Studies of Wheat Flour Grades. II—Buffer Action of Water Extracts^{1,2}

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SIGNIFICANCE OF H-ION CONCENTRATION OF WHEAT FLOUR DOUGHS

Reaction is an important factor in controlling biological phenomena. All living tissues from the simplest to the most complex are affected by significant changes in the H-ion concentration of the fluids which bathe them. Bacteria, yeasts, and related microorganisms are no exception to this rule, and of late much attention has been given to the preparation of substrates with an optimum H-ion concentration for their culture and study. Sörenson³ studied the influence of H-ion concentration on enzymatic activity and concluded that this can be controlled by adjusting the reaction. Clark⁴ showed the effect of reaction upon the activity of bacteria and emphasized the necessity of employing culture media with the proper H-ion concentration. The studies of Euler and Emberg⁵ establish the sensitivity of yeast and its enzymes to H-ion concentration. They found that the fermentation velocity, and the activity of invertase, reached a maximum in the vicinity of pH=5.

Jessen-Hansen⁶ concluded that there is a definite H-ion concentration to which the bread made from any particular wheat flour should best be brought. The optimum corresponds approximately to pH=5.0; for choice flours it appears to be a little higher, and for poorer grades slightly lower. The same author later⁷ suggests that "flour improvers" owe their value to the manner in which they increase the H-ion concentration of the dough.

Cohn and Henderson⁸ state that the proper fermentation of bread dough is related to the increasing H-ion concentration, the dough being in best condition when a concentration is reached which turns methyl red from orange to red (about pH=5.0). At this concentration the elasticity of gluten is at a maximum and the yeast most active; moreover, the development and activity of the organism causing "ropy" bread are depressed.

Observations made in this laboratory, as yet unpublished, show the isoelectric point of wheat gluten to be reached when the pH=5.1±. It thus appears that the production of satisfactory yeast-leavened bread is best effected through a slightly acid condition of the dough. This acidity may result from the formation of organic acids by microorganisms, or by the deliberate addition of acid-reacting substances. Through the proper H-ion concentration the elasticity of the gluten, and the activity of the yeast and of "rope producing" bacteria are affected in the desired direction.

SIGNIFICANCE OF THE BUFFER ACTION OF FLOUR

Jessen-Hansen indicated that the pH of high-grade wheat flours is numerically lower, *i. e.*, they have a higher H-ion concentration, than low-grade flours. This is in spite of the fact that the latter have a higher titrable acidity, the reason being the presence of more buffers in the lower grades. No data are presented in his paper, however, which establish in a definite manner the proportional relation between grade and buffer action. Since the variations in this regard are of significance in controlling the pH of doughs, it was deemed advisable to determine the magnitude of these differences.

Such data would be of value in the preparation of doughs of any desired H-ion concentration by a definite, rather than a cut-and-try, process.

THE INFLUENCE OF TIME AND TEMPERATURE OF EXTRACTION UPON THE BUFFER ACTION OF THE EXTRACT

In developing a standard method of procedure a series of preliminary studies were conducted to determine the effect of time and temperature of extraction upon the buffer action of the extract. Two flours, a high-grade patent and a lower or second clear grade, were employed, in order to afford marked differences in the character and quality of material. A ratio of 1 part of flour to 5 parts of redistilled water was used in all of the determinations of buffer action. The water was first brought to the temperature used for extraction before adding it to the flour, which had also been brought to the same temperature. Extractions were made in water thermostats at 0°, 25°, 40°, and 60°, for periods of 1, 2, 4, and 6 hrs. The flour particles were kept in suspension by intermittent shaking during the extraction period. At the end of the period the flour particles were thrown out of suspension by whirling rapidly in a centrifuge for a few minutes. The clear decantate was divided into 100-cc. portions to which were added 10, 20, 30, 40, and 50 cc. of 0.02 N HCl, and like quantities of 0.02 N NaOH.¹ The H-ion concentrations

FIG. 1.—RELATION OF TIME AND TEMPERATURE OF EXTRACTION TO THE BUFFER ACTION OF WATER EXTRACTS OF PATENT AND CLEAR FLOURS

of the original extracts and of the portions treated as described were then determined electrometrically by means of the hydrogen electrode described by Bailey,² connected through a calomel electrode to a potentiometer in the conventional manner. In addition, a number of colorimetric determinations were made, chiefly to ascertain the accuracy of this method. A later paper will detail certain of the observations made through the use of indicators. All of the data reported in the following tables and graphs were obtained by the electrometric method.

