Electrolytes and Colloids.—The Physical State of Gluten.

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Gluten, as ordinarily prepared by washing wheat flour in tap water, forms a coherent stringy mass insoluble in water. It consists essentially of a mixture of two proteins, gliadin and glutenin, but even when very thoroughly washed it always includes some starch. Gliadin, which forms rather more than half of the total protein, is soluble in dilute alcohol, and gives to the gluten its peculiar physical properties.

The power which dough possesses of retaining the gas formed during fermentation is due to the tenacity and ductility of gluten.* Therefore, the property of forming a light and well-shaped loaf, which is so variable a feature of different flours, is determined by the amount and the physical state of the contained gluten.

The physical state of gluten, like that of other colloids, is conditioned by the electrolytes which are present. Gluten washed out of flour with distilled water obviously is more friable and less tenacious than gluten washed out with tap water which contains salts. It is this influence of electrolytes upon the physical state of gluten which we propose to discuss.

Gluten is peculiarly sensitive to low concentrations of acid or alkali. A tenacious ductile mass suspended in a large volume of, for instance, 0·0001 normal acid, begins almost at once to show signs of disintegration, and is at once dispersed by slight movement to form a stable opaque colloidal solution or hydrosol.

Action of Acids.—This action was investigated quantitatively by suspending a small mass of gluten on a bent glass rod in a beaker containing 120 c.c. of a solution of acid of known strength, and noting the concentration at which cohesion was so far reduced as to allow the protein to fall off the rod and disperse in a cloudy "solution." It was found that while very dilute acid causes dispersion, a solution of a strong acid above a certain concentration maintains the cohesion. Gluten, therefore, is coherent in distilled water, and in strong acids above a certain critical concentration. A weak acid, such as acetic acid, brings about dispersion up to as high as twice normal, the highest concentration tried. Inspection of a series of beakers with concentrations of any strong acid from zero to the critical point makes it clear that, starting from the lowest concentration,

* Wood, 'Journ. Agric. Sci.,' vol. 2, part 2, p. 139, and part 3, p. 267.

dispersion increases to a maximum and then falls to zero at the critical point. In other words, the power of destroying the cohesion and dispersing the gluten as a cloud varies with the concentration of the acid, so that the relation can be shown by a curve. The form of the curve will be seen later.

The dispersion of the gluten is not due to a change in the protein molecule of the nature, for instance, of hydrolysis, since it can be recovered as a tenacious stringy precipitate by neutralising the acid or by the addition of salt.

The following table gives the mean of several determinations of the concentration at which the gluten retains its coherence. The exact point is the concentration at which gluten just breaks under its own weight when suspended in the solution of acid; and the results obtained in different experiments are fairly consistent. It is remarkable that there should be no simple relation between the observed concentrations and the strengths of acid used as measured by electric conductivity. The conductivity of the solutions after the gluten had been immersed in them was measured, and the results are given in the second column of figures, the value of the sulphuric acid solution being taken as unity:—

Table I.

Acid.	Normality of critical concentration.	Relative conductivity.
H_2SO_4	0·017	1·0
Camphorsulphonic	0·02	1·59
HNO_3	0·03	1·9
HCl	0·05	3·8
Oxalic	0·15	3·8
H_3PO_4	2·00	—

Action of Distilled Water.—Gluten breaks up when washed very thoroughly in many changes of ordinary distilled water. The distilled water used was acid to litmus owing to the presence of carbonic acid; and the dispersion of the protein is due to this acidity, since (1) it is precipitated by the addition of a trace of alkali, and (2) the protein when dispersed is electro-positively charged—that is to say, it displays the characteristic relation of protein to acid.

The Influence of Salts.—Salt in small concentration precipitates a hydrosol of gluten whether it be formed by acid or by alkali. Therefore, salts lessen the power which acids or alkalis possess of destroying the cohesion of gluten, and, in sufficient concentration, completely neutralise it. The concentration of salt necessary completely to nullify the dispersive power

of particular acids was investigated in the manner already described, namely, by suspending approximately equal pieces of gluten in varying concentrations of acid and salt, and noting the point at which cohesion was so far reduced as to allow the protein to flow off the rod. The relations appear in the following curves (fig. 1), which show that for all strong acids and for all salts the concentration of the latter needed to balance the former increases to a maximum as the concentration of acid increases, and then declines to zero at the point where the acid alone is sufficient to maintain cohesion. The curves all agree, therefore, in showing that, measured by the concentration of salt needed to prevent dispersion, the dispersive power of an acid increases with increasing concentration, and then falls until the critical concentration is reached, where dispersive action is *nil*.

