds (Mecham 1959; Frater et al. 1960; Mauritzen et al. 1963; Sullivan and Dahle 1966; Bloksma 1972). However, only 1 to 2% of all gluten S-S bonds can be broken by exchange with active accessible SH groups during dough mixing (Bushuk 1961, Mauritzen 1967). Kuninori et al. (1968) and Bloksma (1972) have pointed out that only a small proportion of the S-S bonds in gluten protein are crucial to the rheological properties of dough. Most of the functionally active SH groups appear to be contributed by low molecular weight SH compounds, for example cysteinylglycine and glutathione, which are highly mobile in dough (Frater and Hird 1963; Stewart and Mauritzen 1966; Hird et al. 1968; McDermott et al. 1969; Tkachuk 1969). Similarly low molecular weight S-S compounds can also interchange with S-S bonds of the wheat protein and produce rheological effects very similar to those of reducing agents (Sullivan and Dahle 1966; Jones and Carnegie 1969).

Disulfide reducing agents have long been known to bring about a rapid breakdown of dough. They produce an increase in the extensibility and a decrease in the resistance to extension of doughs (as measured in the

extensigraph) through reduction of protein S-S crosslinks (Merritt and Bailey 1945; Hlynka 1949; Udy 1953; Matsumoto et al. 1960; Tanaka and Bushuk 1973C). Similarly, removal of SH groups from the dough proteins by reaction with oxidizing agents such as iodate or bromate, or with blocking agents such as N-ethylmaleimide, toughens the dough (Tanaka and Bushuk 1972). There are fewer SH groups in such doughs that can initiate the interchange of S-S crosslinkages (Sullivan 1954; Hird 1966) thus the doughs have lesser mobility. However with excessive mixing of doughs containing relatively large amounts of iodate or NEMI, the doughs show a marked breakdown. This has been attributed to hydrolytic or oxidative cleavage of S-S bonds (Bushuk and Hlynka 1962; Tanaka and Bushuk 1973C). Tanaka and Bushuk (1973C) suggested that mixing breakdown of dough can also occur as a result of depolymerization of insoluble glutenin protein which would occur through disulfide interchange reactions involving low molecular weight sulfhydryl peptides and proteins. The lower molecular-weight glutenins, produced by depolymerization would have a lower water binding capacity compared to intact protein. Thus, it would appear S-S and SH groups in gluten protein are very important to the binding of water in dough.

(4) Effect of Other Bread Ingredients. Of the various ingredients added to bread doughs, salt and fat appear to be important relative to water binding. A variety of physical effects have been attributed either directly or indirectly to water binding. Bohn and Bailey (1937) reported that sodium chloride markedly increased the stress readings in their plastometer. Moore

et al. (1942) observed a pronounced decrease in consistency in the farinograph upon the addition of salt to dough. In the extensigraph test, the addition of salt increased both the resistance to extension and the extensibility of doughs (Fisher et al. 1949).

Hlynka (1962) studied the effect of salt on the hydration properties of flour with the farinograph. He showed that when dough consistency was 500 B.U., 1% of salt produced a decrease in absorption of 2.3%; 2% salt produced a decrease of 3%. At a constant absorption of 60%, 1% salt decreased consistency by 70 B.U. and 2% salt by 90 B.U. Hydration studies on prime starch and dry gluten have shown that salt decreases the hydration capacity of gluten but has no effect on the uptake of water by the starch (Bushuk and Hlynka 1964). At 2% salt level, the water absorption of gluten decreased by some eight percent. Bushuk et al. (1964) suggested that salt replaces bound water on some of the sites on gluten protein and in this way increases the amount of free water and thereby dough mobility. Hence less water is required to produce a dough of the same consistency in the presence of salt than in its absence. A similar dough softening effect is produced by calcium ions normally found in yeast foods (Skovholt 1964). However, the effect of calcium ions on the distribution of water in dough has not been investigated.

Fat, when added to dough, decreases the maximum hydration capacity of the flour in dough (Bayfield et al. 1964). It was suggested by Brother et al. (1947) that fats form complexes with starch and protein and thereby make these flour constituents more hydrophobic. Olcott and Mecham (1947) and Davies et al. (1969) showed that there was a rapid drop in the amount of free fat extracted when water is added to flour. Mixing of the dough produced

a further decrease in the amount of extractable fat. These observations suggest a strong interaction of fat with other flour constituents in the presence of water during dough formation. Fat used in the bread formula decreased the optimum water absorption of dough (Bushuk and Hlynka 1964). Herz (1965) postulated that added fat forms contiguous and continuous layers on starch or protein particles and thereby decrease their capacity to absorb water.

(5) Chemistry of Dough. To explain chemical and physical changes that occur during breadmaking, it is essential to understand the nature of the chemical bonds and interacting groups in the dough constituents, (Pomeranz 1966; Wall et al. 1969). Wehrli and Pomeranz (1969) reviewed the four main types of cross links or chemical bonds which are involved in dough structure. These are covalent bonds (two atoms are held together by sharing common electrons), ionic bonds (attraction between opposite charges), hydrogen bonds (affinity of hydrogen for an electronegative atom i.e. oxygen) and Van der Waals forces (long range interaction between non polar groups). Wehrli et al. (1969) postulated that while covalent and ionic bonds primarily affect the cohesiveness and elasticity of doughs, ionic, hydrogen and hydrophobic bonds contribute to viscosity and plasticity. Other Van der Waals interactions are of limited significance in dough structure. Any factor that contributes to dough structure will also have some effect on the binding of water in the dough.

c. Bread. The two main changes that occur during the baking of dough into bread are the partial gelatinization of the starch (Sandstedt et al. 1954; Schoch 1965; Yasunaga et al. 1968) and the heat denaturation of the proteins including inactivation of enzymes (Pyler 1973). These changes exert a marked influence on the distribution of water in the baked product. In addition, there is a direct moisture loss by evaporation from about 45% in the dough to about 35% in bread (Bushuk 1966).

Yamazaki (1955) showed that when starch gelatinized its water absorption capacity increased to about eighteen fold. Gluten, on heat denaturation, loses practically all of its affinity for water. The water absorption capacity of pentosans remains unaffected after exposure to heat of baking temperatures (Pyler 1973). Accordingly, in the bread baking process, the gluten gives up most of its bound water, some of which is taken up by the gelatinized starch and some (about 5%) is lost by evaporation. Moreover, freshly baked loaf exhibits a moisture gradient between its interior and exterior portions because of the greater rate of evaporation at the loaf surface during baking (Yasunaga et al. 1968). This gradient gradually equalizes during post-baking storage when the excess moisture in the interior of the loaf migrates to the drier outer parts.

During post-baking storage, a variety of physical changes occur in bread some of which are related to binding of water. These changes are collectively referred to as staling (Willhoft 1973; Zobel 1973). Concomitant with the physical changes in some of the bread constituents there is a further redistribution of moisture (Herz 1965; Willhoft 1971A, 1971B, 1973; Zobel 1973). Water is either lost to the atmosphere or redistributed within

the loaf (Bechtel 1955). This and other changes related to bread staling are reviewed in the next section.

B. Bread Staling

Bread is a complex and relatively unstable system tending toward a state referred to as stale bread (Bice and Geddes 1954; Prentice et al. 1954; Bechtel 1955, 1961; Pelshenke and Hampel 1962; Schoch 1965; Willhoft 1973). Although there is no specific definition of bread staling that is generally acceptable to cereal chemists, it has usually been defined as a condition which indicates a decreasing consumer acceptance of the bread caused by changes in the crumb other than those resulting from the action of spoilage micro-organisms (Bechtel 1955; Herz 1965; Zobel 1973). Bice et al. (1954) have further defined staling as involving changes in which the texture of the crumb becomes harder, tougher, more crumbly and opaque. The crumb itself shows a reduction in water absorption capacity and a change in the x-ray diffraction pattern.

The degree of staling of bread is usually evaluated by changes in appearance or texture of the crust or the crumb, by flavour changes or by direct consumer sensory evaluation (Bechtel 1955). Compressibility and organoleptic tests on the crumb are also employed to measure staleness of bread (A.A.C.C. 1969; Axford et al. 1968). Use of the amylograph has also been suggested as a possible technique for following changes in the starch during post-baking storage (Yasunaga et al. 1968).

Some scientists (Knjaginciev 1970 and Zobel 1973) have observed that during staling there is detectable change in the distribution of free and bound water and have suggested that this change could be used as an index of bread staling. The next part of the literature review will deal with the roles in bread staling of water, gluten, starch and added fat in separate subsections.

1. Role of Water.

In general, higher water absorption of dough increases crumb softness and retards the firming of bread during post-baking storage (McCormick 1961). Although changes in starch and protein components of bread appear to be chiefly responsible for bread staling, the shift in moisture between these components may play an important role in the staling process (Bechtel 1955; Herz 1965; Willhoft 1971A, 1971B, 1973). It has been suggested that the transfer of moisture within the loaf or the loss from the loaf may accelerate the staling process (MacMasters 1961). However, it has been known for quite some time (Boussingault 1852) that the simple loss of moisture is not the main cause of staling. Bechtel et al. (1953) showed that bread will stale without appreciable loss of moisture. Stale, firm crumb often contains as much moisture as fresh soft crumb (Herz 1965). Cluskey et al. (1959) showed that starch gels can become firm without loss of water. It has been suggested that the firming of the starch is sufficient to account for most of the firming of bread crumb during post-baking storage.

On the other hand, Bachrach and Briggs (1947) found a small but definite increase in water binding capacity of bread crumb during ageing.

However, they concluded that the increase was not sufficient to account for the marked changes in the crumb structure due to the loss in plasticizing (lubricating) action of water. They suggested that the increase in water binding might affect crumb structure only if moisture was actually involved in the structure through hydrogen bonding.

Results obtained from studies on starch and gluten gels (Cluskey et al. 1959; Senti and Dimler 1960) showed that the water absorbing capacity of starch decreased rapidly with ageing, whereas that of gluten remained virtually constant. Accordingly transfer of moisture from starch to gluten may occur as the moisture absorbing capacity of the starch (in the crumb) decreases during ageing of bread. The reverse process has also been suggested (MacMasters et al. 1954; Prentice et al. 1954; Willhoft 1971A, 1971B) wherein moisture migration during staling occurs from the gluten to the starch. Obviously more research is required to clarify this point.

Willhoft (1971A) used a model system to investigate bread staling. The model comprised specially designed pans used to bake starch and gluten doughs in adjacent compartments. He observed that a significant fraction (about 30%) of the water associated with the protein was released from bound state and migrated to the partly gelatinized starch during the five days storage at room temperature. On the basis of these results, he suggested that the loss of moisture from the gluten would result in a significant rigidification of the gluten matrix of the bread. The migration of moisture from the gluten to the starch phase is accompanied by a slight softening of the starch which, however, is masked by negative effects of starch retrogradation (Willhoft 1971A).

2. Role of Gluten.

The concensus among cereal chemists is that staling of bread can be attributed principally to changes in the starch component (Bice and Geddes 1954; Senti and Dimler 1960; MacMasters 1961; Herz 1965; Zobel 1973). However there are some that are of the opinion that changes in the gluten contribute significantly to the staling process (Cluskey et al. 1959; Herz 1965; Willhoft 1971A, 1971B, 1973). Work of Stellar and Bailey (1938) showed that bread from high protein flours remained fresher longer than bread from lower protein flours. These workers concluded that both the quality and quantity of protein in the flour are relevant to staling. Bechtel et al. (1954) and Prentice et al. (1954) studied the effects of protein content of the flour on firming of bread crumb and sensory staling. In the protein content range from 10.5% to 16.5%, a decrease in both crumb firmness and firming rate was observed with increasing protein content. On the other hand, Ponte et al. (1962) found no relation between protein content and rate of firming of crumb for 10 flours over the narrow range of protein from 11.4% to 12.8%. Protein as such has little power to induce staling but its ratio to starch appears to be important in the rate of staling (Herz 1965). It is generally known that bread from higher protein flours stales slower than bread from flours of lower protein content.

3. Role of Starch.

There are a number of excellent reviews dealing with the changes in the starch component during bread staling. (Bice and Geddes 1954; Senti and Dimler 1960; MacMasters 1961; Pelshenke and Hampel 1962; Herz 1965;

Zobel 1973). The concept that bread staling is caused by intermolecular association (crystallization or retrogradation) of the starch molecules was confirmed by observation that bread prepared from flour in which part of the starch had been replaced by cross-linked starch, was firmer than the control bread (Prentice et al. 1954; Bechtel 1959; Zobel and Senti 1959).

Cornford et al. (1964) have provided further evidence that crystallization of starch is mainly responsible for staling of bread from their studies of the relationship between the elastic modulus of bread crumb and time and temperature. They observed that the relative rate of increase in the limiting elastic modulus became greater at temperatures near, but above, the freezing point. They emphasized the importance of the negative temperature coefficient of staling process and showed that the staling process as reflected by changes in the elastic modulus of the crumb could be adequately represented by the equation of Avrami (1941) type. The Avrami equation has been widely applied to the crystallization of high polymers (Avrami 1939, 1940, 1941). Since this equation holds for the staling process, this is taken as evidence that staling results from a crystallization of some component of bread crumbs. It is presumed that the component in question is starch (McIver et al. 1968).

A direct relationship between the ageing of starch gels, as measured by differential thermal analysis (DTA) and the staling of bread, as measured by crumb firmness at storage temperatures between -1°C and 21°C, was obtained by Cornford et al. (1964) and Axford et al. (1967). However, at higher temperatures (32°C and 43°C) the starch gels firmed three to four times more slowly than bread at these temperatures, suggesting a lesser effect of starch

crystallization at the elevated temperatures (Colwell et al. 1969). McIver et al.(1968) proposed that crystallization of starch is a process that can be reversed by heat. They used DTA to record the amount of heat required to melt the retrograded starch. It is common knowledge that stale bread can be partially refreshened by warming in a moist oven.

Preliminary studies with DTA on bread staling have been carried out by Axford and Colwell (1967). They found that while an endothermic peak was not present in fresh bread, a small peak developed during storage. The increase in peak area was similar to the increase in crumb firmness with storage time found earlier by Cornford et al. (1964). They also found that the endothermic peak was absent in a repeat run on the same sample of initially stale bread. This agrees well with the fact that bread can be re-freshened by being heated to above 70°C suggesting that the change which produces the endothermic peak is reversible.

4. Role of Fat.

Fat is added to the bread formula primarily because of its ability to retard staling (Carlin 1947). Added fat gives uniform, softer and more velvety bread texture and the effect is progressive with increasing shortening additions up to 5 - 6% of flour weight (Pyler 1973). Fat may form a continuous layer on starch and protein molecules of flour during dough mixing and thus help in retention of absorbed moisture (bound water) inside these structures (Herz 1965). It has been suggested (Bayfield et al. 1964) that fat, when added to dough, forms complexes with the amylose fraction of the starch (linear molecules) and thereby retards the rate of retrogradation

of these starch molecules, which is primarily responsible for bread staling. Yasunaga et al. (1968) suggested that added fat does not affect the gelatinization of the starch during baking but retards the changes that occur during post-baking storage. Complexing of fat with gluten has also been reported (Brother et al. 1947; Olcott et al. 1947). It may form fine films over gluten strands in bread structure and thus interfere with the transfer of moisture from gluten to starch during post-baking storage of bread.

C. Summary

It may be concluded from the foregoing review of the literature that water plays an extremely important role in the transformation of wheat to bread. Appropriate amount of moisture is necessary in wheat during milling to obtain the maximum amount of flour with optimum baking and keeping quality. Water (moisture) at a level of about 14% forms an integral part of the flour. In the process of dough formation water is necessary for the conversion of flour to dough. Moreover, it controls the rheological properties of the dough required for machine handling and also for the activity of various enzymes essential for optimum breadmaking potential. In the baking stage, water plays an important role in the gelatinization of starch and, therefore, in the production of the desired bread crumb structure. Higher moisture in dough gives a higher yield of bread and also extends the shelf-life by controlling the softness of bread. If the loss of moisture from bread is reduced by binding it to various bread components, the bread will remain softer for longer time. A high level of consumer acceptance can thus be maintained over a much longer period.

MATERIALS

The following four wheat varieties were selected for this study.

A. Red River 68

This is a hard red spring wheat variety developed in the U.S. by World Seeds Inc. Its parentage, as far as it can be established, is Tezanos Pintos Precoz x Sonora 64 A. Red River 68 is a semi-dwarf variety with a strong white stem and red kernels. Its overly strong dough mixing characteristics can be explained on the basis of the physical and chemical properties of its proteins.

B. Manitou

This is a Canadian hard red spring wheat variety developed by the Canada Department of Agriculture for the rust area of Western Canada. Its parentage is (Thatcher ^{7} - Frontana x Thatcher^{6} - Kenya Farmer) x Thatcher^{6} - P.I. 170925. It is essentially the same as the well known variety Thatcher but contains two extra genes for stem rust resistance, Sr 6 from P.I. 170925 (a red Egyptian wheat) and Sr 7 from Kenya Farmer, and a gene for leaf rust resistance from Frontana. Compared with Thatcher, Manitou has better rust resistance, gives higher grain yield, is earlier maturing, is usually slightly higher in protein content, and is, therefore, better in overall breadmaking quality. It is considered to be of medium mixing strength. Six different Manitou wheat samples containing different levels of protein were used in this study.

C. Talbot

This is a soft white winter wheat variety grown mainly in Eastern Canada. Its parentage is (Trumbull-Hope-Hussar) x F. (of Dawsons G.C.^{2} - Ridit Cornell 595). It was licenced in Canada in 1963 because of its superior lodging resistance compared with the then leading soft white winter variety, Genesee. Talbot was selected for the present study because it has very weak mixing characteristics compared with the two hard red spring wheat varieties.

D. Pembina

This is another Canadian hard red spring wheat variety. It was licenced for production in Canada in 1959. This variety was developed by the Rust Area Project Group at the Winnipeg Research Station of the Department of Agriculture. Its parentage is Thatcher x R.L. 2565 (a sister selection of the variety Selkirk). This variety is also known for its overly strong mixing characteristics.

E. Technological Properties of the Flours Used

Some pertinent technological properties of the flours used in this study are given in Table 1 and Table 2. Flours listed in Table 1 and Table 2 were used for studies of water binding in dough and bread respectively. All flours were milled on an experimental Buhler mill using an overnight tempering to 15.5% moisture unless stated otherwise.

TABLE 1. PERTINENT TECHNOLOGICAL DATA FOR FLOURS USED FOR WATER BINDING IN DOUGH

Variety	Talbot	Red River 68	Manitou				
Moisture, %	14.0	13.6	13.6	13.7	14.4	13.4	13.9
Protein*, % (14% m.b.)	11.3	10.9	10.8	10.1	12.4	13.8	14.7
Ash, % (14% m.b.)	0.46	0.57	0.45	0.34	0.38	0.42	0.45
Starch Damage, F.U.[a]	5	33	35	34	30	22	21
Farinograph Absorption, %	52.6	65.0	63.9	66.4	66.1	66.5	66.4
Dough Development Time, min.	2.0	8.0	5.0	2.0	5.5	6.0	6.5

[a]Farrand Units

*N x 5.7

TABLE 2. PERTINENT TECHNOLOGICAL DATA FOR FLOURS USED FOR WATER BINDING IN BREAD

Variety	Manitou			Pembina
Moisture, %	14.5	14.2	13.3	13.9
Protein*, % (14% m.b.)	9.9	12.4	14.1	13.4
Ash, % (14% m.b.)	0.35	0.38	0.51	0.42
Starch Damage, F.U.[a]	34	25	28	31
Farinograph Absorption, %	58.8	61.3	66.2	65.5
Dough Development Time, min.	5.0	5.0	5.5	6.5

[a]Farrand Units

*N x 5.7

METHODS

A. Methods Relative to Water Binding in Dough

The flour data of Table 1 were obtained using the Approved Methods of the American Association of Cereal Chemists (1969).

1. Production of Damaged Starch

To obtain flours of varying levels of starch damage, flour samples were ball milled and pin milled for varying periods. Ball milling was done for about 48 hours at 20°C. Pin milling was achieved by passing the flour once and three times through the Alpine S.L. 160 Z pin mill running at 16,000 r.p.m.

2. Preparation of Water-Soluble Pentosans

Modified Baker's Procedure (Baker et al. 1943) was used to prepare water-soluble pentosans. Wheat flour (150 g.) was first mixed with 300 ml. of water in a Waring Blender for 3 min. at high speed to make a uniform batter. The batter was centrifuged at 1000 x g for 30 min. to separate supernatant I. Supernatant I (containing water-soluble pentosans) was heated to 95°C for 3.5 min. to inactivate any enzymes present and to coagulate the soluble proteins, and then cooled to room temperature. The proteins that remained in the supernatant were precipitated with saturated $CuSO_4$ (16.7 ml. per 100 ml. supernatant) and 5N NaOH (6 ml. per 100 ml. supernatant) as described by Baker et al. (1943). After addition of $CuSO_4$ and NaOH, the solution was stirred until a homogeneous mixture was obtained.

The pH of the mixture was adjusted to 5.5 - 5.6 and the mixture centrifuged at 2,500 x g for 30 min. The precipitate was discarded and supernatant II was retained. Supernatant II was heated and treated with $CuSO_4$ as supernatant I, adjusted to slightly higher pH (5.5 - 6.0) with 5N NaOH and centrifuged to yield supernatant III.

Ammonium sulfate (70 g. per 100 ml. supernatant) was added to supernatant III and stirred at high speed to saturate the solution and thus precipitate the water-soluble pentosans. The dispersion was left to stand for several hours during which time a white layer of pentosans formed on top. This layer was removed, washed once lightly with a small amount of distilled water to remove most of the ammonium sulfate, and then dissolved in distilled water by high speed stirring. This pentosan solution usually contains a small amount of soluble starch. To remove the starch, 10 mg. amyloglucosidase (Grade III, Sigma Chemical Co.) was added to each 100 ml. of pentosan solution and allowed to react with stirring for one hr. at 55°C. The enzyme reaction was stopped by bringing the temperature of the solution to 100°C. The solution was then dialyzed for 72 hr., with several changes of water to remove the sugars and freeze-dried. The dry substance obtained was assumed to be soluble pentosan.

3. Preparation of Dough

Doughs were mixed on a constant weight basis (80 g) at 30°C in a stainless steel Brabender farinograph mixer. Mixing was done at a normal speed of 63 r.p.m. Except in the case of doughs with added chemicals or doughs where the effect of variable mixing time was studied, the mixing

time was 8 minutes. Doughs with added chemical were mixed for 20 minutes to allow sufficient time for the chemical to react with dough components.

N-ethylmaleimide, L-cysteine (free base), L-ascorbic acid and potassium iodate were added to dough at the level of 2 μeq./g flour. This level was selected on the basis of the effects of these chemicals on dough mixing properties observed by Tanaka and Bushuk (1973A). Sodium chloride was added at 2% level.

