HILGARDIA

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOLUME 11 JUNE, 1938 NUMBER 7

CONTENTS

EFFECT OF STORAGE ON LEACHING OF MINERALS AND NITROGEN FROM ASPARAGUS AND PEAS DURING COOKING

HARRIET MORGAN FYLER and J. T. MANCHESIAN

BUFFERING ACTION OF NONACID VEGETABLES

GEORGE L. MARSH

UNIVERSITY OF CALIFORNIA · BERKELEY, CALIFORNIA

BUFFERING ACTION OF NONACID VEGETABLES

GEORGE L. MARSH

BUFFERING ACTION OF NONACID VEGETABLES[1]

GEORGE L. MARSH[2]

JUICES FROM ALL PLANT TISSUES exhibit more or less ability to resist changes in pH value on the addition of strong acids or bases (*15*).[3] The substances responsible for this buffering capacity have not been completely defined largely owing to the complexity of the systems involved. The complex nature of the extracted juices increases the difficulties of identifying the specific buffer substances. In those plant juices which have been investigated most extensively, the buffer capacity has been ascribed to organic acids (*10*), dialyzable acid-salt systems (*14*), proteins (*6*), and acid phosphates (*9*). Probably the buffering is also due to the adsorption reactions of the colloids as well as to dissociation of the weak acids or bases that may be present.

The buffering capacity of nonacid vegetables is of particular importance in connection with the use of acidified brines in canning. Cruess, Fong, and Liu (*4*) found that nonacid vegetables canned with a sufficiently acid brine to bring their pH value below 4.5 may be readily pasteurized in boiling water and need not be processed in steam-heated pressure cookers. These investigators found, however, that the nonacid vegetables exerted a pronounced buffering action when canned in brines acidified with hydrochloric, citric, or acetic acids: a marked rise in pH value of the added brine occurred during heating. The increase was much greater than could be accounted for by diffusion, and they concluded that the rise in pH value was probably caused principally by the action of buffer substances dissolved from the vegetables. Bigelow and Cathcart (*2*) had also noted that when beans were canned with tomato sauce there was an increase in pH value of the sauce which they stated to be "due to diffusion of acids into the beans."

Aside from the work of the above investigators, no detailed study of the buffer capacity of nonacid vegetables is available.

For the past several seasons, some attention has been devoted to a study of this problem. Experiments were undertaken to determine the effect of heat on the buffering capacity of the juices extracted from several vegetables, to determine the buffer index of the vegetable juices, and in addition to investigate in greater detail the changes in pH value during

[1] Received for publication November 18, 1937.

[2] Associate in the Experiment Station, Division of Fruit Products.

[3] Italic numbers in parentheses refer to "Literature Cited," at the end of this paper.

the canning of vegetables in brines acidified to varying degrees with citric, acetic, and hydrochloric acids. The results of these various experiments are reported in this paper.

pH VALUES OF EXPRESSED JUICES

Methods of Determining pH Values.—In the experiment to determine the effect of heat on the buffer capacity of nonacid vegetable juices, green-asparagus, pea, and string-bean juices were extracted from the fresh vegetables of midseason maturity during 1932, by coarsely grinding and pressing. The juice was preserved in the frozen state in cans at a storage temperature of —17° C. Before use, the juices were thawed quickly in the cans in warm water and brought to room temperature. Several 100-cc portions of each vegetable juice were measured into 100-cc volumetric flasks graduated at 100 and 110 cc. Amounts of 0.1*N* or *N* HCl and citric acids corresponding to those shown in tables 1 and 2 respectively and amounts of 0.1*N* or *N* NaOH corresponding to those shown in table 3 were added to the samples. Each sample was then diluted to 110 cc, the contents thoroughly mixed, and the pH value determined on duplicate portions of each sample. The remainder of each sample was heated at 100° C for 1 hour in tightly sealed, 120-cc glass jars. After cooling to room temperature, the pH value of duplicate portions of the thoroughly mixed contents of each jar was again determined.

The pH measurements were made with a Hildebrand-type hydrogen electrode at a temperature of approximately 25° C. The electrodes were frequently replatinized and checked against standard buffer solutions. After each measurement in the alkaline range, the electrode was removed and dipped in dilute hydrochloric acid and thoroughly washed with water before proceeding with the next sample. The electrode became poisoned less rapidly when this procedure was followed. Commercial compressed hydrogen, purified by being passed through an absorption train of alkaline pyrogallol and soda lime, was used. The results obtained are probably accurate to 0.02 pH unit.

pH Values after Heating with Acid or Alkali.—The data obtained are presented in tables 1, 2, and 3. The curves in figure 1 are plotted from the data obtained with pea juice. Heating in the presence of acid lowered the pH value of green-asparagus and string-bean juices acidified with small amounts of hydrochloric acid but raised the pH values of these juices when sufficient acid was added before heating to bring the pH of green-asparagus juice below 3.0 and of string-bean juice below 4.0. Heating raised the pH value of pea juice acidified with hydrochloric acid. The pH changes that occurred on heating pea and green-asparagus juices acidified with citric acid were inconsistent and slight and did not exceed 0.2 pH unit. On the other hand, a marked lowering of the pH occurred on heating juices containing added sodium hydroxide when the initial pH value exceeded 6.5.

Vegetable juices therefore behave like other biological fluids with respect to change in pH value when heated in the presence of acid or alkali.

TABLE 1

EFFECT OF HEAT ON THE pH VALUE OF GREEN-ASPARAGUS, PEA, AND STRING-BEAN JUICES ACIDIFIED WITH VARIOUS AMOUNTS OF HCl

N HCl added	Green asparagus		Pea		String bean	
	Before heating	After heating	Before heating	After heating	Before heating	After heating
cc	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
0.00	6.24	6.04	5.40	5.34	5.98	5.74
0.10	6.06	5.93	5.29	5.31	5.88	5.68
0.20	5.88	5.74	....	....	5.78	5.55
0.30	5.69	5.62	5.20	5.25	5.66	5.34
0.40	5.48	5.47	....	....	5.57	5.38
0.50	5.31	5.25	5.14	5.13	5.49	5.29
0.75	5.00	4.88	....	....	5.33	5.11
1.00	4.66	4.47	4.92	4.99	5.13	4.96
2.00	3.96	3.89	4.65	4.65	4.66	4.57
3.00	....	....	4.43	4.46	4.26	4.32
4.00	2.96	3.14	4.19	4.25	....	....
5.00	....	....	3.95	4.04	3.63	3.68
7.50	....	....	3.51	3.61	3.00	3.07
10.00	....	....	3.09	3.22	2.43	2.45

TABLE 2

EFFECT OF HEAT ON THE pH VALUE OF GREEN-ASPARAGUS AND PEA JUICES ACIDIFIED WITH VARIOUS AMOUNTS OF CITRIC ACID

N citric acid added	Green asparagus		Pea	
	Before heating	After heating	Before heating	After heating
cc	*pH*	*pH*	*pH*	*pH*
0.00	6.24	6.04	5.40	5.35
0.10	6.06	5.89	....	....
0.20	....	....	5.27	5.29
0.30	....	....	5.23	5.24
0.40	....	....	5.17	5.19
0.50	5.45	5.40	5.14	5.17
0.75	....	....	5.08	5.10
1.00	4.97	4.93	5.00	5.03
2.00	4.53	4.47	4.78	4.75
3.00	4.26	4.20	4.62	4.57
4.00	4.05	4.02	4.50	4.42
5.00	3.96	4.00	4.39	4.38
7.50	3.68	3.71	4.17	4.17
10.00	3.48	3.50	4.02	3.95