Since the pH of the untreated extracts of the patent and of the clear flour was not affected by the time and temperature

¹ In actual practice 25- or 50-cc. portions of the extract were used, together with proportional quantities of the 0.02 N HCl and NaOH.

² J. Am. Chem. Soc., 42 (1920), 45.

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² Published with the approval of the Director as Paper No. 216 Journal Series, Minnesota Agricultural Experiment Station.

³ *Ergebnisse Physiol.*, 12 (1912), 393.

⁴ *J. Infectious Diseases*, 17 (1915), 109.

⁵ *Z. Biol.*, 69 (1919), 349.

⁶ *Compl.-rend. trav. lab. Carlsberg*, 1 (1911), 170.

⁷ *Z. ges. Getreidew.*, 4 (1912), 271.

⁸ *Science*, 48 (n. s.) (1918), 501.

TABLE I—INCREASES AND DECREASES IN H-ION CONCENTRATION (IN TERMS OF pH) RESULTING FROM THE ADDITION OF ACID AND ALKALI TO THE WATER EXTRACTS

Duration of Extraction Hours	Original Extract pH	Decrease in pH on Adding 0.02 N HCl					Increase in pH on Adding 0.02 N NaOH						
		10 Cc.	0.02 N HCl per 100 Cc. Extract	20 Cc.	30 Cc.	40 Cc.	50 Cc.	10 Cc.	0.02N NaOH per 100 cc. Extract	20 Cc.	30 Cc.	40 Cc.	50 Cc.
Patent Flour Extracted at 0°													
1	6.10	1.70	2.63	2.99	3.26	3.38	2.86	4.18	4.67	4.90	...	...	
2	6.09	1.56	2.51	2.95	3.17	3.33	2.08	3.85	4.46	4.76	4.97	...	
4	6.10	1.54	2.45	2.89	3.12	3.29	1.68	3.67	4.21	4.74	4.94	...	
6	6.09	1.51	2.40	2.84	3.11	3.27	1.59	3.41	4.22	4.66	4.92	...	
Patent Flour Extracted at 25°													
1	6.05	1.53	2.40	2.84	3.06	3.26	1.36	3.24	4.07	4.58	4.87	...	
2	6.05	1.47	2.33	2.78	3.04	3.18	1.37	3.01	3.87	4.43	4.77	...	
4	6.05	...	2.28	2.74	3.01	3.18	1.27	2.93	3.79	4.40	4.72	...	
6	6.05	1.44	2.26	2.74	3.01	3.18	1.13	2.91	3.86	4.40	4.72	...	
Patent Flour Extracted at 40°													
1	6.00	1.49	2.35	2.77	3.04	3.20	1.52	2.61	3.94	4.47	...	...	
2	6.02	1.47	2.33	2.76	3.02	3.21	1.23	...	3.79	4.33	4.67	...	
4	5.99	1.40	2.20	2.71	3.01	3.18	1.06	2.48	3.45	4.12	4.51	...	
6	5.99	1.37	2.20	2.69	3.01	3.17	1.06	2.45	3.43	4.00	...	...	
Patent Flour Extracted at 60°													
1	6.04	1.34	2.13	2.62	2.96	...	1.06	2.80	3.54	4.24	4.46	...	
2	6.04	1.32	2.15	2.62	2.93	...	0.92	2.70	3.51	4.12	4.37	...	
4	6.02	1.32	2.11	2.61	2.91	...	1.05	2.57	3.51	4.11	...	...	
6	6.04	1.32	2.13	2.64	2.93	...	1.04	2.26	3.49	3.98	...	...	
Clear Flour Extracted at 0°													
1	6.29	1.20	2.01	2.63	3.03	3.25	1.47	3.08	3.92	4.36	4.62	...	
2	6.29	1.17	1.93	2.57	2.94	3.21	1.20	2.75	3.65	4.20	4.28	...	
4	6.29	1.07	1.81	2.42	2.84	3.21	0.95	2.26	3.23	3.80	4.19	...	
6	6.27	0.98	1.67	2.28	2.72	3.02	0.78	1.69	2.88	3.43	3.99	...	
Clear Flour Extracted at 25°													
1	6.30	0.75	1.55	2.11	2.65	3.00	0.53	1.12	2.17	2.90	3.50	...	
2	6.29	0.69	1.47	2.06	2.55	2.83	0.47	0.91	1.64	2.52	3.08	...	
4	6.30	0.65	1.43	2.02	2.53	2.85	0.46	0.89	1.41	2.22	2.88	...	
6	6.31	0.63	1.37	1.97	2.57	2.79	0.41	0.80	1.24	...	...	...	
Clear Flour Extracted at 40°													
1	6.29	0.69	1.51	2.10	2.54	2.87	0.47	0.90	1.54	2.50	3.11	...	
2	6.30	0.67	1.47	2.06	2.51	2.85	0.43	0.85	1.39	2.22	2.98	...	
4	6.30	0.64	1.41	2.04	2.51	...	0.40	0.82	1.38	2.15	2.78	...	
6	6.30	0.65	1.40	1.98	2.40	2.72	0.41	0.80	1.24	...	...	...	
Clear Flour Extracted at 60°													
1	6.25	0.72	1.48	2.06	2.46	...	0.44	0.92	1.46	2.42	2.83	...	
2	6.25	0.72	1.43	1.89	2.38	...	0.43	0.85	1.34	2.22	2.88	...	
4	6.24	0.68	1.39	1.95	2.40	...	0.40	0.83	1.30	2.14	2.77	...	
6	6.24	...	1.37	...	...	...	0.45	0.84	1.27	1.94	...	...	