Immediately after mixing, the dough samples were sealed in aluminum foil and kept in a closed container to prevent loss of moisture, until subsamples were taken for differential thermal analysis (DTA). Dough moisture was determined from the weight loss after drying overnight at 105°C.

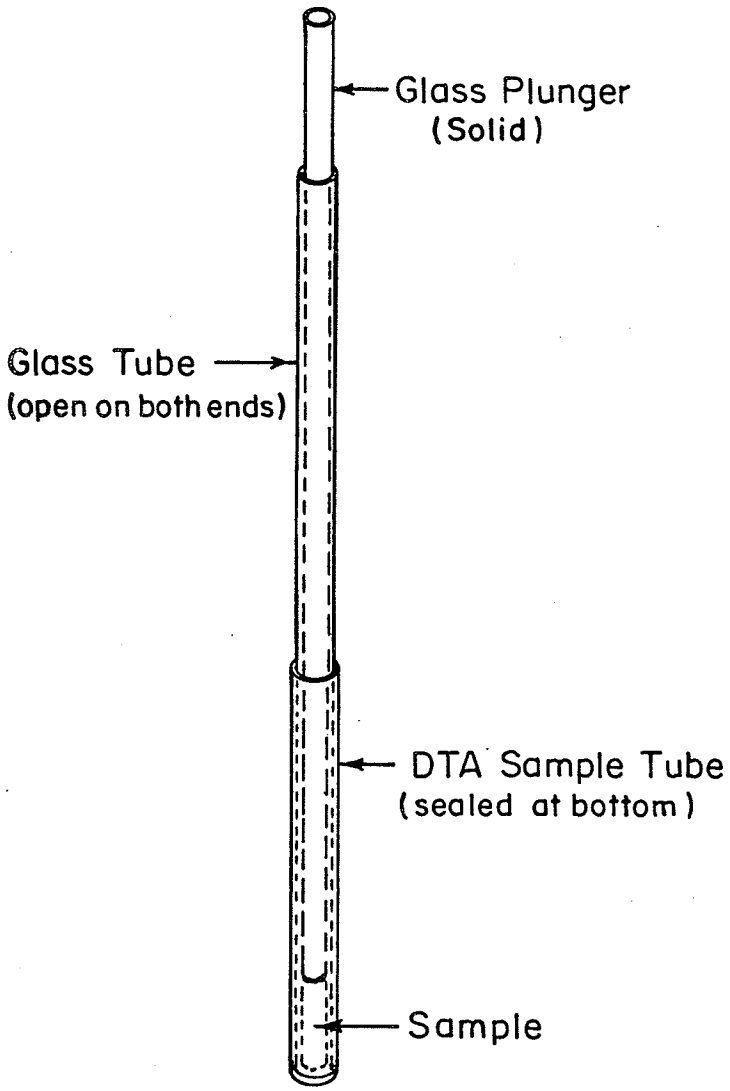
Dough subsamples of approximately 25 mg. were cut from the centre of each dough piece using a specially designed glass tube and plunger assembly (Fig. 1). The subsample was placed at the bottom of a DTA macro sample tube with the help of plunger assembly and was immediately weighed before thermal analysis.

4. Differential Thermal Analysis

A brief description of the Differential Thermal Analyser is included in this part of the thesis to outline its mode of operation.

Thermal analysis (TA), in its broadest sense, is the measurement of changes in physical or chemical properties of materials as a function of temperature (Levy 1970). Depending on the nature of the temperature-dependent variable e.g. energy, weight, dimensions, etc., the thermal

FIG. 1 GLASS-TUBE AND PLUNGER ASSEMBLY USED FOR HANDLING DOUGH SAMPLES.


Glass Plunger
(Solid)
Glass Tube
(open on both ends)
DTA Sample Tube
(sealed at bottom)
Sample

analysis technique has been divided into four groups: 1) differential thermal analysis (DTA), 2) differential scanning calorimetry (DSC), 3) thermogravimetric analysis (TGA), and 4) thermomechanical analysis (TMA). In the present study only the DTA mode was used.

Differential thermal analysis is a record of the difference in temperature between the experimental material and a reference substance as the two specimens are subjected to identical temperature changes (heating or cooling) at a controlled and programmed rate. The record obtained is called the DTA curve and, provided the substance is thermally active in the temperature range used, shows a series of peaks. The area of these peaks is related to the energy involved in the reaction or phase change that occurs at the temperature of the peak (Mackenzie 1970).

A schematic of a typical DTA apparatus is shown in Fig. 2. It consists of temperature programmer, amplifier, and a recorder, common to all TA systems. In DTA, the transducers are two thermocouples placed directly in the sample and reference materials. The reference material chosen is a substance that has no thermal transitions in the temperature range used in the experiments. The thermocouples are connected in series opposition (i.e. back to back) so that if the sample and reference are at identical temperatures, the resulting e.m.f. of the pair is zero. If the sample (Ts) is lower in temperature (endothermic process) than the reference (Tr), the output e.m.f. will be positive; if the sample is higher in temperature (exothermic process) the e.m.f. will be reversed. Normally, the amplifier and recorder are wired so that exothermic peaks ($Ts > Tr$) are displayed upward and endothermic peaks ($Ts < Tr$) are displayed downward.

FIG. 2 SCHEMATIC DIAGRAM OF A TYPICAL DIFFERENTIAL THERMAL ANALYZER.

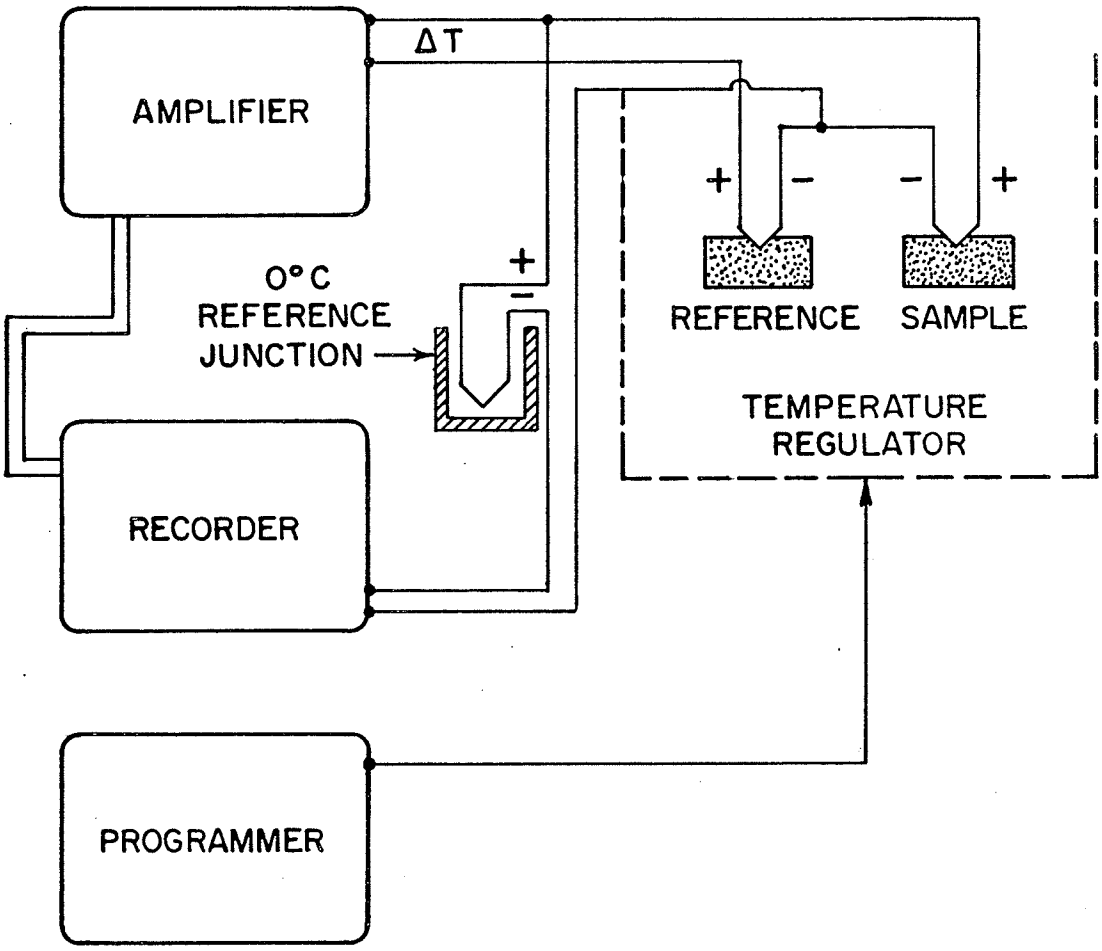
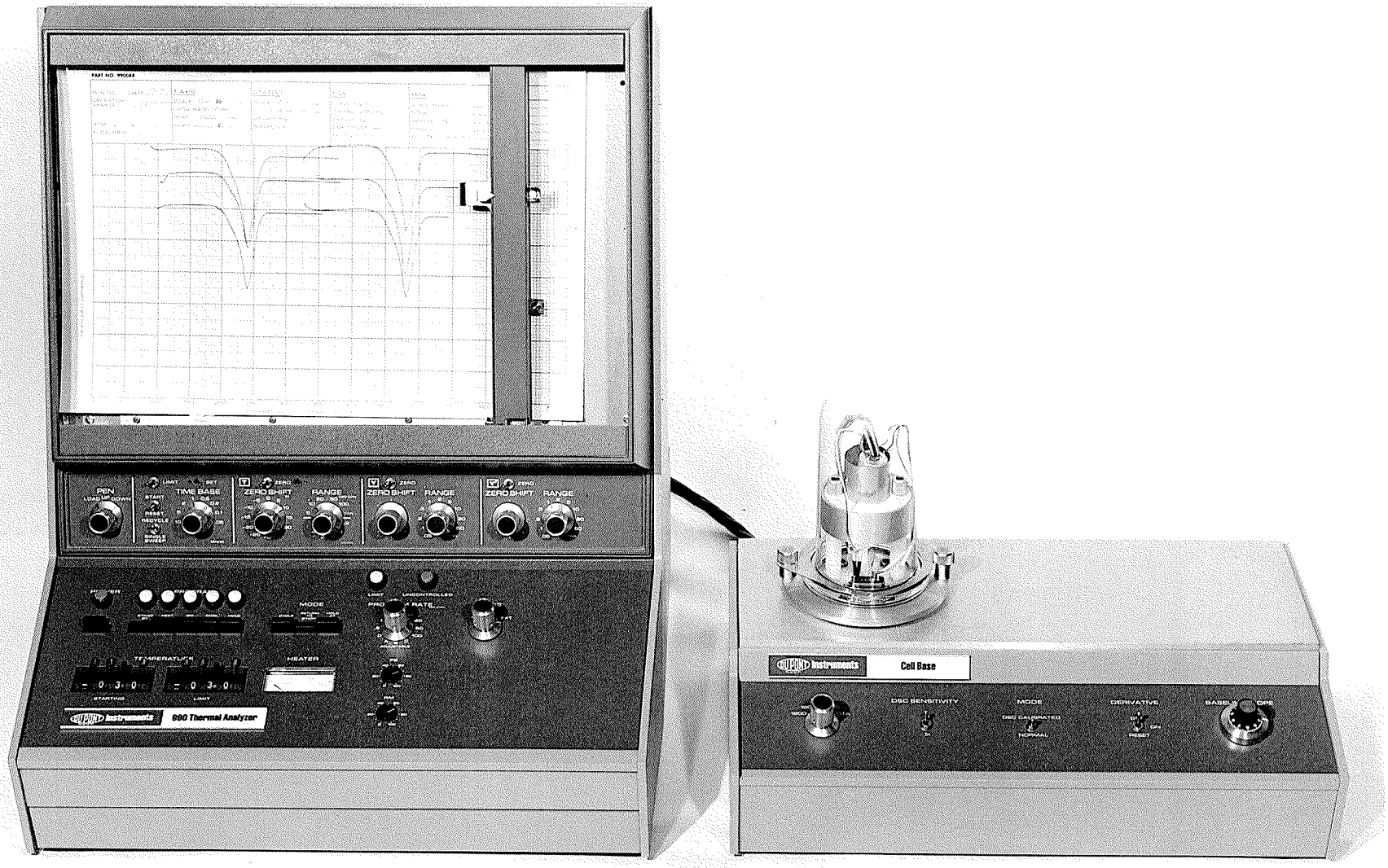

AMPLIFIER
ΔT
+
−
−
+
0° C
REFERENCE
JUNCTION
+
−
REFERENCE
SAMPLE
TEMPERATURE
REGULATOR
RECORDER
PROGRAMMER

FIG. 3 DUPONT 990 MODEL DIFFERENTIAL THERMAL ANALYZER EQUIPPED WITH A STANDARD (500°C) DTA CELL (RIGHT).


PEN
TIME BASE
ZERO SHIFT
RANGE
ZERO SHIFT
RANGE
ZERO SHIFT
RANGE
MODE
TEMPERATURE
HEATER
STARTING
LIMIT
DuPont Instruments
990 Thermal Analyzer
DuPont Instruments
Cell Base
DSC SENSITIVITY
MODE
DSC CALIBRATE
NORMAL
DERIVATIVE
PRESET
BASELINE
DIFF

Since the thermocouple is placed directly in the sample, the DTA technique provides the highest thermometric accuracy of all the thermoanalytical methods. The area under the output curve, is proportional to the amount of energy transferred in or out of the sample.

The temperature range covered by DTA is quite extensive. Cells are available that will allow the detection of transitions from liquid nitrogen boiling temperature (-190°C) to 1600°C. Typical sample sizes run from 0.1 to 100 mg. The actual size used should be as small as possible in order to reduce thermal gradients within the sample.

The DTA instrument used in the present study was Dupont Model 990 (Fig. 3) equipped with the standard (500°C) DTA cell. Glass beads were used in both reference and control tubes as it has no thermal transitions in the temperature range studied. The beads were not changed throughout the whole study. In the part of the study dealing with dough, both the "boiling" and "melting" DTA modes were used. For bread studies, only the melting mode was used. The methods of the two modes of DTA will be discussed briefly.

a. Boiling Curve. The macro sample tube containing subsample of dough was placed in the DTA cell and heated from 30°C to 160°C at a programmed rate of 5°C per min. The x-axis and y-axis sensitivities were 50°C per inch and 5°C per inch respectively. At about 100°C an endothermic peak appears (Fig. 4) due to the boiling-off of the dough moisture. In practice, each dough sample was heated only once. To increase the accuracy of the experiment, three to six replicate analyses of fresh subsamples were

made for each dough. The results were expressed as peak area per mg. dry flour.

b. Melting Curve. The macro sample tube containing the dough subsample was first cooled to -30°C with liquid nitrogen after placing in DTA cell. Subsequently, the sample was heated to 30°C at a programmed rate of 5°C per min. to obtain the endothermic peak at about 0°C (Fig. 5). In these experiments, the x-axis and y-axis sensitivities were 10°C and 1°C per inch respectively. Each subsample was frozen only once and three replicate analyses were made for each dough.

In both (boiling and melting) procedures, the area under the peak was measured to $\pm$ 0.1 cm^2. using a planimeter.

5. Determination of Bound Water

The bound water content of dough was determined by substracting the freezable or free water from the total moisture of the sample. The results are expressed as mg. bound water per mg. dry flour. The freezable water content of the sample was determined by using the procedure of Davies and Webb (1969).

The DTA was first calibrated to correlate the area of the recorded endotherm of melting ice with the amount of water involved in the ice-water transition at 0°C. A calibration constant E was calculated from the equation:

$$E = \frac{H.M.R.}{A.T.S.} \text{ ------(1)}$$

where H = heat of fusion of ice (79.71 m.cal./mg.)

M = weight of water, 10 mg.

R = heating rate, $5^{o}C$/min.

A = area of the endotherm, sq. inches

T = Y-axis sensitivity, $1^{o}C$/inch

S = X-axis sensitivity, $10^{o}C$/inch

The value of E for the instrument used in the present study was 131.188 m.cal. per ^{o}C min.

The weight of freezable water (W) in each dough sample was calculated from the data obtained using the following equation:

$$W = \frac{A.E.T.S.}{H.R.N.} \text{ -------(2)}$$

All variables in equation 2 except N are as defined for equation 1. N is the weight in mg. of the dough (or bread) subsample.

B. Methods Relative to Water Binding in Bread

The flour data of Table 2 were obtained using the Approved Methods of A.A.C.C. (1969).

Loaves were baked by "Remix" experimental baking procedure described by Irvine and McMullan (1960). The bread formula used is given in Table 3. Modifications to this formula will be indicated where used. The loaves were individually sealed in polyethylene bags immediately after measuring their volume. Six loaves from each dough mix were stored at room temperature ($26^{o}C$) except in the case of the loaves where the effect of storage temperature was studied. For the temperature study, loaves were stored at $26^{o}C$ (room temperature), $4^{o}C$ (refrigerator) and $-20^{o}C$ (deep freezer) for

FIG. 4 A TYPICAL 'BOILING THERMOGRAM' OF A DOUGH SAMPLE CONTAINING 45% MOISTURE.

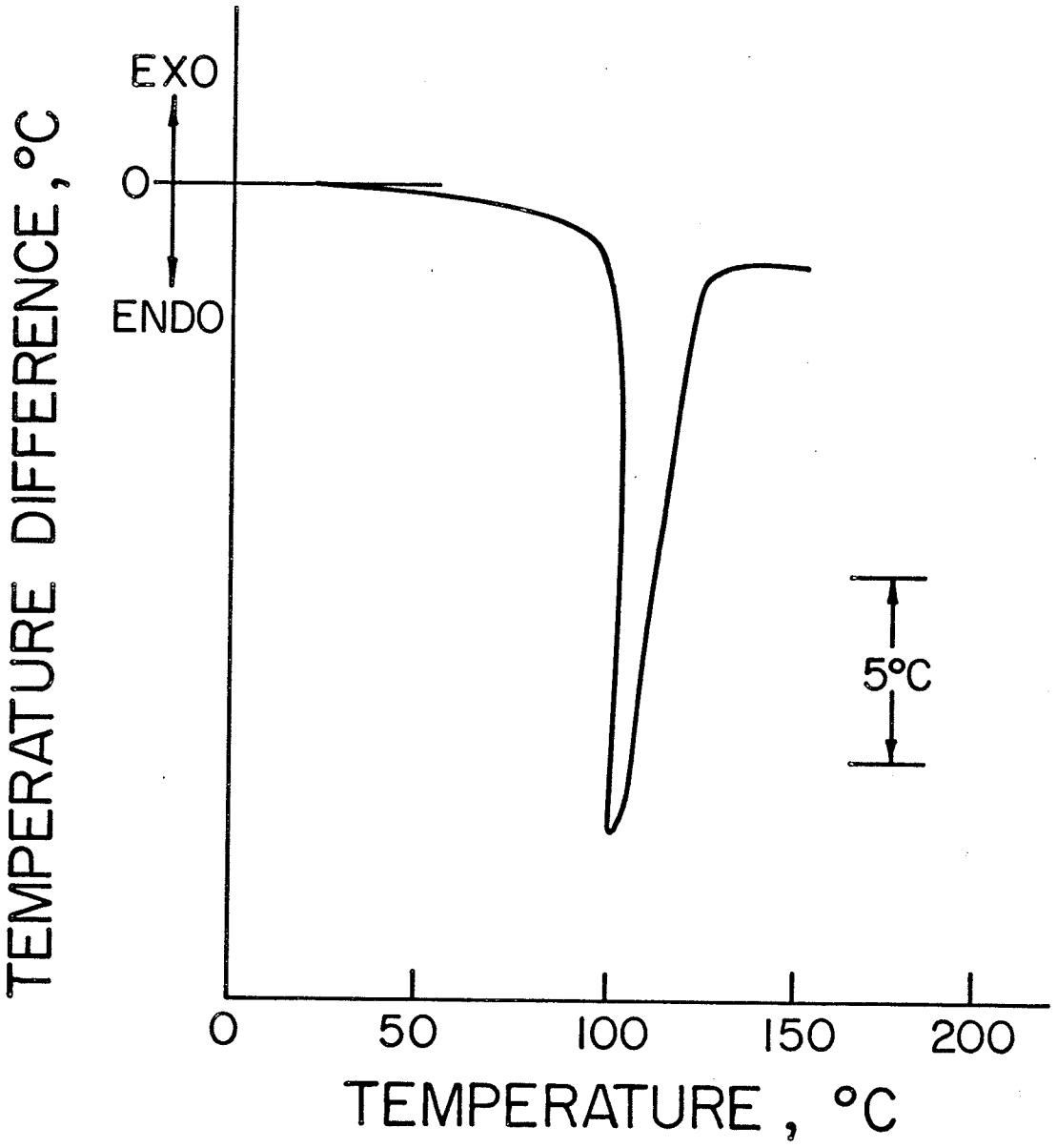

EXO
0
ENDO
TEMPERATURE DIFFERENCE, °C
5°C
0
50
100
150
200
TEMPERATURE , °C

FIG. 5 A TYPICAL 'MELTING THERMOGRAM' OF A DOUGH SAMPLE CONTAINING 45% MOISTURE.

