THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Anna C. Peterson final oral examination for the degree of Master of Science . We recommend that the degree of Master of Science be conferred upon the candidate.

Minneapolis, Minnesota

June 1 1920

C. A. Bailey
Chairman

F. H. MacDougall

F. J. Alway

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THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Anne C. Peterson for the degree of Master of Science. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

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April 19 1920

A STUDY OF THE BUFFER VALUES OF WHEAT FLOUR EXTRACTS BY MEANS OF THE HYDROGEN ELECTRODE.

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF MINNESOTA.

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

JUNE 1920.

INTRODUCTION

The most successful methods used hitherto for estimating quality or grade in wheat flour have been summarized by Bailey (1918). Much of the later work has been directed to the problem of ascertaining the fundamental properties which constitute grade, and the factors which tend to modify them. The hydration capacity, viscosity, elasticity and like properties of gluten are known to be profoundly affected by the presence of small quantities of acids, alkalis and salts. One should consider then, the effect on bread making quality of certain acid forming, and acid combining or regulating substances which are normally present in water extracts of flour to a degree which varies with the method of milling.

Wood (1907) states that the difference between strong and weak flours lies in the physical properties of their glutens. Small quantities of acid, alkali or salt have a marked effect on these properties. Wood and Hardy (1909) found the cohesiveness of gluten to be destroyed by low concentrations of acid or alkali, an effect which was counteracted by addition of salt. They found the hydration capacity of gluten to be greatly modified by acid or alkali. Upson and Calvin (1915) showed that wheat gluten as a typical vegetable colloid behaves toward acids and salts in solution like the animal colloids investigated by Fischer (1915).

Jessen-Hansen (1911) found that production of acid as in aging of flour improved its baking quality as did the addition of small quantities of old "sour" flour. He observed that the consistency of gluten was much improved by washing with a mixture of acid which had been found most favorable for baking. For the best consistency the hydrogen ion concentration must fall within rather narrow

limits. This was observed by Gortner and Doherty (1918) and others. Henderson, Fenn and Cohn (1918) found that increasing the acidity of dough to certain limits will increase its elasticity.

Jessen-Hansen's (1911) work would indicate that there is an optimum hydrogen ion concentration for a bread-dough. This was found to be approximately 10^{-5} normal, being slightly higher for the best and lower for low-grade flours. This indication of the importance of reaction in doughs has been confirmed by the work of Cohn, Cathcart and Henderson (1918) and Landenberger (1918). In addition to its influence on the properties of gluten, the reaction of the dough bears a relation to the growth and activity of yeast and other microorganisms which function in fermentation. (Henderson 1918).

This optimum acidity for the baked loaf is higher than that of a mixture of freshly milled flour and water, or of fresh milk, according to the findings of Jessen-Hansen (1911) and others.

Several factors contribute to the attainment of this optimum acidity for baking. The original acidity of the water extract of the flour has been found to vary. Jessen-Hansen (1911) reported that in flour from the same wheat, the top grade flour has the highest, the seconds and bran the lowest hydrogen ion concentration. In titrating these same extracts however, the low-grade flour extracts are found to neutralize a greater amount of alkali, other things being equal. These results have been confirmed frequently by later workers. Jessen-Hansen (1911) found also that the addition of increasing quantities of bran caused a regular decrease in the hydrogen ion concentration of the dough. This is a result of the soluble buffers or reaction regulators which are present in amounts increasing directly as the amount of bran or fibrous material. White (1913) found that acid extracts of bran added in bread making improved the quality of the gluten.

By the action of yeast on sugar, CO_2 is produced. Thus by increasing the

quantity of yeast or of sugar the production of acid will be hastened. Wahl (1915) found an improvement in bread due to the production of lactic acid by Bacillus Delbruecki.

Swanson and Tague (1919) showed that the amount of NaOH required to titrate equal amounts of the extracts of ground wheat to a given P_H increased with time and temperature up to a certain limit, the original P_H of the extract remaining constant. This suggests that buffers are produced by hydrolysis of more complex organic compounds.

Since the production of an optimum acidity improves baking quality and hastens the process of fermentation, and since this acidity is a function, not alone of the acid present, added, or developed in the dough but also of the acid combining power of the buffers or reaction regulators present in the flour, the estimation of these becomes important. These are present to a varying degree, depending on the method of milling, but they modify the effect of acid directly as the amounts present. To know the buffer value of the flour then is to have an index to the quantity of acid needed to produce the desired effect. Jessen-Hansen (1911) called attention to the fact that the value of various "flour improvers" is due to their increasing the hydrogen ion concentration of the dough. If such are used the most favorable quantity must be determined from a knowledge of the buffer value of the flour.

Cohn and Henderson (1918) determined the optimum reaction by the use of Methyl Red on the baked loaf. They also found that development of acidity was dependent in part on the quality of the flour.

It would be of advantage to ascertain, by means of the buffer action of a flour extract, that treatment to which a dough made from it should be subjected in the manufacture of yeast leavened bread. Our problem was the development of a method for judging the effective value of the buffers present in the water extracts of various wheat flour grades. This method might also serve as a means of indicating grade. A colorimetric method developed for this purpose would be more

rapid and easy than any method in present use.

HYDROGEN ION CONCENTRATION

In 1887, Svante Arrhenius announced the theory that the molecules of acids, bases and salts when dissolved in water dissociate wholly or partly into positively and negatively charged particles or "ions". This, the electrolytic dissociation theory, is the basis for explanations of observed facts regarding the behavior of acids, bases and salts, "electrolytes", in solution.

These charged particles will conduct an electric current. Kohlrausch and Heydwiller have demonstrated that the purest water obtainable will do so, a result which has been frequently confirmed. We assume therefore, that water dissociates according to the reversible equation; $H_2O \rightleftharpoons H^+ + OH^-$. Stated in terms of the law of mass action $\frac{H^+ \times OH^-}{(H_2O)} = K$. Since the concentration of water is constant, the equation may be written $H^+ \times OH^- = Kw$. The dissociation constant for water has been determined and at 25° C, $Kw = 1.012 \times 10^{-14}$.

The active characteristics of electrolytes in solution depend on the degree to which the molecules dissociate and acids and bases are classified as strong and weak on this basis. The terms acidity, alkalinity and neutrality in terms of true reaction are terms defined quantitatively on the basis of H^+ or OH^- ions present. When $H^+ = OH^-$ the solution is neutral; when $H^+ > OH^-$ it is acid; when $OH^- > H^+$ it is basic. True reaction is proportional to the ionic concentration, not to the total acid or base present. This concentration must be determined in order to know the true reaction. Since $H^+ = \frac{Kw}{OH^-}$ and $OH^- = \frac{Kw}{H^+}$, it is seen that if either H^+ or OH^- is determined, the other may be calculated, Kw being known. Also the concentrations of H^+ and OH^- vary inversely, and if OH^- is increased, H^+ must decrease and vice versa. Since pure water is a neutral solution $H^+ = OH^- = 1 \times 10^{-7}$ normal.

The concentrations met with in physiologic solutions are generally very small and it is simpler to avoid the use of large figures by adopting Sörenson's

logarithmic notation, wherein $\frac{1}{CH}$ = PH = the exponent of the reciprocal of the hydrogen ion concentration. For example, if CH = 1×10^{-7}, PH = 7, and if CH = 0.25×10^{-5}, CH = $10^{-.602} \times 10^{-5.6}$ and PH = 5.6.

THE IMPORTANCE OF REACTION

That reaction is an important factor in controlling biological processes has frequently been demonstrated. It has been found by Lundsgaard (1912) and others that the reaction of the blood plasma in normal and in most pathological conditions is maintained at a constant level. Henderson (1911), Henderson and Palmer (1912) and others have demonstrated the diagnostic value of reaction in clinical findings. Again, Michaelis (1914) demonstrated the significance of hydrogen ion concentration in blood and urine.

Sörenson (1912) studied the influence of hydrogen ion concentration on enzymatic activity and concluded that this can be adjusted by controlling the reaction. Itano (1916) found certain hydrogen ion concentrations to measure the exact influence both inhibitory and prohibitory and to indicate the exact limits of the proteolytic activity of Bacillus Subtilis, also, that the reaction of the medium converged toward the optimum as proteolysis proceeded. Fred and Loomis (1917) say that alfalfa bacteria apparently bring about changes in reaction favorable for their reproduction. Clark (1915c) demonstrated the influence of reaction upon the activity of Bacillus Coli and indicated that bacterial processes are markedly affected by the reaction of the medium, some organisms being much more sensitive than others. McClendon (1916) reviews much of the work done. Van Slyke and Baker (1918) studied the relation of hydrogen ion concentration to the souring of milk.

Jessen-Hansen (1911) showed that a definite relation exists between the concentration of hydrogen ions of water extracts of wheat flour and its baking value. Wahl (1915) found an improvement in bread due to the presence of bacterial lactic acid.

Many other researches on the value of knowing and controlling reaction in biological fluids are reviewed by Michaelis (1914b).

Haas (1916) observed that when extracts prepared from colored plant tissues were added to buffer solutions of varying hydrogen ion concentration, the color of the living tissue was generally obtained in acid solutions, less frequently in neutral or alkaline ones. Many pigments gave the color of the living tissue at $H^+ = 10^{-3}$. Haas (1917), Hoagland (1918) and others have found that plant protoplasm unlike animal organisms exhibits a great variation in reaction.

BUFFERS

The resistance to change in reaction which characterizes certain solutions has been frequently observed. Fernbach and Hubert (1900) commenting on this property in phosphate solutions likened it to a "tampon". Sörensen (1909) adopted the word which in the German translation of his paper became "puffer", then in English, "buffer".

In many of the titrimetric estimations of hydrogen ion concentration, no account has been taken of buffers whose presence might produce a true reaction very different from that expected from the known amounts of acid or alkali present. Unbuffered solutions may be changed in reaction even due to the presence of neutral salts according to Harned (1915) and others. Fernbach and Hubert (1900) say that the beneficial influence of phosphates on proteolytic enzymes is to prevent changes in the acidity or alkalinity of the medium.

The mechanism for the maintenance of neutrality in the animal organism has received increasing attention. This is ascribed to the buffer action chiefly of bicarbonates less to phosphates and but slightly to proteins. The theory of this protective mechanism which in spite of wide fluctuation in acid-base intake and production maintain a constant slight alkalinity has been discussed by Henderson (1908,1909), Robertson (1909,1910), McClendon (1916), Palmer and Henderson (1913) and others.

Carbonates and phosphates are salts of polybasic acids which give acid salts, $NaHCO_3$, NaH_2PO_4, approximately neutral in reaction. These will neutralize considerable amounts of acid and alkali without materially changing the reaction. Proteins being amphoteric substances may exert some buffer action.

A mixture of a weak polybasic acid with its alkali salt will act as a buffer. This was discussed by Sörenson (1909), Henderson (1909) and Michaelis (1914b). Koppel and Spiro (1914) discuss the theoretical aspects of reaction regulators or buffers. Henderson and Webster (1907) found phosphates useful in neutrality regulation of media.

It is known that a solution of NaH_2PO_4 is slightly acid, while a solution of Na_2HPO_4 is slightly alkaline. A mixture of the two is practically neutral and will take up large quantities of acid or alkali with small change in reaction. Clark and Lubs (1917) say that phosphates have relatively little buffer action at hydrogen ion concentrations lower than that of blood, while at and above neutrality phosphates exert a strong buffer action. Clark and Lubs (1917) also show that the buffer action of a solution depends upon the nature and concentration of the constituents; upon the PH region in which their action is measured; upon the nature of the acid or alkali added, and upon the fact that the presence of substances which tend to adsorb any constituent of the solution must also be taken into consideration. Many solutions whose buffer action must be studied for biological purposes, contain undissolved or colloidal material of this nature and this fact must be reckoned with if buffer action is to be considered in its broadest and most practical sense. Charcoal will exert such buffer action. Sörenson and Palitzch (1913) discuss what they term the "salt error" and Lubs and Clark (1917) discuss "protein and salt errors" the nature of which is not clearly understood; although it is known that the presence of protein and even of neutral salt will frequently modify reaction.

THE USE MADE OF BUFFER MIXTURES

Substances which will exercise a definite buffer action have been used in the preparation of standard comparison solutions for use with indicators in determining hydrogen ion concentration colorimetrically and for checking electrometric apparatus. These mixtures must have sufficient buffer action to resist change from unavoidable slight contamination.