TABLE 3

EFFECT OF HEAT ON THE pH VALUE OF GREEN-ASPARAGUS, PEA, AND STRING-BEAN JUICES ALKALIZED WITH VARIOUS AMOUNTS OF NaOH

N NaOH added	Green asparagus		Pea		String bean	
	Before heating	After heating	Before heating	After heating	Before heating	After heating
cc	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
0.00	6.24	6.04	5.40	5.34	5.98	5.74
0.10	6.37	6.21	5.46	5.44	6.11	5.95
0.20	....	....	5.50	5.49	6.22	6.04
0.30	6.68	6.50	5.58	5.54	6.33	6.12
0.40	....	....	5.63	5.57	6.45	6.23
0.50	6.91	6.76	5.69	5.59	6.57	6.36
0.75	....	....	5.80	5.70	6.84	6.62
1.00	7.43	7.06	6.03	5.91	7.14	6.90
2.00	8.33	7.61	6.70	6.29	8.15	7.62
3.00	8.80	7.80	7.37	6.67	8.75	7.90
4.00	....	....	7.93	7.13	9.18	8.36
5.00	....	....	8.21	7.41	9.59	9.00
7.50	....	....	8.53	7.92	....	....
10.00	....	....	8.68	8.30	10.94	10.50

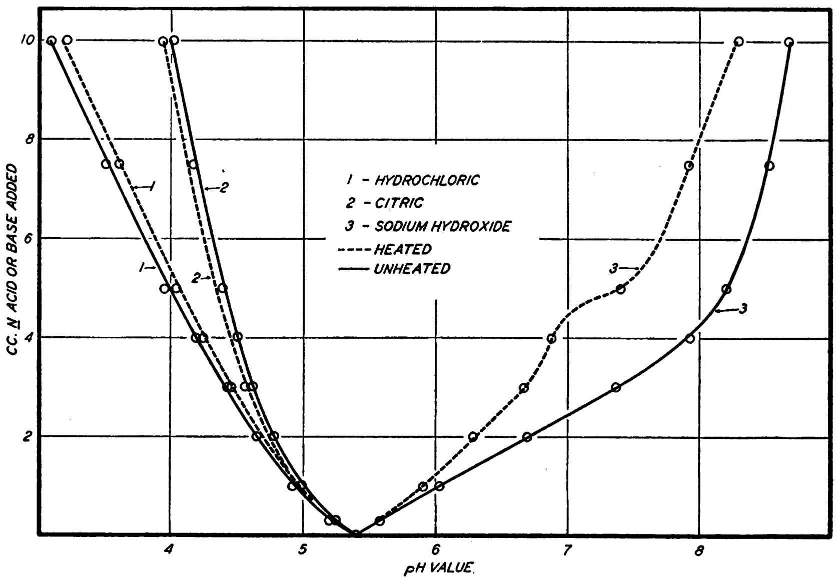

Fig. 1.—The effect of heat upon the pH value of pea juice after the addition of acid or base.

It is well known that prepared bacteriological media change in pH on heating, particularly when their initial pH values are above 7.0 (*1*). Cruess, Richert, and Irish (*5*) give data showing the changes in pH value upon sterilization of media prepared from bacto-peptone, Libby's beef extract, glucose, and small amounts of inorganic salts, and adjusted to various pH values with citric and sodium hydroxide. Below pH 4.0, the media, on heating, increases slightly in pH, while above pH 4.0, it decreases, the decrease becoming more marked the higher the original pH value. McClung (*11*) has also called attention to the occurrence of these phenomena in certain vegetable-extract bacteriological media. The change in pH value occurring upon heating in biological fluids adjusted to high pH values is probably largely due to the formation of acidic materials by the decomposition of sugars by the alkali—a change known to occur in pure sugar solutions at high pH values (*3, 7, 12*).

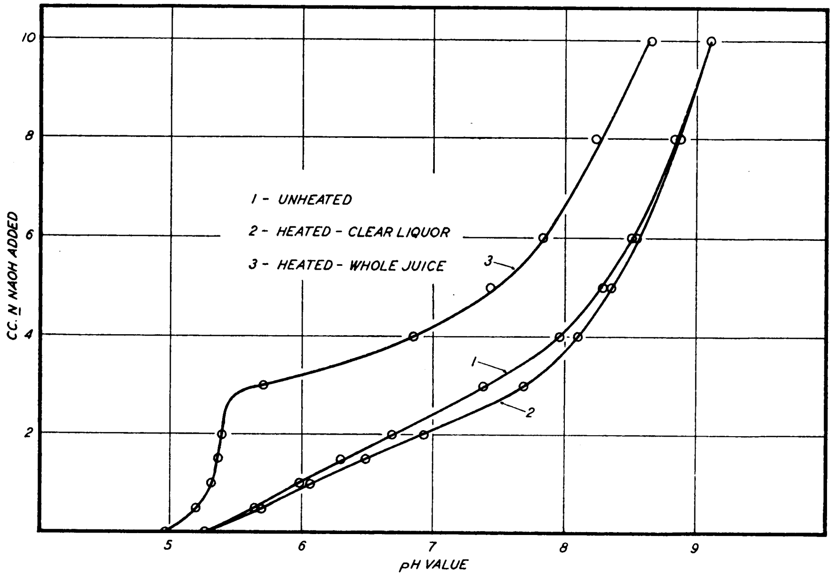

Fig. 2.—The effect of heat upon the potentiometric titration of different fractions of pea juice with NaOH.

pH Values of Supernatant Liquor of Pea Juice.—During the heating of the samples, certain substances contained in the raw juices coagulated and precipitated from solution. The supernatant liquor was straw-colored and brilliantly clear. The buffer capacity of pea juice free from its heat-coagulable colloidal material was tested in comparison with the unheated juice and with the entire, well-mixed, heated juice containing the resuspended coagulum.

A 500-cc portion of the previously prepared pea juice was placed in a tightly sealed glass jar, heated for 1 hour at 100° C, and cooled to room temperature. The heat-coagulated material was resuspended by shaking and stirring and two 100-cc aliquots were withdrawn. The remainder was filtered. Alkaline potentiometric titrations were then conducted on duplicate portions of each of the above samples and on duplicate portions of the unheated raw juice. The pH values were recorded after each addition of *N* NaOH measured from a 10-cc microburette. Great difficulty was encountered in the determination of the titration curve of the heated sample containing the resuspended coagulum, because the electrode poisoned rapidly. Therefore, a freshly platinized electrode was used to determine the pH value after each addition of *N* NaOH.

The average values of the results obtained are given in figure 2. Heat caused little change in the initial pH value of the clear liquor, whereas it appreciably lowered the initial pH value of the whole juice. The clear liquid shows less buffer action toward added NaOH than does the unheated juice. The heated whole juice shows a very strong buffering action in the range pH 5.0 to 5.7; markedly more than that of the unheated juice.

BUFFER INDEX OF EXPRESSED NONACID VEGETABLE JUICES

Methods of Calculating Index and of Determining pH Values.—Van Slyke (*16*) has established the buffer index as a measure expressing buffer capacities of various solutions. The unit adopted by Van Slyke is the differential ratio $\frac{dB}{dpH}$, or β, which is defined as the rate of change in amount of strong base added with change in pH value produced. If an acid is added, the values dB and dpH are negative; hence the ratio, the Van Slyke β, always has a positive value. This differential ratio is usually difficult to measure, and the ratio $\frac{\Delta B}{\Delta pH}$, or B, where each of these values is a measurable increment, is commonly used. This differs from β in being the slope of the intercept between any two points of a B-versus-pH graph instead of the slope of the tangent to the curve at one pH value. The smaller the pH interval used in calculating B, the closer will it approach β in value. In the results reported herein, the data obtained were plotted on large-scale graphs and for each $\Delta pH = 0.2$, the corresponding ΔB was measured, and plotted against the average pH.