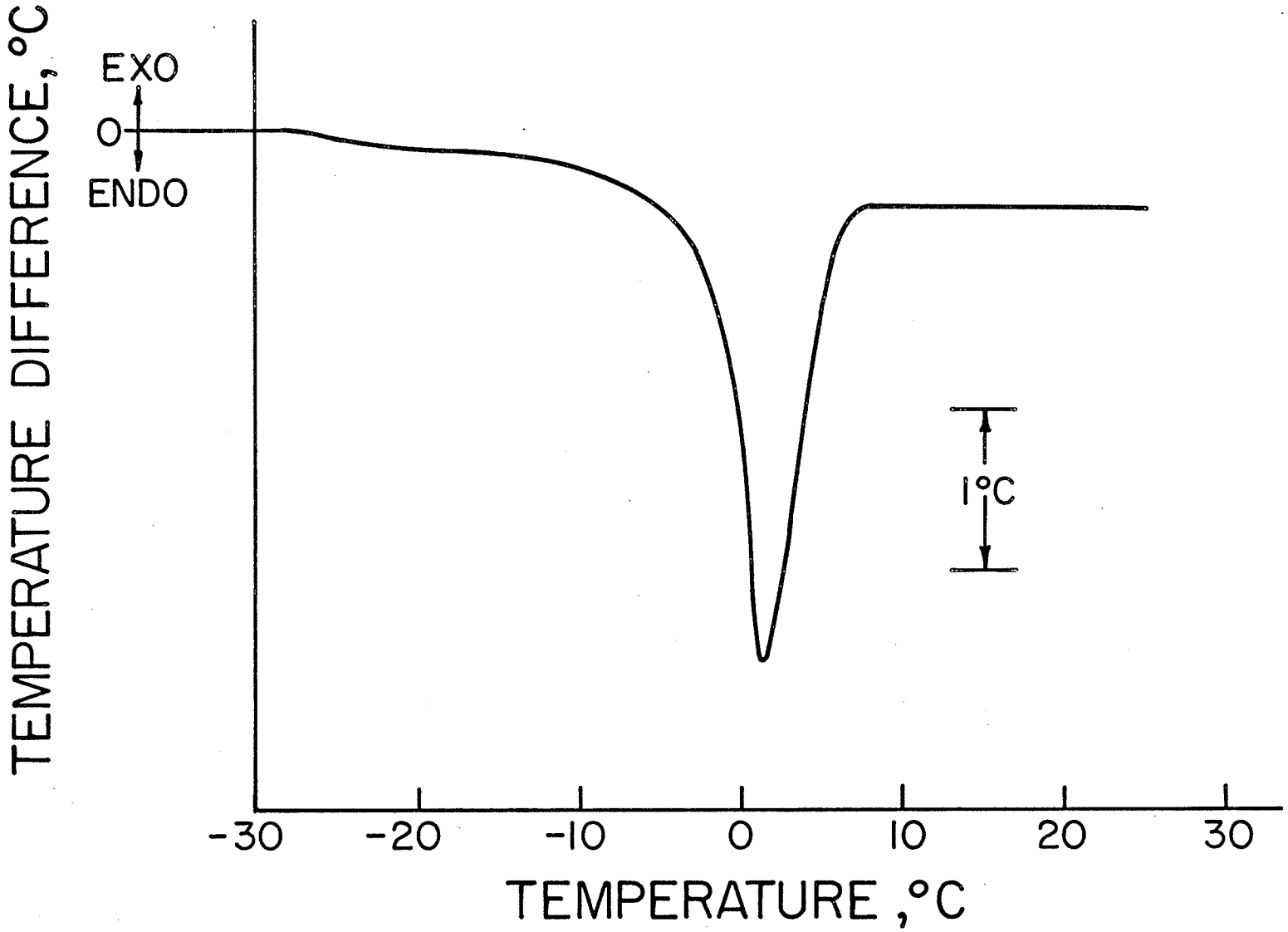

TEMPERATURE DIFFERENCE, °C
EXO
0
ENDO
1°C
-30
-20
-10
0
10
20
30
TEMPERATURE ,°C

TABLE 3. BREAD FORMULA AND PROCEDURE USED

Flour	-	100 g (14% m.b.)
Yeast	-	3 g
Salt	-	1 g
Sugar	-	2.5 g
Potassium Bromate	-	1.5 mg
Ammonium Phosphate	-	0.1 g
Malt	-	0.3 g
Water	-	4% less than farinograph absorption
Mixing	-	3.5 min.
Fermentation	-	165 min. at 86°F
Re-mixing	-	2.5 min.
Re-fermentation	-	25 min. at 86°F
Proofing	-	55 min. at 86°F
Baking	-	25 min. at 446°F
Determination of Bread Volume	-	25 min. after baking

different periods of time. The loaves stored at $4^{o}C$ and $-20^{o}C$ were warmed to room temperature (enclosed in polyethylene bags) by exposing them to room temperature for three hours before thermal analysis.

For DTA, one loaf of each mix was used for each measurement. The loaf was sliced vertically into two pieces and approximately 25 mg. of bread crumb was taken from the central region of the bread as the experimental subsample. Immediately after sampling, the sliced loaf was repacked in polyethylene bags. The subsample was placed at the bottom of the DTA macro sample tube and was compressed with a glass rod. The sample was immediately weighed before DTA.

Bound water in bread samples was determined using the procedure already described under subheadings melting curve and determination of bound water in dough. Moisture content of the crumb was calculated from the weight loss on heating overnight at $105^{o}C$.

For studying the effect of storage time on bound water content of bread, flour from the hard red spring variety Pembina was used but for all other studies a bread flour from a related hard red spring variety (Manitou) was used. It was felt that this change of variety would not affect the general trend of post-baking changes in the bread. For experiments at different absorption levels, loaves were baked at absorptions of 4% above and below the optimum baking absorption of the flour. Loaves were also baked from three different Manitou flours containing 9.9, 12.4 and 14.1% protein for studying the effect of protein content on water binding in bread after various storage periods.

The effect of starch damage was studied using flour milled from Manitou wheat. The protein content of this flour was 12.4%. Before milling this sample of wheat, one batch was conditioned to produce flour of low starch damage and another to produce flour of high starch damage. For the low starch damage flour, the wheat was conditioned by soaking in water for 8 hours at $4^{\circ}C$. This wheat was subsequently freeze-dried to 10.4% moisture, then tempered to 15.5% moisture before milling. For the high starch damage flour, the wheat was tempered at 15.5% moisture for 20 minutes.

RESULTS AND DISCUSSION

As indicated in the introduction, the main purpose of this study was to examine some of the factors that contribute to water binding in dough and bread using differential thermal analysis (DTA). Accordingly, most of the experiments were designed with this purpose in mind. The results of the investigation will be presented and discussed in two sections: (1) water binding in dough, and (2) water binding in bread. The first section will be further divided into two subsections: (1) boiling curve results, and (2) melting curve results.

A. Water Binding in Dough

1. Boiling Curve Results

The method of determining water binding from the DTA boiling curve is based on the assumption that size of the endothermic peak at about 100°C, due to the boiling-off of the dough moisture, is directly proportional to the amount of heat energy involved in the endothermic process. Accordingly, at constant dough moisture, samples that give a larger peak area require more heat energy than samples with smaller peak area to boil-off the same quantity of water. Presumably the endothermic process involves the removal of both, bound water and the vaporization of free water. The boiling curve method gives only quantitative results in terms of the energy of water binding and therefore, it cannot be used to determine the amount of free or bound water.

The results obtained in this part of the study are presented and discussed in a series of subsections that follow.

a. Effect of Mixing Strength of Flour. Flours differ widely in mixing strength as measured by the farinograph mixing curve. Generally strong mixing flours give better results in terms of baking compared with weak mixing flours. Accordingly, it was of interest to examine if there was any relationship between the mixing strength of flour and the binding of water in dough.

The effect of mixing strength was studied using flours from the three varieties, Red River 68, Manitou and Talbot, which varied widely in mixing characteristics from very strong through medium strong to weak. Farinograms of the three varieties are shown in Fig. 6 to demonstrate the range of mixing strength investigated.

For DTA, doughs from the three flours were mixed for 8 minutes in a farinograph mixer at a constant water absorption of 60% (on a 14% moisture basis). It was observed that the size of the endothermic peak for the doughs of the three varieties differed considerably (Fig. 7) although the samples were given identical mixing treatment and had the same moisture content. Red River 68, which is a very strong mixing variety had the largest peak area. Manitou, the medium strength variety, had an intermediate peak area and Talbot, the weakest variety, had smallest peak area.

Since the size of the DTA peak obtained due to the boiling-off of the dough moisture, is a measure of the amount of heat energy used in the endothermic process, it is evident from Fig. 7 that the energy of water

FIG. 6 FARINOGRAMS OF THE THREE WHEAT VARIETIES USED IN THIS STUDY.

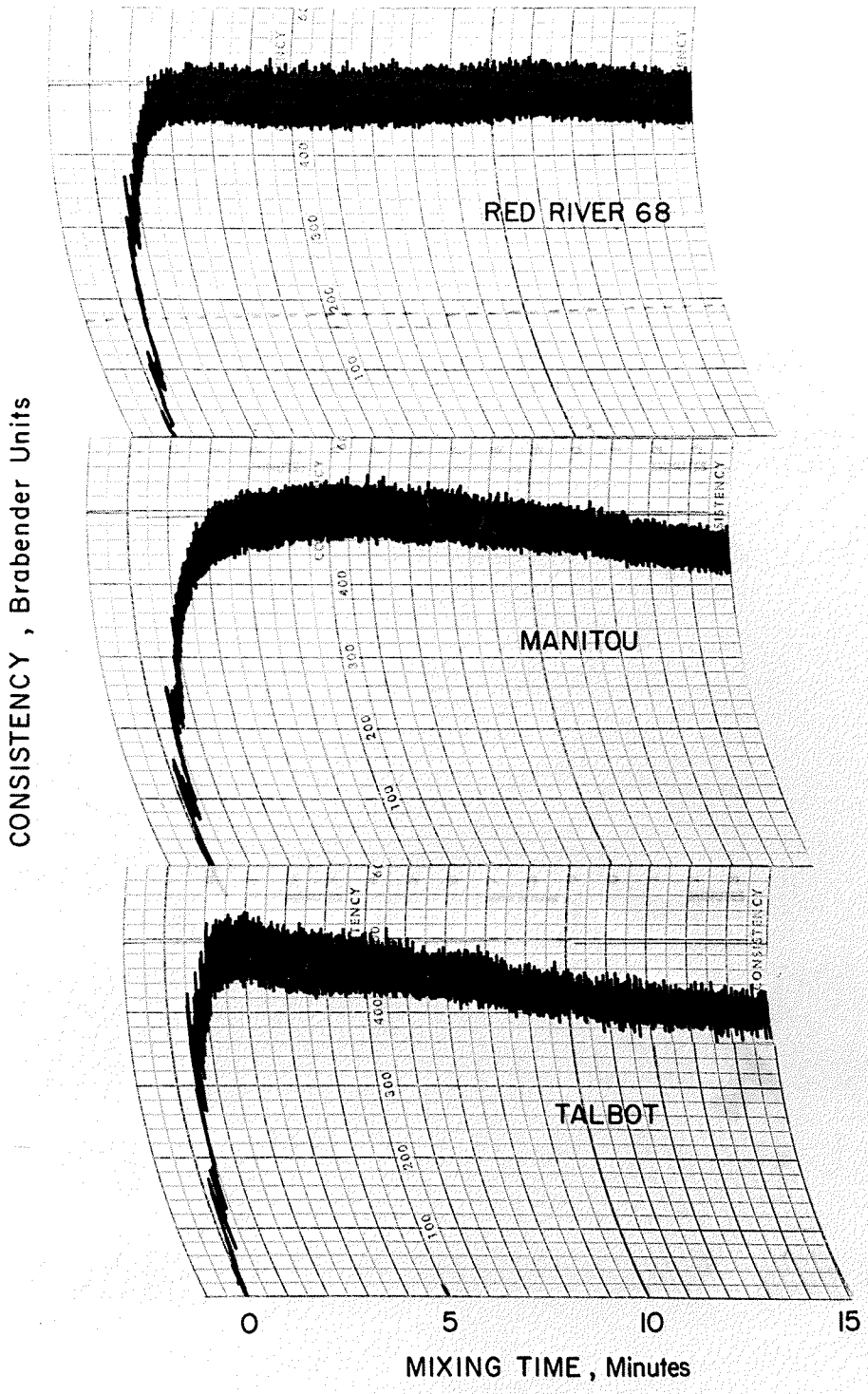
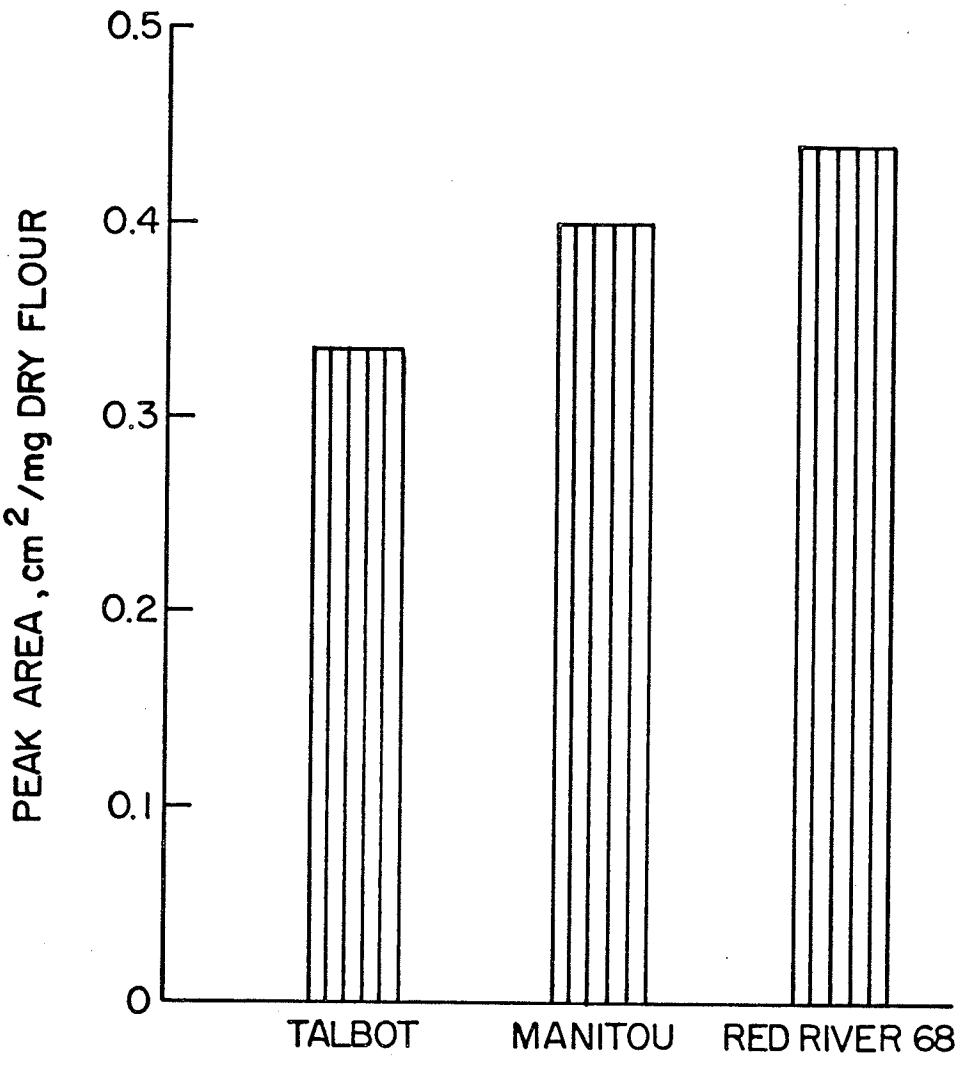

RED RIVER 68
MANITOU
TALBOT
CONSISTENCY , Brabender Units
MIXING TIME , Minutes
0
5
10
15

FIG. 7 BOILING ENDOTHERM PEAK AREAS OF DOUGHS AT A CONSTANT WATER ABSORPTION OF 60% FOR THE THREE WHEAT VARIETIES USED IN THIS STUDY.


0.5
0.4
0.3
0.2
0.1
0
PEAK AREA, cm 2/mg DRY FLOUR
TALBOT
MANITOU
RED RIVER 68

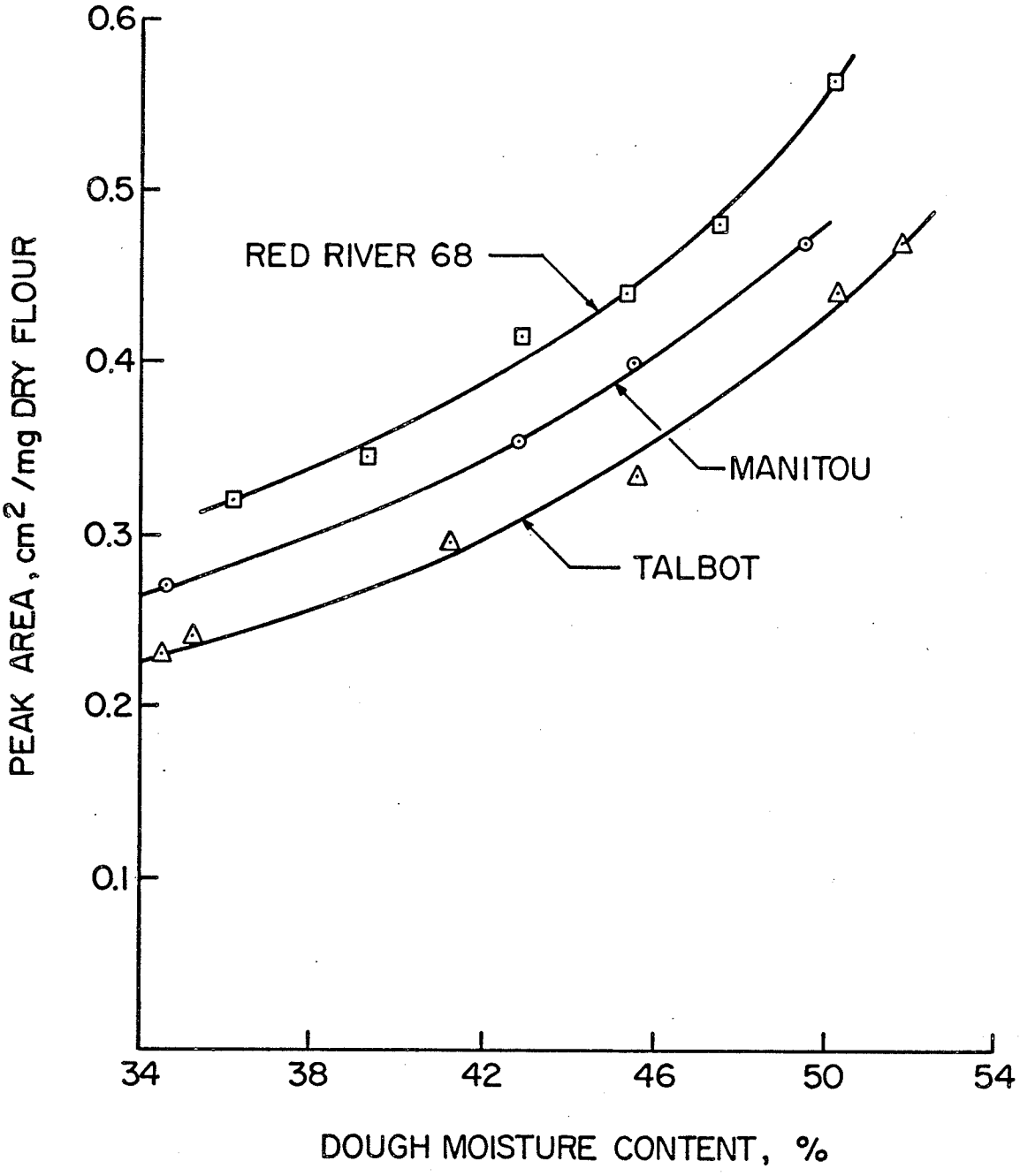
binding differs markedly in the doughs of the three wheat varieties. Strong mixing flours bind water more strongly (tightly) than weaker mixing flours. The chemical and physical properties of the flour (or its constituents) that determine the energy of water binding in dough remain to be established. It is speculated that the level of starch damage in the flour, the content of pentosans and the nature of the protein may be important factors.

b. Effect of Water Absorption. The results on the effect of mixing strength were further expanded by varying the moisture in the doughs of the three wheat varieties over a fairly wide range from about 35% to 52%. This moisture range spans the range of water absorption normally encountered in breadmaking. Observations beyond this moisture range could not be made as the dough became either too tight or too slack for proper handling in DTA. The results are shown graphically in Fig. 8.

Over the range of absorption investigated, Red River 68 (the strongest variety used), showed a larger peak compared with the peaks for the weaker varieties Manitou and Talbot. These results confirmed the findings of the constant absorption experiment (above) that water is bound more strongly in doughs of stronger mixing varieties than of weaker varieties.

Since the three flours used had approximately the same protein content, it appears that protein quality may be affecting the water binding of flour and which, in turn controls the mixing strength of flour. There is also the possibility that the observed differences may be due to differences in the amount of water soluble pentosans in the three flours. The effect of pentosans will be examined later.

FIG. 8 EFFECT OF WATER ABSORPTION ON DTA PEAK AREA FOR THE THREE WHEAT VARIETIES.


0.6
0.5
0.4
0.3
0.2
0.1
PEAK AREA, cm² /mg DRY FLOUR
RED RIVER 68
MANITOU
TALBOT
34
38
42
46
50
54
DOUGH MOISTURE CONTENT, %

c. Effect of Flour Protein Content. It is generally accepted by cereal chemists that the quantity of protein in a flour is the main factor that determines the value of the flour for bread production. The greater the amount of protein, the better is the flour's potential for breadmaking (Bushuk et al. 1969). It is also commonly accepted that flour absorption varies directly with protein content (Finney 1945). With the above facts in mind, the role of protein content on water binding was investigated.

The effect of protein was examined for one variety, Manitou, for which grain samples of different protein content were available and also for all three varieties by adding commercial vital wheat gluten (73.0% protein on 0% m.b.) at different levels to increase the total protein content. All doughs used in these experiments were mixed for 8 minutes at a constant water absorption of 60%.

When the boiling curve peak area per mg. protein was plotted against protein content of Manitou flour (without vital gluten), as shown in Fig. 9 a linear relationship was obtained showing a slight but a significant decrease in water binding energy with increasing protein content. Similar results were obtained when the peak area values for the three flours supplemented with different amounts of vital gluten were plotted against the protein content (Fig. 10). These results suggest that flour proteins bind water with less energy than starch (or other constituents) and therefore, the total binding energy decreases with increasing protein content.

When the flours used in this experiment were analyzed for damaged starch, it was found that the level of damaged starch (flour basis)

FIG. 9 EFFECT OF PROTEIN CONTENT ON DTA PEAK AREA OF MANITOU FLOURS.