Von Szily suggested that solutions near the neutral point might be prepared by using phosphate mixtures. Salm (1904) made measurements of such mixtures. Schmidt and Finger (1908) prepared mixtures of NaOH and $H_3 BO_3$ as standards. Sörenson's (1909,1912) wellknown standards were sets of phosphate, thallate and borate mixtures. Walpole (1914a and b) investigated acetic acid - sodium acetate mixtures. Palitzsch (1915) studied combinations of boric acid and borax. Clark and Lubs (1917) recommend the following which are said to be easily prepared and accurate:

Acid potassium o-phthalate — HCl
" " " — NaOH
" " phosphate — NaOH
Boric acid — KCl — NaOH

These, they say, possess the advantage that all the solids crystallize without water of crystallization. These mixtures are made up for use to a known PH as determined by previous investigators, and further tested with the hydrogen electrode to ensure accuracy. They are used as standards with indicators for colorimetric comparison in determining reaction. Because of its rapidity and simplicity this method has found increasing favor in work where PH may be determined by an approximate method. Clark and Lubs (1917) used the somewhat wide interval of 0.2 PH in their colorimetric comparison solutions, interpolating to 0.1 PH. They consider hydrogen electrode measurement standard however, and recommend its use in inventigations where slight changes in PH will seriously disturb equilibrium conditions.

In the use of these standards for determining hydrogen ion concentration by the colorimetric method a demand has been created for an increasing variety of useful indicators.

INDICATORS

A great number of substances are known to have indicator properties. The colorimetric method is based on the fact that an indicator is sensitive to a particular range of hydrogen ion concentration in which it exhibits a color change characteristic of the given indicator. One cannot therefore determine the exact neutral point of a solution with an indicator unless its color change is known to take place at the neutral point. The color change means simply that a reaction has been reached to which the particular indicator is sensitive.

Indicators therefore serve two distinct purposes: to mark the end point in titrations and to measure by color changes the concentrations of H^+ or OH^-. Indicators most useful for titration change color sharply at a particular known reaction and otherwise not. For measuring H^+ or OH^- concentration an indicator should have a well defined color range to correspond to a changing reaction. The limits of this color range varies with the nature of the indicator. It has been determined for a great number of indicators. This work was first studied thoroughly and systematically by Salm (1904,1906), Salessky (1904), Fels (1904), and Friedenthal (1904). Sörenson (1909) made a more complete study of the indicator method making his selection on the basis of comparative freedon from "protein and salt error". Clark and Lubs (1917) contributed further to the list of useful indicators, and suggested those which could be used through the usual range. Walpole (1910) presented graphically the relation between the logarithm of the hydrogen ion concentration of certain mixtures and the related color changes of indicators.

Natural indicators, of which litmus, alizarin and cochineal are examples, although valuable in indicating reactions in plant tissues are being rapidly

displaced by synthetic indicators of increasing variety and brilliancy, although Bjerrum (1914) states that for most titrimetric purposes methyl red and phenolphthalein are all that are necessary, and methyl red and phenol red have been used for covering the whole range of standard comparison solutions commonly employed.

The first important step in the preparation of useful synthetic indicators was made by Sohon (1898) who prepared phenolsulfonphthalein and other sulfonphthalein derivatives. Their use has been rapidly developed. Tizard (1910) showed that the degree of hydrolysis of aniline salts can be accurately determined by measuring the depth of color produced with methyl orange. Levy, Rowntree and Marriott (1915), devised a method for determining the hydrogen ion concentration of blood, by the use of phenolsulfonphthalein in dialyzed blood serum, and standard comparison solutions. Hurwitz, Meyer and Ostenburg (1915,1916) employed the colorimetric method in adjusting the reaction of bacteriologic culture media. Marriott (1916) used the method to determine the alkali reserve of the blood. Lubs and Clark (1917) give improved methods for the preparation of the sulfonphthalein indicators and add new ones. They made electrometric comparisons to ensure accuracy in preparing their standard comparison solutions. Dugger and Dodge (1919) review the work done and add a note on the use of the Kober colorimeter for closer approximation.

THEORY OF INDICATORS

The early attempts to explain color changes in indicators made by Meyer, Spängler, Baeyer, Fischer, Nietzki, Friedlander and others was generally based on a theory of quinone structure, on Witt's theory of chromophore groups, etc.

Ostwald (1890) proposed an explanation based on the electrolytic dissociation theory, and assuming indicators to be very weak acids and bases. Thus adding alkali to phenolphthalein, a weak acid, gives a salt which is highly dissociated, to give a colored anion. The physical constants of phenolphthalein show a close

relationship between Ostwald's theory and the observed color changes. The theory has been found insufficient however, to account for all the phenomena observed. While true that the anions are colored, the change seems due to a change in the constitution of the compound, that is, to a different ionic structure or molecular rearrangement.

Stieglitz (1903) concluded that change of color and ionization is merely a coincidence, the formation of the strongly chromophoric quinoid complex ($:C_6H_4:O$) in phenolphthalein being a sufficient explanation for the color change.

Acree and Brunel (1906) began work on indicators as part of their investigations on tautomerism. Acree (1907,1908) concludes that the chief source of color in both acidic and basic indicators is not the quinone group but one or more inter- or intra-molecular combinations of a quinone group with a salt of phenol or with an aniline-like group. Noyes (1910) discusses the equilibrium relation between the two differently colored structural forms in which all indicators probably exist and under which these tautomeric substances can show sharply differentiated colors in acid and alkaline solutions. He shows that under the conditions described the indicator can be treated as if it were a single acid or base, having an ionization-constant which, though a function of three equilibrium-constants, can be determined directly from the color changes exhibited in solutions of varying hydrogen ion concentration, or by measurement of the conductivity of the indicator acid or base, or by studying the hydrolysis of its salt.

According to Hildebrand (1913) the color change is produced by a change in ionization and also by a tautomeric change. He calls attention to the fact that if the dissociation constant is known, the reaction corresponding to the color change can be calculated. Lubs and Acree (1916), Lubs and Clark (1915,1916) have discussed proposed theories in connection with indicators in use. Wegscheider (1915) presents a theory of color change on the ground that undissociated compounds and ions are similarly colored, the color change depending on change in constitution.

Lubs and Clark (1917) basing their assumptions on the simple theory of electrolytic dissociation as Ostwald (1890) did, have shown its applicability in using data thus derived, for the construction of curves to show the PH range of usefulness of several indicators. These curves are valuable as a guide in the selection of the proper indicator for a particular range. They also discuss the theory of tautomerism in indicators. A review is given of the methods of compensating for turbidity and color in solutions tested by the indicator methods.

Many of the older conflicting opinions regarding the influence of acidity upon biologic processes are the result of determination of titratable acidity and not of true reaction. Henderson (1909), Sörenson (1909), Michaelis (1914b), Clark (1915a) and others have discussed the objections to applying the titration method to biological problems. The method of titration designed for the estimation of strong acids and bases in analytical work is not applicable to fluids which contain weak acids and bases and other substances which tend to modify the reaction or obscure the end-point. This end-point shows merely that a hydrogen ion concentration to which the indicator is sensitive, has been reached. It gives no information concerning the original reaction. In order to determine the true reaction the method requires that the original hydrogen ion concentration remains unchanged.

The importance of measuring the true reaction in biological fluids has been sufficiently emphasized. The two methods employed, the colorimetric and the electrometric, have become accurate and reliable enough for use in biologic research.

DEVELOPMENT OF THE ELECTROMETRIC METHOD

In spite of the rapidity and convenience of the indicator method and its increased efficiency due largely to the work of Clark and Lubs (1917), the electrometric still remains the fundamental method. The standard solutions used in the colorimetric method must be frequently checked by the electrometric method in

order to ensure their accuracy.

The evolution of the electrometric method we owe originally to Nernst. The first applications of the principles evolved by Arrhenius, Nernst (1889) and Kohlrausch and Heydwiller (1894), was made by Böttger (1897) who titrated many acids and bases and discussed the theoretical grounds and apparatus. They were first applied to biological fluids by Bugarsky and Liebermann (1898) and by Höber (1900).

Kohlrausch and Heydwiller from measurements of the electrical conductivity of normal acids and bases calculated the dissociation of pure water and its concentration of H^+ and OH^-. This method is available for determination of reaction provided no other conductors are present in solution. A more direct way may be used however, depending on electrolytic solution tension.

Any metal if placed in contact with pure water tends to send off ions into solution at a rate which depends on the solution tension of the metal or its tendency to go into the ionic state. As metal ions are positive they carry away positive electrical charges and leave the metal negatively charged. The ions tend to diffuse out, due to their kinetic energy. They tend to be pulled back due to attractions between opposite charges on ions and metal. When the pull on the ions becomes equal to their solution pressure a state of equilibrium is reached in which the number of ions leaving and returning are equal. If the metal be placed in a solution containing its own ions, these will be deposited in greater number, giving the metal a positive charge. The charge on a piece of metal then is proportional to the concentration of its ions in the solution in which it is placed, and its electric potential in a gram equivalent solution of its ions is an index taken as a standard for its electrolytic solution tension. This value varies for every metal. The charge on a zinc electrode in a gram equivalent solution of zinc ions will be less positive than that on a copper electrode in the same concentration of copper ions, because zinc has a greater electrolytic solution tension. If the two are joined by a conductor the difference in potential may be measured.

Changing the concentration of either zinc or copper ions, will change the potential difference between the metals. By determining the change in potential difference at different known concentrations of zinc ions, the ionic concentration of copper remaining constant, a set of values is obtained from which an unknown concentration of zinc ions may be calculated.

Instead of using different metals the electrodes may be of the same metal placed in unequal concentrations of its ions, or a gaseous element may be introduced wherein the electrode is an inactive metal (platinum, palladium or gold) with the gas condensed on its surface. The constancy of such an element was determined by Smale (1894). The electrode is placed in a solution containing the gas in an ionic state.

Nernst's (1889) formula for calculating the ionic concentration from the electromotive force produced, depends upon the work done by the ions in producing this electromotive force, and its relation to the osmotic pressure of the ions in solution, this being equal to their gaseous pressure at the same concentration. The gas pressure can be calculated from the formula $PV = RT$ where P = pressure, V = volume, T = absolute temperature and R = the gas constant = 8.316. Applying this formula to finding the work done by the ions which is equal to their osmotic pressure the following equation is derived: $W = RT \log_e \frac{P1}{P2}$, or since the pressure varies as the concentration, $W = RT \log_e \frac{C1}{C2}$. This work is changed to electrical energy if two similar electrodes placed in unequal concentrations of their ions are connected by an electric conductor.

To express electrical energy by means of this equation we divide by one Faraday, 96500 being the number of coulombs necessary to discharge one gram equivalent of ions. $\log_e$ is converted to the common logarithm by multiplying by $\frac{1}{0.4343}$. We also divide by the valence of the metal. E is the electromotive force.

The equation then becomes $E = \frac{RT}{nF} \log_e \frac{C1}{C2} = \frac{8.316\ T}{96500 \times 0.4343} \log 10 \frac{C1}{C2} = .000198427\ T \log 10 \frac{C1}{C2}$.

If C1 is a gram equivalent solution of hydrogen ions, $\log \frac{C1}{C2} = \log \frac{1}{CH} = -\log CH = PH$.

$$\text{Log } \frac{1}{CH} = PH = \frac{E}{0.0001984\,T}$$

Using this formula the hydrogen ion concentration of an unknown solution connected to a gram equivalent solution of ionic hydrogen may be calculated.

A standard calomel electrode has been found more useful than the normal hydrogen electrode as it can be maintained more readily at a constant value. A correction is applied for the difference between the two. From the work of Ostwald, succeeded by Coggeshall (1895), Wilsmore (1900), Loomis and Acree (1911) and Lipscomb and Hulett (1916), the preparation of the normal calomel electrode has become standardized and its e.m.f. found to have the mean value, 0.283 volt, based on the adoption as zero potential that of a hydrogen electrode in a normal solution of hydrogen ions. In addition to this potential there is a potential set up at the surface of contact of the two liquids. According to Bjerrum (1911) this can be calculated using Planck's formula. A discussion of the method and principles involved are given by Clarke, Myers and Acree (1916).

It has been found more convenient however, to eliminate this contact potential by interposing between the two solutions a saturated solution of a very soluble salt whose anion and cation have approximately equal migration velocities. The one most commonly employed is KCl.

With variations in temperature the e.m.f. of a tenth normal KCl calomel electrode is found to be much more constant than the normal electrode. Recently, Lewis, Brighton and Sebastian (1917) have made measurements fixing the values for the normal and tenth normal KCl calomel electrodes. They found for the normal electrode, E = 0.283 and for the difference between the two E = 0.053. This gives a value of 0.336 volt for the $\frac{N}{10}$ KCl calomel electrode. This is .001 volt less than the value 0.337 usually adopted, but is accepted and used by Schmidt and Hoagland (1919) in their calculations. For the $\frac{N}{10}$ KCl calomel electrode the

equation then becomes: $E - 0.336 = \frac{RT}{nF} \log 10 \frac{1}{CH}$, or, $\log 10 \frac{1}{CH} = PH = \frac{E-0.336}{0.0001984T}$.