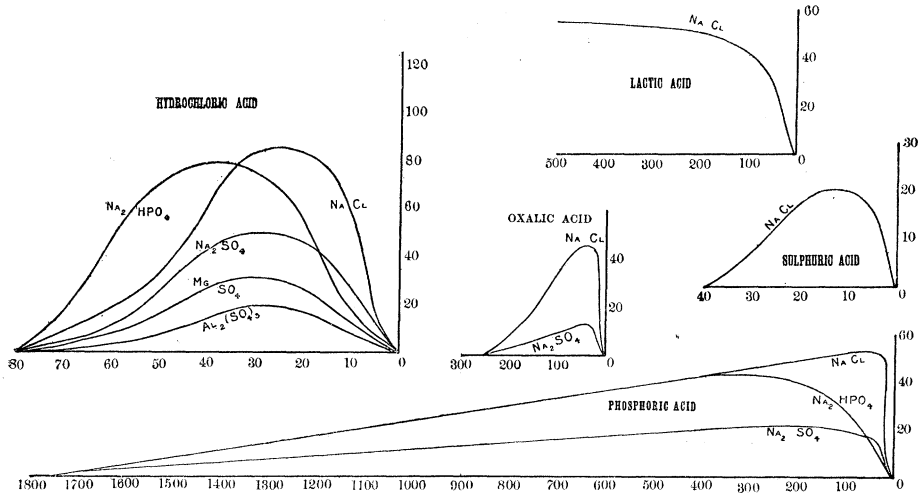

FIG. 1.

These curves are so characteristic that they afford a means of testing a point of general theoretical interest. One great class of colloidal solutions, the aqueous solutions of characteristically insoluble bodies such as metals, some proteins, sulphides, and gums, are characterised by the fact that round each particle of the solute there is an electric double layer, and on the potential difference between which the stability of the solution depends. Coagulation or precipitation of such a solution is approximately coincident with the reduction of the potential difference to zero, the most complete coagulation, *i.e.*, mechanically the densest and most coherent coagulum, being formed at the isoelectric point.*

* Hardy, 'Roy. Soc. Proc.,' vol. 66, p. 110, 1900; Picton and Linder, 'Chem. Soc. Trans.,' 1905—1906, vol. 87; Perrin, 'Journ. de Chim. Physique,' vol. 2, p. 601, 1904; vol. 3, p. 50, 1905.

On this view the formation of the hydrosol of gluten is due to the development of electric charges round the particles of the protein owing to chemical interaction between the protein, the acid or alkali and the water; and the tenacity, ductility, and water-content of a solid mass of moist gluten depends upon the total or partial disappearance of these electric double layers, and the reappearance of what is otherwise obscured by them, namely, the adhesion or "idio attraction," as Graham called it, of the colloid particles for each other, which makes them cohere when they come together.

It is possible to put this hypothesis to the proof. We can measure the potential difference between the water face and the protein face of each particle in the hydrosol of gluten by determining the rate of transport of the particles in a uniform electric field. The method adopted has been described by one of us.* Briefly it consists in the use of a graduated U-tube, the opalescent hydrosol is introduced as the lower layer, the upper layer in each limb being a clear solution of the same electrical resistance. Electrodes are immersed in the upper layer, a field established, and the rate of movement of the boundaries between upper and lower layers observed.

The hydrosol was prepared either by washing gluten in distilled water containing carbonic acid, a process which occupied at least two days, or in a few hours by washing in a few changes of 0·0001 normal sulphuric acid. It was freed from all starch by centrifuging. To successive lots of the hydrosol, acid was added in varying amounts, and water when necessary, so that the concentration of protein was constant, while the concentration of acid varied. Finally the resistance was measured, and a fluid to form the upper layer was prepared either by adding the same acid to water or by adding sodium chloride. Hydrochloric, sulphuric, and acetic acids were used, and the results were in all cases the same. The figures for hydrochloric acid are plotted in the following curve, the ordinates being specific conductivity of the solution $\times 10^{-6}$, the abscissæ the specific velocity in centimetres per second for unit potential gradient $\times 10^{-}$ (fig. 2).

The curve agrees in form with those already given for the effect of salt upon cohesion, and we may therefore conclude that acids, and by inference alkalis also, destroy the cohesion of gluten by forming double electric layers round the particles, and that the potential difference between these layers rises with increasing concentration of acid to a maximum, and then falls.

Action of Alkalis.—The action of alkali in destroying the cohesion of gluten is essentially similar to that of acid, except that the electric sign is reversed. In a hydrosol of gluten formed by carbonic acid or any other acid

* Hardy, 'Journ. Physiol.,' vol. 33, p. 251, 1905.

the protein is charged positively; when formed by any alkali it is charged negatively.