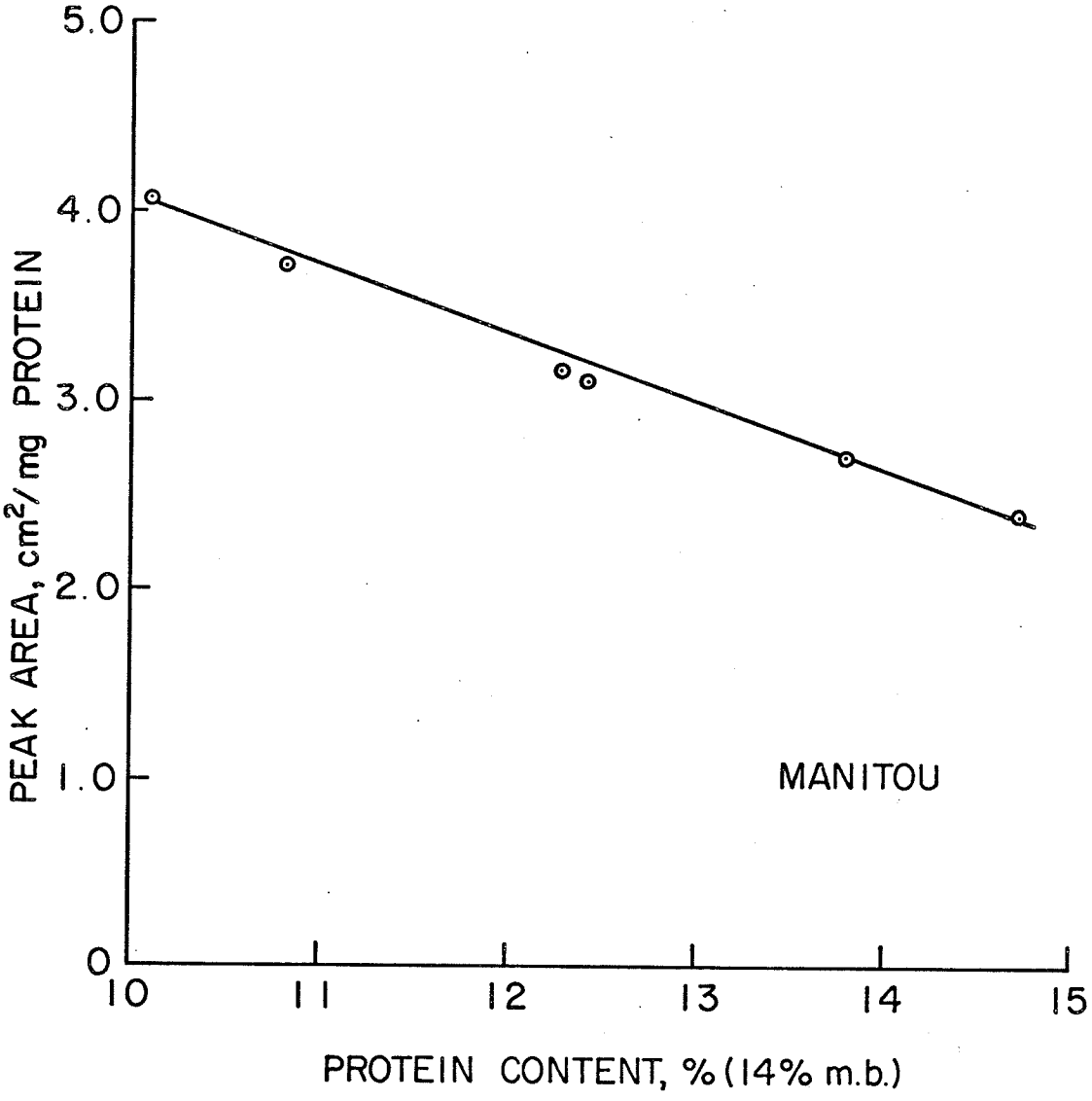
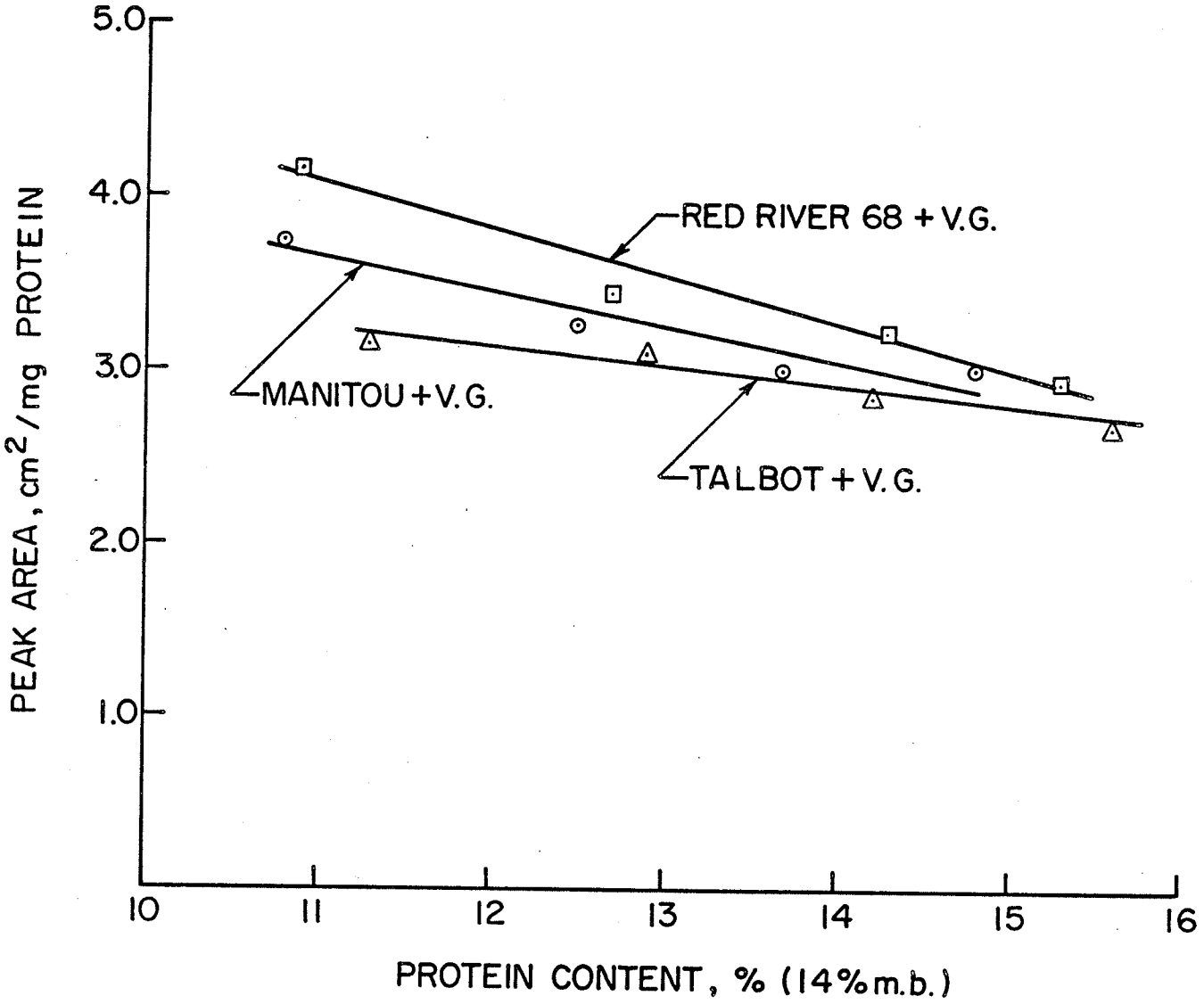

5.0
4.0
3.0
2.0
1.0
0
PEAK AREA, cm²/mg PROTEIN
MANITOU
10
11
12
13
14
15
PROTEIN CONTENT, % (14% m.b.)

FIG. 10 EFFECT OF PROTEIN CONTENT ON DTA PEAK AREA OF THREE FLOURS CONTAINING ADDED VITAL GLUTEN (V.G.).


5.0
4.0
3.0
2.0
1.0
PEAK AREA, cm²/mg PROTEIN
RED RIVER 68 + V.G.
MANITOU + V.G.
TALBOT + V.G.
10
11
12
13
14
15
16
PROTEIN CONTENT, % (14% m.b.)

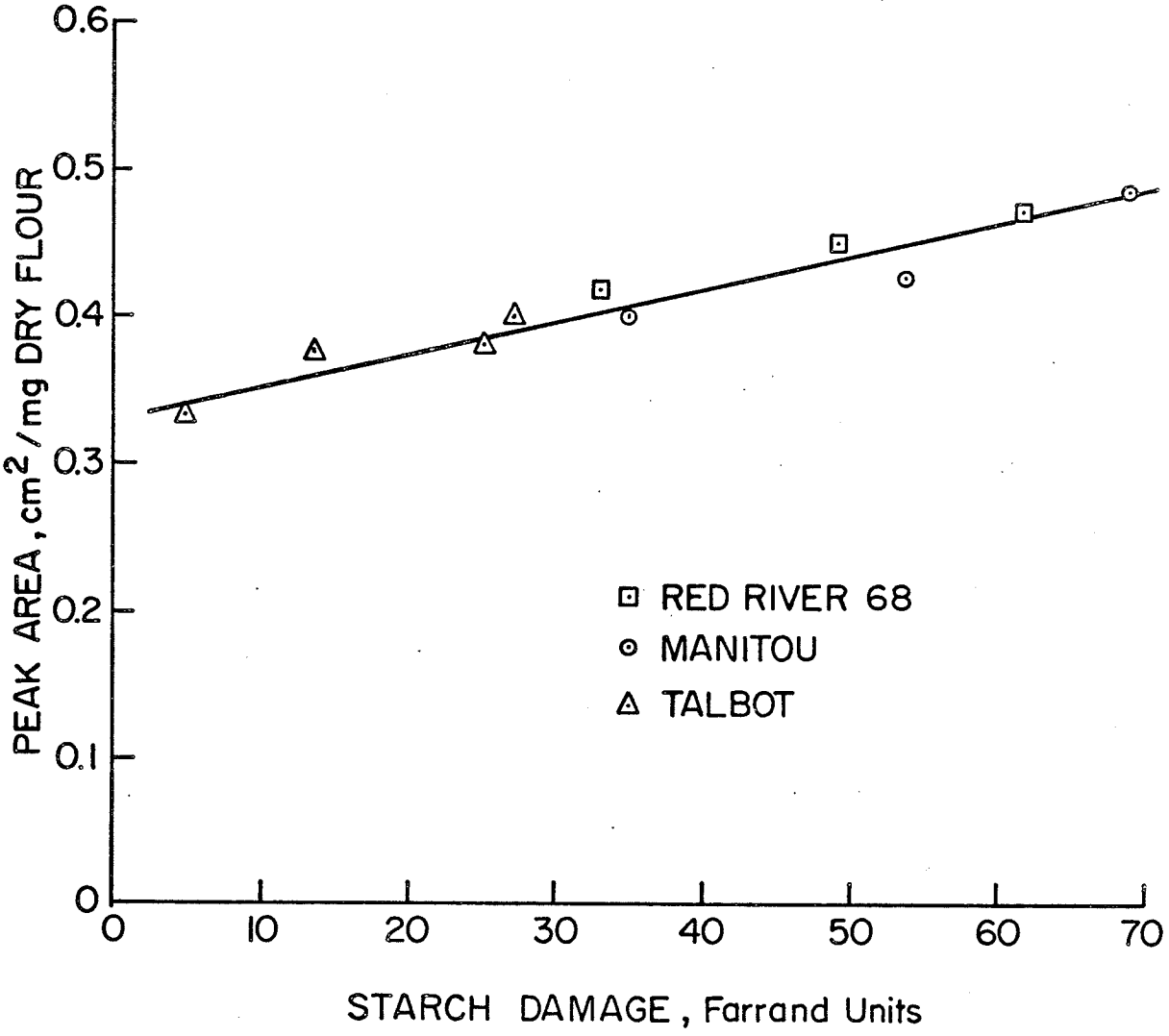
decreased with increasing protein content. Accordingly it would appear that indirect relationship shown in Fig. 9 and 10 results from the decrease in the percentage of damaged starch (flour basis). These results show that flour proteins bind water (in dough) more strongly than granular starch but less strongly than physically damaged starch. It would be useful to confirm this conclusion by measurements on granular and damaged wheat starch and vital gluten.

d. Effect of Starch Damage in Flour. The above observations on the possible role of damaged starch were examined further using flours of the three wheat varieties with different levels of starch damage produced by ball and pin milling. The results obtained are shown in Fig. 11.

Figure 11 shows that with increase in damaged starch, the peak area increased for three varieties. Furthermore, the binding energy increased essentially linearly with increasing level of damaged starch, irrespective of the mixing strength of the flour. It can therefore be concluded that damaged starch is extremely important in water binding in dough particularly since it appears to bind water more strongly than protein. This fact was not apparent from previous studies of water binding in flour and dough although it is generally known that damaged starch can absorb considerably more water than granular starch and slightly more than vital gluten.

e. Effect of Water-Soluble Pentosans. The three flours (varieties) used for DTA study of water binding were analyzed for water-soluble (WS)

FIG. 11 EFFECT OF DAMAGED STARCH ON DTA PEAK AREA FOR THE THREE WHEAT VARIETIES.


0.6
0.5
0.4
0.3
0.2
0.1
0
PEAK AREA, cm² / mg DRY FLOUR
RED RIVER 68
MANITOU
TALBOT
0
10
20
30
40
50
60
70
STARCH DAMAGE, Farrand Units

pentosans using the modified Baker's method (Baker et al. 1943). The purpose of these analyses was to examine if the difference in water binding energy of the three flours could be related to their WS pentosans content.

The analyses showed that Red River 68 had 0.67%, Manitou had 0.60% and Talbot had 0.51% WS pentosans. These results are consistent with those of Neukom et al. (1967) who have reported that the WS pentosans of wheat flour range between 0.5 to 0.8%.

Although the three varieties do not differ markedly in pentosans content, the trend parallels the trend of the boiling curve peak areas (Fig. 7). The variety that had the highest peak area also had highest WS pentosans content. These results are in general agreement with the hypothesis that the pentosans content of wheat flour is an important factor in water binding in dough.

The effect of WS pentosans was examined further by incorporating different amounts of WS pentosans in doughs from flours of the strongest and the weakest varieties (Red River 68 and Talbot). The amount of pentosans available was sufficient only for experiments with two of the three wheat varieties. The difficulty of blending cotton-like WS pentosans material with flour was solved by prior rehydration of the pentosans as suggested by Kulp (1968). The doughs were mixed at a constant water absorption of 60%. Farinograph results for both varieties showed that dough consistency increased markedly with increasing amount of pentosans added. At the higher consistencies, longer development times and greater stabilities than for control dough were obtained. Similar results on the

effect of pentosans on farinograph properties of doughs were published by Jelaca and Hlynka (1971).

Figure 12 shows that the boiling curve DTA peak area increased essentially linearly with the amount of pentosan added. The rate of increase for the two varieties investigated was about the same. The stronger variety retained the initial advantage over the weaker variety at the supplemented level of pentosan. These results show that pentosans not only increase the water binding capacity of flour (as is well known) but they also increase the binding energy. Accordingly this minor component of flour appears to be extremely important in relation to water absorption in doughs.

Studies on water-insoluble pentosans were not included in this thesis project because there is no method available for preparing relatively pure insoluble pentosan. One preparation was carried out but the insoluble pentosan obtained contained too high a proportion of starch and was considered unsuitable for DTA studies.

f. Effect of Mixing Time. This experiment was included to investigate the possibility that mixing breakdown (over-mixing) of dough produces an increase in the amount of free water or water of mobility in dough as suggested by Hlynka (1959). For these experiments, doughs were subjected to different mixing times in the farinograph mixer at a constant water absorption of 60%.

The effect of mixing on the boiling curve DTA peak areas is shown in Figure 13. Peak areas per mg. of dry flour are plotted as a function

FIG. 12 EFFECT OF ADDED WATER-SOLUBLE PENTOSANS ON DTA PEAK AREA OF RED RIVER 68 AND TALBOT FLOURS.

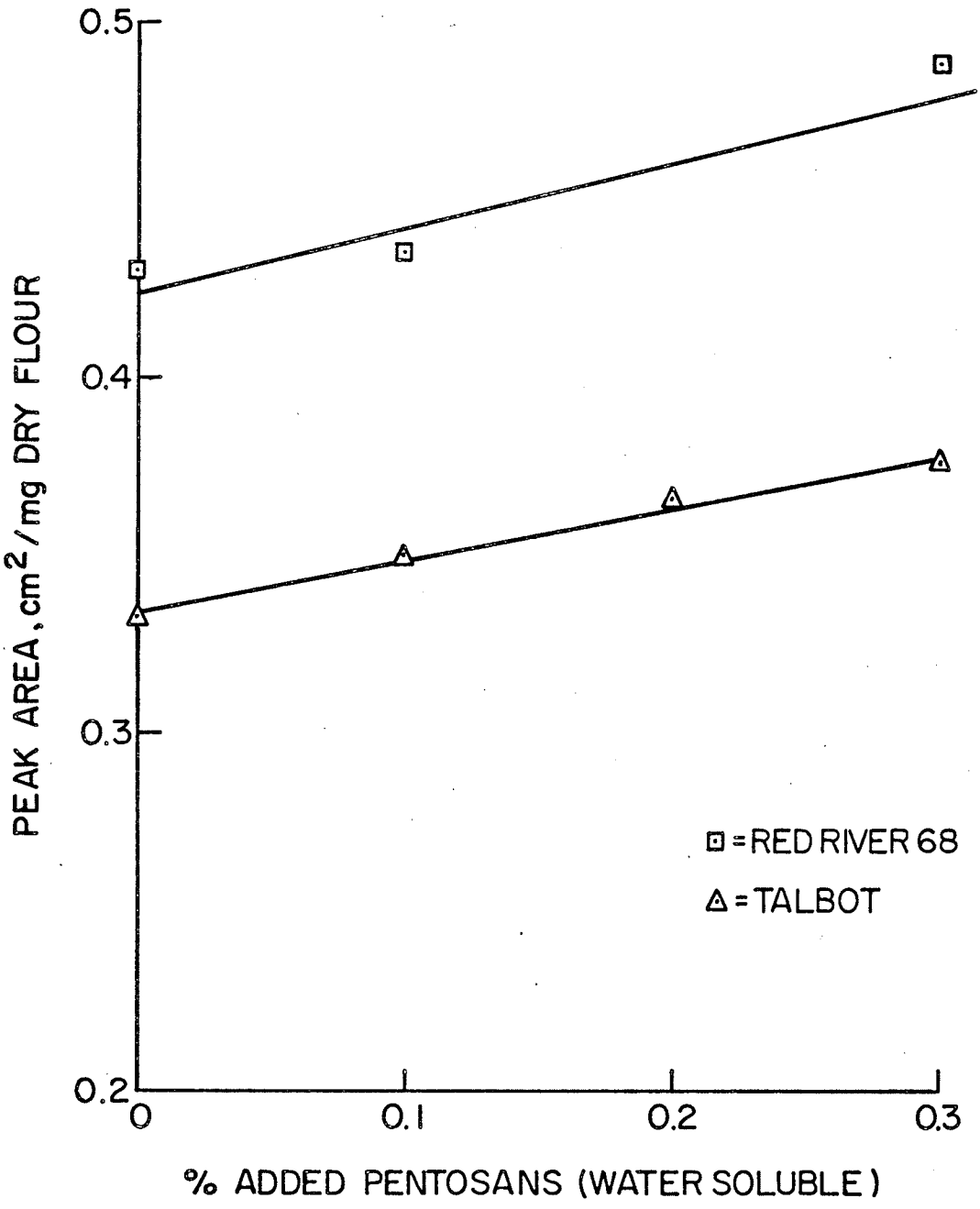
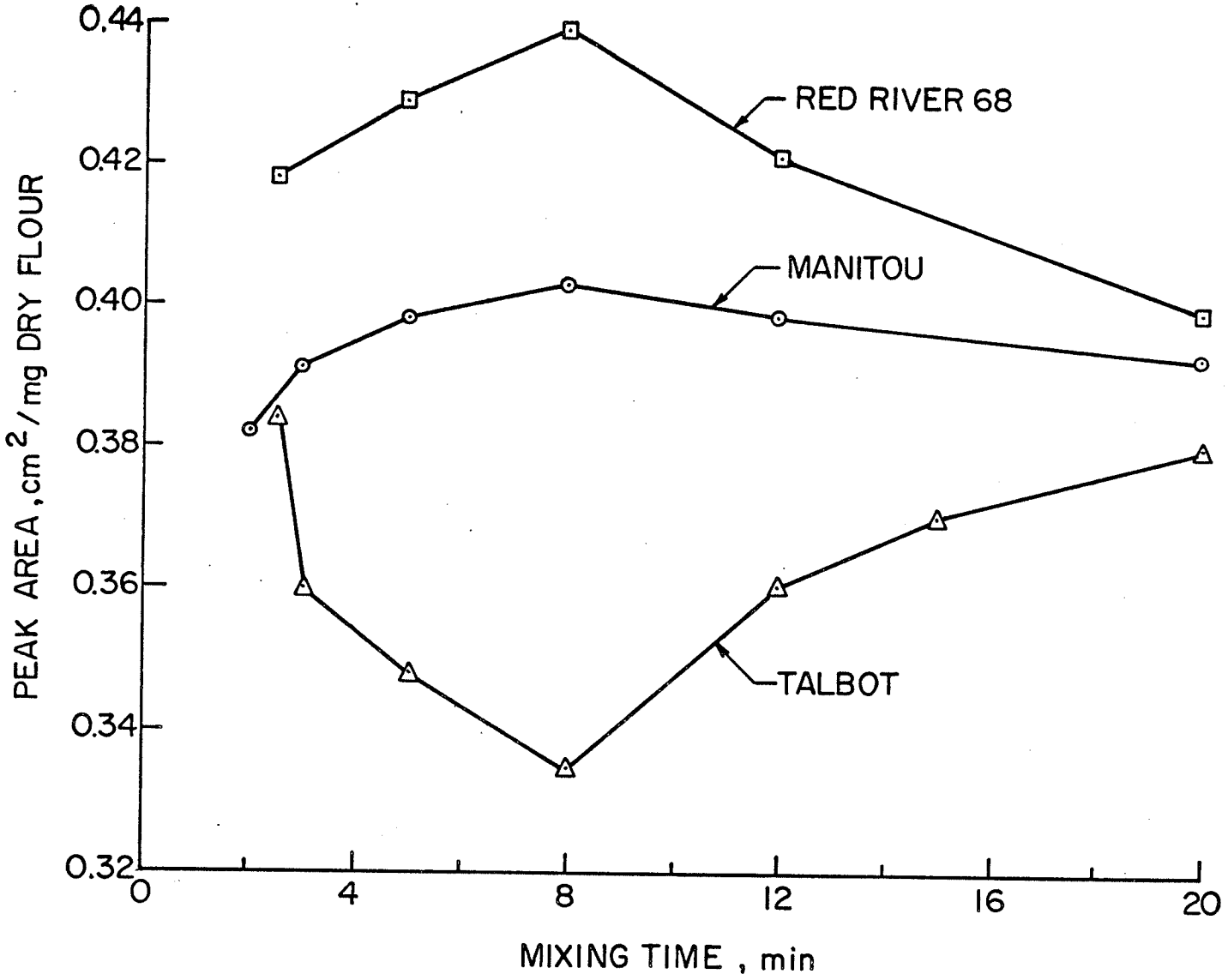

0.5
0.4
0.3
0.2
PEAK AREA, cm²/mg DRY FLOUR
⊡ = RED RIVER 68
△ = TALBOT
0
0.1
0.2
0.3
% ADDED PENTOSANS (WATER SOLUBLE)

FIG. 13 EFFECT OF FARINOGRAPH MIXING TIME ON DTA PEAK AREA FOR THE THREE WHEAT VARIETIES.


0.44
0.42
0.40
0.38
0.36
0.34
0.32
PEAK AREA, cm² / mg DRY FLOUR
RED RIVER 68
MANITOU
TALBOT
0
4
8
12
16
20
MIXING TIME , min

of mixing time.