At 20°C, $E-0.336 = 0.058155 \log 10 \frac{1}{CH} = 0.058155\ PH$. $PH = \frac{E-0.336}{0.058155}$ and at 25°C,

$PH = \frac{E-0.336}{0.059152}$.

Since T is the variable it is evident that PH will vary with temperature. The values for 25° and the factors for converting from 25° to temperatures ranging from 18° to 30° are given by Schmidt and Hoagland (1919).

THE HYDROGEN ELECTRODE VESSEL AND THE ATTAINMENT OF CONSTANT POTENTIALS.

Several workers have discussed the difficulties in the way of obtaining constant potentials in hydrogen electrode measurements. The time required to obtain equilibrium has seemed longer than necessary and the voltages obtained have not remained constant.

In the older methods hydrogen was bubbled through a solution in which the electrode was wholly or partly immersed.

Deeks and Acree (1911) in order to follow the course of a reaction (hydrolysis) wished to develop a rapid method. They say that their experiments show that a preliminary saturation of the electrode and solution to be tested with hydrogen, shortens the time required to obtain equilibrium from five to ten minutes.

Michaelis and Rona (1913) obtained constant potentials quickly by allowing the platinized electrode merely to touch the surface of the solution. Hasselbalch (1913) suggests that this is probably due to a sharply localized equilibrium at the point of contact. He describes an apparatus which permits the solution to be shaken constantly during the determination. Konikoff (1913) used a modification of Hasselbalch's apparatus and confirmed his conclusion that repeated shaking with hydrogen was necessary in order to bring the e.m.f. to a constant value.

Meyers and Acree (1913) state that constant potentials cannot be obtained as long as the portion of the solution connecting with the saturated KCl is left

unsaturated with hydrogen.

Clark (1915a) suggests that Kidmelis and Kenu's method is successful because diffusion is probably hindered by the density of the surface layer and that it seems precarious to rely on a device which takes advantage of a sharply localized, hence a pseudo-equilibrium which makes no allowance for a possible difference in reaction between the surface film and the solution. Clark adopted a modification of Hasselbalch's electrode, eliminating certain features such as the wide angle of rotation and the large dead spaces in the tubes adjacent to the body of the electrode vessel.

The advantages claimed for the shaking electrode were: a more rapid interchange of gas between electrode and solution; a more rapid reduction of oxygen if present. Exposing the electrode periodically required the hydrogen to penetrate only a thin film of liquid before reaching the platinum black. With preliminary shaking Clark found the voltage to be constant.

McClendon and Magoon (1916) described a two-compartment electrode, modified from Hasselbalch's, the solution being shaken in one compartment and transferred to the other for the determination. We gave this a trial but found it troublesome because for the low conductivity of our preparations the distance was too great between the electrode and the saturated KCl.

Hildebrand (1913) describes a dipping electrode which has been modified for use by several workers. Van Slyke and Baker (1918) say that Hildebrand's electrode is not applicable to solutions containing proteins. They used a modification, the new features relating to the assemblage of parts and the arrangement of the electrode. Their device included a mechanical stirrer and a U-tube carrying saturated KCl, the end dipping into the solution to be tested being drawn to a small opening and plugged with filter paper to prevent diffusion. They displace the air from the vessel before lowering the electrode into the solution thus aiming to prevent interference by dissolved oxygen. Equilibrium is obtained in three to five minutes.

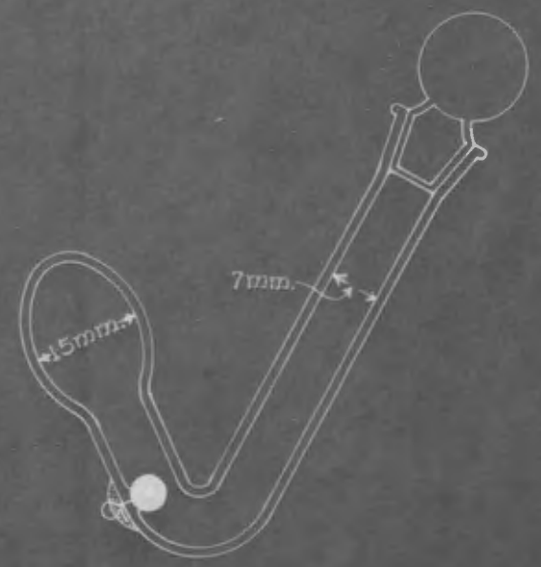
Swanson and Tague (1919) used Hildebrand's electrode in their work with ground wheat extracts. They bubbled hydrogen through until equilibrium was reached which they found took about an hour unless the electrode were previously saturated with hydrogen when the period was somewhat shortened.

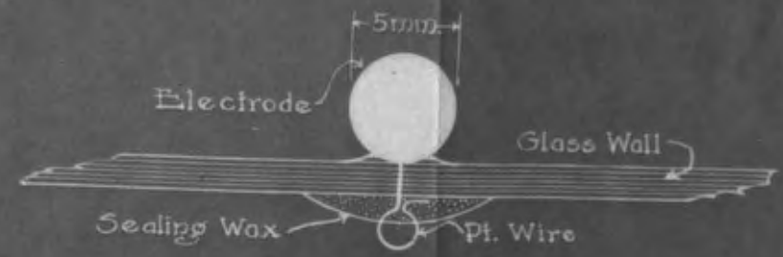
We tried a modification of Hildebrand's electrode fitting a small vial with a rubber stopper carrying an electrode similar to Hildebrand's, a trap and a bent glass tube carrying the saturated KCl made like Van Slyke and Baker's. Hydrogen was bubbled through until constant readings were obtained, the hydrogen bubbles serving as a stirrer. Our chief difficulty lay in the foaming of the solution, this being carried out through the trap during the time required to obtain equilibrium, (one-half to one hour). We tried foam breakers such as toluene with very slight advantages in this type of electrode vessel. The results when obtained were not sufficiently consistant and all the work was repreated with the special electrode finally adopted.

Schmidt and Hoagland (1919) use glass U-tubes filled by placing in a heated solution of two percent washed agar saturated with KCl, for making the connection between saturated KCl and solution to be tested. On cooling, the agar solidifies and for each new determination a fresh boundary is obtained by cutting off a small portion from the end of the glass tube.

The electrode which we finally adopted was one designed by Bailey. It was a V-shaped glass tube closed at one end and fitted at the other with a ground-glass stopper. The platinum electrode was sealed in just above the bend on the closed side. The electrode is pictured in detail in figure 1.

For use, the electrode was filled by placing the solution to be tested in the open end and tilting to transfer it to the closed end. Hydrogen was admitted through a glass tube drawn to a long capillary reaching to the bend in the electrode vessel, and displaced the solution in the enlarged oval. The electrode was completely filled with solution to exclude air bubbles and the ground glass stopper fitted in. It was shaken for two minutes by hand, or it was


7mm.
15mm.


5mm.
Electrode
Glass Wall
Sealing Wax
Pt. Wire

given 200 to 250 double shakes by count. The hydrogen was finally transferred to the cleaned and hot leaving the electrode wholly immersed in the manner suggested by McClendon and Thary (1919). The stopper was removed and part of the solution thrown out by a quick motion of the hand. This was done to prevent overflow when the glass tube carrying saturated KCl was inserted. The latter was bent and drawn to a capillary bore at one end. This end reached into the solution to the bend in the electrode and was plugged with filter paper to prevent diffusion. The other end of the bent tube dipped into the saturated KCl. The electrode was rinsed outside with distilled water and wiped dry. When connections were made, readings were obtained instantly which remained constant.

The electrode was cleaned with hot chromic acid before using, washed thoroughly and given a uniform coating of platinum black. We used two dry cells in series. The electrode was soaked at least one-half hour and tested frequently with standard buffer mixtures following Clark's (1919d) directions. We used the chain Hg - $HgCl\left|\frac{N}{10}\right.$ KCl | saturated KCl | unknown solution | H_2 -- Pt. The mercury was prepared according to Hulett's (1911) method. In the construction of the tenth-normal KCl Calomel electrode certain special features were introduced to prevent diffusion and changes in concentration.

Our hydrogen supply was first obtained from a Kipp generator which did not prove very reliable. We tried an electrolytic generator developed by McClendon, but finally adopted as the most satisfactory the use of compressed hydrogen from a cylinder fitted with a reducing valve. The gas was bubbled through a bottle of distilled water to ensure its saturation with water vapor.

The other parts of the apparatus consisted of an Edison storage battery to supply the working current, a Leeds and Northrup special potentiometer and a high sensitivity galvanometer used as a null instrument. We found that with this combination and the special electrode, readings could be made accurately to $\frac{1}{4}$ millivolt. Thinking this unnecessary we recorded our results to the nearest millivolt.

METHOD OF WORK

For use as standards in the colorimetric comparisons mixtures were selected from those recommended by Clark and Lubs (1916). The following were prepared:

Buffer Mixtures Prepared

With 50 c.c. 0.2 MKH phthalate + x c.c. 0.2 N NaOH diluted to 200 c.c.

c.c. 0.2 N NaOH	PH (theoretical)	PH found
3.70	4.2	4.29
7.50	4.4	4.46
12.15	4.6	4.63
17.70	4.8	4.82
23.05	5.0	5.07
29.95	5.2	5.24
35.45	5.4	5.48
39.85	5.6	5.65
43.00	5.8	5.92

With 50 c.c. 0.2 MKH_2PO_4 + x c.c. 0.2 N NaOH diluted to 200 c.c.

3.72	5.8	5.80
5.70	6.0	6.00
8.60	6.2	6.20
12.60	6.4	6.40
17.80	6.6	6.60
23.65	6.8	6.80
29.63	7.0	7.00
35.00	7.2	7.20
39.50	7.4	7.40
42.80	7.6	7.54
45.20	7.8	7.61
46.80	8.0	7.93

With 50 c.c. 0.2 MH_3BO_3 and 0.2 MKCl + x c.c. 0.2 N NaOH diluted to 200 c.c.

5.90	8.2	8.20
8.50	8.4	8.40
12.00	8.6	8.60
16.30	8.8	8.80
21.30	9.0	9.00
26.70	9.2	9.20
32.00	9.4	9.30
36.85	9.6	9.60

The indicators were chosen from the selections of Clark and Lubs (1917). We found it more useful to prepare a higher concentration of indicator, however. Below are given the PH range, color change and concentration of each indicator used.

Indicator Solutions Prepared#

Name	PH range	Color change	Concentration	
Phenol Sulfon Phthalein	6.8-8.4	yellow-red		.05%
Ortho Cresol Phthalein	8.2-9.6	colorless-red	.05 gm in 50 c.c. alcohol	.10%
Thymol Sulfon Phthalein	8.0-9.6	yellow-blue	.05 gm in 50 c.c. alcohol	
	1.2-2.8	red-yellow	+12.5 c.c. water	.06%
Tetrabromphenol Sulfon Phthalein	3.0-4.6	yellow-blue	.05 gm in 62.5c.c. alcohol	.08%
Orthocresol Sulfon Phthalein	7.2-8.8	yellow-red	.05 gm in 50 c.c. alcohol	.10%
Dibrom Cresol Sulfon Phthalein	5.2-6.8	yellow-purple	.05 gm in 50 c.c. alcohol	.10%
Dibrom Thymol Sulfon Phthalein	6.0-7.6	yellow-blue	.05 gm in 50 c.c. alcohol	.10%

10 c.c. of standard buffer mixture+6 drops of indicator were sealed in small Jena glass test tubes of uniform bore. Sets were prepared covering the entire range of each indicator except for mixtures below PH = 4.2. The PH actually found for each buffer mixture with the hydrogen electrode was taken as the true value.

Our preparations were all turbid, but none were colored. We adopted the "Comparator" method of Hurwitz, Meyer and Ostenburg (1916) described also by Clark and Lubs (1917). This device consists of a block of wood bored to hold four test tubes of equal diameter, so that they can be viewed in pairs. Standard+indicator — unknown, form one pair; unknown+indicator -- clear water, forms the other.

After approximating the color match outside, the test tubes were placed in the "Comparator" and the colors matched more closely. We interpolated to .1 PH after the manner of Clark and Lubs. Our best results were obtained with phenol sulfon phthalein which was found particularly satisfactory. Also, any two-colored indicator served better than a one-colored one, because in our preparations colloidal and suspended material tended to adsorb the indicator and change its color intensity.