The juices used for the determination of the buffer index were prepared from vegetables of midseason maturity during 1933 by coarsely grinding and pressing in an American Utensil expeller press. Finely

TABLE 4

CHANGES IN pH VALUE OF VEGETABLE JUICES ON ADDITION OF HCl

HCl per liter	String-bean juice	Green-asparagus juice	Spinach juice	Pea juice	White-asparagus juice	Artichoke juice
mols	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
0.000	5.92	5.40	6.56	5.37	6.20	5.58
0.001	5.70	5.37	6.46	5.30	5.83	5.56
0.002	....	5.28	6.40	5.23	5.68	5.47
0.003	5.49	5.19	6.32	5.22	5.45	5.40
0.004	....	5.10	6.24	5.18	5.41	5.32
0.005	....	5.05	6.15	5.13	5.29	5.25
0.006	5.24	4.96	6.06	5.10	5.17	5.19
0.008	....	4.81	5.87	5.01	4.97	5.05
0.010	5.01	4.70	5.65	4.94	4.79	4.97
0.015	4.76	4.43	5.21	4.77	4.49	4.68
0.020	4.60*	4.22	4.86	4.63	4.16	4.53
0.030	4.19	3.87	4.38	4.37	3.65	4.18
0.040	3.85	3.56	4.05	4.15	3.20	3.87
0.050	....	3.28	3.77	3.95	3.87	3.57
0.060	3.23	3.01	3.53	3.74	2.59	3.27
0.080	2.75	2.59	3.11	3.36	2.12	2.80
0.100	2.31	2.25	2.68	3.04	1.81	2.41

* 0.021 mols.

TABLE 5

CHANGES IN pH VALUE OF VEGETABLE JUICES ON ADDITION OF NaOH

NaOH per liter	String-bean juice	Green-asparagus juice	Spinach juice	Pea juice	White-asparagus juice	Artichoke juice
mols	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
0.000	5.92	5.40	6.56	5.37	6.20	5.58
0.001	6.05	5.42	6.74	5.41	6.42	5.59
0.002	6.16	5.47	6.84	5.47	6.59	5.65
0.003	6.27	5.53	6.95	5.53	6.72	5.70
0.004	6.39	5.58	7.09	5.58	6.89	5.76
0.005	6.50	5.65	7.21	5.65	7.03	5.82
0.006	6.62	5.71	7.34	5.71	7.17	5.86
0.008	6.84	5.86	7.61	5.86	7.49	5.97
0.010	7.08	5.99	7.88	5.99	7.77	6.08
0.015	7.60	6.30	8.45	6.30	8.23	6.37
0.020	8.07	6.63	8.84	6.68	8.53	6.56
0.030	8.61	7.38	....	7.41	8.97	7.21
0.040	9.04	7.89	....	7.97	9.34	7.52
0.050	9.43	8.26	....	8.29	9.72	7.82
0.060	9.80	8.50	....	8.51	10.13	8.04
0.080	10.51	8.79	....	8.84	10.72	8.46
0.100	10.96	9.02	....	9.11	10.97	8.87

ground suspended material was separated by sieving through several layers of cheesecloth. They were preserved by freezing until used. The frozen samples were thawed, and duplicate 100-cc portions of each vegetable juice were titrated with N HCl and N NaOH added from a 10-cc microburette. The pH values after each addition of acid or base were determined by means of a hydrogen electrode.

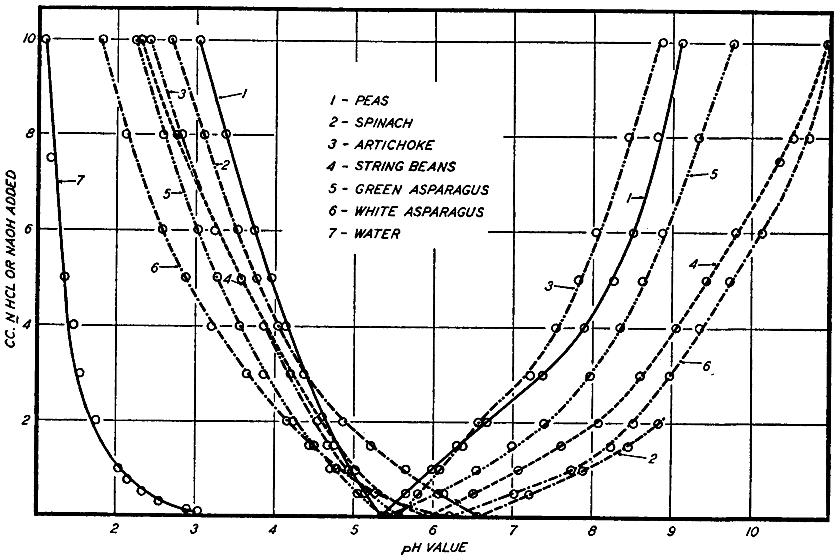

Fig. 3.—The potentiometric titration curves of 100-cc portions of vegetable juices with HCl and NaOH.

Comparison of Buffer-Index Curves.—The results of the potentiometric titrations on the expressed juices of the various vegetables are recorded in tables 4 and 5 and shown graphically in figure 3. The vegetables used differ markedly in their buffer capacity. The juices of peas and of artichokes resist change in pH upon addition of acid or base to far greater extent than do those of white asparagus and spinach.

The Van Slyke B values plotted against pH are shown in figure 4. These curves indicate that there are present in the various vegetable juices certain substances that exert a definite buffering action in the pH range 3.5 to 4.2, also in the range from pH 6.0 to 7.5, and again in the range from pH 8.5 to 10.0. The type of curve obtained would indicate that the buffering action is not due to a simple system but is in all probability due to a very complex mixture of buffer substances.

Oakley and Krantz (*13*) report for tomato juice at pH 3.5 a B value of 0.047 and a β value of 0.033. This latter value, however, is in error and when calculated from the data they present should be no lower than 0.045. Their curve for tomato juice also shows the presence of definite buffer substances in the range of pH from 3.5 to 4.0. Pea juice, therefore, very closely approaches tomato juice in its buffer capacity toward added strong acid.

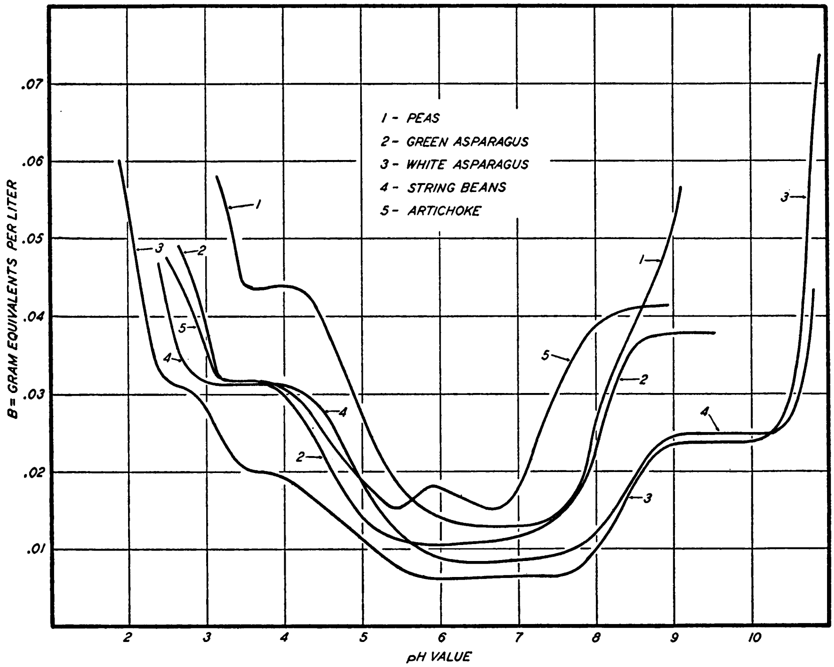

Fig. 4.—The buffer index of vegetable juices at various pH values.

pH VALUES AND TOTAL ACIDITY OF VEGETABLES CANNED IN ACIDIFIED BRINES

Review of Previous Work.—Cruess, Fong, and Liu (*4*) and many others have shown that the death temperature and death time of heat-resistant spore-bearing bacteria are greatly affected by the pH of the medium. Their results indicate that if it is feasible to reduce the pH value of the vegetable below 4.5 by the addition of acid to the brine, the present industrial method of sterilizing vegetables of low hydrogen-ion concentration by steam under pressure—which results in injury to texture, flavor, and color—need not be used.