The top curve is for the strongest wheat variety, the middle curve for the medium strength variety, and the bottom curve is for the weakest variety. The two stronger varieties behaved more or less as expected; the endothermic peak area curve followed the mixing curve (farinogram) indicating that the energy of water binding at first increased with mixing time but later decreased with additional mixing. The peak area-mixing time curve for these varieties is analogous to the farinograph consistency-mixing time curve. In the case of Talbot, the weakest variety, the peak area decreased sharply from the beginning of mixing, later levelled-off, and then showed an increasing tendency. There is no obvious explanation for the behaviour of the weakest variety except that it may be related to the extremely low level of damaged starch in this flour. This point is open to further investigation.

The results for the two stronger varieties are generally consistent with the published work of Webb et al. (1970) who showed (with the extensigraph) that the input of mechanical energy into dough by mixing decreased the resistance to extension and presumably decreased the binding of water. The actual relationship between free and bound water in dough and its extensigraph properties has not been investigated.

g. Effect of Added Chemicals. It has been suggested by various workers (Mecham et al. 1962, Tsen 1967, Tanaka and Bushuk 1973C) that over-mixing of wheat-flour dough may decrease the water holding capacity of dough because of the breakdown of the gluten complex. Furthermore this

effect appears to be more severe in the presence of thiol-blocking agent N-ethylmaleimide (NEMI), reducing agent L-cysteine, oxidizing agent potassium iodate, ascorbic acid (shows both oxidizing and reducing actions in dough) and sodium chloride.

In these experiments, the chemicals except salt were added to dough before the beginning of mixing at a rate of 2 μeq. per g. flour. This concentration was selected to represent approximately 3 times the molar equivalent of accessible -SH content of wheat flours in dough (0.7 μeq. per g. of flour according to Bushuk (1961)). Salt (sodium chloride) was added at 2% level (flour basis). The doughs were mixed at a constant water absorption (60%) for 20 min. to allow sufficient time for the added chemicals to react. Figures 14, 15 and 16 show the mixing curves (farinograms) for Talbot, Manitou and Red River 68 flours respectively, with and without added chemicals. The effects of the chemicals on water binding will be discussed in separate sections that follow.

(1) Effect of Sodium Chloride. As can be seen in Figs. 14, 15 and 16, addition of sodium chloride (salt) tends to displace the farinograph curves downward. In otherwords, it tends to decrease dough consistency. These observations on the effect of salt on the farinogram are the same as those reported by Bennett et al. (1965) and Hlynka (1962). To explain these farinograph results, it was suggested that salt competes with bound water for some of the interaction sites and thereby displaces some of the bound water. The net effect of this is an increase in the amount of free water or water of mobility and hence an increase in mobility. If this is the mechanism, then it should be possible to detect a change in water

FIG. 14 FARINOGRAMS OF TALBOT FLOUR AT 60% ABSORPTION, WITH AND WITHOUT ADDED CHEMICALS.

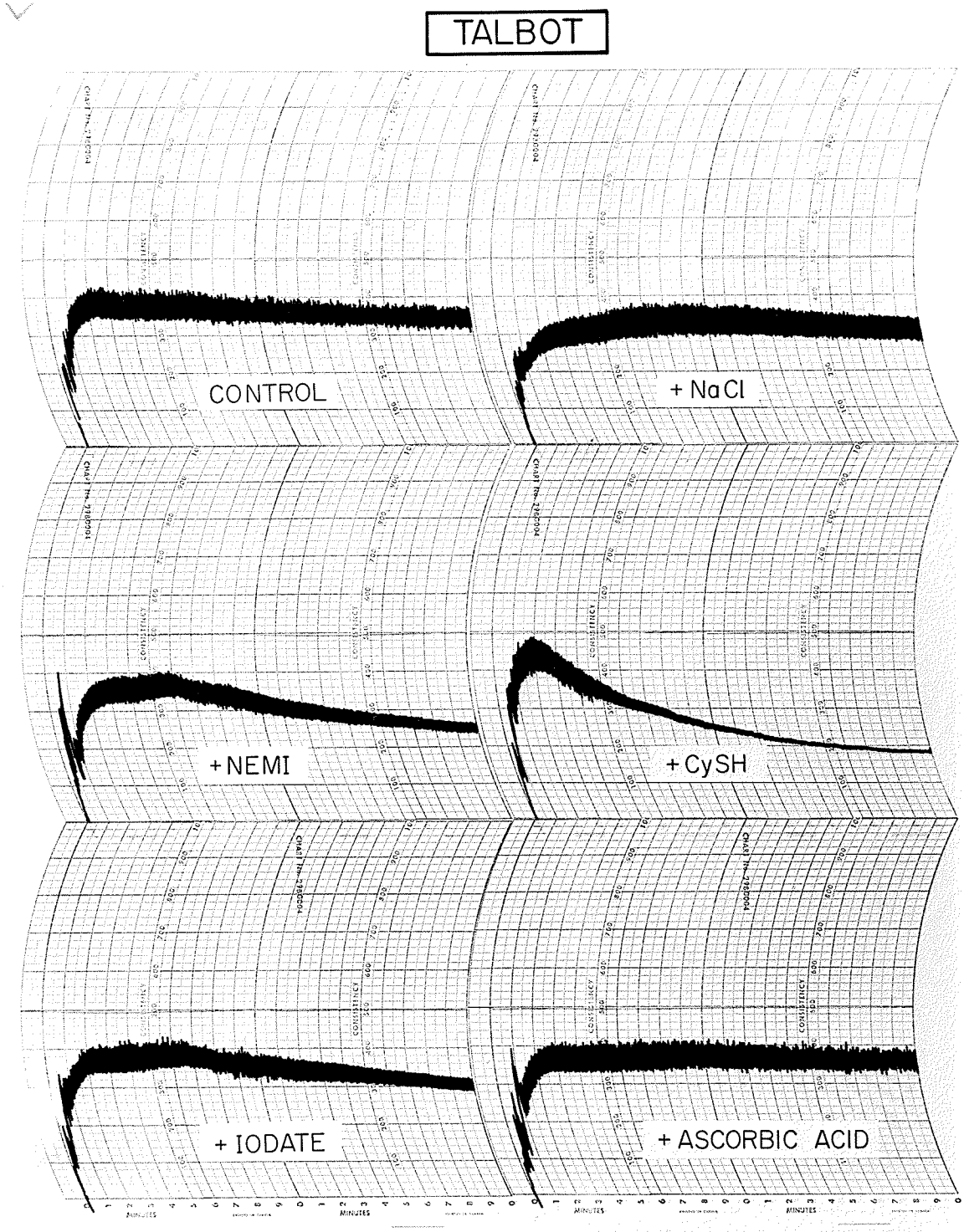

TALBOT
CONTROL
+ NaCl
+ NEMI
+ CySH
+ IODATE
+ ASCORBIC ACID
MINUTES

FIG. 15 FARINOGRAMS OF MANITOU FLOUR AT 60% ABSORPTION, WITH AND WITHOUT ADDED CHEMICALS.

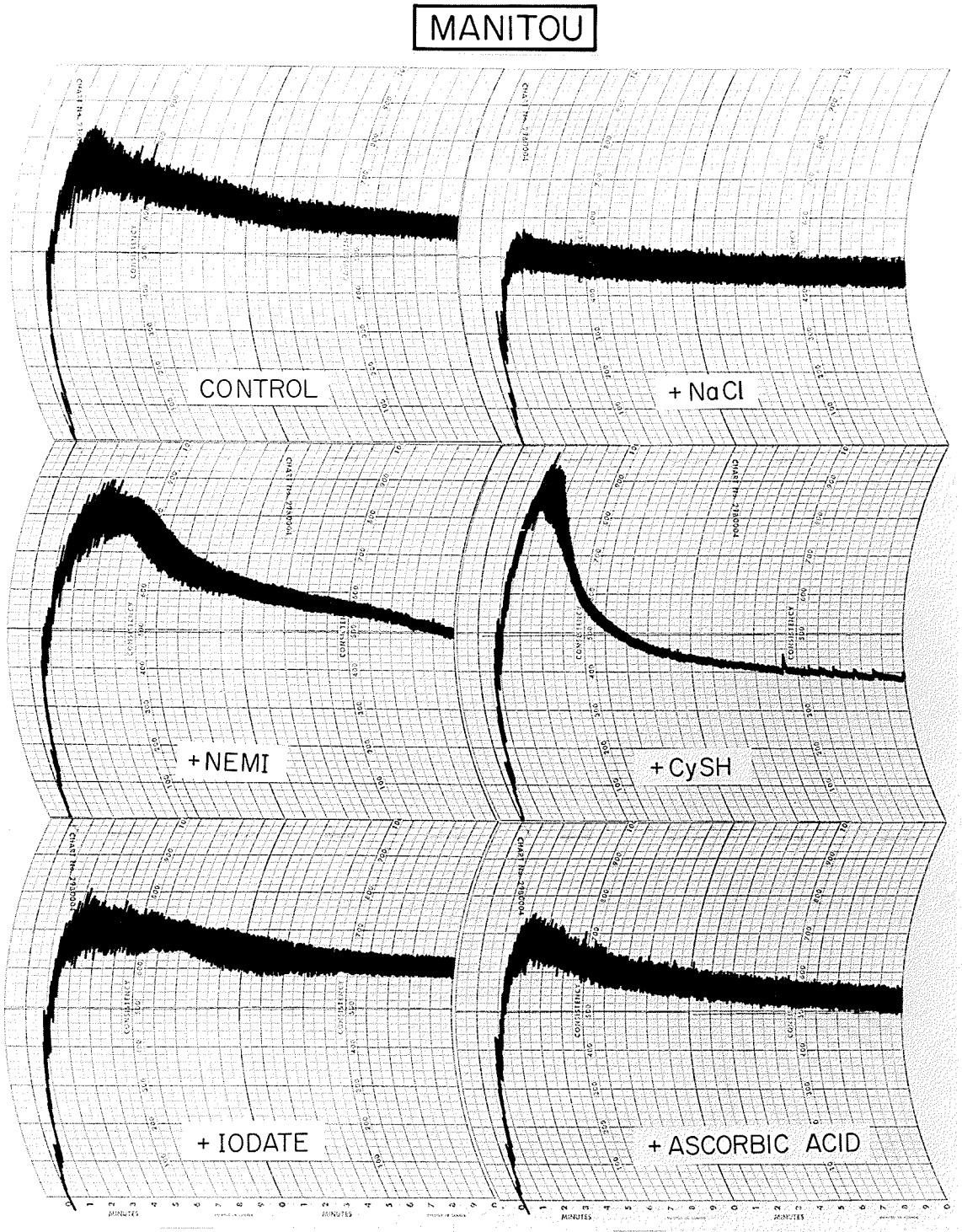
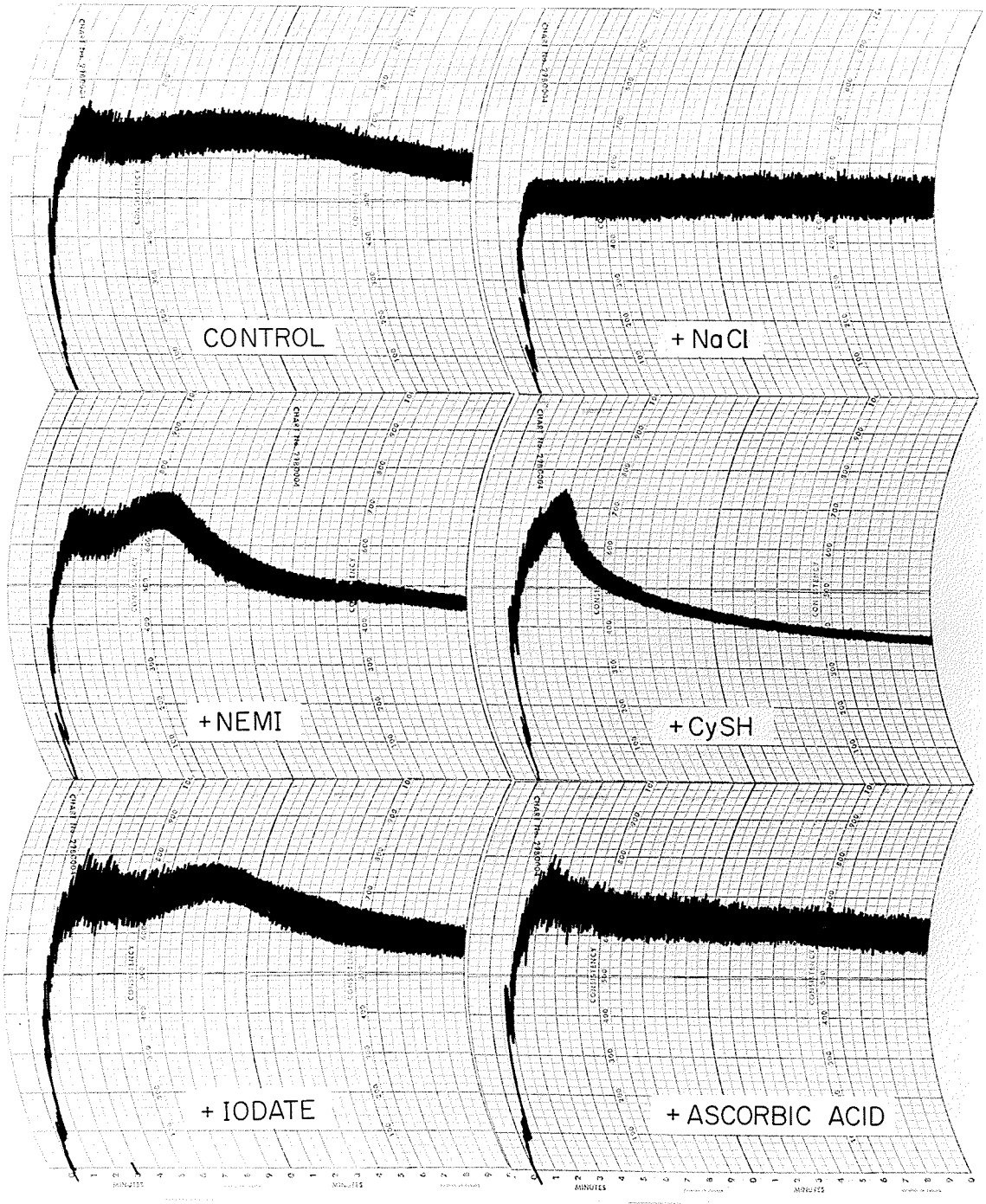

MANITOU
CONTROL
+ NaCl
+ NEMI
+ CySH
+ IODATE
+ ASCORBIC ACID
MINUTES

FIG. 16 FARINOGRAMS OF RED RIVER 68 FLOUR AT 60% ABSORPTION, WITH AND WITHOUT ADDED CHEMICALS.

RED RIVER 68



binding (on the addition of salt) by DTA.

Results for the three flours (Table 4) showed a considerable drop in boiling curve peak area on the addition of 2% salt. According to the basic assumption, salt decreases the energy of water binding in dough. The effect was more pronounced in the dough of the stronger flours than in that of weaker flour. The decrease in peak area for Talbot, Manitou and Red River 68 was 8.5, 10.1 and 12.7% respectively. It is obvious that the magnitude of the effect of added salt in doughs is strongly dependent on variety. It is not possible to formulate an all-inclusive theory on the salt effect in doughs on the basis of current knowledge. This should be a fruitful area for further research.

(2) Effect of N-ethylmaleimide. The effect of N-ethylmaleimide (NEMI) on farinograph mixing properties (see Figs. 14, 15 and 16) was the same as obtained by Tanaka (1972). Addition of NEMI increased the rate of dough breakdown after optimum development. In stronger doughs, it also slowed down the rate of attainment of maximum consistency.

Table 4 shows that the addition of NEMI decreased the endothermic peak area by 0.9%, 3.2% and 3.2% for Talbot, Manitou and Red River 68 doughs respectively. It is noteworthy that the decrease is substantially greater for the two stronger flours than for Talbot. These observations are analogous to those of Tanaka and Bushuk (1973A) who showed that the effect of NEMI on depolymerization of glutenin during mixing was greater in stronger varieties.

TABLE 4. EFFECT OF ADDED CHEMICALS ON PEAK AREAS OF THE THREE FLOURS
(Standard deviation of peak areas is 5%)

	TALBOT		MANITOU		RED RIVER 68	
	P.A.[a]/mg.D.F.[b]	% decrease or increase	P.A./mg.D.F.	% decrease or increase	P.A./mg.D.F.	% decrease or increase
Control	0.3797	-	0.3925	-	0.3988	-
Sodium chloride	0.3473	- 8.5	0.353	- 10.1	0.348	- 12.7
NEMI	0.3764	- 0.9	0.380	- 3.2	0.3861	- 3.2
Cysteine	0.3679	- 3.1	0.3784	- 3.6	0.3712	- 6.9
Iodate	0.3646	- 4.0	0.3727	- 5.1	0.377	- 5.5
Ascorbic acid	0.4167	+ 9.7	0.4209	+ 7.2	0.4284	+ 7.4

[a] P.A. = peak area

[b] D.F. = dry flour

The effect of NEMI on water binding in dough can be readily explained on the basis of the deploymerization mechanism postulated by Tanaka and Bushuk (1973C). The depolymerization of glutenin, which is greater in the presence of NEMI, would decrease the energy of water binding by this protein.

(3) Effect of Cysteine. Addition of cysteine (which acts as a S-S reducing agent) produced the most extensive dough breakdown of all chemicals studied in all three varieties (see Figs. 14, 15 and 16). The decrease in the peak areas relative to control were 3.1, 3.6 and 6.9% for Talbot, Manitou and Red River 68. It appears that here too, there is a notable varietal effect. This may be due to possible differences in the physicochemical properties (quality) of gluten proteins of different common wheat varieties.

The observed decrease in peak area was greater with cysteine than with NEMI. On an equivalent concentration basis, cysteine affects water binding much more than NEMI. This is not surprising since cysteine acts directly on S-S bonds whereas NEMI reacts first with -SH and the excess NEMI catalyzes the reduction of S-S bonds (Tanaka and Bushuk 1973C).

The doughs from the stronger varieties showed a higher decrease in peak area (greater decrease in energy of water binding) suggesting that proteins in the stronger flours are more highly crosslinked (probably by disulfide bonds). Confirmation of this suggestion must await further results on the secondary and tertiary structure of glutenins and varietal differences in these structures.

(4) Effect of Iodate. The effect of iodate on farinograph mixing properties was the same as reported by others (Meredith and Bushuk 1962; Tanaka 1972). Like NEMI, iodate also increased the rate of mixing breakdown after the optimum. As noted before (Meredith and Bushuk 1962; Tanaka 1972) iodate-treated doughs showed the additional effect of oxygen which was not apparent with NEMI.

Table 4 gives the DTA peak areas per mg. dry flour for the three varieties. Addition of iodate in all three produced about 5% decrease in peak area. The effect of iodate (in the presence of atmospheric oxygen) is similar to that of NEMI and can be explained by the same mechamism involving oxidative cleavage of S-S bonds of glutenin and thereby decreasing the ability of the protein network to bind water.

On the basis of the above results, it is postulated that the decrease in the energy of water binding caused by iodate and NEMI involves breakdown of S-S bonds. The similarity of the effects of iodate and NEMI and cysteine indicates that S-S bonds are probably involved (directly or indirectly) in the binding of water in wheat flour doughs.

(5) Effect of Ascorbic Acid. Ascorbic acid is also used as a bread improver especially in "no time" dough methods. It has certain advantages over other bread improvers such as potassium bromate, potassium iodate, etc. Zentner (1968) observed that doughs with ascorbic acid are generally softer, but feel drier to the touch than doughs containing other improvers. Because of these differences between the effects of ascorbic acid and oxidizing improvers (eg. potassium bromate or potassium iodate), it was of

interest to compare the effect of ascorbic acid on water binding as measured by DTA with that of potassium iodate presented above.

Results in Table 4 show that the addition of ascorbic acid increased the DTA peak area for all three varieties. The increase was slightly greater for the weakest flour than for the two stronger flours. The results obtained with ascorbic acid are quite interesting because the addition of the other chemicals used in this study generally produced a decrease in water binding energy.

The farinograms for doughs containing ascorbic acid in Figs. 14, 15 and 16 do not show very significant decrease in consistency after 20 min. of mixing. Indeed the final consistency of ascorbic acid-treated doughs are actually slightly higher than that of the appropriate control dough. Obviously the action of ascorbic acid in doughs is quite different from that of the other chemicals used in this study. It was quite surprising that its effect was different from that of iodate since the two chemicals have a similar positive effect on loaf volume although their effects on the handling properties of dough appear to be different (Zentner 1968). The mechanism of the ascorbic acid effect in dough deserves further work.

Relative to the apparent difference in the effects of ascorbic acid and iodate (and NEMI), Zentner (1968) showed that ascorbic acid in dough does not reduce S-S bonds in flour proteins nor does it block -SH groups. The present study indicates that it opens up some new sites for binding of water in dough. The nature of these sites remains to be investigated.

The foregoing results of added chemicals (except ascorbic acid) on DTA water binding show that the magnitude of the effect of these chemicals depends on the mixing strength of the flour (wheat variety). In doughs of the weakest variety, the additional effect of the additive was small. It was greatest in doughs of the strongest flour, and intermediate in the case of the medium strength flour. These findings are in general consistent with the hypothesis that there are qualitative differences in the proteins of different wheat varieties that are partly responsible for differences in baking quality. The effects of ascorbic acid are extremely interesting but any explanation of its action in dough at this time would be quite speculative.

2. Melting Curve Results.

The method of determining bound water in dough (or bread) samples using the DTA melting curve is based on the assumption that bound water is that fraction of the total moisture in the sample which does not freeze at sub-zero temperatures. This method is used basically to determine the amount of freezable (or free) water in the sample and the amount of bound water is then determined by subtracting it from total moisture of the sample.