The colorimetric comparisons were made on a number of the solutions tested

We added 6 drops indicator solution to 10 c.c. buffer mixture in all cases except with Thymol Sulfon Phthalein — of this we added 12 drops to 10 c.c.

electrometrically. The purpose was to test the applicability and accuracy of the colorimetric method in working with flour extracts and to find which indicators would prove most useful.

THE COMPARATIVE BUFFER EFFECTS OF A PATENT AND A CLEAR GRADE FLOUR

The first sets of determinations were made on extracts prepared from a high grade patent and from a clear grade flour. We aimed to use a somewhat high concentration of flour in water in order to obtain a comparatively high concentration of buffers in the extract and chose one part of flour by weight to five parts of water, as the best ratio. Our first purpose was to ascertain the effect of time and of temperature of extraction on the buffer value of the two flours. The buffer value was measured by determining the comparative resistance to change in PH shown by the extracts when unit quantities of acid or of alkali were added.

Flour and redistilled water were taken in the proportion of one gram of flour to five c.c. of water at room temperature. The water was in each case brought to the temperature used for the extraction before mixing it with the flour in Jena glass, Florence or Erlenmeyer flasks. Extractions were made at 0°, 25°, 40° and 60°C, for periods of 1, 2, 4 and 6 hours in a thermostat, the flour particles being kept in suspension during the extraction by periodic shaking. The particles were then thrown out of suspension by centrifuging for about seven minutes and the extract decanted into a beaker. To different 100 c.c. portions of this extract were added 10, 20, 30, 40 and 50 c.c. of $\frac{N}{50}$ HCl and 10, 20, 30, 40 and 50 c.c. of $\frac{N}{50}$ NaOH*. The PH value of the original extract and that of each of the above preparations was determined with the hydrogen electrode. Several colorimetric comparisons were also made.

A summary of the PH values obtained for the various temperatures and periods

* In actual practice 25 or 50 c.c. of the extract were used depending on the quantities available, with the acid or alkali solutions in the proper proportions.

of extraction is given in tables I and II.

Table I.

Time and Temperature Series.
PH Values of Patent Flour Extracts.

	c.c. N/50 HCl + 100 c.c. extract						c.c. N/50 NaOH + 100 c.c. extract				
	50	40	30	20	10	0	10	20	30	40	50
Duration of extraction				Extracted at 0° C.							
1 hr.	2.72	2.84	3.11	3.47	4.40	6.10	8.96	10.28	10.77	11.00	--
2 "	2.76	2.92	3.14	3.58	4.53	6.09	8.17	9.94	10.55	10.85	11.06
4 "	2.81	2.98	3.21	3.65	4.56	6.10	7.78	9.77	10.31	10.84	11.04
6 "	2.82	2.98	3.25	3.69	4.58	6.09	7.68	9.50	10.31	10.75	11.01
				Extracted at 25° C.							
1 hr.	2.79	2.99	3.21	3.65	4.52	6.05	7.40	9.28	10.11	10.62	10.91
2 "	2.87	3.01	3.27	3.72	4.58	6.05	7.42	9.06	9.92	10.48	10.82
4 "	2.87	3.04	3.31	3.77	--	6.05	7.32	8.98	9.84	10.45	10.77
6 "	2.87	3.04	3.31	3.79	4.61	6.05	7.18	8.96	9.91	10.45	10.77
				Extracted at 40° C.							
1 hr.	2.87	2.96	3.23	3.65	4.51	6.00	7.52	8.61	9.94	10.47	11.06
2 "	2.81	3.00	3.26	3.69	4.55	6.02	7.25	--	9.81	10.35	10.69
4 "	2.81	2.98	3.28	3.79	4.59	5.99	7.05	8.47	9.44	10.11	10.50
6 "	2.82	2.98	3.30	3.79	4.62	5.99	7.05	8.44	9.42	9.99	--
				Extracted at 60° C.							
1 hr.	--	3.08	3.42	3.91	4.70	6.04	7.10	8.84	9.58	10.28	10.50
2 "	--	3.11	3.42	3.89	4.72	6.04	6.96	8.74	9.55	10.16	10.41
4 "	--	3.11	3.41	3.91	4.70	6.02	7.07	8.59	9.53	10.13	--
6 "	--	3.11	3.40	3.91	4.72	6.04	7.08	8.30	9.53	10.02	--

Table II.

Time and Temperature Series.
PH Values of Clear Flour Extracts.

	c.c. N/50 HCl + 100 c.c. extract						c.c. N/50 NaOH + 100 c.c. extract				
	50	40	30	20	10	0	10	20	30	40	50
Duration of extraction				Extracted at 0° C.							
1 hr.	3.04	3.16	3.66	4.28	5.09	6.29	7.76	9.37	10.21	10.65	10.91
2 "	3.08	3.35	3.72	4.36	5.12	6.29	7.49	9.04	9.94	10.49	10.57
4 "	3.08	3.45	3.87	4.48	5.22	6.29	7.24	8.55	9.52	10.09	10.48
6 "	3.25	3.55	3.99	4.60	5.29	6.27	7.05	7.96	9.15	9.70	10.26
				Extracted at 25° C.							
1 hr.	3.30	3.65	4.19	4.75	5.55	6.30	6.83	7.42	8.47	9.21	9.80
2 "	3.46	3.74	4.23	4.82	5.60	6.29	6.76	7.20	7.93	8.81	9.37
4 "	3.45	3.77	4.28	4.87	5.62	6.30	6.76	7.19	7.71	8.52	9.18
6 "	3.52	3.74	4.34	4.94	5.68	6.31	6.78	7.17	7.69	8.42	8.89

Extracted at 40°C.

1 hr.	3.42	3.75	4.19	4.78	5.60	6.29	6.76	7.19	7.83	8.79	9.40
2 "	3.45	3.79	4.24	4.83	5.63	6.30	6.73	7.15	7.69	8.52	9.28
4 "	--	3.79	4.26	4.89	5.66	6.30	6.70	7.12	7.58	8.45	9.08
6 "	3.58	3.90	4.32	4.90	5.65	6.30	6.71	7.10	7.54	8.08	9.18

Extracted at 60° C.

1 hr.	--	3.79	4.19	4.77	5.53	6.25	6.69	7.17	7.71	8.67	9.08
2 "	--	3.87	4.36	4.82	5.53	6.25	6.68	7.10	7.59	8.47	9.13
4 "	--	3.84	4.29	4.85	5.56	6.24	6.64	7.07	7.54	8.38	9.01
6 "	--	--	--	4.87	--	6.24	6.69	7.08	7.51	8.18	--

It is seen that for the same flour the temperature and period of extraction have no effect on the original PH of the extract. That the PH of ground wheat extracts remained constant with varying periods and temperatures of extraction was found by Swanson and Tague (1919). The hydrogen ion concentration of the extract of patent flour is higher than that of the clear grade. The titratable acidity is higher for the clear however. It was found for example, that 50 c.c. of patent flour extract neutralized 1.85 c.c. of N/10 NaOH while for 50 c.c. of clear flour extract prepared in the same manner 2.50 c.c. were required. These data confirm Jessen-Hansen's (1911) conclusions. A larger quantity of buffers in the clear flour extract tend to keep it nearer to the neutral point in spite of its higher content of acid reacting material.

At 60° considerable hydration of the patent took place especially on longer periods of digestion, a smaller volume of centrifuged extract being obtained. This fact may have modified the buffer value of this extract by removal of buffers as adsorbed material or by concentrating these due to removal of water through hydration of the colloidal material. The changes from the original PH produced in these extracts in the same series of determinations are tabulated in tables III and IV.

Table III

Time and Temperature Series

Duration of extraction	PH decrease on adding N/50 HCl — c.c. N/50 HCl + 100 c.c. extract: 50	40	30	20	10	0	PH increase on adding N/50 NaOH — c.c. N/50 NaOH + 100 c.c. extract: 10	20	30	40	50
Patent Extracted at 0° C.											
1 hr.	3.38	3.26	2.99	2.63	1.70	6.10	2.86	4.18	4.67	4.90	--
2 "	3.33	3.17	2.95	2.51	1.56	6.09	2.08	3.85	4.46	4.76	4.97
4 "	3.29	3.12	2.89	2.45	1.54	6.10	1.68	3.67	4.21	4.74	4.94
6 "	3.27	3.11	2.84	2.40	1.51	6.09	1.59	3.41	4.22	4.66	4.92
Patent Extracted at 25° C.											
1 hr.	3.26	3.06	2.84	2.40	1.53	6.05	1.36	3.24	4.07	4.58	4.87
2 "	3.18	3.04	2.78	2.33	1.47	6.05	1.37	3.01	3.87	4.43	4.77
4 "	3.18	3.01	2.74	2.28	--	6.05	1.27	2.93	3.79	4.40	4.72
6 "	3.18	3.01	2.74	2.26	1.44	6.05	1.13	2.91	3.86	4.40	4.72
Patent Extracted at 40° C.											
1 hr.	3.20	3.04	2.77	2.35	1.49	6.00	1.52	--	3.94	4.47	--
2 "	3.21	3.02	2.76	2.33	1.47	6.02	1.23	2.82	3.79	4.33	4.67
4 "	3.18	3.01	2.71	2.20	1.40	5.99	1.06	2.48	3.45	4.12	4.51
6 "	3.17	3.01	2.69	2.20	1.37	5.99	1.06	2.45	3.43	4.00	--
Patent Extracted at 60° C.											
1 hr.	--	2.96	2.62	2.13	1.34	6.04	1.06	2.80	3.54	4.24	4.46
2 "	--	2.93	2.62	2.15	1.32	6.04	0.92	2.70	3.51	4.12	4.37
4 "	--	2.92	2.61	2.11	1.32	6.02	1.05	2.57	3.51	4.11	--
6 "	--	2.93	2.64	2.13	1.32	6.04	1.04	2.26	3.49	3.96	--

Table IV

Time and Temperature Series

Duration of extraction	PH decrease on adding N/50 HCl — c.c. N/50 HCl + 100 c.c. extract: 50	40	30	20	10	0	PH increase on adding N/50 NaOH — c.c. N/50 NaOH + 100 c.c. extract: 10	20	30	40	50
Clear Extracted at 0° C.											
1 hr.	3.25	3.03	2.63	2.01	1.20	6.29	1.47	3.08	3.92	4.36	4.62
2 "	3.21	2.94	2.57	1.93	1.17	6.29	1.20	2.75	3.65	4.20	4.28
4 "	3.21	2.84	2.42	1.81	1.07	6.29	0.95	2.26	3.23	3.80	4.19
6 "	3.02	2.72	2.28	1.67	0.98	6.27	0.78	1.60	2.88	3.43	3.99
Clear Extracted at 25° C.											
1 hr.	3.00	2.65	2.11	1.55	0.75	6.30	0.53	1.12	2.17	2.90	3.50
2 "	2.83	2.55	2.06	1.47	0.69	6.29	0.47	0.91	1.64	2.52	3.08
4 "	2.85	2.53	2.02	1.43	0.65	6.30	0.46	0.89	1.41	2.22	2.80
6 "	2.79	2.57	1.97	1.37	0.63	6.31	0.41	0.80	1.24	--	--

	Clear Extracted at 40° C.										
1 hr.	2.87	2.54	2.10	1.51	0.69	6.29	0.47	0.90	1.54	2.50	3.11
2 "	2.85	2.51	2.06	1.47	0.67	6.30	0.43	0.85	1.39	2.22	2.96
4 "	--	2.51	2.04	1.41	0.64	6.30	0.40	0.82	1.38	2.15	2.78
6 "	2.72	2.40	1.98	1.40	0.65	6.30	0.41	0.80	1.24	--	--
	Clear Extracted at 60° C.										
1 hr.	--	2.46	2.06	1.48	0.72	6.25	0.44	0.92	1.46	2.42	2.83
2 "	--	2.38	1.89	1.43	0.72	6.25	0.43	0.85	1.34	2.22	2.88
4 "	--	2.40	1.95	1.39	0.68	6.24	0.40	0.83	1.30	2.14	2.77
6 "	--	--	--	1.37	--	6.24	0.45	0.84	1.27	1.94	--

Buffer value is measured on a comparative basis as resistance to change in PH. The buffer value of the patent digested at 0° increases slowly up to six hours. At 25° increase is much more rapid, a slightly higher value being reached in one hour at 25° than in six hours at 0°. If increase in buffer value is due to hydrolysis, then this is practically complete in two hours at 25°. At 40° and 60° the acid titrations indicate a completion of hydrolysis at the end of one hour, possibly in less time, as no determinations were made for shorter periods. The alkali titrations however, indicate a longer period of buffer increase.