As was pointed out by these investigators and confirmed by the results already presented, however, nonacid vegetables exert pronounced buffering action toward added acids. During processing, a marked increase in pH value of the added acidified brine occurs as a result of the intimate mixing of the juice of the vegetable and the acidified brine. Mixing is made possible by the heat applied during processing, which kills the living cellular tissue and thereby causes it to lose its selective permeability. Since the pH value after heating was found by the above investigators to be more significant in relation to the effect on the heat resistance of spores than was the initial pH value of the brine and since they did not make a direct study of the changes occurring in pH value of acidified brines during the canning procedure, it was thought that a detailed study of the factors responsible for the observed changes was desirable. The following data were obtained from samples packed experimentally during three different seasons. Each season the vegetables used for the tests were of approximately the same maturity as those used the preceding season but were grown under different climatic and soil conditions.

Methods of Canning and Acidifying.—The vegetables were prepared for canning in the usual manner, and in the tests made during the first two seasons approximately 150-gram portions were placed in 8-ounce cans. There were then added approximately 100-cc portions of brine containing 2 per cent of NaCl and acidified with various amounts of acid. After the addition of the acidified brine, the cans were heated for 3 minutes in steam at 100° C, sealed, and processed for 1 hour in water at 100° C.

During the third season, the samples were prepared in a more accurate manner for the purpose of attempting to establish an acid balance in the samples before and after canning. Therefore an approximately constant amount of vegetable was placed in previously tared 8-ounce cans, the samples were weighed, brine was then added, and the samples reweighed. The lids were crimped in place by a light first roll, the cans were then exhausted 10 minutes at 100° C, given a second roll to seal them airtight, and pasteurized for 30 minutes at 100° C. After canning, all samples were cooled quickly in running water and were subsequently stored at 0° C until removed for pH determinations. All samples were packed in triplicate, two samples for pH determinations and the third for visual examination and organoleptic tests.

Samples the first season were prepared from green asparagus, string beans, and peas packed with brines containing 0.00, 0.10, 0.20, 0.30, 0.40, 0.50, 0.75, 1.00 and 2.00 grams of citric acid per 100 cc. The following season, green asparagus, string beans, peas, spinach, and a mixed-vegetable sample were packed with brine prepared by adding 0.0, 2.5, 5.0, 7.5, 10.0 and 15.0 grams of citric acid and 25, 50, 75, 100, 125, 150 and 200 cc of 40-grain vinegar to 1,000-cc portions of 2 per cent NaCl brine. The mixed-vegetable pack, referred to as "vegetable salad," was composed of peas, string beans, green asparagus, and carrots in equal proportion by weight, with a small piece of pimiento in each can. The actual acid concentration of the various brines are shown in table 7. Only pea and string-bean samples were prepared for the tests made during the third season. These vegetables were canned in brines prepared by dissolving 20 grams of NaCl and the required amount of acid in a small volume of water, transferring to 1,000-cc volumetric flasks, and making up to volume. The total acid concentration and the pH value of the brines prepared for this test are given in table 8, page 331.

In addition, a large quantity of the peas and string beans used during the third season were coarsely ground, frozen and thawed, and the juice extracted by pressure. The juice obtained was preserved in freezing storage at —17° C.

Preliminary tests showed that after canning and storage the pH values of the brine and that of the juice expressed from the vegetables were practically identical. On this account, only the pH values of the brines drained from the vegetables were determined in most cases. The following values indicate the agreement in pH values of the juice expressed from the vegetables and that of the drained brines.

	Drained brine, pH	Expressed juice, pH
Peas	5.96	6.00
String beans	5.90	5.98
Asparagus	5.51	5.54

The pH determinations on the first two seasons' samples were made with the hydrogen electrode, but those on the last set were made with the quinhydrone electrode. Tests indicated that the latter gave results that were just as reliable. It has the advantages that equilibrium is more quickly attained and that, unlike the hydrogen electrode, it is not easily poisoned by the solutions used in these studies.

Amount of Change in pH after Processing, First Two Seasons' Tests.—The results of the pH determinations on the first two season's samples are reported in tables 6 and 7, and part of the data of table 7 is graphically presented in figures 5 and 6.

An examination of table 6 indicates that during the canning and processing of vegetables in acidified brines, there occurs a decrease in pH value of the vegetable tissue, which is directly related to the total amount of acid added; and an increase in pH value of the brine, which is related to the initial pH value of the brine—the nature of the vegetable under examination determining the magnitude of the changes. Peas exert a

TABLE 6

CHANGE OF pH VALUE OF ASPARAGUS, STRING BEANS, AND PEAS CANNED IN BRINES ACIDIFIED WITH CITRIC ACID

Total acid of brines before canning	pH value of added brine	Green asparagus (initial pH=5.60)			String beans (initial pH=5.90)			Peas (initial pH=6.40)		
		pH after processing	Change in pH of vegetable	Change in pH of brine	pH after processing	Change in pH of vegetable	Change in pH of brine	pH after processing	Change in pH of vegetable	Change in pH of brine
grams per 100 cc	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
0.00	....	5.45	0.15	....	5.57	0.33	....	6.38	0.02	....
0.10	2.70	5.10	0.50	2.40	5.18	0.72	2.48	5.96	0.44	3.26
0.20	2.44	4.79	0.81	2.35	4.80	1.10	2.36	5.48	0.92	3.04
0.30	2.36	4.58	1.02	2.22	4.65	1.25	2.29	5.30	1.10	2.94
0.40	2.28	4.47	1.13	2.19	4.48	1.42	2.20	5.06	1.34	2.78
0.50	2.23	4.23	1.37	2.00	4.19	1.71	1.96	4.77	1.63	2.54
0.75	2.12	4.07	1.53	1.95	4.09	1.81	1.97	4.57	1.83	2.45
1.00	2.05	3.98	1.62	1.93	3.83	2.07	1.78	4.36	2.04	2.31
2.00	1.87	3.23	2.37	1.36	3.27	2.63	1.40	3.72	2.68	1.85

TABLE 7

CHANGES IN pH VALUE OF VEGETABLES CANNED IN BRINES ACIDIFIED WITH CITRIC ACID AND IN THOSE ACIDIFIED WITH VINEGAR (ACETIC ACID)

Acid added to 1,000 cc of brine	Total acid of brines before canning	pH value of added brine	pH values after processing*				
			Peas	Green asparagus	String beans	Vegetable salad	Spinach
	grams per 100 cc	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
No added acid	....	....	6.35	5.61	5.49	5.56	5.73
2.5 grams citric acid	0.22	2.54	5.55	4.56	4.71	4.76	5.32
5.0 grams citric acid	0.44	2.31	5.02	4.31	4.24	4.35	5.05
7.5 grams citric acid	0.68	2.17	4.69	3.95	4.00	4.10	4.80
10 grams citric acid	0.89	2.12	4.50	3.75	3.81	3.86	4.64
15 grams citric acid	1.34	2.03	4.19	3.35	3.49	3.62	4.40
25 cc vinegar	0.24	2.99	5.28	4.65	4.64	4.68	....
50 cc vinegar	0.48	2.87	4.96	4.39	4.32	4.53	....
75 cc vinegar	0.70	2.80	4.84	4.25	4.18	4.36	....
100 cc vinegar	0.90	2.73	4.65	4.14	4.12	4.32	....
125 cc vinegar	1.10	2.70	4.57	4.03	4.02	4.27	....
150 cc vinegar	1.32	2.66	4.44	4.00	3.98	4.13	....
200 cc vinegar	1.66	2.61	4.28	3.92	3.86	4.08	....

* Average of the determinations on duplicate samples.

greater buffer effect than string beans or green asparagus, which two vegetables show nearly identical buffering power.