of extraction, the data secured are given in Table I in terms of the decrease and increase in pH effected by the progressive addition of acid and alkali, respectively. These show an increase in buffer action with an increase in time of extraction, and an even greater increase in buffer action when the temperature of extraction was elevated from 0° to 40°. The differences between the extract prepared at 40° and 60° are small. The changes in pH on addition of 20 cc. of 0.02 N HCl and 20 cc. of 0.2 N NaOH are shown graphically in Fig. 1.

EFFECT OF BOILING UPON BUFFER ACTION OF EXTRACT

The water extract of wheat flour contains a variety of complex substances, and the question arises as to which of these are responsible for the buffer action observed. Several proteins are usually present in such extract, notably leucosin (an albumin), gliadin (a prolamin), and some globulin. The leucosin is the typical water-soluble protein, and since it is coagulated by heat, an experiment was conducted to determine the effect of removing it by boiling. Portions of the original extracts prepared by shaking patent and clear flours with redistilled water at 25° for 1 hr. were immersed in boiling water for several minutes. After cooling, these preparations were made up to their original volume by replacing the water lost by evaporation. Their buffer action was then determined in comparison with the original extract which had not been heated. The results, given in Table II, show that the effect of thus coagulating the leucosin was slight, and that consequently it played an insignificant role in determining the buffer action of extracts in which it was present. It apparently functions to a small extent, however, as indicated by the fact that in the clear flour extract which contained the most soluble protein, the differences in buffer action between the original and the boiled extract were greatest.

Further, to verify the observations recorded in Table II, the buffer action of a series of flours of differing quality was compared with the soluble protein in the extract. The specific electrical conductivity of a 1:10 extract of these same flours had been previously determined by Bailey and Collatz.¹

These data will be found in Table III, and they appear to establish the fact that no relation exists between the percentage of protein in the extract and the buffer action of that extract as shown by the change in pH on addition of 20 cc. of 0.02 N NaOH to 100 cc. of the extract. The buffer action increases regularly with the electrical conductivity, however, which suggests that the buffers may be chiefly electrolytes. A study of phytase by Collatz and Bailey¹ showed that enzyme to respond to variations in temperature in hydrolyzing phytin, as evidenced by the rate of change in electrical conductivity of phytin-phytase solutions. A similar