The results obtained using the melting curve method are presented and discussed in a series of subsections that follow.

a. Effect of Mixing Strength and Water Absorption. The effect of these two variables on bound water was studied using the same three wheat

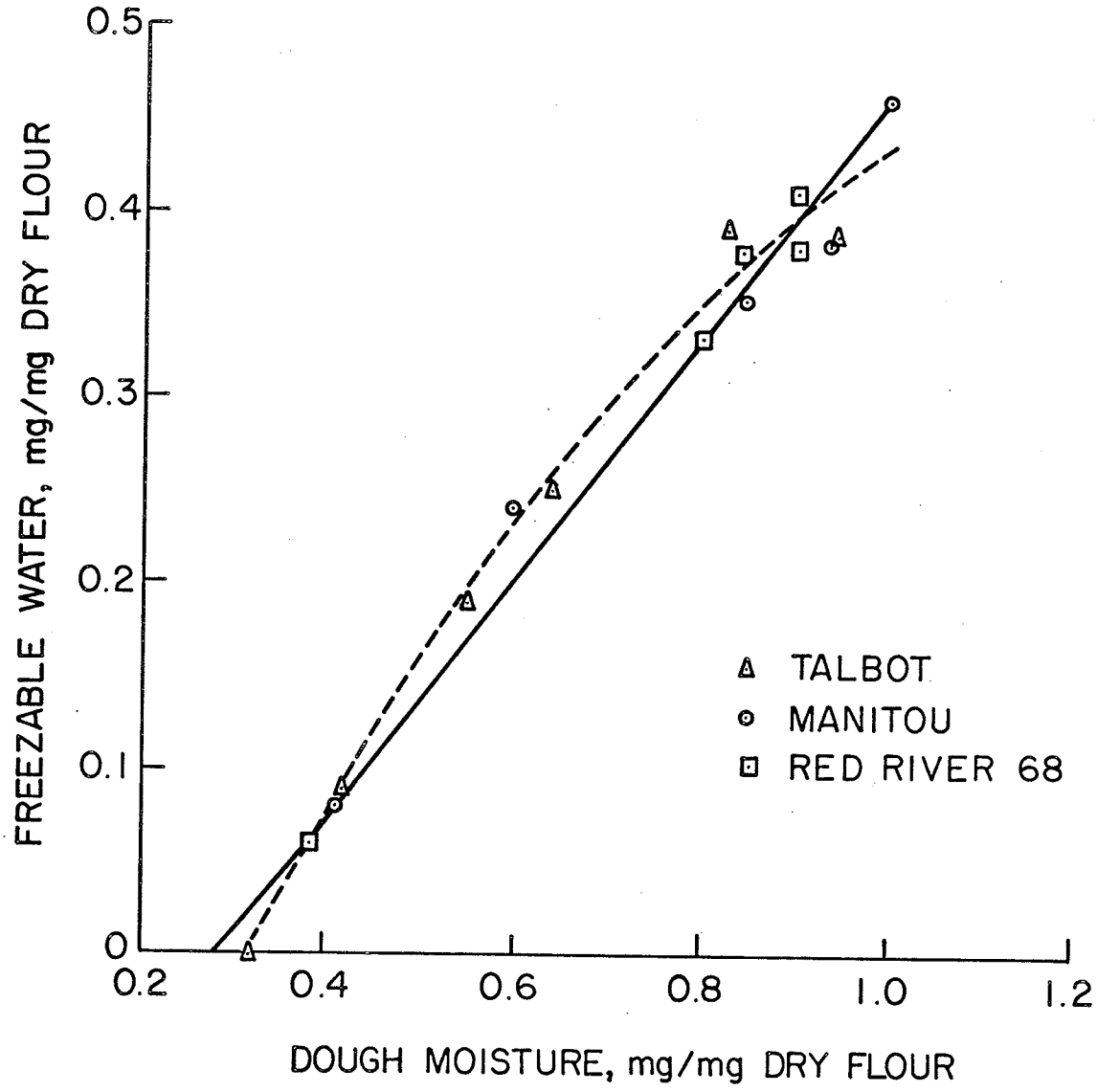
varieties, Talbot, Manitou and Red River 68, used for the boiling curve study. The doughs were mixed in the farinograph mixer for 8 min. using a fairly wide range of water absorption, the range being limited by the feasibility of mixing and ease of dough handling. The results obtained are presented graphically in Figs. 17 and 18.

Figure 17 shows that with increase in the amount of dough moisture, the amount of freezable water is zero up to a dough moisture of about 0.3 mg per mg. (dry flour basis) and then increases essentially linearly with moisture content. There is an indication that the data can also be fitted by a curve of slightly decreasing slope. The points for the three varieties, which differed markedly in their farinograph mixing strength, fell essentially on the same curve. That is, dough mixing strength had no effect on the relationship between the amount of freezable water and total moisture in the dough.

According to Fig. 17, the curve intersects the x-axis (dough moisture) at a value of about 0.3 mg per mg. Below this moisture level, all of the water is of the bound type. Actually when the dough containing 24.7% moisture (0.33 mg. per mg dry flour) was subjected to DTA, it did not show any melting peak indicating that there was no freezable water in this dough. This amount of bound water is considered to be bound to initially available binding sites on the flour.

If all of the moisture in the dough above 0.3 mg per mg was free water then the slope of the line in Fig. 17 should be equal to one. However this is not the case; the actual slope is about 0.65. This indicates that for each gram of water added to the dough, only two thirds is of the

FIG. 17 EFFECT OF WATER ABSORPTION ON FREEZABLE WATER CONTENT OF DOUGHS FROM THREE DIFFERENT WHEAT VARIETIES.

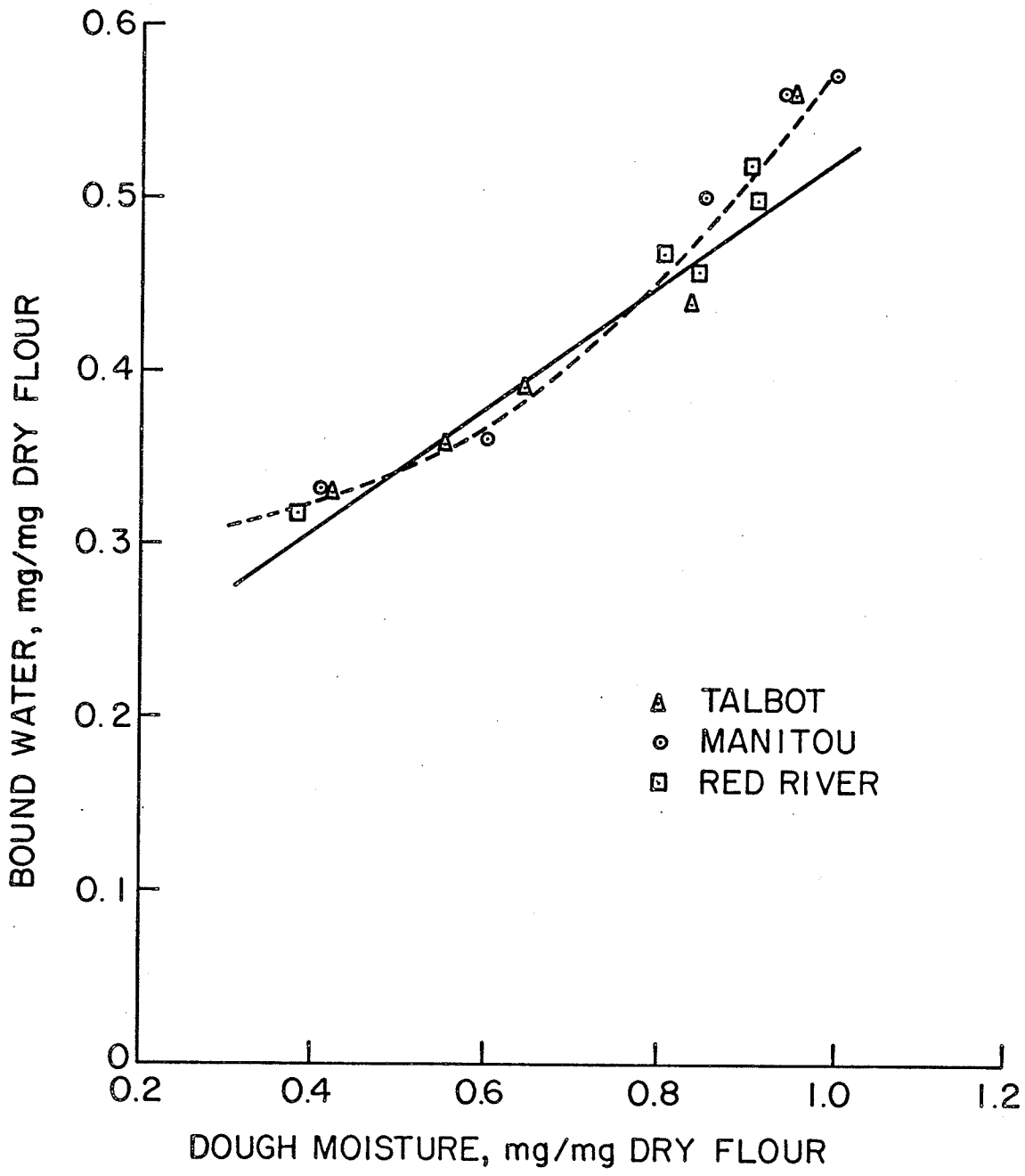

0.5
0.4
0.3
0.2
0.1
0
FREEZABLE WATER, mg/mg DRY FLOUR
0.2
0.4
0.6
0.8
1.0
1.2
DOUGH MOISTURE, mg/mg DRY FLOUR
TALBOT
MANITOU
RED RIVER 68

free type and the rest is bound water. That is, in the region of moisture above 0.3 mg/mg (dry flour basis) the amount of bound water increases linearly with moisture. This increase in bound water with additional moisture can be explained on the hypothesis that the additional moisture makes available new sites for water binding that are not accessible initially. Moisture seems to be the key factor in opening up the new binding sites. Dough mixing (at a constant water absorption) does not produce any additional water binding sites (see below).

The results of the present study agree with those of Davies and Webb (1969), obtained by differential scanning calorimetry, in two aspects and disagree in one. The values obtained for the amount of bound water agree reasonably well (0.33 mg/mg by Davies and Webb and the minimum value of 0.3 mg/mg from the present study). Davies and Webb (1969) found that mixing strength (or baking quality) had no effect on the free water value; this was confirmed by the present study. The points for the three wheat varieties used in the present study fell on the same curve (see Fig. 17).

The two studies disagree on the effect of additional dough moisture on bound water beyond the minimal level obtained by extrapolation. Davies and Webb (1969) reported that all of the moisture above the minimal value was of the freezable or free type. In contrast, the present study showed that only about two thirds of the additional water was of freezable type and the remaining was of the bound type. The only reason that can be given for this discrepancy is that the two studies used different modes of thermal analysis. Further research is necessary to clarify this point.

FIG. 18 EFFECT OF WATER ABSORPTION ON BOUND WATER CONTENT OF DOUGHS FROM THREE WHEAT VARIETIES.


0.6
0.5
0.4
0.3
0.2
0.1
0
BOUND WATER, mg/mg DRY FLOUR
0.2
0.4
0.6
0.8
1.0
1.2
DOUGH MOISTURE, mg/mg DRY FLOUR
TALBOT
MANITOU
RED RIVER

Another way of presenting DTA data on water binding is by plotting the amount of bound water against dough moisture. This is obtained by subtracting the freezable water shown in Fig. 17 from the total moisture in the dough. Figure 18 shows such a plot of the data obtained in the present study. In this presentation of the data, the relationship is definitely curvilinear with the slope increasing as the dough moisture increases. Since the sum of the curves in Figs. 17 and 18 must give a straight line with a slope of one, it would be more correct to use a curved line, instead of a straight line for the data of Fig. 17. However whether one uses a curve or a straight line does not alter the conclusions that can be drawn from the results obtained. Figure 18 shows that bound water increases with dough moisture somewhat more dramatically than Fig. 17.

b. Effect of Mixing Time. The effect of mixing time (in the farinograph mixer) was examined for the three wheat varieties using two different mixing times, 8 min. and 20 min. The results obtained (not shown in thesis) showed that mixing time does not affect the amount of bound water (at the one level of absorption investigated) as determined by the melting mode of DTA. This result was rather surprising in view of the results obtained in the experiments described in the previous subsection, however the line of research was not pursued any further.

c. Effect of Flour Protein Content. The effect of protein content on bound water by DTA was examined for one variety, Manitou, for which grain samples of different protein content were available and also for all three varieties where the protein content was increased by adding vital wheat gluten.

FIG. 19 EFFECT OF FLOUR PROTEIN CONTENT ON BOUND WATER IN MANITOU DOUGHS.

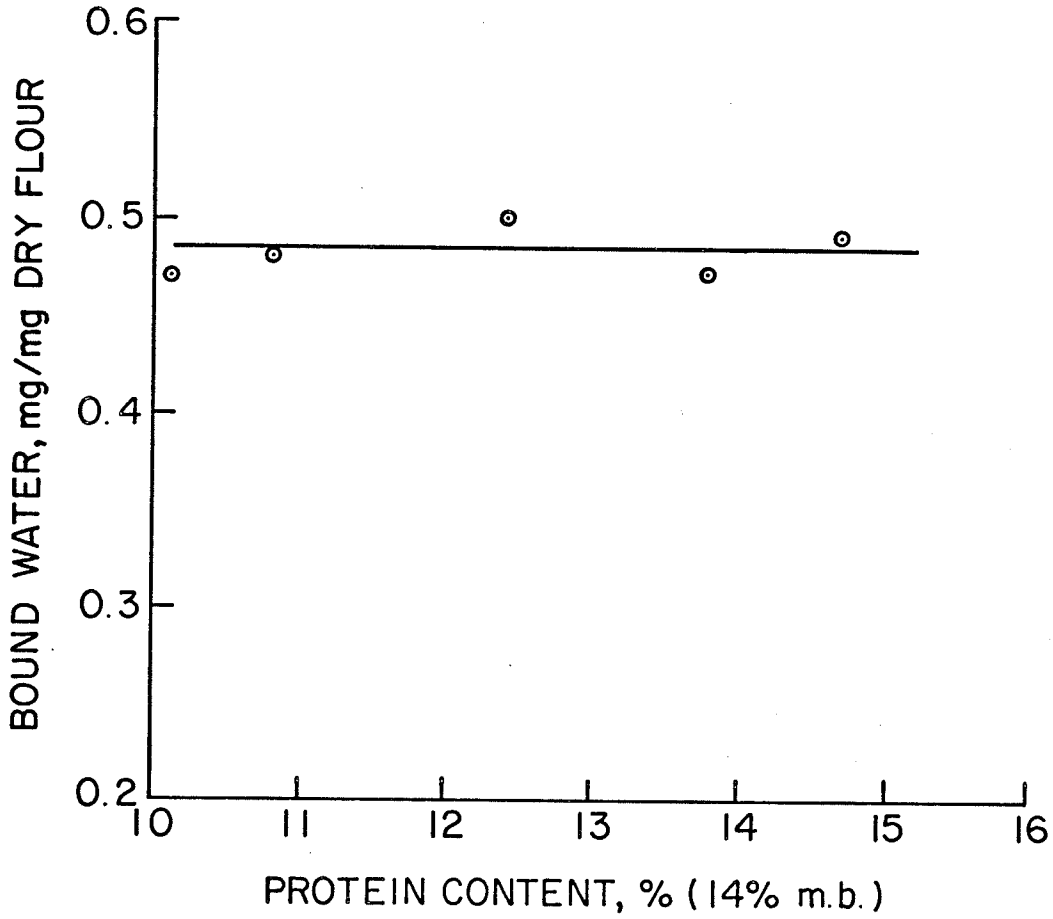
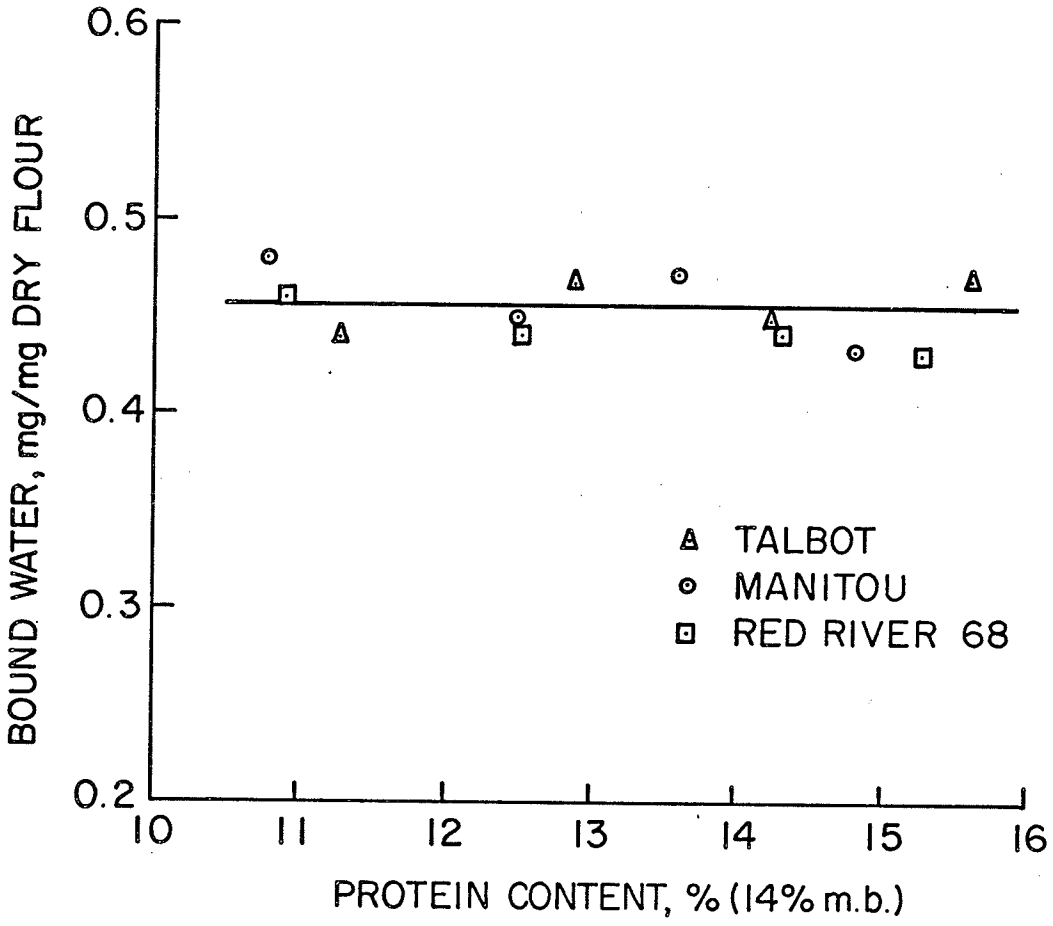

0.6
0.5
0.4
0.3
0.2
BOUND WATER, mg/mg DRY FLOUR
10
11
12
13
14
15
16
PROTEIN CONTENT, % (14% m.b.)

FIG. 20 EFFECT OF FLOUR PROTEIN CONTENT ON BOUND WATER IN DOUGHS OF THREE WHEAT VARIETIES WITH ADDED VITAL GLUTEN.

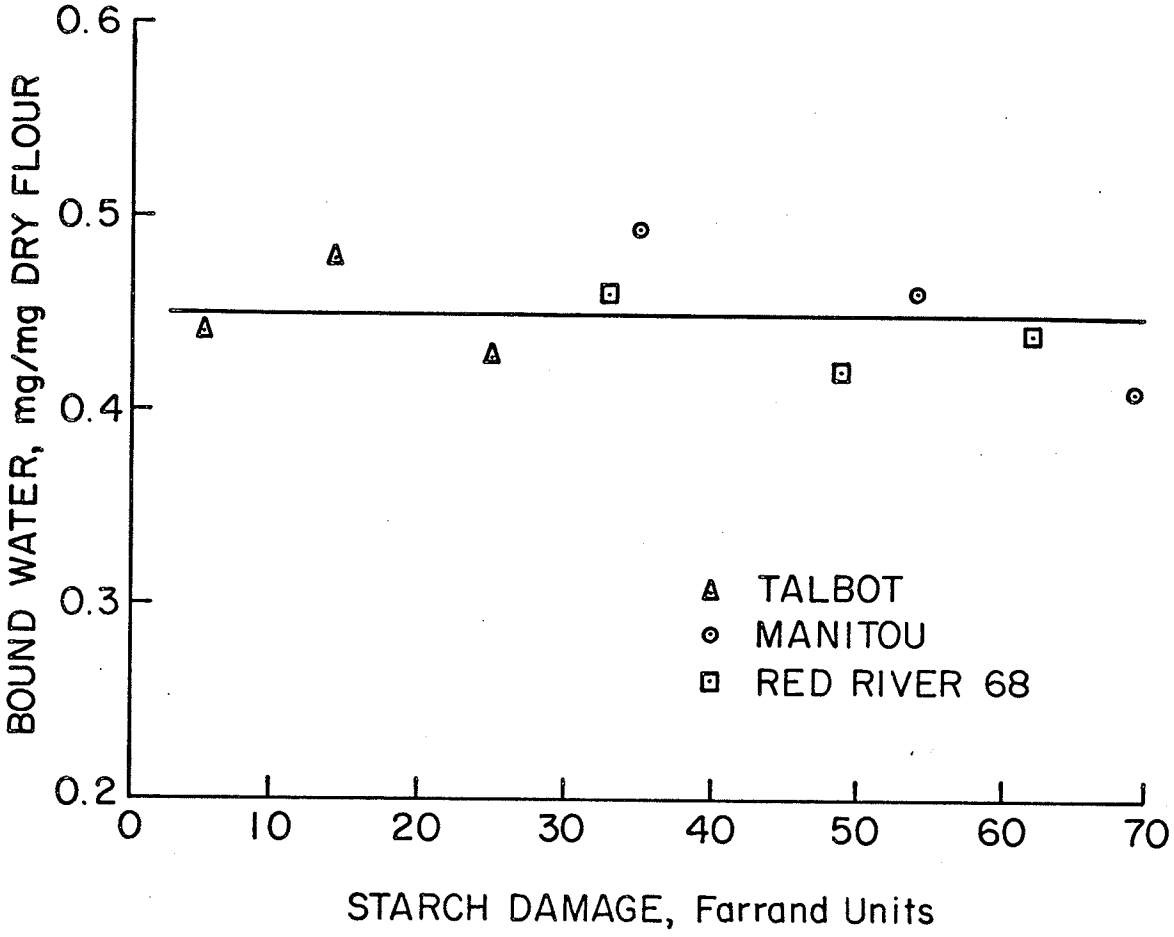

0.6
0.5
0.4
0.3
0.2
BOUND WATER, mg/mg DRY FLOUR
10
11
12
13
14
15
16
PROTEIN CONTENT, % (14% m.b.)
TALBOT
MANITOU
RED RIVER 68

When bound water content per mg. dry flour was plotted against flour protein content for the Manitou variety (Fig. 19) a straight line of zero slope was obtained indicating that there is no change in the amount of bound water with increase in protein content. Similar results were obtained when bound water content of the three flours supplemented with different amounts of vital gluten were plotted as a function of protein content (Fig. 20). It was rather surprising to observe that protein content of the flour had no effect on the amount of bound water in the dough particularly since it is well known that baking absorption usually increases with protein content. On the basis of results obtained, it would appear that the higher baking absorption of higher protein content flours results from the requirement of additional free or mobility water to maintain the desirable level of dough consistency.

d. Effect of Starch Damage. This effect was examined using pin milled flours of the three wheat varieties. The results obtained (Fig. 21) did not show any definite trend in the amount of bound water with increasing level of damaged starch. Accordingly, it appears that the higher farinograph (or baking) absorption required by flours with higher levels of damaged starch results from the requirement for more water of mobility rather than from an increase in the amount of bound water. The situation with respect to the effect of starch damage appears to be the same as that of increasing protein content.

e. Effect of Salt, Cysteine, N-ethylmaleimide and Iodate. Preliminary DTA analyses were made of doughs containing these chemicals. Salt

FIG. 21 EFFECT OF DAMAGED STARCH ON BOUND WATER CONTENT OF DOUGHS FROM THREE WHEAT VARIETIES.