The pH value of the string beans and green asparagus canned in nonacidified brine likewise decreased in pH value during processing, but very little change occurred in the peas. This observation is in accordance with the findings of Bigelow and Cathcart, in which all vegetables studied by them—peas included, however—decreased in pH value dur-

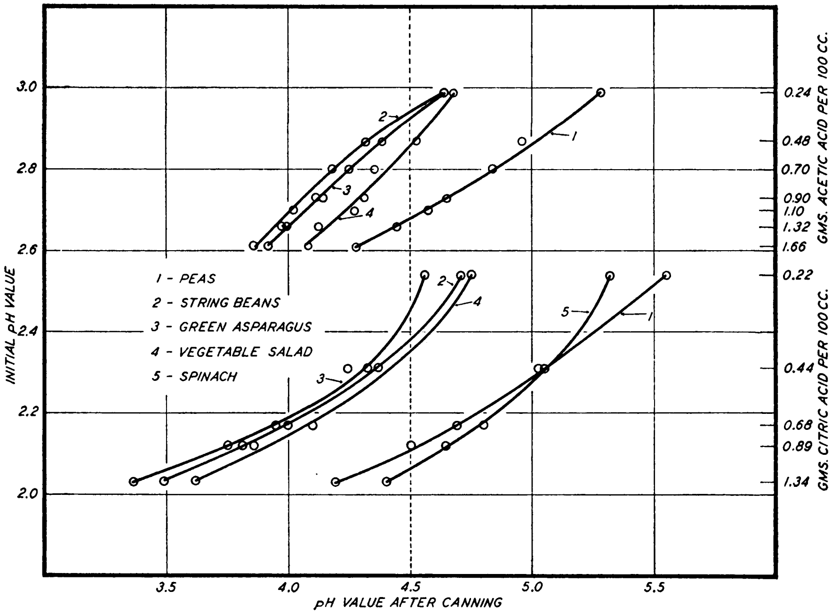

Fig. 5.—Changes in pH value occurring in the acidified brines during processing at 100° C.

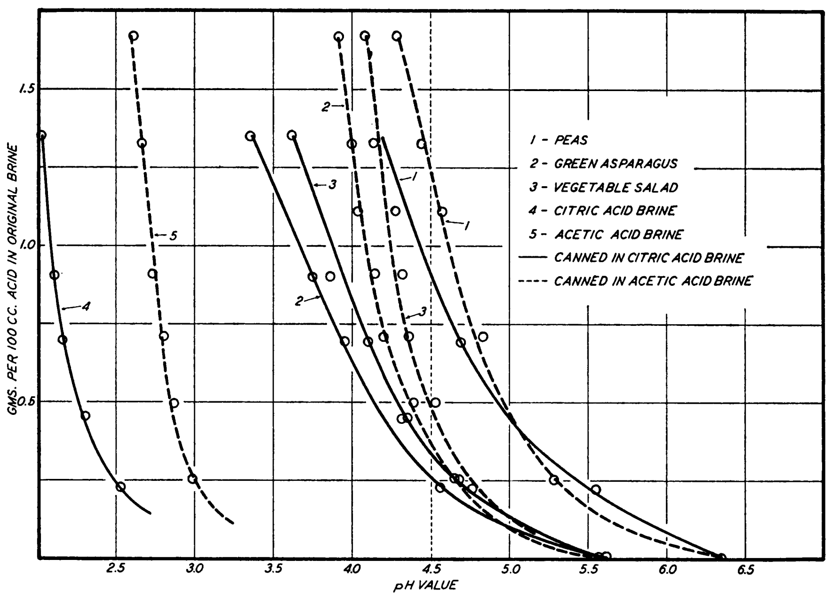

Fig. 6.—The pH value of vegetables canned in various concentrations of acidified brine after processing at 100° C.

ing processing; they believed this to be due to the formation of acidic substances such as carbon dioxide or hydrogen sulfide, to a reaction between the proteins and aldehydes rendering the former more acidic, or to the precipitation of buffer substances by heat. Data will be presented to show that acidic constituents are formed during heating of string-bean juice but not during the heating of pea juice.

As shown in figure 5, acetic acid brines of low initial total acid concentration produce slightly lower pH values after processing than citric acid brines of the same total acid concentration but of lower pH value. This observed difference in behavior, in which the weaker acid causes a greater change in pH value of the product, is in this instance probably due to the buffering effect of compounds other than acetic acid contained in the 40-grain cider vinegar used as a source of acetic acid. Other tests in which acetic acid brines were prepared from glacial acetic acid show that citric and acetic acid brines of similar low total acid concentration affect the pH value of the vegetable tested to the same extent. At higher total acid concentrations, however, the decrease in pH value is greatest in the vegetables canned in citric acid brines.

On the other hand, owing to the differences in degree of ionization, acetic acid brines lower the pH value of the vegetable to a greater extent than citric or hydrochloric acid brine of the same initial pH value; and citric acid in turn decreases the pH value to a greater extent than hydrochloric. Since at any pH value in which a comparison is made of the three acids, the concentrations of citric and hydrochloric acids in solution are less than the concentration of acetic acid, a removal of any portion of the hydrogen ions present, through neutralization of basic constituents produced by heat or combination with constituents already present, would affect the acetic acid brine to a less extent than the citric or hydrochloric acid brines.

Besides the direct buffer action of constituents of the vegetable tissues, there are other possible explanations for the observed phenomena of increase of pH value of the acid brines during processing. All or part of the observed change may be due to the neutralization by, or chemical combination or loose conjugation of a part of the acid added with, basic constituents that either are naturally present or are elaborated during the heating process. Or some of the effect noted might be due to the relatively simple phenomena of dilution.

Amount of Change in pH after Processing, Third Season's Tests.— Since definite conclusions as to the cause of the observed changes in pH values could not be drawn from the results of the first two seasons' tests, the samples during the third season were prepared for the purpose of determining whether loss of acid through neutralization, chemical combination, or adsorption might not play a rôle in the observed phenomena. As previously indicated, the samples were carefully prepared in order that an accurate acid balance might be undertaken. The ingoing weight of vegetable and brine, the total acid content, and the pH value of the vegetables and the brines used in the test, and the specific gravity of the brines were determined before canning (table 8). Total acid was determined on the brines and vegetable juices by direct titration and by the indirect-titration method of Hartmann and Hillig (*8*). The pH values of the brines and vegetable juices were determined by means of the quinhydrone electrode.

TABLE 8

TOTAL ACID CONCENTRATION AND pH VALUE OF BRINES USED IN CANNING PEAS AND STRING BEANS, THIRD SEASON'S TESTS

Acetic acid		Citric acid		Hydrochloric acid	
Grams per 100 cc	pH	Grams per 100 cc	pH	Grams per 100 cc	pH
0.103	3.18	0.103	2.69	0.103	1.58
0.205	3.02	0.202	2.50	0.204	1.29
0.404	2.87	0.400	2.33	0.415	0.98
0.762	2.72	0.754	2.16	0.765	0.73
1.250	2.61	1.257	2.04	1.277	0.50
2.000	2.50	2.010	1.92	2.038	0.30

After storage for six months at 0° C, duplicate sets of cans were removed from storage and allowed to reach room temperature. The drained weights of the vegetables were determined by the usual procedure, after which pH values, total acid concentrations, and specific gravity of the drained brine were determined by the methods previously used.

Analysis of the pea and string-bean juices gave pH values of 6.50 and 5.70 respectively. The pea juice required 26.0 cc 0.1 *N* NaOH and the string-bean juice 31.0 cc 0.1 *N* NaOH per 100 grams to neutralize the acids present. The above values are calculated from those obtained by direct titration of 10-gram samples. There was little difference between the results of the direct titrations and those obtained by the indirect method of Hartmann and Hillig. All the following calculations are based upon the results obtained by direct titration.