TABLE II—COMPARISON OF THE BUFFER ACTION OF THE ORIGINAL AND BOILED EXTRACTS OF PATENT AND CLEAR FLOURS

TREATMENT	H-Ion Concentration		
	Not Boiled pH	Boiled pH	Difference pH
<i>Patent flour extracts</i>			
Original extract of patent flour.....	6.05	6.07	0.02
100 cc. extract + 20 cc. 0.02 N HCl.....	3.68	3.65	0.03
100 cc. extract + 20 cc. 0.02 N NaOH.....	9.28	9.35	0.07
100 cc. extract + 40 cc. 0.02 N NaOH.....	10.62	10.67	0.05
<i>Clear flour extracts</i>			
Original extract of clear flour.....	6.32	6.37	0.05
100 cc. extract + 20 cc. 0.02 N HCl.....	4.75	4.61	0.14
100 cc. extract + 20 cc. 0.02 N NaOH.....	7.42	7.66	0.24
100 cc. extract + 40 cc. 0.02 N NaOH.....	9.21	9.53	0.32

response in terms of buffer action to variations in the conditions of extraction has been shown in Table I. These facts suggest that the buffer action of flour extracts is probably due in large part to phosphates produced from phytin through the action of phytase during the period of extraction with water.

TABLE III—THE BUFFER ACTION, ELECTRICAL CONDUCTIVITY, ASH, AND WATER-SOLUBLE PROTEIN CONTENT OF A SERIES OF FLOURS

SAMPLE	Ash Per cent	Specific Conductivity $K_{sp} \times 10^{-4}$	Increase in pH on Adding 20 Cc. 0.02 N NaOH to 100 Cc. Extract		Soluble Proteins Per cent
First middlings....	0.44	5.38	3.21		1.26
Second middlings..	0.45	5.49	2.62		1.45
Third middlings...	0.55	6.26	2.37		1.57
Second break.....	0.58	6.67	2.27		1.40
Fifth middlings....	0.61	6.69	2.07		1.35
Third break.....	0.67	7.59	1.67		1.59
Fourth middlings..	1.17	10.00	0.87		2.45
First break.....	1.34	11.24	0.83		2.09
Fourth break.....	1.62	12.18	0.72		2.09

¹ THIS JOURNAL, 13 (1921), 319.¹ THIS JOURNAL, 13 (1921), 317.

TABLE IV—H-ION CONCENTRATION AFTER ADDITION OF ACID AND ALKALI TO THE EXTRACT OF FLOURS OF DIFFERENT GRADES

Flour Grade	Ash Per cent	0.02 N HCl Added per 100 Cc. Extract				Original Extract pH	0.02 N NaOH Added per 100 Cc. Extract			
		40 Cc. pH	30 Cc. pH	20 Cc. pH	10 Cc. pH		10 Cc. pH	20 Cc. pH	30 Cc. pH	40 Cc. pH
Patent ¹	0.40	3.01	3.25	3.72	4.55	6.02	7.69	9.40	10.41	10.82
1st middlings	0.44	2.87	3.11	3.60	4.48	6.07	7.47	9.28	9.89	10.62
2nd middlings	0.45	2.96	3.25	3.64	4.65	6.10	7.27	8.72	9.79	10.32
3rd middlings	0.55	3.04	3.45	3.96	4.94	6.22	7.22	8.59	10.00	10.35
2nd break	0.58	3.15	3.45	4.11	5.00	6.25	7.12	8.52	9.53	10.21
5th middlings	0.61	3.16	3.53	4.14	5.04	6.31	7.15	8.38	9.62	10.22
3rd break	0.67	3.46	3.62	4.18	5.19	6.22	6.96	7.89	9.25	9.77
1st clear ¹	0.83	3.48	3.96	4.60	5.49	6.40	6.97	7.64	8.81	9.62
4th middlings	1.17	4.06	4.48	5.11	5.85	6.42	6.86	7.29	7.91	8.79
1st break	1.34	4.04	4.56	5.15	5.86	6.34	6.77	7.17	7.66	8.59
2nd break	2.38	4.80	5.26	5.73	6.12	6.44	6.73	7.02	7.27	7.66

¹ Flours from Mill C. All others from Mill A.