The effect of temperature was much greater than that of time, the buffer value after one hour at 0° being much lower than after one hour at 25°, 40° or 60°. This indicates that hydrolysis is slow at 0°, much more rapid at 25° and slightly more rapid at 40° and 60°. The results at 40° and 60° are almost identical, the variations probably being within experimental error. Results obtained by Bailey and Collatz (1919) in conductivity studies indicate that hydrolysis is slower at 60° than at 40°. They suggest that this may be due to the inhibition of an enzyme, phytase, which is present in wheat as shown by Anderson (1915).

Clear flour extract after one hour at 0° has a comparatively low buffer value but is still higher than patent flour extract after six hours at 0°. In the clear buffer increase is rapid at 0°, and much more rapid at 25°, at which temperature it reaches a maximum in about two hours, and at 40° and 60° after one hour. Here also time has less effect than temperature on hydrolysis. One hour at 25° gave a

higher value than six hours at 0°.

Titration curves showing the difference in PH between patent and clear flour extracts after addition of the same amounts of N/50 acid and alkali to 100 c.c. are shown in figure 2. The maximum difference is produced when 20 c.c. of N/50 NaOH were added. The curves tend to converge as the quantities of acid or alkali used are increased.

Figures 3 and 4 show graphically the effect of varying periods of digestion, and figures 5 and 6 show the effect of temperature on the buffer value. For the clear flour this increases more rapidly and to a higher maximum then for the patent. The difference between patent and clear is greatest after one hour at 25° when hydrolysis is nearing completion in both. Adding 20 c.c. N/50 NaOH to 100 c.c. extract at this stage, changes the patent through a PH of 3.24 units, the clear through only 1.12 units or a difference of 2.12 units. This very striking difference in buffer value is shown clearly in figures 4 and 6. This very striking difference is greater with alkali then with acid as may be seen by comparing figures 3 and 4, (or 5 and 6).

The maximum PH differences between patent and clear are shown when these are extracted for one hour at 25° C. One hour is a convenient period in which to get material and apparatus ready for use and at 25° C the temperature control of the thermostat is more easily maintained because closely approximating room temperature constant attention is required in maintaining temperatures unvarying at 0°, 40° or 60° and more especially for longer periods. There being no obvious advantage in doing so, extractions under these conditions were no longer made and one hour at 25° C was chosen as standard in succeeding work.

The effect of adding equal volumes (20 c.c.) of HCl of increasing concentration to 100 c.c. of extract was determined for the two flours. In this way the concentration of buffers in the extract remained constant when increasing amounts of acid were added. The normality of the mixture was calculated on the basis of HCl added. The results are given in Table V.

FIGURE 2.

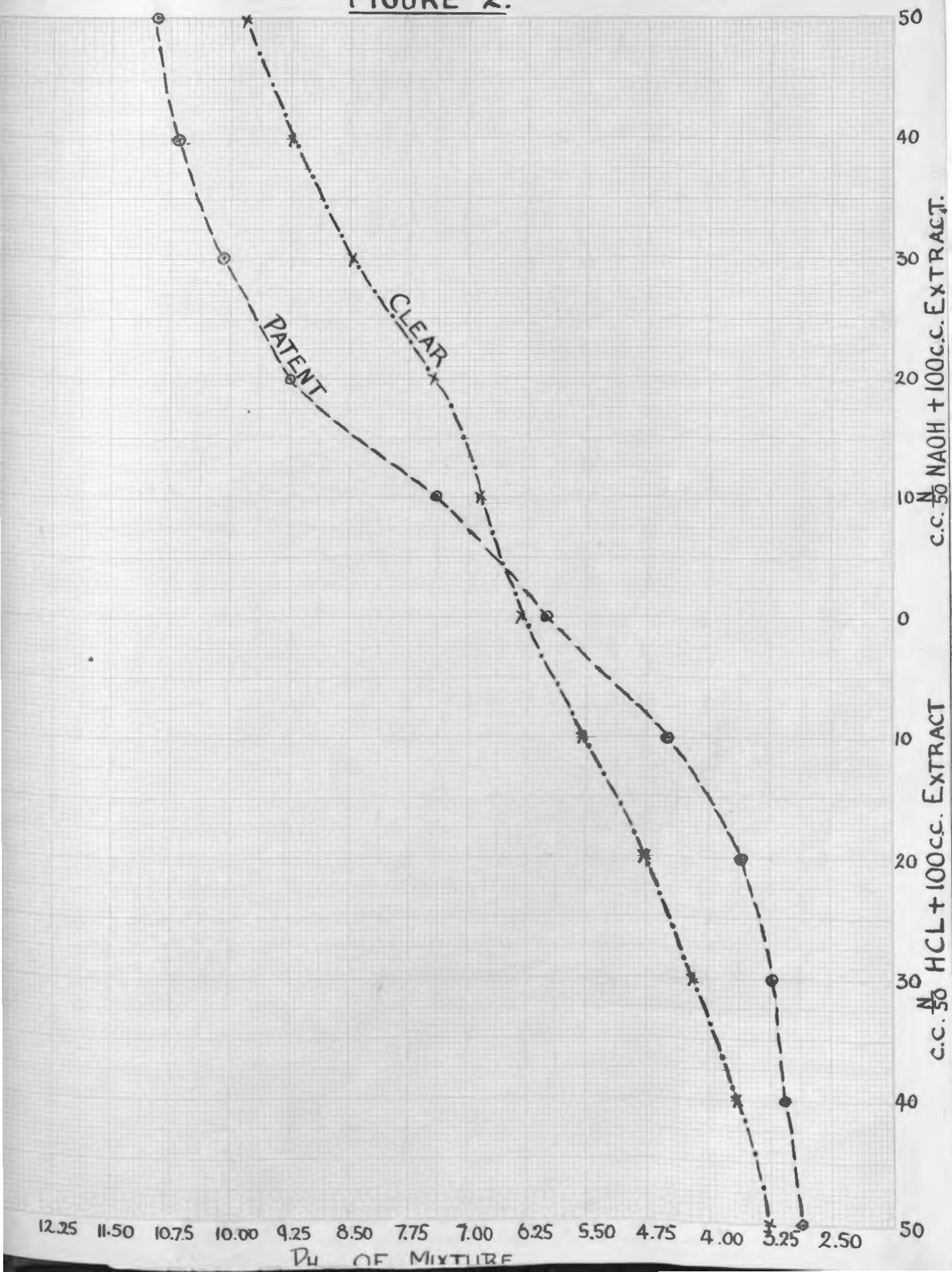
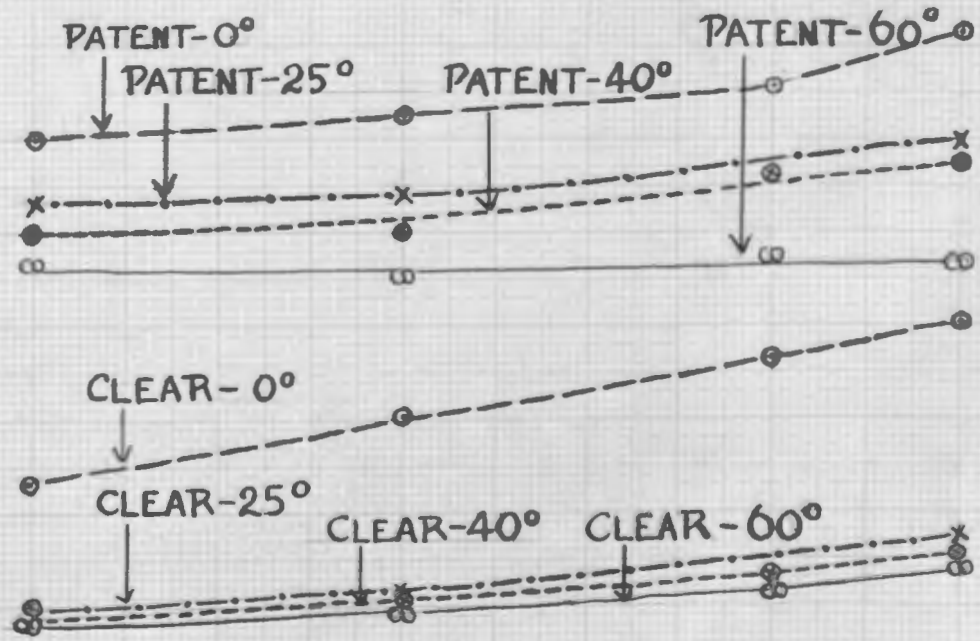

CLEAR
PATENT
50
40
30
20
10
0
10
20
30
40
50
c.c. N/50 NAOH + 100c.c. EXTRACT.
c.c. N/50 HCL + 100c.c. EXTRACT
12.25
11.50
10.75
10.00
9.25
8.50
7.75
7.00
6.25
5.50
4.75
4.00
3.25
2.50
PH OF MIXTURE

FIGURE 3.



DECREASE IN PH ON ADDITION OF 20cc $\frac{N}{50}$ HCL. TO 100 c.c. EXTRACT.

4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 8 6 4

6 4 2 1

TIME - HOURS

FIGURE 4.

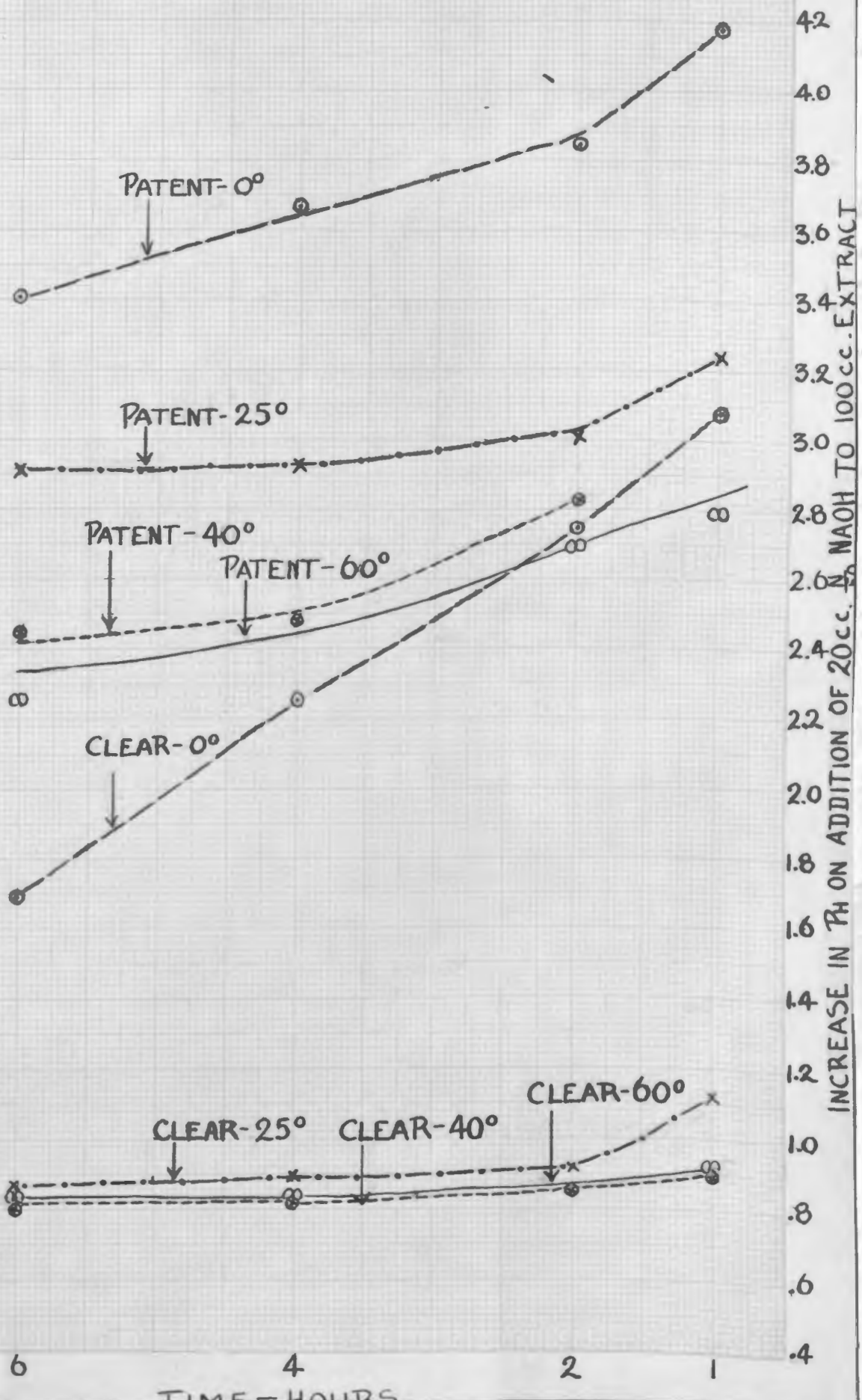
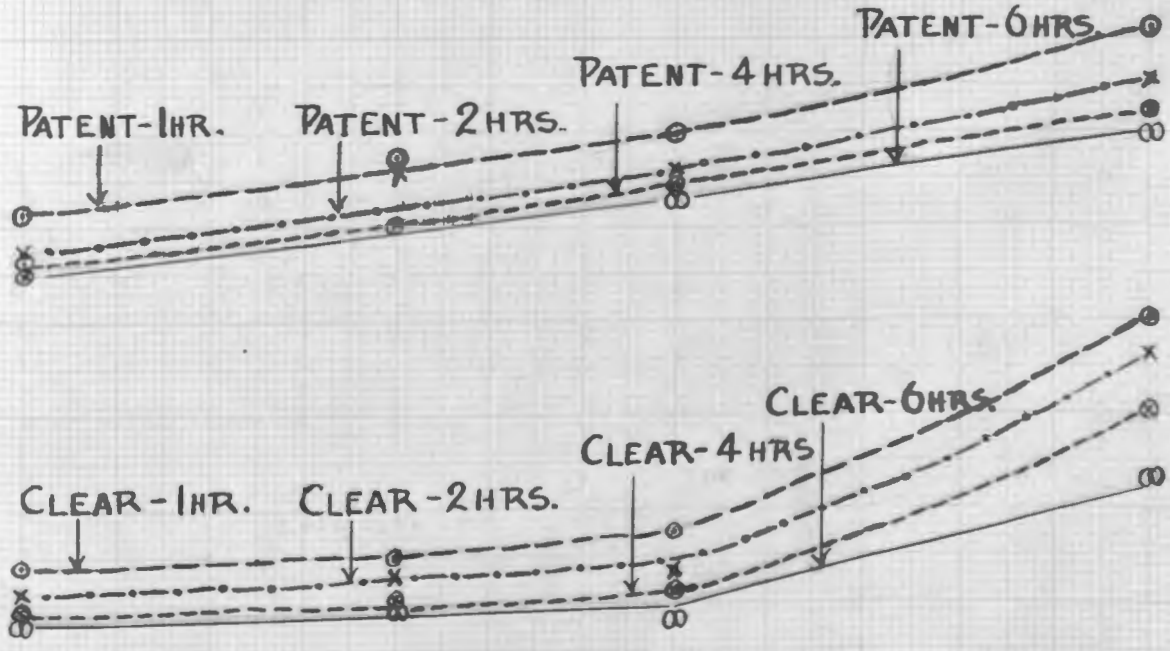