It is interesting to note that, when alkali is added, it not only neutralises any acid present, but also reacts directly with the protein as though the latter were itself an acid. The alkali, therefore, disappears as such; it is, in point

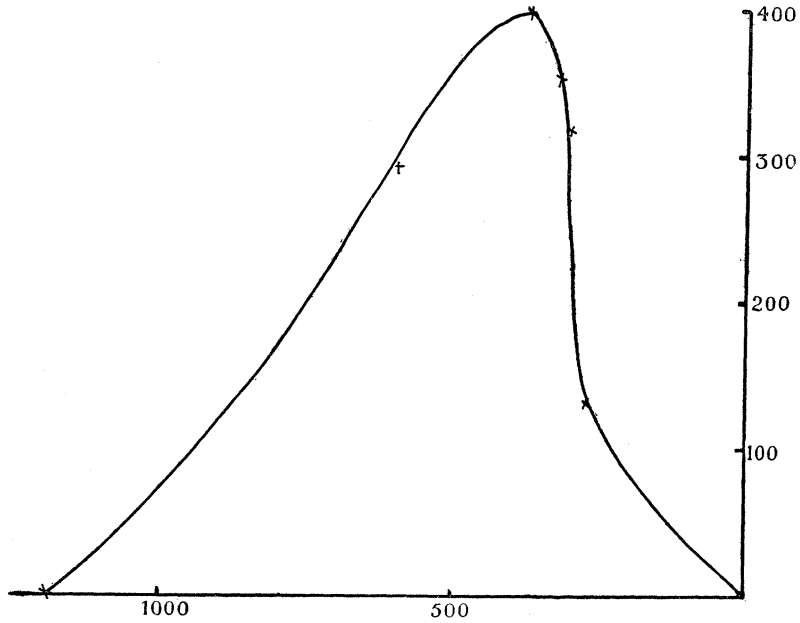

FIG. 2.

of fact, neutralised by the protein with the formation of new ions. For instance, in a particular hydrosol formed by carbonic acid, the particles of gluten were charged positively, and had a specific velocity 46×10^{-6} cm. per second. Sodium hydroxide was added in quantity sufficient to make the entire solution contain N/1600 of NaOH. The fluid was not alkaline to phenolphthalein, but in spite of this the protein was now charged negatively and had a specific velocity of 23×10^{-6} cm. per second.

Approximately pure gliadin, dissolved in 70-per-cent. alcohol, shows relations to acid and alkali the same as those described for gluten. Dropped into 98-per-cent. alcohol or distilled water, it forms an opalescence, and is then electro-positive; in presence of N/4160 of NaOH it is electro-negative.

Conclusions.—The experimental results seem to prove beyond question that the physical state of gluten—that is to say, the degree of coherence or dispersion as a hydrosol, is determined by the potential difference between the particles of the protein and the fluid.

The development of such a potential difference between colloid particles and fluid has been accounted for in two ways. The first, which may be described as a purely physical hypothesis, ascribes it to differences in the speed of the ions of electrolytes present. The colloid particles at any moment contain within themselves an excess of the most penetrating and rapidly moving ions present, and they therefore have the charge of that ion. In presence of acid they will have the charge of the hydrogen ion, in the presence of alkali that of the hydroxyl ion. This hypothesis was advanced by one of us to explain the properties of certain proteins of the globulin class when in solution.* It was also advanced independently by Perrin to explain the electrical properties of colloidal solutions in general.†

The second hypothesis is frankly chemical in nature, and, as applied to proteins, it may be put as follows:—The protein molecule contains H and OH groups. Proteins, therefore, as a class are, like their chemical allies the amino-acids, amphoteric electrolytes. They react with acids and alkalis to form salts, but the reactions are not precise, an indefinite number of salts of the form $(B)_nBHA$ being formed where the value of n is determined by conditions of temperature, and concentration, and of inertia due to electrification of internal surfaces within the solution.

The salt so formed is ionised by the water. Positive or negative ions, as the case may be, leave the protein face to enter the water face, and form an electric layer there, while the protein face is left charged respectively negative or positive.‡ On this view, in the particular case under consideration, the decrease and final disappearance of the potential difference which occurs when the concentration of acid rises above a certain value would be due to a suppression of the feeble ionisation by the excess of acid.

The first view seems to be incompatible with certain experimental facts—such, for instance, as the fact that salts such as LiCl or LiBr, the velocities of whose ions are in the ratio of about 1 to 2, do not confer any change on proteins, nor, as Perrin noticed, do they produce any contact difference of potential between a water and a solid wetted by it. It also ignores the purely chemical nature of the conditions which govern the formation of colloidal solutions of metals.§

* Hardy, 'Journ. Physiol.,' vol. 29, p. xxvii, 1903.

† Perrin, 'Journ. de Chim. Physique,' vol. 2, p. 601, 1904; vol. 3, p. 50, 1905.

‡ Hardy, 'Journ. Physiol.,' vol. 33, p. 251, 1905; 'Roy. Soc. Proc.,' vol. 79, p. 413, 1907.

§ Burton, 'Phil. Mag.,' vol. 11, p. 425, 1906.