The pH value of the brines drained from the peas and string beans after canning and six months' storage are recorded in table 9. They are in close agreement with those obtained during the preceding two seasons'

TABLE 9

pH Value of the Brines Drained from String Beans and Peas After Canning in Brines Acidified with Acetic, Citric, and Hydrochloric Acids

Total acid per 100 cc of brine	Acetic acid		Citric acid		Hydrochloric acid	
	Peas	String beans	Peas	String beans	Peas	String beans
grams	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
0.0	6.40	5.52	6.40	5.52	6.40	5.52
0.1	6.11	5.19	6.13	5.21	5.79	4.73
0.2	5.72	4.86	5.80	4.76	5.14	3.95
0.4	5.21	4.56	5.26	4.38	4.32	2.91
0.75	4.78	4.29	4.73	4.02	3.21	1.98
1.25	4.50	4.10	4.32	3.64	2.16	1.43
2.0	4.28	3.93	3.94	3.33	1.23	1.10

TABLE 10

Calculation of Acid Balances in Peas Canned in Acid Brines

Brine	0.1 N NaOH required to neutralize acid in product							Per cent acid lost during canning
	Before canning			After canning			Difference ($A-B$)	
	In vegetable	In brine	Total (A)	In vegetable	In brine	Total (B)		
	cc	*cc*	*cc*	*cc*	*cc*	*cc*	*cc*	*per cent*
Nonacidified......	55.4	00.0	55.4	35.0	16.7	51.8	3.6	6.5
Acetic acid brine..	55.5	15.8	71.3	33.4	18.4	51.8	19.5	27.3
	55.4	30.6	86.0	37.9	20.8	58.7	27.3	31.7
	55.5	64.3	119.8	48.7	29.8	78.5	41.3	34.5
	55.4	121.8	177.2	78.0	48.1	126.1	51.1	28.8
	55.5	202.0	257.5	121.8	78.2	200.0	57.5	22.3
	55.4	316.0	371.4	196.0	122.8	318.8	52.6	14.2
Citric acid brine..	55.4	15.0	70.4	29.4	16.6	46.0	24.4	34.7
	55.4	27.8	83.2	33.2	17.9	51.1	32.1	38.7
	55.4	54.0	109.0	48.7	25.5	74.2	34.8	31.9
	55.4	109.0	164.4	67.5	37.2	104.7	59.7	36.3
	55.4	178.0	233.4	107.0	59.9	166.9	66.5	28.5
	55.5	286.0	341.5	175.0	94.3	269.3	72.2	21.1
Hydrochloric acid brine...........	55.4	27.3	82.7	34.8	20.9	55.7	27.0	32.6
	55.3	48.2	103.5	40.7	20.2	60.9	42.6	41.2
	55.3	84.2	139.5	58.9	21.8	80.7	58.8	42.2
	55.2	153.1	208.3	98.1	35.5	133.6	74.7	35.9
	55.4	214.8	270.2	192.8	66.3	259.1	11.1	4.1
	55.3	388.8	444.1	321.8	139.0	460.8	−16.7	−3.8

tests but are included because they show the buffering effect of a similar lot of vegetable material towards the three acids.

Changes in Total Acidity.—From the data obtained the third season, acid balances were calculated. The results of these calculations are recorded in tables 10 and 11 and are the averages of duplicate samples in all cases.

An examination of tables 10 and 11 reveals that no significant change in total acid content occurs during the canning of peas or string beans in nonacidified brines, although significant changes did occur in pH value (table 9). The deviations in results obtained in calculating the acid balance are within the limits of experimental error.

TABLE 11

CALCULATION OF ACID BALANCES IN STRING BEANS CANNED IN ACID BRINES

Brine	0.1 N NaOH required to neutralize acid in product							Per cent acid lost during canning
	Before canning			After canning			Difference ($A-B$)	
	In vegetable	In brine	Total (A)	In vegetable	In brine	Total (B)		
	cc	*cc*	*cc*	*cc*	*cc*	*cc*	*cc*	*per cent*
Nonacidified......	44.4	00.0	44.4	26.2	17.6	43.8	0.6	1.4
Acetic acid brine..	44.6	17.0	61.6	24.7	15.9	40.6	21.0	34.1
	44.4	34.4	78.8	31.1	20.7	51.8	27.0	34.3
	44.6	65.8	110.4	48.5	30.9	79.4	30.8	28.0
	45.3	126.8	172.1	87.0	56.2	143.2	28.9	16.8
	43.5	207.0	250.5	129.8	86.8	216.6	33.9	13.5
	44.3	327.0	371.3	207.0	129.0	336.0	35.3	9.5
Citric acid brine..	44.6	16.1	60.7	24.6	16.3	40.9	19.8	32.6
	44.6	31.5	76.1	31.0	19.6	50.6	25.5	33.5
	44.6	61.0	105.6	46.1	28.9	75.0	30.6	29.0
	44.6	109.8	154.4	75.2	45.0	120.2	34.2	21.2
	44.3	193.0	237.3	118.1	74.5	192.6	44.7	18.8
	44.5	306.0	350.5	179.0	120.0	299.0	51.5	14.7
Hydrochloric acid brine...........	44.5	25.6	70.1	27.8	16.6	44.4	25.7	36.7
	44.5	53.0	97.5	37.2	23.0	60.2	37.3	38.3
	44.4	111.2	155.6	64.7	41.6	106.3	49.3	31.7
	44.4	202.0	246.4	118.0	80.2	198.2	48.2	19.6
	44.5	336.0	380.5	210.0	131.0	341.0	39.5	10.4
	44.5	548.0	592.5	360.0	194.5	554.5	38.0	6.4

On the other hand, rather large losses of total acid occur when these vegetables are canned in brines acidified with acetic, citric, and the lower concentrations of hydrochloric acids. The loss of acid increases with the concentration of acid added to the product, except in those samples canned in high concentrations of hydrochloric acid brine. In the pea samples canned in brine containing 1.25 grams of hydrochloric acid per 100 cc, little or no acid is lost, while in those canned with brine containing 2.0 grams of hydrochloric acid per 100 cc, there is evidence that a production of acid occurs. In general, the loss of acid is greater in canned peas than in canned string beans.

Loss of acid therefore seems to account for part of the increase in pH value that occurs when vegetables are canned in acidified brines. Although the loss of acid is insufficient to account for the entire change, it nevertheless contributes to the final result. The results of this experiment, however, do not indicate whether the acid was used in neutralization of some basic constituents, or in decomposition of some of the more complex constituents, or whether it is bound by colloidal constituents present in the vegetable tissues.

TABLE 12

Total Acidity and pH Relations of Acidified Pea Juice, before and after Heating

Brine	Milliequivalents of acid present in original sample*	Milliequivalents of acid recovered		Difference		pH value	
		Before heating	After heating	Before heating (col. 1 – col. 2)	After heating (col. 1 – col. 3)	Before heating	After heating
	1	2	3	4	5	6	7
Control (H_2O)	0.650	0.643	0.540	0.007	0.110	6.60	6.72
Acetic acid brine	0.997	0.855	0.675	0.142	0.322	6.33	6.29
	1.330	0.878	0.882	0.452	0.448	6.00	5.93
	1.991	1.440	1.372	0.551	0.619	5.28	5.31
	3.179	2.520	2.564	0.659	0.615	4.76	4.78
	4.794	4.095	3.982	0.699	0.812	4.47	4.46
	7.310	6.638	6.435	0.672	0.875	4.23	4.22
Citric acid brine	0.979	0.890	0.585	0.089	0.394	6.33	6.39
	1.280	1.056	0.810	0.224	0.470	6.11	6.17
	1.888	1.373	1.283	0.515	0.605	5.36	5.43
	2.989	2.349	2.204	0.640	0.785	4.68	4.74
	4.570	3.780	3.622	0.790	0.948	4.25	4.27
	6.950	5.985	5.875	0.965	1.075	3.87	3.86
Hydrochloric acid brine	1.213	0.945	0.810	0.268	0.403	5.95	6.07
	1.775	1.598	1.620	0.177	0.155	5.30	5.37
	2.930	2.399	1.980	0.531	0.950	4.24	4.22
	4.860	3.713	3.726	1.147	1.134	2.99	2.98
	7.692	6.480	6.772	1.212	0.920	1.84	1.90

* That present in pea juice plus that added.