PATENT-0°
PATENT-25°
PATENT-40°
PATENT-60°
CLEAR-0°
CLEAR-25°
CLEAR-40°
CLEAR-60°
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
INCREASE IN PH ON ADDITION OF 20cc. N/10 NAOH TO 100cc. EXTRACT
6
4
2
1
TIME - HOURS

FIGURE 5.



4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4

PH DECREASE ON ADDITION OF HCL TO 100 cc EXTRACT.

60° 40° 25° 0°

TEMPERATURE = °C

FIGURE 6.

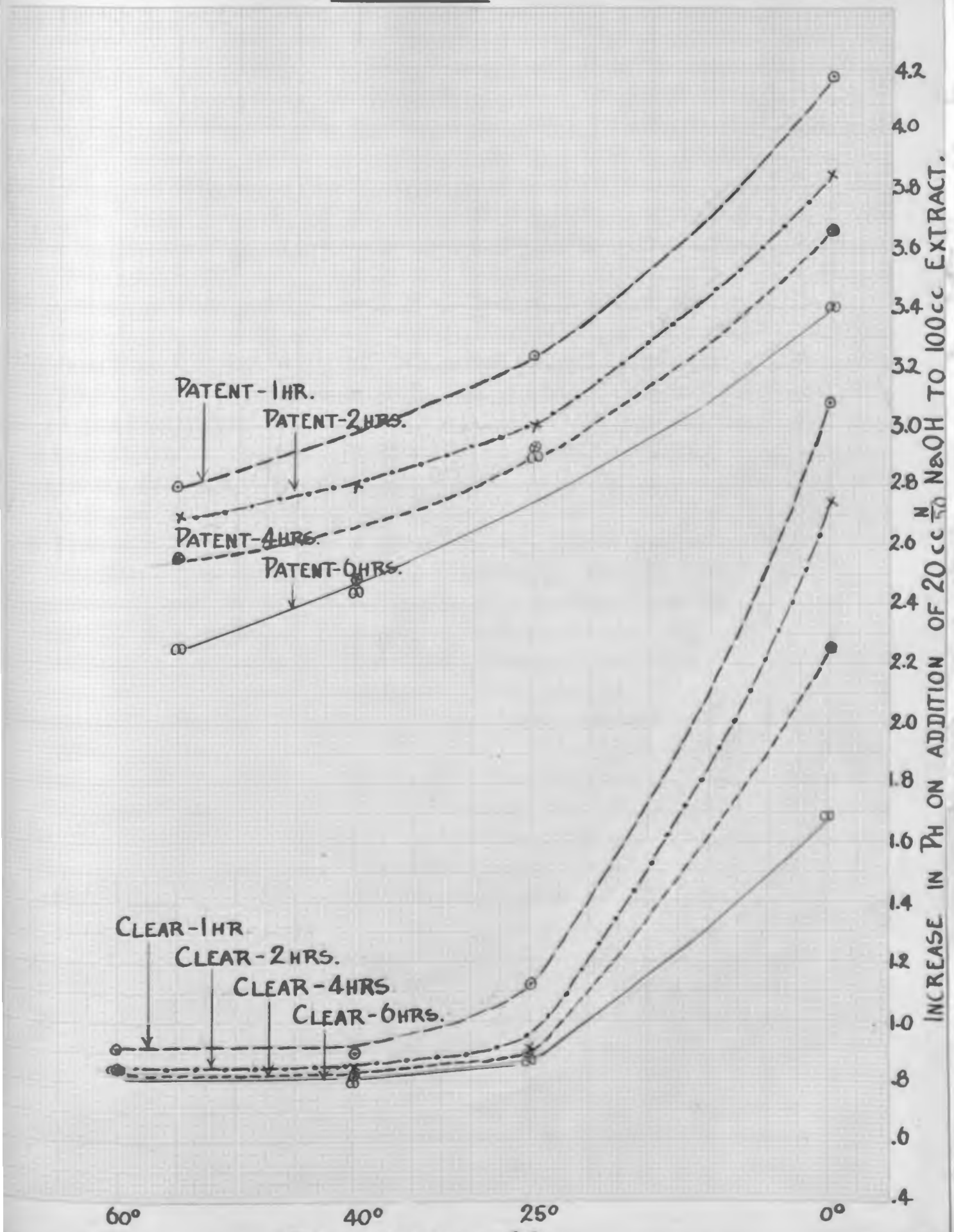

PATENT-1HR.
PATENT-2HRS.
PATENT-4HRS.
PATENT-6HRS.
CLEAR-1HR
CLEAR-2HRS.
CLEAR-4HRS
CLEAR-6HRS.
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
INCREASE IN PH ON ADDITION OF 20 cc N/50 NaOH TO 100 cc EXTRACT.
60°
40°
25°
0°
TEMPERATURE - °C

Table V

PH of Patent and Clear extracted one hour at 25° C after addition of equal volumes of HCl of increasing normality

Volume HCl added	Normality of HCl solution	Normality of Mixture as HCl	PH of Patent	PH of Clear	Difference in PH between Patent & Clear
10 c.c.	1/160=(.00625)	.00104	5.06	5.85	0.99
10 "	3/320=(.00938)	.00156	4.60	5.60	1.00
10 "	1/80 =(.01250)	.00209	4.30	5.34	1.04
10 "	3/160=(.01875)	.00312	3.65	4.79	1.14
10 "	1/50 =(.02000)	.00333	3.65	4.75	1.10
10 "	1/40 =(.02500)	.00417	3.50	4.43	0.93
20 "	3/160=(.01875)	.00536	3.07	--	--
10 "	3/80 =(.03750)	.00625	3.03	3.73	0.70
10 "	1/20 =(.0500)	.00833	2.75	3.28	0.53
10 "	3/40 =(.07500)	.01250	2.46	2.74	0.28
10 "	1/10 =(.1000)	.01667	2.25	2.48	0.23
10 "	3/20 =(.1500)	.02500	2.00	2.15	0.15
10 "	1/5 =(.2000)	.0333	1.86	1.96	0.10
10 "	1/2 =(.5000)	.08333	1.40	1.50	0.10
10 "	1. =(1.000)	.16667	1.15	1.25	0.10
20 "	1. =(1.000)	.2857	0.93	0.99	0.06
No HCl added		.0000	6.03	6.27	0.24

Curves plotted from these data are shown in figure 8. The maximum difference between the two flours is shown at a normality of the mixture of 0.00312 N. This corresponds to an addition of 20 c.c. of 3/160 normal HCl (approximately N/50) to 100 c.c. of extract. This concentration we had arbitrarily selected before as the best after trials with N/5 and N/10 acid. The curves (figure 7 tend to converge at higher concentrations.

BUFFER VALUES OF FLOUR GRADES

For further study a series of flours representing several streams produced in a commercial mill was chosen to determine relative buffer value and a possible relation of this as indicated in our previous work, to the method of milling and grade of the flour. Again a standard period and temperature of extraction was adopted; one hour at 25°. The results of this study are summarized in Table VI.

FIGURE 7.