0.6
0.5
0.4
0.3
0.2
BOUND WATER, mg/mg DRY FLOUR
0
10
20
30
40
50
60
70
STARCH DAMAGE, Farrand Units
TALBOT
MANITOU
RED RIVER 68

(sodium chloride) was added at 2% level and the other chemicals at 2 μeq. per g. flour. All doughs were mixed for 20 min. at 60% absorption.

Comparison of the DTA melting curves for these doughs with that of the control dough showed that the chemicals used had no effect on the amount of bound water as determined by the DTA technique. Accordingly this line of investigation was not pursued any further.

B. Water Binding in Bread

It has been suggested by several previous workers (Knjagincievv 1970; Zobel 1973) that detectable changes occur in the distribution of free and bound water during the post-baking storage of bread. Furthermore, it was emphasized that these changes could be used as an index of bread staling. Accordingly, it was of interest to investigate if the DTA melting curve method could be used successfully to determine the amount of bound water in bread and also to follow any change that occurs in the amount of bound water during post-baking storage. It was hoped that results of these experiments would be useful in providing information on the mechanism of water binding in bread and also on the extremely important commercial problem of bread staling.

Bound water in bread was determined using the DTA melting curve procedure already described. The results obtained are discussed in the following subsections.

a. Effect of Post-Baking Storage Time. To investigate the effect of post-baking storage time on bound water, freshly baked loaves were sealed

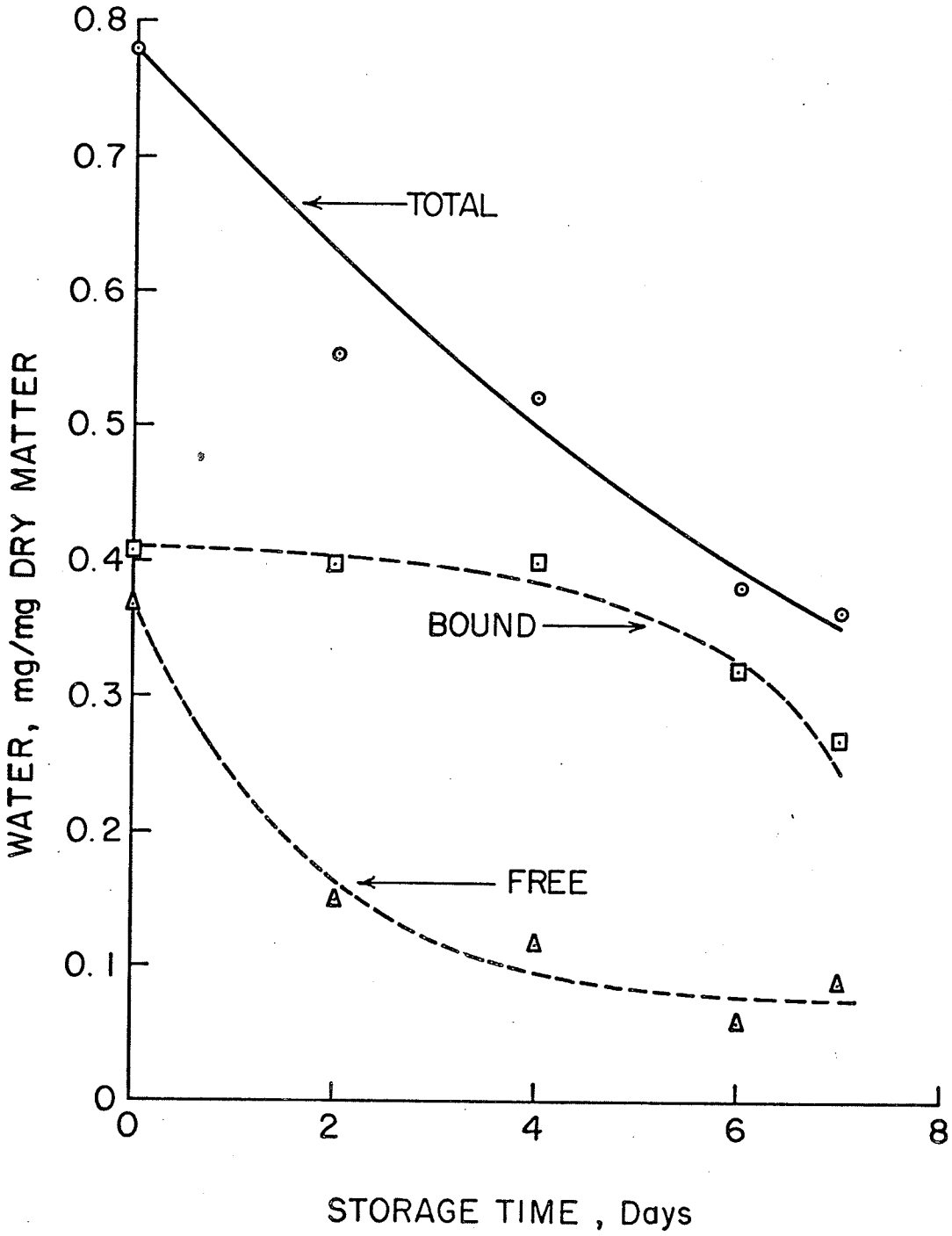
in polyethylene bags and were stored at room temperature (26°C). Unfortunately, the sample of flour that was used in the dough experiments was completely used-up and it was necessary to select another flour from a different variety of similar class. The only flour that was available at that time was that milled from hard red spring variety Pembina. This variety has slightly stronger farinograph mixing characteristics than Manitou, the previously used variety. Although it was realized that this change introduced another variable, it was hoped that the trend in DTA results would not be affected by variety (it will be seen later that this assumption is not entirely valid).

Bound water was determined in one set of bread samples at 0, 2, 4, 6 and 7 days after baking. The data obtained are presented in Fig. 22.

Figure 22 shows that with increase in post-baking storage time, the moisture content of the bread crumb shows an almost linear decrease. This decrease is probably due to the transfer of moisture from the crumb to the crust region of the loaf as reported by Yasunaga et al. (1968). Similarly, the amount of free water in the crumb also decreased with increasing storage time. The decrease was quite rapid during the first two to three days and then levelled-off so that there was essentially no change in the amount of free water from about four to seven days. The amount of bound water showed very little change (a small decrease) during the initial four days of storage. Beyond four days' storage there was a marked decrease in the amount of bound water.

The results of this experiment indicate that during post-baking storage, bread crumb continuously loses moisture. Initially, it is pri-

FIG. 22 EFFECT OF STORAGE TIME ON FREE, BOUND AND TOTAL WATER OF BREAD CRUMB FROM PEMBINA FLOUR.


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
TOTAL
BOUND
FREE
WATER, mg/mg DRY MATTER
0
2
4
6
8
STORAGE TIME , Days

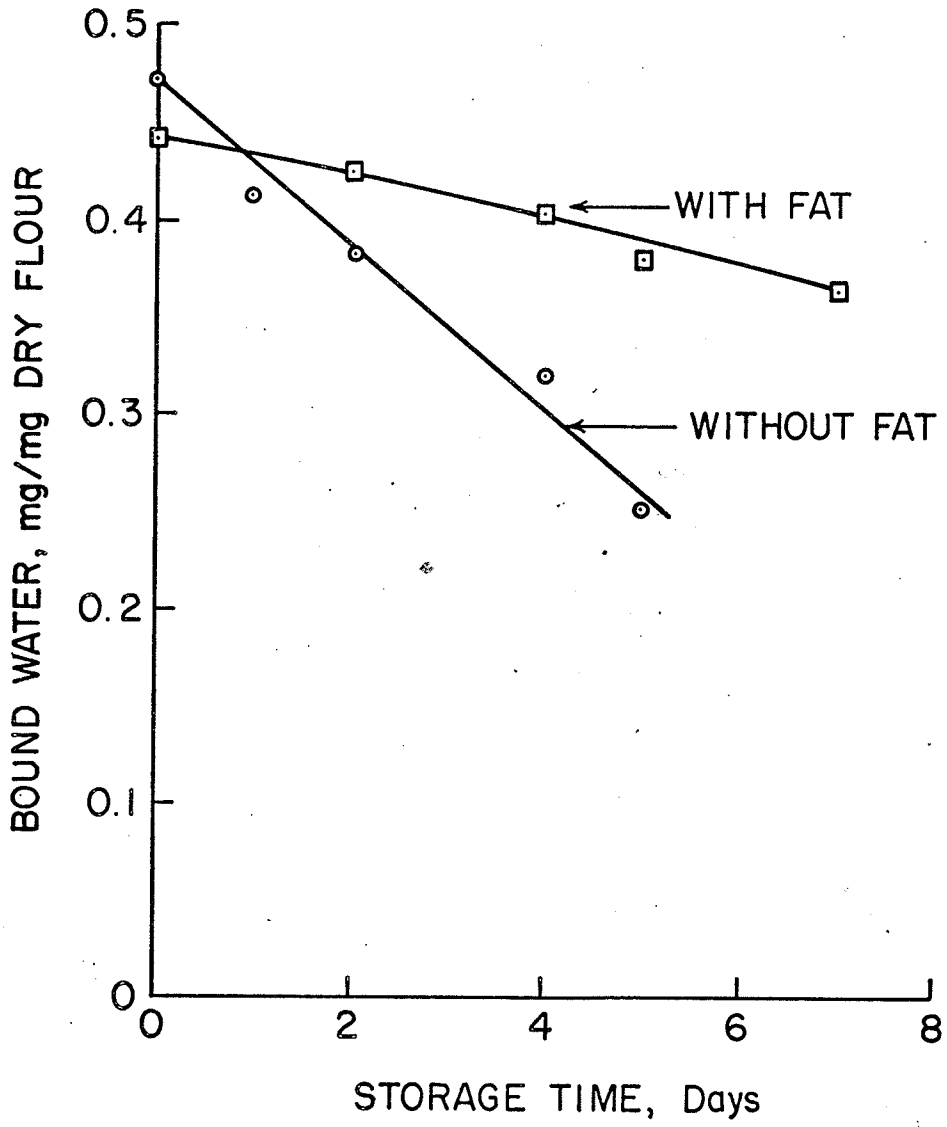
marily the free water of bread crumb which contributes to this loss of moisture but later when the amount of free water reaches a low level, bound water contributes to the total moisture lost. It is speculated that at the stage when there is a sharp decrease in the amount of bound water that staling of bread becomes particularly significant. It appears that free water must reach a critical low value before bound water is lost.

Further research is needed to show that staling of bread is related to the loss of bound water. If indeed this is the case then the DTA melting curve procedure can be used successfully as an index of bread staling. Furthermore, any factor that can slow down the rate of decrease of bound water in bread should be useful as an anti-staling agent. Some of these factors will be examined in the sections that follow.

b. Effect of Added Fat. It is common knowledge that inclusion of fat in bread formula decreases the rate of bread staling. Accordingly, the effect of fat on the amount of bound water, as determined by DTA melting curve, was investigated in bread stored up to seven days after baking. For these experiments, loaves were baked from Manitou flour (not Pembina as in the above experiment) containing 2% Crisco shortening and stored in polyethylene bags at room temperature ($26^{o}C$). Control loaves (without fat) were treated similarly.

Figure 23 shows that the loaves without added fat showed a more rapid drop in bound water over the range of storage time investigated when compared with bread containing added fat. The drop in bound water was quite small in loaves with added fat. That is, the loaves with fat retained com-

FIG. 23 EFFECT OF FAT AND STORAGE TIME ON BOUND WATER CONTENT OF BREAD CRUMB FROM MANITOU FLOUR.


0.5
0.4
0.3
0.2
0.1
0
BOUND WATER, mg/mg DRY FLOUR
WITH FAT
WITHOUT FAT
0
2
4
6
8
STORAGE TIME, Days

paratively more bound water and for longer time. This may be the main reason why loaves with added fat remain fresher for a longer time.

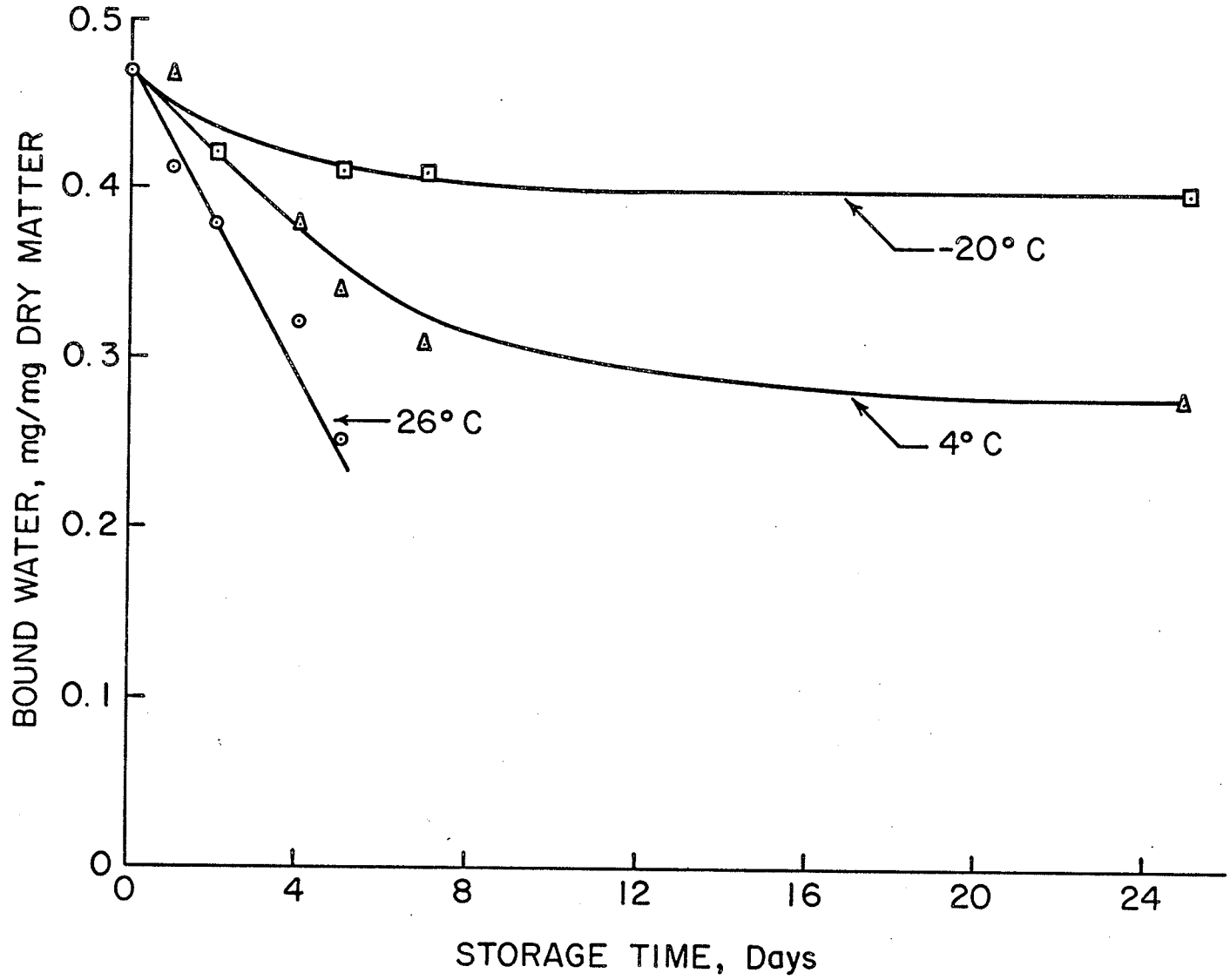
The effect of fat on the amount of bound water in bread crumb can be best explained by mechanism first proposed by Herz (1965). He suggested that fat forms a continuous layer on starch and protein particles of flour during dough mixing and thus helps to retain absorbed moisture (bound water) inside these structures.

If the results of Fig. 23 are compared with those of Fig. 22, it will be noted that the rates of decrease in the amount of bound water in the control loaves are quite different. Loss of bound water in crumb of bread from Pembina flour (Fig. 22) was considerably slower than that obtained for the bread from Manitou flour. The significance of this varietal effect was not investigated further in the present study.

c. Effect of Storage Temperature. The effect of storage temperature was studied using loaves stored at room temperature (26°C), in the refrigerator (4°C), and in a deep freezer (-20°C) for different periods. The results obtained are shown in Fig. 24. The top curve is for loaves stored at -20°C, the middle curve for loaves stored at 4°C, and the bottom one for the loaves stored at 26°C.

The effect of storage temperature on the decrease of bound water with storage time is quite marked. All loaves showed a fairly rapid initial loss which was highly temperature dependent. The initial rates for the three temperatures were 0.20, 0.25 and 0.50 mg. per mg. dry matter per day for bread stored at -20°, 4° and 26°C, respectively. From the data for the two

FIG. 24 EFFECT OF STORAGE TEMPERATURE AND TIME ON BOUND WATER CONTENT OF BREAD CRUMB.


0.5
0.4
0.3
0.2
0.1
0
BOUND WATER, mg/mg DRY MATTER
0
4
8
12
16
20
24
STORAGE TIME, Days
-20° C
26° C
4° C

lower temperatures, it is obvious that the equilibrium level of bound water is also temperature dependent. At -20°C, the equilibrium value was about 0.4 mg. per mg. dry matter and at 4°C, the equilibrium value was 0.28 mg. per mg. dry matter. If the amount of bound water can be directly related to freshness of bread (this hypothesis remains to be verified), the results of this experiment showed that bread stored for 25 days at -20°C is of equal freshness to bread kept at room temperature for about one and a half days.

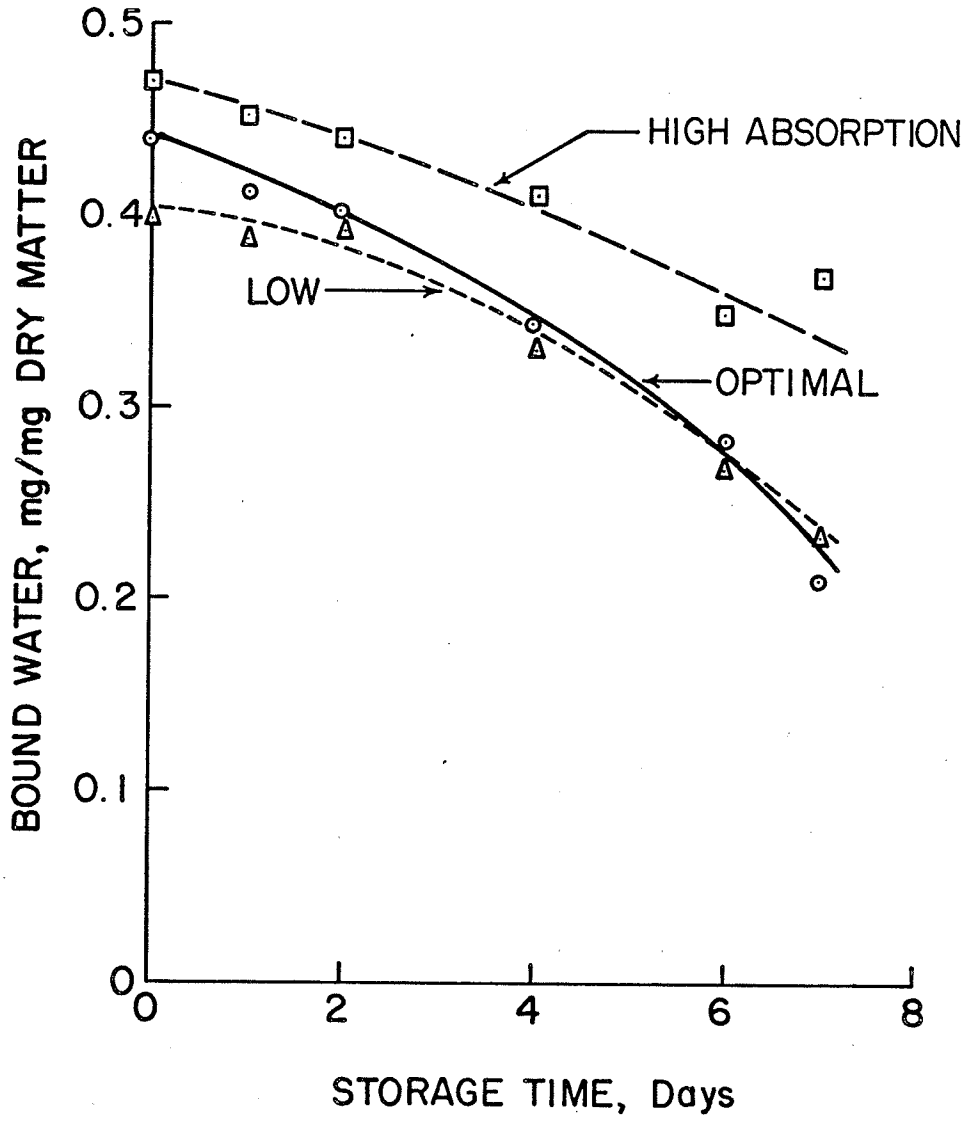
The effect of added fat was also examined in loaves stored at 4°C and -20°C in addition to the room temperature experiment discussed above. The results obtained (not shown) showed that fat did not have any additional effect on the change in the amount of bound water compared with control loaves stored at these low temperatures.

d. Effect of Water Absorption. It is generally known that the initial moisture content of the bread, to some extent, controls the rate of post-baking firming or staling. Loaves with higher initial moisture contents will remain fresher (softer) for longer time compared with loaves with a lower initial moisture.

The effect of moisture content on the amount of bound water in the crumb was investigated using loaves from Manitou flour baked at optimum baking absorption and at 4% above and below the optimum absorption. The results obtained are shown in Fig. 25.

All three sets of loaves showed a gradual decrease (essentially linear) in the amount of bound water with increasing storage time. The curve for

FIG. 25 EFFECT OF BAKING ABSORPTION AND STORAGE TIME ON BOUND WATER CONTENT IN BREAD CRUMB.


0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
BOUND WATER, mg/mg DRY MATTER
STORAGE TIME, Days
HIGH ABSORPTION
LOW
OPTIMAL

the super optimal absorption was consistently above the curve for optimal absorption. It is of practical interest to note that the amount of bound water in the super optimal absorption crumb after 2 days' storage was equal to that of freshly-baked crumb at optimal absorption. If the amount of bound water can be taken as an index of freshness, then these two loaves can be considered to be of equal freshness. Accordingly it might be concluded that four percent additional water in the dough (if it can be handled by the bakery equipment) will increase the shelf life of bread by about two days. This would be a significant economic advantage in the baking industry.