TOTAL ACIDITY OF EXPRESSED JUICES MIXED WITH ACIDIFIED BRINES

Since the experiment just described yielded little evidence concerning the mechanism of the reactions involved, largely because the changes occurring during the canning operations cannot be observed, a series of samples were prepared using the juices obtained from the same lot of vegetables, in which the ratio of vegetable to brine was approximately the same as in the canning tests. The series consisted of 25-cc aliquots of pea and string-bean juice plus 20-cc portions of the acidified brines used in the canning tests. Total acidity was determined by direct titration and pH values by the quinhydrone electrode on each sample before and after heating for 1 hour at 100° C. The results of this experiment are given in tables 12 and 13.

When an aliquot of pea juice is acidified (table 12), stirred, and allowed to stand for some minutes, subsequent titration does not account for all the acid added plus that present in the juice itself. A portion of

TABLE 13

TOTAL ACIDITY AND pH RELATIONS OF ACIDIFIED STRING-BEAN JUICE BEFORE AND AFTER HEATING

Brine	Milliequivalents of acid present in original sample*	Milliequivalents of acid recovered		Difference		pH value	
		Before heating	After heating	Before heating (col. 1—col. 2)	After heating (col. 1—col. 3)	Before heating	After heating
	1	*2*	*3*	*4*	*5*	*6*	*7*
Control (H_2O)	0.825	0.833	1.058	−0.008	−0.233	5.74	5.62
Acetic acid brine	1.171	1.237	1.270	−0.066	−0.099	4.98	5.01
	1.504	1.530	1.570	−0.026	−0.066	4.72	4.75
	2.121	2.160	2.178	−0.039	−0.057	4.42	4.47
	3.354	3.352	3.398	0.002	−0.044	4.16	4.21
	4.968	4.950	4.975	0.018	−0.007	3.96	4.03
	7.485	7.422	7.460	0.063	0.025	3.79	3.86
Citric acid brine	1.153	1.170	1.170	−0.017	−0.017	4.99	5.00
	1.455	1.462	1.463	−0.007	−0.008	4.65	4.67
	2.062	2.057	2.048	0.005	0.014	4.26	4.30
	3.168	3.082	3.117	0.086	0.051	3.86	3.95
	4.745	4.648	4.680	0.097	0.065	3.52	3.60
	7.125	6.975	7.085	0.150	0.040	3.20	3.30
Hydrochloric acid brine	1.328	1.417	1.440	−0.089	−0.112	4.55	4.59
	1.950	1.845	1.935	0.105	0.015	3.83	3.90
	3.103	2.835	2.993	0.268	0.110	2.80	2.92
	5.033	4.838	4.972	0.195	0.061	1.88	1.94
	7.865	7.560	7.875	0.305	−0.010	1.24	1.25
	12.125	11.750	12.280	0.375	−0.155	0.87	0.86

* That present in string-bean juice plus that added.

the acid evidently becomes so bound that it no longer furnishes hydrogen ions detectable by titration. The extent of the reaction is dependent upon the kind and amount of acid added, being greater the higher the concentration and the stronger the acid added. Although the reaction by means of which the acid is fixed occurs in the cold, it is increased in extent by heating except in the samples containing high concentration of hydrochloric acid, where the data would indicate that a liberation of part of the bound acid occurs during heating.

String-bean juice (table 13) behaves somewhat differently from pea juice when treated with acidified brines. When acetic acid brines containing up to 0.4 gram per 100 cc, citric acid brines containing up to 0.2 gram per 100 cc of acid, or hydrochloric acid brine containing 0.1 gram per 100 cc, are added to the juice subsequent titration indicates more acid than the total of that originally added plus the amount present in the vegetable tissue. When brines of higher acidity than those mentioned are added, less acid is found than was originally present, and the amount of acid lost depends upon the kind and concentration of the acid added, as was previously found for pea juice. The data in table 13 show that the increase in total acid occurred only in those samples in which the amount of acid added was insufficient to lower the pH below 4.42. Apparently string beans contain a colloidal system having an isoelectric point of approximately pH 4.3, which is capable of binding a portion of the acid added in excess of the amount necessary to lower the pH beyond the isoelectric point. This colloidal system is precipitated by hydrogen ions.

Heating the samples resulted in an increase in total acid in nearly all the samples over the amounts found in the unheated samples (table 13). This increase in acid was greatest in the samples acidified with hydrochloric acid brines, in which it was large enough at the higher acid concentrations to account for all the acid originally bound and to increase the total acid above that originally present. The increase in total acid as a result of the heating was accompanied by a slight increase in pH value of the samples.

The behavior of the samples of pea juice during preparation indicates that the results obtained were due largely to changes induced in the colloidal systems of the juices by the added acids. The addition of acid to pea juice first causes some of the suspended material to settle out, and leaves the supernatant liquid light green and cloudy. Further addition causes the liquid to become colorless but to remain cloudy and the suspended material to become grayish green in color. The next change is a coagulation and complete flocculation of all suspended material, upon which the liquid becomes colorless and clear. Further addition of acid causes color changes in both the suspended matter and the liquid, with the latter increasing in cloudiness as the acid is increased.

These changes occur at definite pH values and are not functions of the kind of acid used. The change of the liquid from green to colorless occurs at a pH value of 5.3 in the pea juice, and coagulation and clearing of the liquid occurs at approximately 4.5.

Heating caused the suspended material in all the pea-juice samples to coagulate, except those to which the three highest concentrations of hydrochloric acid brine were added. These samples were cloudy and rather viscous. The liquid in all the samples turned brown upon heating and was brilliantly clear. The coagulated sediment was yellowish green in color.

The changes induced by acid added to string-bean juice are likewise a function of the pH value. A very small amount of acid added to string-

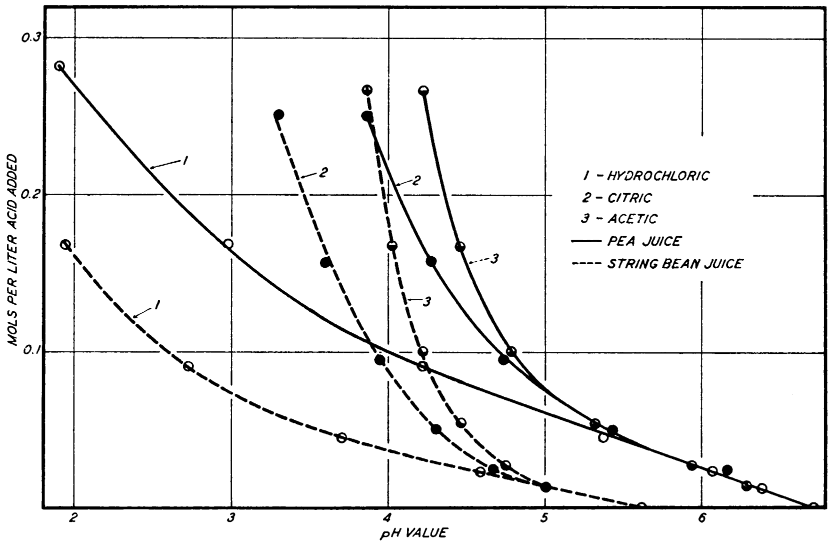

Fig. 7.—The buffering action of string-bean and pea juice toward hydrochloric, citric, and acetic acid.

bean juice resulted in some coagulation of the suspended material. However, the liquid did not become clear until its pH value was lowered to 4.7, and it reclouded at pH 3.5. The changes brought about by heat were similar in all respects to those already recorded for pea juice.

The difference in buffer capacity of the respective vegetable juices previously noted is therefore partially explained by the nature of the colloidal system present and the degree to which it is affected by the added acid. Peas, which have as strong a buffer capacity as any vegetable tested, owe a large proportion of their ability to resist changes in pH value upon acidification, to the presence of a colloidal system which binds a portion of the acid added. String beans, which are among the vegetables having a low buffer capacity, do not contain a colloidal system comparable to that of peas, and the buffer effect noted is undoubtedly due in large part to the presence of acid-salt systems.