THE RELATION OF BUFFER ACTION TO THE GRADE OF FLOUR

The grade of wheat flour is quite definitely related to the percentage of ash which it contains, and the ash determination is extensively employed in commercially estimating the grade of flour. The data reported in Tables I and III indicate a parallelism between ash content, flour grade, and the buffer action of flour extracts. To verify this parallelism further, the series of flours employed by Bailey and Collatz, in studying the specific conductivity of water extracts, were extracted with water at 25° for 1 hr., and the buffer action of the extracts determined. In Table IV, the results are arranged in order of the ash content of the flour. Since the pH of the original extracts of the several flours varies, the actual, rather than the change in, pH is here recorded. It will be observed that as the ash content increases, and the grade diminishes, the buffer action of the extracts increases. Consequently, it requires appreciably more hydrochloric acid to change the pH of the lower grades through a given range than it does in case of the patents. The best index to the flour

isfactory yeast-leavened bread from the lower grades may be due to failure to obtain the proper H-ion concentration of the dough. Further experimentation with these lower grades of wheat flour, containing more than 0.60 per cent of ash, should be conducted to ascertain the possibilities in this direction. Since conditions generally are unfavorable to the production of choice bread from clear flour, it is probable that a more careful adjustment, within narrower limits, is necessary than in case of doughs made from patent flour.

SUMMARY

1—Varying the time and temperature of extraction does not appreciably alter the H-ion concentration of the extract. The buffer action of such extracts is, however, increased somewhat with increased time of extraction, and quite appreciably increased as the temperature during extraction is increased from 0° to 40°.

2—Boiling the extract reduces its buffer action very slightly, indicating that the soluble proteins which coagulate on boiling are not the principle buffers of the extract. A lack of correlation of the buffer action of extracts of several flours with the percentage of soluble proteins in the extracts further confirms the belief that the proteins are not chiefly responsible for the buffer action.

3—Since the buffer action parallels the specific conductivity of water extracts, and varies with modified conditions of extraction in the same direction and to about the same degree as the activity of phytase varies in hydrolyzing phytin, it appears that phosphates, produced by the hydrolysis of phytin by phytase during extraction with water, may be the principal buffers in the extract.

4—Electrometric titration curves show the extracts of high-grade flours to be buffered less than the extracts of lower grades. It consequently requires less acid to bring the H-ion concentration of a high-grade or patent flour dough to the optimum for bread production than is required for a low grade flour. Possibly some of the difficulties experienced in making satisfactory bread from the lower grade or clear flours may be due to failure to obtain the optimum H-ion concentration of the dough.

FIG. 2—ELECTROMETRIC TITRATION CURVES OF WATER EXTRACTS OF FIVE TYPICAL FLOURS REPRESENTING DIFFERENT GRADES

grade is afforded by the extracts to 100 cc. of which 20 cc. of 0.02 N NaOH were added. In Column 9 of Table IV, where these data are recorded, it will be observed that the pH of the extract so treated diminishes regularly with the increase in ash content. Fig. 2 shows graphically the electrometric titration curves of five characteristic flours of different ash content selected from the several reported in Table IV.

In view of the decided differences in the buffer action of high-grade or patent, and low-grade or clear flours, it is possible that some of the difficulties experienced in producing sat-

Copper and Brass Research Association

The formation of the Copper & Brass Research Association an unincorporated, voluntary organization of the copper, brass, and copper alloy interests, was recently announced by its president, R. L. Agassiz, also president of the Calumet & Hecla Mining Co. The purpose of the Association, given in its by-laws, is to stimulate coöperative effort in the use of copper, brass, and copper alloy products. Membership in the Association is open to producers of copper sold here or selling agents of copper in the United States; to fabricators of copper, brass, and copper products generally in the United States and Dominion of Canada, and to others directly engaged in, or connected with the copper and brass industries. The following are the officers of the Association: R. L. Agassiz, *president*; Fred S. Chase and C. F. Kelley, *vice presidents*; Stephen Birch, *treasurer*; W. S. Eckert, *secretary*. Mr. William A. Willis has been appointed manager of the Association, which has offices in New York City.