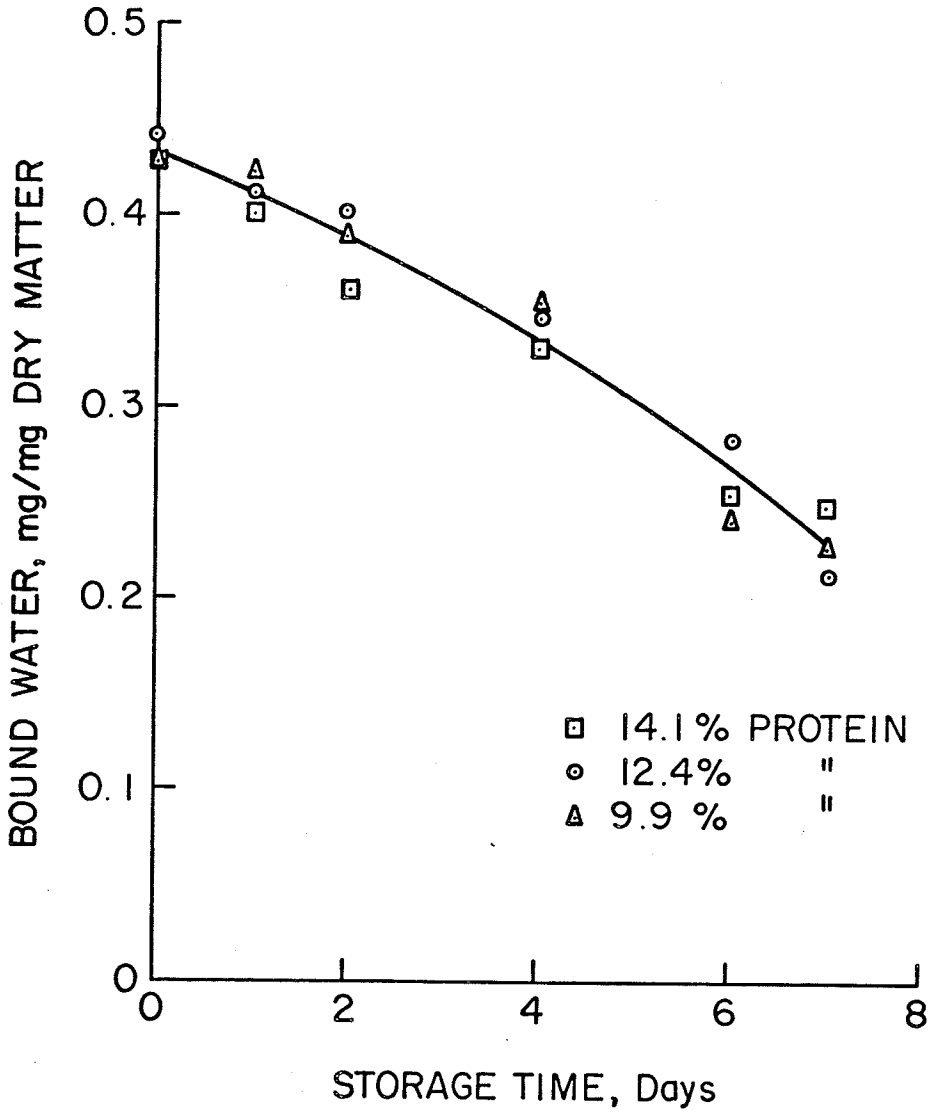
The bound water-storage time curve for the suboptimal absorption was below that for the optimal absorption loaves during the initial two days of storage. Beyond two days, the two curves were essentially the same.

Comparison of the three curves in Fig. 25 shows that the decrease in bound water with storage time was slightly slower in the bread baked at the higher absorption than in bread baked at the two lower absorptions.

In summary, results from the experiment on the effect of absorption indicate that it would be advantageous to use the maximum possible baking absorption from the point of view of slowing down the rate of staling. This is an additional benefit to that related to the yield of bread from a given weight of flour.

e. Effect of Protein Content. Loaves were baked from three different flours milled from the variety Manitou having protein contents of 9.9%, 12.4% and 14.1%. A constant baking absorption of 57.3% was used. These loaves

FIG. 26 EFFECT OF FLOUR PROTEIN CONTENT AND STORAGE TIME ON BOUND WATER CONTENT OF BREAD CRUMB.


0.5
0.4
0.3
0.2
0.1
0
BOUND WATER, mg/mg DRY MATTER
0
2
4
6
8
STORAGE TIME, Days
14.1% PROTEIN
12.4% "
9.9 % "

were used to investigate the effect of protein content on the change in bound water in bread crumb during post-baking storage. The results obtained are shown in Fig. 26.

Although there was some fluctuation in the points for the three protein levels for each storage time, there was no specific trend with protein content. All of the points fit a single curve. As observed in the previous experiment described in this section, the amount of bound water in the crumb of bread from the flours of different protein content decreased continually with storage time over the period investigated.

The observed similarity in water binding of crumb from flours of different protein content is presumed to be due to the fact that all the loaves were baked at the same baking absorption (57.3%). This is the optimal absorption for the flour of medium protein (12.4%) content. Generally baking absorption of similar flours increases with protein content. Accordingly, the well known fact that bread from flours of higher protein content does not stale as fast as bread from flours of lower protein is probably due to differences in baking absorption. It was shown in the previous section that the crumb of bread baked at higher absorption had a higher bound water value initially than crumb of bread baked at lower absorption. Also the decrease in bound water after baking is slower in bread baked at higher absorption.

On the basis of results described in this section, it is concluded that the effect of protein content on the rate of staling is probably indirect through its effect on baking absorption.

f. Effect of Starch Damage. This effect on water binding was investigated using bread from a series of flours from the variety Manitou which were milled to different levels of starch damage by over-tempering and under-tempering the wheat before milling (described in the section on methods). The range of starch damage thus obtained was quite narrow, from 17 to 25 Farrand units. The loaves for this experiment were baked at a constant baking absorption of 57.3% to eliminate any possible effect of variable absorption on bound water content. However, this may not be the best experimental approach from practical baking viewpoint since flours of higher starch damage usually have higher baking absorption.

Bread crumb from the three flours used in this experiment had the same initial level of bound water and followed the same rate of decrease with post-baking storage (actual results not shown). Accordingly it appears that the level of starch damage in the flour has no direct effect on the amount of bound water in the crumb of baked bread or the rate of decrease of bound water with storage time. Starch damage could have an indirect effect on bound water (and presumably on staling) through its effect on baking absorption. However, to generalize this conclusion, it would be necessary to investigate a much wider range of starch damage than was done in the present study.

g. Summary. It is evident that the melting curve DTA can be used successfully to measure and to follow any change in bound water content of bread. The bound water content of bread decreases with increasing post-baking storage time. This decrease was significantly affected by tempera-

ture, added fat and baking absorption. The bound water content of freshly baked bread crumb increased with increasing baking absorption. These results suggest that there is a relationship between bound water content and staling of bread. It is postulated that staling and decrease in the amount of bound water result from the same physical changes in bread crumb constituents. According to this hypothesis, it should be possible to use bound water content of bread as an index of bread staling.

GENERAL DISCUSSION

When water is added to flour to make dough, a certain proportion of it is strongly absorbed by the flour components and is therefore, unavailable in the dough system for any other chemical or physical functions. This fraction of the added water is referred to as bound water. It is generally assumed that this bound water does not contribute directly to the rheological properties of dough.

When dough is converted into bread, a fraction of the bound water still remains bound to bread components. The amount of water thus held by dough or bread is influenced by various factors like flour constituents, added bread ingredients, and physical treatments to which the dough (or bread) is subjected.

The prime objective of the project on which this thesis is based was to study water binding in dough and bread as influenced by the various factors and also to examine the possible implications of bound water in bread staling. Differential thermal analysis (DTA) was adopted as the experimental method for this investigation.

The boiling curve method of DTA which has been applied in the present study for the determination of water binding in dough gives results in terms of the energy of water binding. This statement assumes that the area of the endothermic peak obtained is due to the boiling-off of the dough moisture and is directly proportional to the amount of heat energy involved in

the endothermic process. Accordingly, boiling curve DTA does not give a measure of the "amount" of bound water (i.e. the binding capacity). In spite of this shortcoming, a limited investigation was carried out with this mode of DTA.

Results on the effect of mixing strength of flour on DTA of water binding obtained by the boiling curve method showed that the energy of water binding was dependent on the intrinsic mixing strength of the wheat variety (Fig. 7). Results for three varieties of widely different mixing strength showed that the strongest variety had the largest endothermic peak area, the medium variety had an intermediate peak area, and the weakest variety had the smallest peak area. Since, according to the basic assumption adopted, the size of the DTA peak, is a measure of the amount of energy involved in the endothermic process, it is evident that doughs from strong mixing flours bind water more strongly (tightly) than weaker mixing flours. This observation was further confirmed by the results on the effect of water absorption (Fig. 8) where the three varieties maintained the difference in their peak areas over the range of absorption examined. From the practical viewpoint, it would be of interest to analyze doughs from many different wheat varieties to determine if the endothermic peak area correlates significantly with dough mixing strength.

At this stage of our investigation, it was speculated that the observed difference in the energy of water binding of the three flours could be due to either the differences in the amount of starch damage, pentosan or protein content, or in the qualitative nature of the flour proteins or a combination of these factors. Accordingly, each of these factors was

investigated separately.

The results for doughs from flour of different protein content showed that with increase in protein, the energy of water binding showed a linear decrease irrespective of the variety. These results showed that with increase in protein content the total energy of water binding decreased. This finding was not expected. The only possible explanation of these results that can be offered is based on the level of starch damage. As is well known (and confirmed by actual analysis of flours used in this study), the level of starch damage (flour basis), in wheat flours usually decreases with increasing protein content. Accordingly, if damaged starch binds water (in dough) more strongly than flour protein, then the binding energy should decrease with increasing protein content (i.e. decreasing starch damage). A possible future extension of the present study would be the determination of the energy of water binding of intact and damaged wheat starch and vital wheat gluten.

The contribution of damaged starch (in the flour) to the energy of water binding in dough is demonstrated by the results of Fig. 11. It is seen that the endothermic peak area (binding energy) increased essentially linearly with increase in the level of starch damage irrespective of the mixing strength of the flour. These results confirm the hypothesis advanced above that, in doughs from the same flour, damaged starch binds more strongly than protein. These results are in general agreement with the boiling curve DTA results for the three varieties of different mixing strength. Here it was observed that the stronger mixing varieties (Red River 68 and Manitou) bound water more strongly than the weaker variety

(Talbot). The starch damage values for the three varieties Red River 68, Manitou and Talbot are 33, 35 and 5 Farrand Units, respectively. On the basis of starch damage values alone, the Manitou flour should bind water slightly more strongly than the Red River 68 flour. However the difference between their starch damage values is quite small. The apparent discrepancy for these two varieties might be due to other factors (see below).

Analyses of the three flours for water-soluble pentosan content showed that the trend in endothermic peak area followed the trend of pentosan content. That is, the variety that had the largest peak area (highest binding energy) also had the highest pentosan content. Accordingly, it appears that differences in pentosan content in wheat flours might account for some of the differences in water binding energy in doughs from these flours.

The effect of pentosan on water binding in dough was examined in greater detail using two flours which were supplemented with additional pentosan isolated from the same flour. It was found that the endotherm peak (energy of water binding) increased linearly, in both weak and strong flour doughs, with increase of added pentosan. However, when the peak area for the weak flour was calculated for equivalent quantity of pentosan present in strongest flour (0.67%) (using the rates of increase in peak area with pentosan content from Fig. 12), it was still considerably below that for the strongest flour. Accordingly, only part of the difference in binding energy of strong and weak flours can be explained on the basis of pentosan content. Obviously other factors (e.g. starch damage, and some that have not been investigated) are probably involved.

The change in the DTA boiling endotherm peak area with mixing time followed the mixing curve (farinogram) for the two stronger varieties. These results appear to be straightforward; the energy of water binding increases to the point when the dough reaches optimum development and then decreases with excessive mixing beyond the optimum. The decrease in binding energy in this case can be explained on the basis of the breakdown of dough structure. However, the results for the weakest flour appear to be anomalous. In this case, there was a drop in peak area (binding energy) to a minimum followed by a gradual increase with further mixing. At this stage, the author cannot offer a plausible explanation of this somewhat different result for the weakest variety except that its flour had an extremely low level of starch damage. This point requires further investigation.

Results on the effect of sodium chloride are consistent with the hypothesis that the size of the peak area is proportional to water binding in dough. Apparently salt competes with bound water for some of the interaction sites and thereby displaces some of the bound water. The boiling curve results obtained in this study showed that the addition of salt decreased the peak area substantially for all the three flours.

Results of the effect of NEMI and cysteine indicate that S-S and -SH groups of the flour protein are involved in the binding of water in dough. The addition of these chamicals caused a decrease in peak area indicating a decrease in the energy of water binding. The effect of these chemicals could result from the depolymerization of flour glutenin during mixing of doughs containing these chemicals as suggested by Tanaka and Bushuk (1973C).

The depolymerization of glutenin has been shown to be more extensive in presence of an equivalent concentration of cysteine than of NEMI (Tanaka and Bushuk 1973C). DTA results showed a similar trend; the decrease in binding energy in the presence of cysteine was greater than for an equal amount of NEMI. The effect of these chemicals was greater in doughs of stronger varieties. This is in accord with the generally held hypothesis that glutenin of stronger mixing wheat flours are more highly crosslinked than that of weaker flours.

Addition of iodate also decreased the peak area for all the three flours. Since the effect of iodate in presence of oxygen on mixing properties of dough is similar to that of NEMI (Tanaka and Bushuk 1973C), it is suggested that the decrease in water binding is probably due to oxidative cleavage of S-S bonds of glutenin.

The effect of added ascorbic acid on the endotherm peak area is quite different from that of the other chemicals used in this study. Ascorbic acid increased the energy of water binding in doughs of all three wheat varieties. This suggests that ascorbic acid, in some way, can promote the binding of water in dough. It is now known that ascorbic acid behaves somewhat differently than bromate or iodate as a flour improver. The results of the present study suggest that the difference in improver effect might be partially through the effects of these improvers on the binding of water in dough. This point has important commercial implications and should be investigated further.

The results of added chemicals on water binding showed that the magnitude of the effect is dependent on the mixing strength of flour. In

other words, it is varietal characteristic. These results are generally consistent with the hypothesis that constituents (pentosans, proteins, starch, etc.) of different wheat varieties differ qualitatively. These are the subtle factors that give rise to differences in the functional (breadmaking) properties of wheat flours. The contribution of these factors to the binding of water in dough appears to be important to functional quality.

Experiments on water binding in doughs using the melting curve mode of DTA did not show any significant differences in the amount of bound water in doughs mixed at constant absorption using flours of the three wheat varieties which varied widely in mixing strength. These results are essentially the same as those of Davies and Webb (1969) who obtained the same water binding curves for weak and strong bread flours using the differential scanning calorimetry (DSC) mode of thermal analysis.

The results on the effect of water absorption using DTA melting curve showed that with increasing water absorption, the amount of bound water in dough increased. This was observed for all three varieties. These results are explained on the assumption that increasing water in dough uncovers additional sites (by disaggregating flour particles) which are initially inaccessable for binding water molecules. This effect of water absorption is contrary to published results of Davies and Webb (1969) who showed by DSC that absorption did not affect the amount of bound water. There is no obvious explanation for this discrepancy except that different modes of thermal analysis were used in the two studies.

The present study showed that water added to flour to a limit of about 24.7% (0.33 mg/mg dry flour) was completely bound to initially available binding sites on the flour. This initial value for bound water agrees with the value for bound water in dough obtained by DSC by Davies and Webb (1969). They reported a value of 0.3 mg./mg. dry flour.

Effects of mixing time, flour protein content, starch damage, and added chemicals were investigated using DTA melting curve method for the determination of bound water. Surprisingly, these variables did not show any significant effect on the amount of bound water in doughs of the three flours. The main factor that seems to affect bound water, as determined by DTA, is the amount of water that is added to the flour to form the dough.

The DTA melting curve results differ somewhat from the results obtained by the boiling curve mode of DTA. The latter technique showed a significant effect of mixing strength (Fig. 7). Obviously, the two different modes of DTA measure different properties. The boiling curve mode gives a measure of the total energy of water binding (for bound water) and energy of vaporization (for free water), whereas the melting curve mode gives a measure of the heat of melting and therefore depends directly on the amount of free water (indirectly on the amount of bound water). In otherwords, the boiling curve mode can reflect differences, in both the intensity and capacity (amount) of water binding whereas the melting curve mode reflects only the water binding capacity. The two sets of data are considered to be complimentary.

In the third part of the present study, melting curve DTA was used to follow the change in bound water in bread crumb during post-baking storage. The effects of a number of technologically pertinent factors on this change were investigated in the hope of obtaining information on the bread staling process.

The effect of post-baking storage time (Fig. 22) showed that the bound water content of bread crumb generally decreased with increase in storage time. The rate of decrease of bound water with time was strongly dependent on whether or not fat was included in the bread formula (Fig. 23). Addition of 2% fat slowed down the decrease in bound water from 0.05 mg./mg./day for the control crumb to 0.01 mg./mg./day for fat-containing crumb.

The initial rate of decrease of bound water was strongly dependent on storage temperature. It was much slower at lower temperatures. Moreover, the equilibrium level of bound water, after a prolonged storage time, for the two lower temperatures ($-20^{o}C$ and $4^{o}C$) was also temperature dependent. It was significantly higher for loaves stored at $-20^{o}C$ compared with loaves stored at $4^{o}C$. The loaves stored at room temperature did not reach a constant level of bound water during the storage period investigated.

The amount of bound water in bread crumb was strongly dependent on baking absorption used. As found previously for dough, the amount of bound water in fresh bread crumb also increased with increasing absorption. Furthermore, the rate of decrease of bound water during post-baking storage was slower for the crumb with the higher absorption.

Flour protein content and starch damage did not have any significant effect on the change of bound water in addition to that of storage time.

These results are similar to those obtained for dough where flour protein content and starch damage did not affect the amount of bound water.

It is concluded from DTA results on bread, that the physical changes in bread crumb that affect the binding of water during post-baking storage, are also responsible for the changes that are collectively referred to as staling. Accordingly, the amount of bound water in bread crumb determined by DTA melting curve, might be a useful index of bread staling in fundamental and technological studies of this extremely important commercial problem.

SUMMARY OF RESULTS AND CONCLUSIONS

1. The boiling curve mode of DTA was used to study water binding in doughs from three wheat varieties of different mixing strength but of similar protein content. The three varieties and their mixing strength classification are: 1) Red River 68 - strong, 2) Manitou - medium, and 3) Talbot - weak.

2. At constant water absorption, the dough of the strongest variety (Red River 68) had the highest endotherm peak area, the medium strength variety (Manitou), had an intermediate peak area, and the weakest variety (Talbot), had the lowest peak area. This indicates that the energy of water binding in the three doughs increased with mixing strength of wheat variety.

3. Results of this study indicate that boiling curve endotherm peak area may be a useful index of mixing strength; mixing strength is one of the important parameters of breadmaking quality.

4. Results of experiments on the effect of water absorption confirmed the findings of the constant absorption experiment that endotherm peak area increases with increasing mixing strength. These results also showed that the water binding capacity of all three flours increased in curvilinear manner with increase in water absorption of the dough suggesting that addition of more water to the flour opens up additional sites for water binding.

5. The effect of flour protein content on water binding in dough was investigated using flours of different protein content of the variety Manitou and flours of the three varieties of different mixing strength supplemented with vital wheat gluten to give different levels of protein. With all flours, the total energy of water binding decreased with increasing protein content.

6. The results of experiments on the effect of starch damage showed a linear increase in binding energy with increase in damaged starch content of flour.

7. According to boiling curve DTA results, it appears that damaged starch binds water more strongly than protein in flour-water doughs.

8. For the three varieties used, the content of water soluble pentosan increased in the same order as the energy of water binding; the strongest variety had the highest pentosan content. These results are consistent with the hypothesis that pentosans play a significant role in the binding of water in doughs.

9. Using flours of the strongest and weakest varieties supplemented with different levels of water soluble pentosan separated from the same flours, it was shown that the intensity and capacity of water binding in dough increased directly with pentosan content.

10. In doughs of the two stronger varieties, the binding energy curve versus mixing time showed the same trend as farinograph consistency curve. The binding energy and consistency increased to a maximum at about the same mixing time. Beyond this time, both parameters showed a gradual

decrease. For the weakest variety, the energy-time curve was anomalous; it showed a drop from 0 to 8 min. mixing followed by a gradual rise with mixing time from 8 to 20 min.

11. The addition of salt to the dough decreased the boiling endotherm peak area; this effect was more pronounced in doughs of stronger flours. It appears that salt displaces some of the bound water from its binding sites causing an increase in free water.

12. Addition of NEMI and cysteine reduced the peak area for all three varieties but the effect was more pronounced with cysteine. Depolymerization of glutenin brought about by the addition of these chemicals is cited as being indirectly responsible for the decrease in energy of binding. The effect was generally greater in doughs from the stronger varieties suggesting that proteins of these varieties are more highly crosslinked. Addition of iodate also decreased the energy of water binding in dough.

13. Results of the effect of iodate, NEMI and cysteine indicate that S-S bonds are probably involved in binding of water in doughs.

14. In contrast to the effect of cysteine, iodate and NEMI, ascorbic acid increased the energy of water binding in doughs of all three varieties; the increase was slightly greater for the weakest flour than for the two stronger flours. The action of ascorbic acid in dough appears to be quite different from that of the other chemicals investigated.

15. Melting curve DTA was used to determine the amount of bound water in doughs and in bread crumb subjected to various conditions and treatments.

16. The amount of bound water in doughs prepared at constant water absorption was not affected by the mixing strength of the flour (wheat variety).

17. Experiments on doughs with variable water absorption showed that all of the water added to dough up to a moisture of about 25% was completely bound. On further addition of water, about one third of the additional water was bound and two thirds remained as free water. Presumably additional water disaggregates flour particles and thereby uncovers additional water binding sites.

18. Mixing time, flour protein content, starch damage and added chemicals did not have any effect on the amount of bound water as determined by melting curve DTA.

19. Results of experiments on crumb of bread stored for various periods after baking, showed that the bound water content of the crumb decreased with increasing storage time.

20. Addition of fat to the bread formula slowed down the rate of decrease of bound water with storage time.

21. The initial rate of decrease of bound water in crumb during storage was strongly dependent on the storage temperature; the decrease was much slower at lower temperatures.

22. The amount of bound water in crumb increased with increasing baking absorption. The decrease of bound water with storage time was slower in crumb of bread baked at higher water absorption.

23. Flour protein content and starch damage did not have any significant effect on the change in bound water content of crumb of bread baked at the same absorption.

24. On the basis of DTA melting curve results on bread crumb, a direct relationship between bound water content and staling of bread was postulated. Accordingly, bound water content of bread crumb could be used as an index of bread staling.

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