In figure 7 the pH values recorded in this experiment have been plotted against the calculated mols per liter of acid added, for each vegetable and each acid used. The curves show that at low concentrations each acid is buffered to the same extent by the vegetable under examination. At higher concentrations, the buffering effect of the acid itself becomes apparent and is responsible for the differences noted. The Van Slyke B value for peas at pH 6 taken from this graph is 0.034 and the string beans at pH 5 is 0.022. These values are in agreement with the previously reported values for these two vegetable juices.

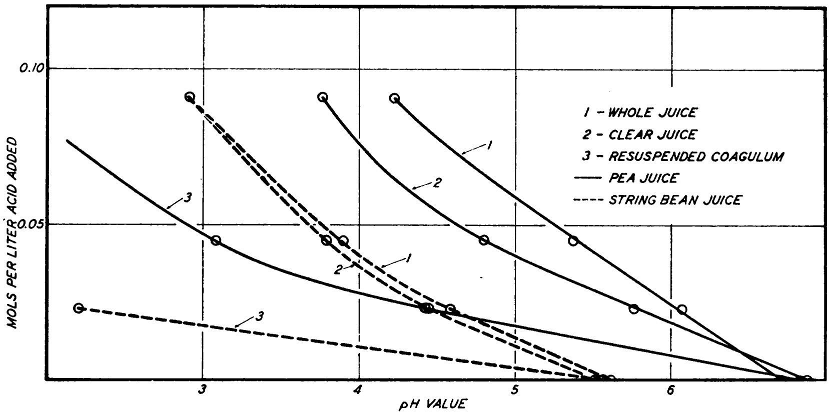

Fig. 8.—The buffering action of various fractions of string-bean and pea juice toward hydrochloric acid.

BUFFER CAPACITY OF HEAT-PRECIPITABLE SUBSTANCES

As previously stated, heating causes a coagulation of suspended matter in both these juices, which readily flocculates and leaves the remaining liquor brilliantly clear. There was evidence that these heat-precipitable substances played a rôle in the capacity of the juice to act as buffers.

Portions of both juices were prepared from the previously ground and frozen material, placed in tightly sealed containers and pasteurized for 1 hour at 100° C. The coagulum was filtered out and the filtrate obtained was set aside. The coagulum was then washed and was resuspended in a volume of distilled water equivalent to the quantity of juice from which it was obtained. Aliquot portions of both the filtrate and the resuspended coagulum were then treated with aliquot portions of the acidified brines used in the previous experiments in the ratio of 25 cc of juice to 20 cc of brine and pH determinations were made upon each sample. Only the data obtained from the pH determinations made upon the samples acidified with hydrochloric acid brine are presented in figure 8, since they are illustrative of the points of chief interest.

The changes in pH resulting from the addition of acid to the samples calculated as mols per liter are shown for the unheated whole juice, the clear filtrate, and the resuspended coagulum for both vegetables. The curves show that the clear filtrate from the heated pea juice has much less buffer capacity than the whole juice, and likewise that very little change in buffer capacity has occurred in the string-bean juice. The curves further show that the resuspended coagulum of both peas and string beans has a definite, although somewhat limited buffer capacity.

The Van Slyke B values for the unheated pea juice, the clear filtrate, and the resuspended coagulum from pea juice are 0.034, 0.021, and 0.010 respectively at a pH value of 6.0, and for the same materials from string-bean juice, 0.022, 0.021, and 0.007 at a pH value of 5.0. The filtrate from pea juice, therefore, has a buffer capacity of the same magnitude as the string-bean juice. This fact would indicate that the materials responsible for the buffer capacity of peas in excess of that shown by other vegetable juices are chiefly composed of heat-coagulable substances. These are probably colloidal protein or carbohydrate. The heat-coagulable substance present in string-bean juice has no influence upon the buffer capacity of the juice, although it does exert a small but definite buffering action when free of juice. The chemical composition of these substances was not determined.

That dilution might account for part of the increase in pH value of the acidified brines has been previously suggested. A considerable dilution does occur when the contents of the cans are caused to mix intimately by heating. The 150-gram portions of vegetable material used contain approximately 125 cc of water which mixes with the 100-cc portions of the acidified brines. However, when 25-cc quantities of water were mixed with 20-cc portions of the acidified brines tested, the change in pH value that occurred was only 0.40 pH unit for hydrochloric acid, 0.24 pH unit for citric acid, and 0.20 pH unit for acetic acid. Dilution is therefore not an important factor in the phenomena observed.

CONCLUSIONS

The buffer capacity of the expressed juices of the vegetables tested was effected to only a slight extent by heat when the juices were heated in contact with acid but marked changes occurred when the juices were heated in contact with base. Heating prior to the addition of acid causes a marked change in the buffer capacity of pea juice as determined by potentiometric titration with base.

Peas and artichokes have the highest buffer indexes of the various vegetables tested, followed in order by green asparagus, string beans, and white asparagus.

Acidified brines increase markedly in pH value during canning with nonacid vegetables. The change in pH value is less with acetic acid brines than with citric acid brines of the same low initial total acid concentration. With brines of the same initial pH value markedly less change occurs in citric acid brines than in acetic acid brines. Peas exert a greater buffering effect on acidified brines than any of the other vegetables tested.

A definite loss of acid occurs when vegetables are canned with acidified brines which accounts for a part, at least, of the increase in pH values noted. Probably the acid lost is involved in adsorption reactions with the colloidal systems of the vegetable tissues. The total buffer effect noted, however, cannot be ascribed to any single substance or system but is complex in nature.

LITERATURE CITED

[1]ANONYMOUS.
1926. Laboratory manual in general bacteriology. Univ. of California Syllabus Ser. **182**:1–302.

[2] BIGELOW, W. D., and P. H. CATHCART.
1921. Relation of processing to the acidity of canned foods. National Canners' Assoc. Bul. **17–L**:1–46.

[3] BROWNE, C. A.
1937. Report on sugars and sugar products. Jour. Assoc. Off. Agr. Chem. **20**: 394–99.

[4] CRUESS, W. V., W. Y. FONG, and T. C. LIU.
1925. The rôle of acidity in vegetable canning. Hilgardia **1**(13):275–93. (Out of print.)

[5] CRUESS, W. V., P. H. RICHERT, and J. H. IRISH.
1931. The effect of hydrogen-ion concentration on the toxicity of several preservatives to micro-organisms. Hilgardia **6**(10):295–314.

[6] GORDEN, W. J., and F. B. DENNY.
1926. Factors influencing the equilibrium known as the iso electric point of plant tissue. Amer. Jour. Bot. **13**:743–53.

[7] GORTNER, R. A.
1929. Outlines of biochemistry. 793 p. John Wiley & Sons, Inc., New York.

[8] HARTMANN, B. G., and FRED HILLIG.
1930. The use of lead acetate in the determination of the acidity of fruit products. Jour. Assoc. Off. Agr. Chem. **13**:259–65.

[9] NIGOLD, T. C.
1929. Hydrogen concentration of plant tissues. X. Buffers of potato tuber. Protoplasma **4**:51–59.

[10] LEUTHARDT, F.
1927. Buffer capacity and plant juices. Kolloidchem. Beihefte **25**:1–68.

[11] MCCLUNG, L. S.
1932. A note on reaction changes during sterilization of vegetable extract culture media. Jour. Bact. **24**:457–59.

[12] NELSON, E. K., and C. A. BROWNE.
1929. Properties and chemical constitution of glucic acids. Jour. Amer. Chem. Soc. **51**:830–36.

[13] OAKLEY, M., and J. C. KRANTZ, JR.
1936. The buffer capacity of tomato juice. Indus. and Engin. Chem. **8**:205–6.

[14] RUSSO, G.
1930. Factors which determine or modify the buffer value of certain vegetables. Bul. Soc. Ital. Biol. Sper. **5**:490–2.

[15] SMALL, J.
1929. Hydrogen ion concentrations in plant cells and tissues. 421 p. Borntraeger Bros., Berlin.

[16] VAN SLYKE, D. D.
1922. The measurement of buffer values and the relationship of buffer value to the disassociation constant of the buffer and the concentration and reaction of the buffer solution. Jour. Biochem. **52**:525–70.

4500-8,'38(2850)