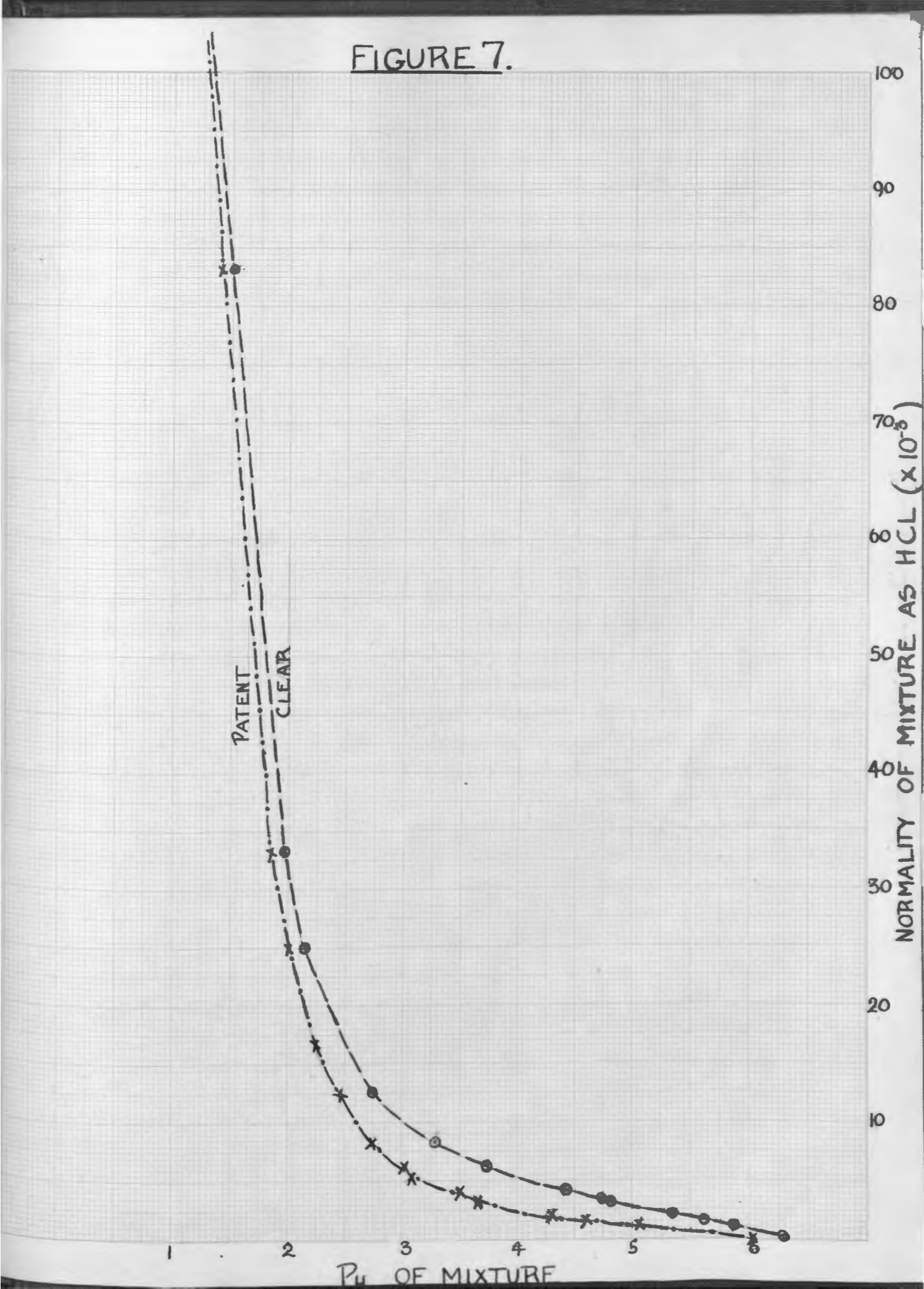

PATENT
CLEAR
100
90
80
70
60
50
40
30
20
10
NORMALITY OF MIXTURE AS HCL (X 10^{-3})
1
2
3
4
5
6
Pu OF MIXTURE

Table VI

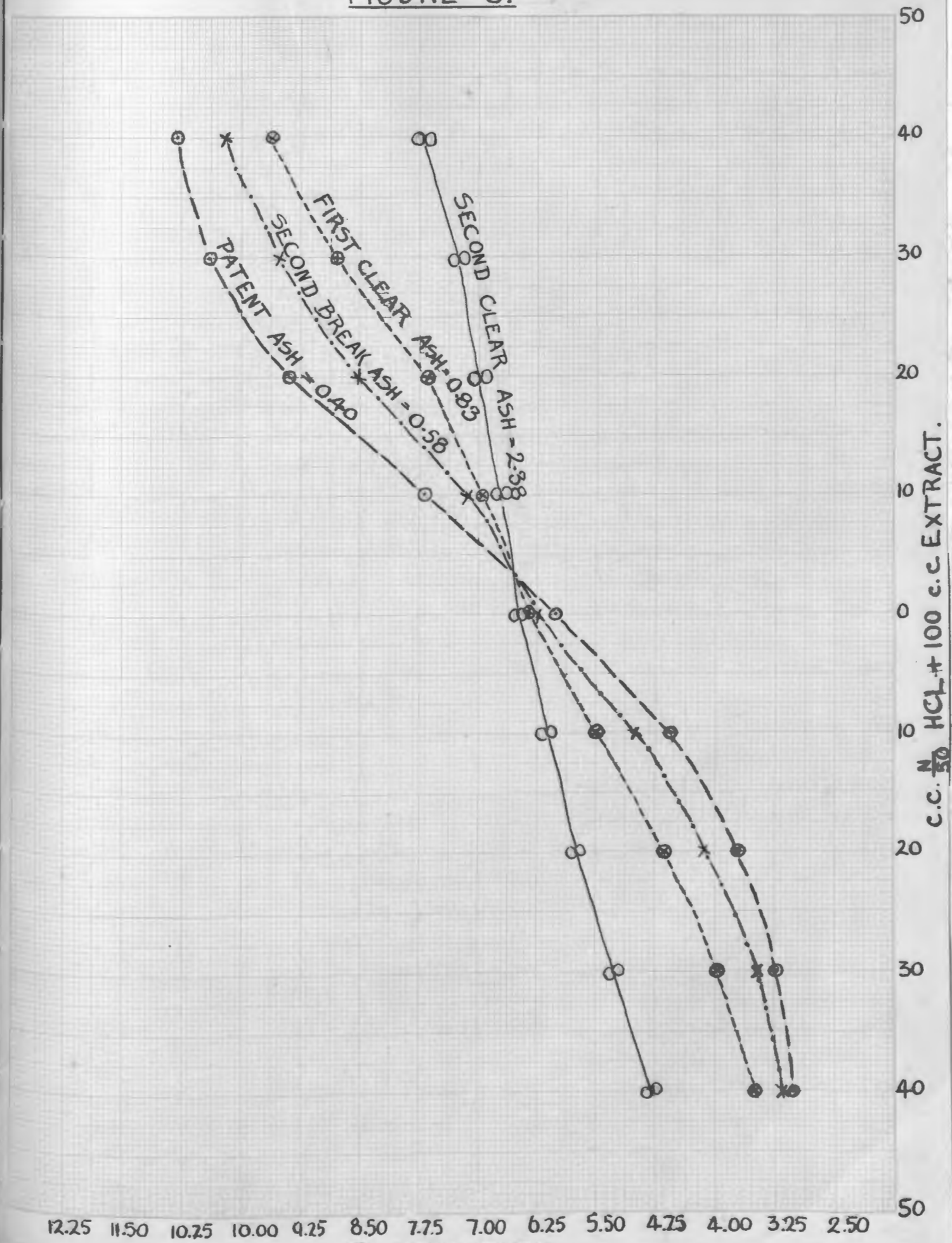
Comparisons between Ash Percent and effect on PH Value of adding Unit Quantities Acid or Alkali to Extracts of Various Flour Grades

	c.c.N/50 HCl + 100c.c.extract					c.c.N/50 NaOH + 100c.c.extract			
Normality of Mixture as HCl or NaOH	.00571	.00461	.00333	.00182	0	.00182	.00333	.00461	.00571
Flour grade	Ash%			Hastings Mill Flours					
First Break	1.34	4.04	4.56	5.15	5.86	6.34	6.77	7.17	7.66	8.59
Second "	0.58	3.15	3.45	4.11	5.00	6.25	7.12	8.52	9.53	10.21
Third "	0.67	3.46	3.62	4.18	5.19	6.22	6.96	7.89	9.25	9.77
Fourth "	1.62	4.26	4.78	5.43	6.02	6.36	6.75	7.08	7.49	8.20
First Middlings	0.44	2.87	3.11	3.60	4.48	6.07	7.47	9.28	9.89	10.62
Second "	0.45	2.96	3.25	3.64	4.65	6.10	7.27	8.72	9.79	10.32
Third "	0.55	3.04	3.45	3.96	4.94	6.22	7.22	8.59	10.00	10.35
Fourth "	1.17	4.06	4.46	5.11	5.85	6.42	6.86	7.29	7.91	8.79
Fifth "	0.61	3.16	3.53	4.14	5.04	6.31	7.15	8.38	9.62	10.22
				Columbia Mill Flour					
Patent	0.40	3.01	3.25	3.72	4.55	6.02	7.69	9.40	10.41	10.82
First Clear	0.83	3.48	3.96	4.60	5.49	6.40	6.97	7.64	8.81	9.62
Second "	1.38	4.80	5.26	5.73	6.12	6.44	6.73	7.02	7.27	7.66
				"Alpha" used for Soda Crackers -- Milled in 1905					
Patent	0.41	2.81	2.99	3.35	4.04	5.17	6.69	8.05	9.74	10.41
				Hastings Mill Flours					
Patent	0.42	2.99	3.21	3.65	4.52	6.05	7.40	9.28	10.11	10.62
Clear	0.80	3.65	4.19	4.75	5.54	6.32	6.93	7.54	8.59	9.35

THE RELATION OF BUFFER VALUE TO ASH CONTENT

The percentage of ash was determined in each flour of this series. Vidrédi (1893) was probably the first to establish a relation between grade and ash content. Swanson (1912) found that the percentage of ash increases directly as the proportion of bran or fibrous material. The ash content is probably the method most frequently employed for determining the relative grade of flour. Bailey (1918) and Bailey and Collatz (1919) found that the electrical conductivity of the extracts of flour grades paralleled their ash content. An inspection of table VI shows the consistent manner in which buffer value increases as the ash content. The titration curves figure 8 plotted for four flours of varying ash content

FIGURE 8.


SECOND CLEAR ASH = 2.38
FIRST CLEAR ASH = 0.93
SECOND BREAK ASH = 0.58
PATENT ASH = 0.40
50
40
30
20
10
0
10
20
30
40
50
c.c. N/10 HCL + 100 c.c EXTRACT.
12.25 11.50 10.25 10.00 9.25 8.50 7.75 7.00 6.25 5.50 4.75 4.00 3.25 2.50
PH OF MIXTURE

selected from this series show clearly this relation.

The change from the original PH produced on addition of 20 c.c. N/50 NaOH to 100 c.c. of extract were plotted against ash content for this series. To obtain more points on the curve similar data were obtained on a series of twentytwo flours from Duluth Universal Mill. Table VII and figure 9 show these results and also a series obtained on adding 10 c.c. N/40 NaOH and one obtained on adding 30 c.c. N/50 NaOH to extracts prepared in the same manner.

Table VII a

Change in PH on addition of Alkali to Extracts of various Flour Grades

Sample	Ash	Initial PH	10 c.c. N/50 NaOH	20 c.c. N/50 NaOH	30 c.c. N/50 NaOH
Patent	0.40	6.02	1.67	3.38	4.39
Alpha Patent	.41	6.17	1.52	2.88	4.57
First Middlings	.44	6.07	1.40	3.21	3.82
Second "	.45	6.10	1.56	3.35	3.69
Third "	.55	6.22	1.00	2.37	3.78
Second Break	.58	6.25	1.07	2.27	3.28
Fifth Middlings	.61	6.31	0.84	2.07	3.31
Third Break	.67	6.22	0.74	1.67	3.03
First Clear	.83	6.40	0.57	1.24	2.41
Fourth Middlings	1.17	6.42	0.44	0.87	1.49
First Break	1.34	6.34	0.43	0.83	1.32
Fourth "	1.82	6.36	0.39	0.72	1.13
Second Clear	2.38	6.44	0.29	0.58	0.83

Table VII b

Change in PH on addition of Alkali to Extracts of various Flour Grades (I)

Sample	Ash	Original PH	Color PH(PSP)	10 c.c. N/50 NaOH	20 c.c. N/50 NaOH	30 c.c. N/50 NaOH
Stone Stock	.35	6.00	# 8.4	# 3.23	3.96	4.60
Second Middlings	.38	6.00	# 8.4	# 2.81	3.84	4.60
First "	.41	6.04	# 8.4	# 2.62	3.39	4.32
Third "	.42	6.05	# 8.4	# 2.45	3.40	4.28
Sixth "	.42	6.20	# 8.4	# 2.39	3.42	4.23
Fifth "	.43	6.19	8.4	2.25	3.36	4.29
Patent "	.44	6.22	8.3	2.05	3.16	4.02

(I) Samples from Duluth Universal Mill B 802 to B 812 inclusive. Determinations made by C. H. Bailey.

Too alkaline for PSP.

FIGURE 9.

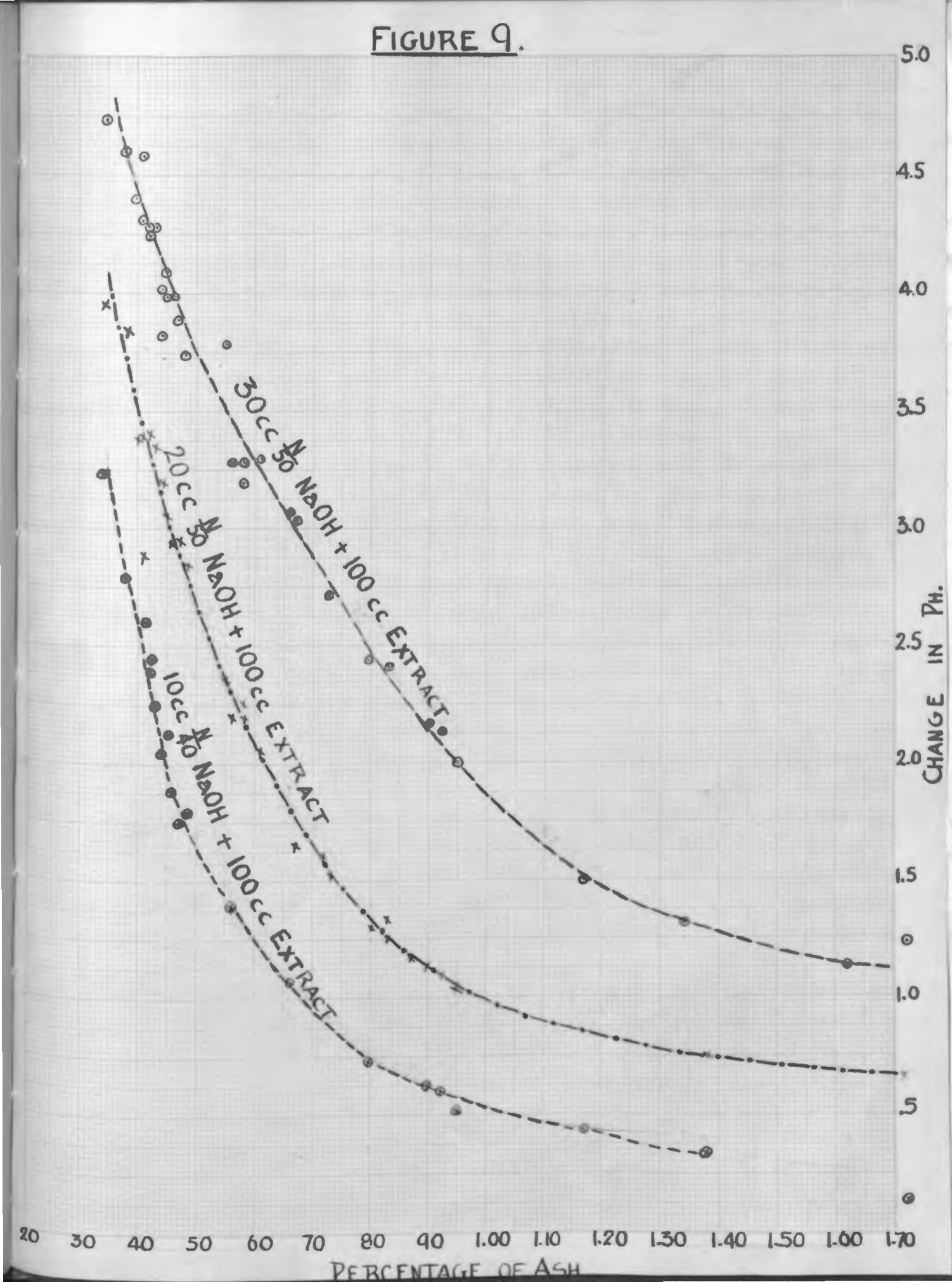

30cc N/50 NaOH + 100 cc EXTRACT
20cc N/50 NaOH + 100cc EXTRACT
10cc N/40 NaOH + 100cc EXTRACT
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
CHANGE IN PH.
20 30 40 50 60 70 80 90 1.00 1.10 1.20 1.30 1.40 1.50 1.00 1.70
PERCENTAGE OF ASH

Table VII b continued

Sizings	.45	6.32	8.3	2.13	3.06	3.97
Fourth Middlings	.46	6.20	8.1	1.88	2.93	3.98
Seventh "	.47	6.29	8.1	1.77	2.96	3.89
Second Break	.48	6.24	8.0	1.79	2.85	3.72
First "	.56	6.39	7.8	1.40	2.20	3.28
Third "	.58	6.41	7.5	1.12	2.19	3.19
First coarse tailings	.66	6.41	7.5	1.08	1.91	3.07
" fine "	.73	6.46	7.4	0.93	1.52	2.72
Fourth Break	.80	6.51	7.2	0.73	1.30	2.45
First Clear	.90	6.54	7.1	0.63	1.14	2.17
Second fine tailings	.92	6.41	7.0	0.61	1.10	2.13
Fifth Break	.95	6.39	6.9	0.52	1.05	2.01
Dust Flour	1.38	6.58	6.9	0.32	0.76	1.96
Second Clear	1.73	6.63	6.8	0.13	0.67	1.23

Figure 9 shows how consistent the relation is between buffer value and ash content. A determination of the buffers may serve as readily and accurately to indicate grade. It is seen from the curve that as ash increases to higher value, rate of change in PH diminishes. In lower grade flours therefore, the buffer value does not so accurately define grade as in higher grade flours. The method becomes less accurate as ash increases because the relative difference in buffer content becomes increasingly small per unit difference in ash. This fact does not lessen the usefulness of the method in grading flours with comparatively low ash content, or in distinguishing between those which vary rather widely.

THE NATURE OF THE BUFFERS PRESENT IN WHEAT FLOUR EXTRACTS

The composition of these acids and acid regulators is still somewhat a matter of conjecture. Swanson (1912) found a variation in soluble phosphates, amino-compounds and ash in relation to the portion of the wheat kernel from which the flour was milled. The percentage of ash has been used as a standard for determination of grade, because it has been found to vary directly as the amount of branny or fibrous material present. This ash contains approximately fifty percent P_2O_5. On the basis of the inorganic elements present, Swanson postulates that the phosphorous is present as K_2HPO_4 and KH_2PO_4, both being soluble. Clark and Lubs

(1917) say that phosphates have very little buffer action below the neutral point. In our work the difference between slightly and heavily buffered extracts was more marked above the neutral point, that is, upon addition of alkali. Evidence points to acid phosphates as the chief buffers in flour extracts. Whether these are present in the inorganic form in the flour and increase due to solubility as affected by time and temperature, or whether they are present in organic combination and increase due to hydrolysis aided by an enzyme, we have not yet sufficient data to prove definitely. The conductivity studies of Bailey and Collatz (1919) indicate however, that probably the phosphorous is originally in organic (and non-dissociable) combinations, which are hydrolysed. The change in conductance with time and temperature support this hypothesis.

BUFFER VALUE OF PROTEINS EXTRACTED IN ONE HOUR AT 25° C

An attempt was made to determine the buffer effect of proteins extracted under the given conditions. Portions of the extract of patent and of clear prepared as usual were boiled for five minutes to precipitate heat coagulable protein. The clear flour extract gave a small curdy precipitate with a very clear solution, the electrolytes present precipitating all colloidal material. The patent gave a milky solution and no precipitate, this extract becoming more turbid on boiling. The extracts were made up to volume with redistilled water and the PH determinations given in Table VIII were made to find the effect of this treatment.

It is seen that the buffer effect of soluble protein removed by boiling, is slight or none. The original reaction of the extracts remain unchanged. PH difference after addition of acid or alkali, tend to show a slightly decreased buffer value, much more noticeable in the clear flour extract. Whether this may not fall within the limits of experimental error is a question.

Table VIII

PH Values of Boiled Extracts

Clear

	boiled	not boiled	difference
Original extract ----------	6.37	6.32	0.05
100 c.c. " 20 c.c. N/50 HCl	4.31	4.75	0.14
100 c.c. " 20 c.c. N/50 NaOH	7.66	7.42	0.24
100 c.c. " 40 c.c. N/50 NaOH	9.53	9.31	0.32

Patent

	boiled	not boiled	difference
Original extract ----------	6.07	6.05	0.02
100 c.c. " 20 c.c. N/50 HCl	3.68	3.65	0.03
100 c.c. " 20 c.c. N/50 NaOH	9.35	9.28	0.07
100 c.c. " 40 c.c. N/50 NaOH	10.67	10.62	0.05

The determination of soluble protein from total nitrogen present in a series of mill streams, would indicate that no consistent relation exists between buffer value and proteins soluble under the conditions of these experiments. Nitrogen determinations were made on extracts prepared from the same series previously used for determinations of comparative buffer effect, to find whether or not the percentage of soluble protein paralleled the buffer content. Since we had already found a very close agreement between the percentage of ash, the electrical conductivity, K X 10^{-4}, and the buffer value, these quantities were also included in Table IX. The values for K X 10^{-4} are from Bailey and Collatz (1917). That there is no consistent relation between percentage of soluble protein and the other values, is seen from these comparisons.

Table IX

Comparisons between Buffer Value, Ash, Electrical Conductivity, and Percentage of Soluble Protein

Mill Stream	Ash percent	Electrical Conductivity	PH change with 20c.c. N/50 NaOH+100c.c.extract	Soluble Protein Percent
First Middlings	0.442	5.360	1.21	1.26
Second "	0.446	5.492	2.62	1.45
Third "	0.555	6.257	2.37	1.57
Second Break	0.585	6.675	2.27	1.40
Fifth Middlings	0.613	6.687	2.07	1.35
Third Break	0.660	7.587	1.67	1.59
Fourth Middlings	1.171	10.000	0.87	2.45
First Break	1.340	11.241	0.83	2.09
Fourth "	1.520	12.177	0.72	2.09

COMPARISON OF METHODS

Returning to a consideration of a number of determinations made to check the applicability and accuracy of the colorimetric against the electrometric method we compiled from our work the data presented in Table X.

The hydrogen electrode was always taken as standard since it was found to give consistent results and correct values when tested with standard buffer mixtures. The method of carrying out the colorimetric comparisons has been previously described. To distinguish between flour grades by the colorimetric method was much more easily done on the alkaline side, that is, if alkali were added to the extract rather than acid. Of all the indicators tried, phenolsulfonphthalein gave the best results. The maximum differences between flour grades were found when extractions were made for one hour at 25° C. and when 20 c.c. of N/50 NaOH were added to 100 c.c. of extract. This statement holds for trials made thus far.

Table X

Comparisons of Electrometric and Colorimetric Methods

100 c.c. extract + N/50 NaOH or N/50 HCl

Flour grade	Acid or Alkali added	Electrode PH	Color PH	Indicator
First Middlings	0	6.07	6.0	Dibrom C S P
" "	20 c.c. NaOH	9.28	9.1	T S P
Second Middlings	10 c.c. NaOH	7.56	7.6	P S P
" "	10 c.c. HCl	4.50	4.4	Tetrabrom P S P
Third Middlings	10 c.c. NaOH	7.22	7.2	P S P
" "	20 c.c. NaOH	8.59	8.6	T S P
Fourth Middlings	0	6.42	6.4	Dibrom C S P
" "	20 c.c. NaOH	7.29	7.2	P S P
" "	30 c.c. NaOH	7.91	8.0	P S P
" "	40 c.c. NaOH	8.79	8.6	T S P
" "	10 c.c. HCl	5.05	5.3	Dibrom C S P
" "	20 c.c. HCl	5.11	5.2	" "
Fifth Middlings	0	6.31	6.2	Dibrom T S P
" "	10 c.c. NaOH	7.15	7.1	P S P
" "	20 c.c. NaOH	8.38	8.4	T S P
" "	30 c.c. NaOH	9.62	9.6	T S P
" "	10 c.c. HCl	5.04	5.1	Dibrom C S P
First Break	0	6.34	6.4	" "
" "	10 c.c. HCl	5.86	5.9	" "
" "	20 c.c. HCl	5.15	5.2	" "
" "	10 c.c. NaOH	6.77	6.7	" "
" "	20 c.c. NaOH	7.17	7.2	P S P

Table X continued

Third Break	0	6.22	6.2	Dibrom C S P
" "	10 c.c. HCl	5.19	5.2	" "
" "	20 c.c. HCl	4.18	4.2	Tetrabrom P S P
" "	10 c.c. NaOH	6.96	7.0	P S P
" "	20 c.c. NaOH	7.89	7.9	P S P

Our results indicate that the colorimetric method may become useful in comparing reaction and buffer value of flour grades. In cases where buffer content varies as widely as between a high grade patent and a clear flour similar to those studied by us, this method may become readily applicable. The relative change in buffer action per unit difference in ash content steadily diminishes as the ash content increases and in the lower grade flours buffer action is not as accurate an index of grade as in high grade flours.

SUMMARY AND CONCLUSIONS

1. Varying the temperature and period of extraction for the same flours does not alter the initial hydrogen ion concentration of the extract.

2. Extracts of low-grade flours have a higher titratable acidity, but a lower hydrogen ion concentration than extracts of high-grade flours. Large quantities of buffers in low-grade flours tend to maintain the reaction nearer the neutral point in spite of a higher content of acid reacting material.

3. Buffer value in wheat flour extracts is proportional to content of branny or fibrous material; consequently it rises to a greater maximum in a low-grade than in a high-grade flour.

4. Increase in buffer content is slow at 0°, much more rapid at 25°, and slightly more so at 40° and 60°. Buffer increase is accelerated much more by increasing the temperature than by prolonging the period of digestion.

5. Titration curves show maximum variations between patent and clear when 20 c.c. N/50 alkali are added to 100 c.c. extract.

6. Buffer value increases directly as percentage of ash and electrical conductivity.

7. To indicate grade, buffer value may replace ash determination. Rate of change in buffer content decreases however as the percentage of ash reaches higher values, thus diminishing the accuracy of the method for low-grade flours.

8. Proteins extracted in one hour at 25° exert no apparent buffer action, nor is there any consistent relation between quantity of soluble protein and ash, electrical conductivity or buffer value.

9. It appears that buffer increase is due chiefly to production of inorganic phosphates from decomposition of complex organic compounds by hydrolysis aided by an enzyme.

10. Colorimetric comparisons suggest a rapid easy method for determining grade based on the use of color standards.

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