

THE DETERMINATION OF THE ORGANIC ACIDS IN
FERMENTING DOUGH, OVEN VAPORS AND BREAD

by

FRED FUNSTON BARRETT, JR.

B. S., Kansas State College
of Agriculture and Applied Science, 1951

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Flour and Feed Milling Industries

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1955



LD
2668
T4
1955
B36
c.2

Documents.

11

TABLE OF CONTENTS

INTRODUCTION	Page 1
MATERIALS AND METHODS	10
EXPERIMENTAL RESULTS AND DISCUSSION	13
SUMMARY	24
ACKNOWLEDGMENTS	26
LITERATURE CITED	27
APPENDIX	30

INTRODUCTION

Commercial bread, today is attractively wrapped and marketed. This bread, also, is fortified with vitamins and minerals making it more nutritious. The flavor is mild yet has universal appeal.

There has been no concentrated study to analyze bread flavor. This probably is because the sensation known as flavor is difficult to define. Also, the knowledge of the components of flavor is lacking. There is an obvious need for research to separate and identify the constituents that may be responsible for bread flavor. Among these flavor constituents are organic compounds classed as ketones, aldehydes, alcohols, esters, and acids and a group known as "browning reaction products".

The problem of relating flavor to chemical constituents is complex. This is due to the large number of components which are present in minute quantities. One of the limitations in studies of this kind has been the lack of adequate methods of analysis. Furthermore, although certain constituents are known to be present in bread, there still remains the problem of associating their presence with flavor.

Investigations on the flavor components of cheeses and wines have shown alcohols, aldehydes, esters, and acids to be present. The production of the acids during fermentation of these food products seems to be necessary particularly for the development of a full flavor.

Baker et al. (1) studying the flavor of bread, analyzed baking oven vapors and volatile reducing substances in bread crumb. This analysis of the oven condensate demonstrated the presence of organic acids at 0.02 per cent concentration. Also present were aldehydes, ketones, esters, and diacetyl together with other substances.

Pence (19), also, studied the baking oven vapors and found alcohols, esters, aldehydes, ketones and acids to be present. Due to the high hydrogen-ion concentration in the condensate, formic and acetic acid were presumed to be the predominant acids. It is assumed that organic acids contribute to flavor of bread.

Johnson (13) found that the increase in acidity of a fermenting dough was due mainly to lactic acid and to a minor extent acetic acid.

The relationship of flavor to content of acetylmethylcarbinol in dough was studied by Visser't Hooft and de Leeuw (26). They found the flavor to be primarily due to the diacetyl produced from the acetylmethylcarbinol.

Studies (Krebs, 15) pertaining to yeast technology and alcoholic fermentation reactions have shown that pyruvic, succinic, and fumaric acids are produced although the primary action of the yeast is to convert sugars into carbon dioxide and alcohol. The nature of sugar fermentation, however, is an involved chain of reactions which forms many intermediate compounds and by-products. The Embden-Meyerhof-Parnas scheme for sugar fermentation presented in Fig. 1 represents the compounds that result

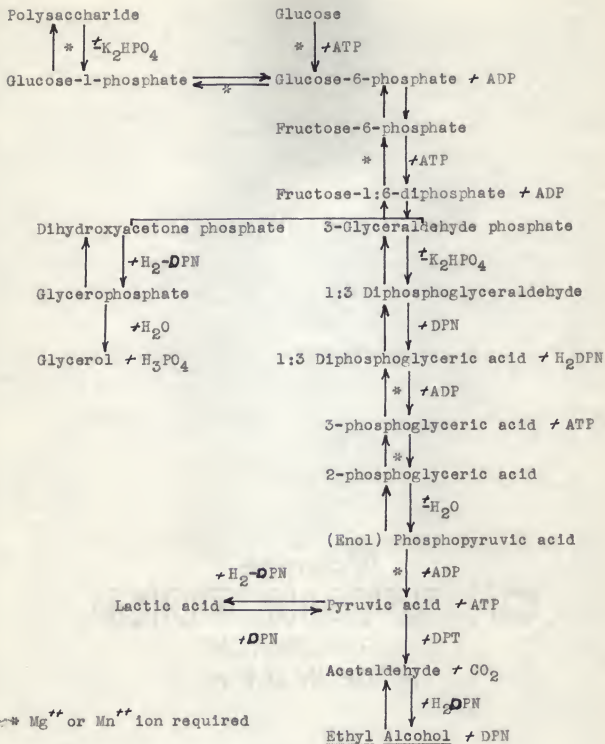
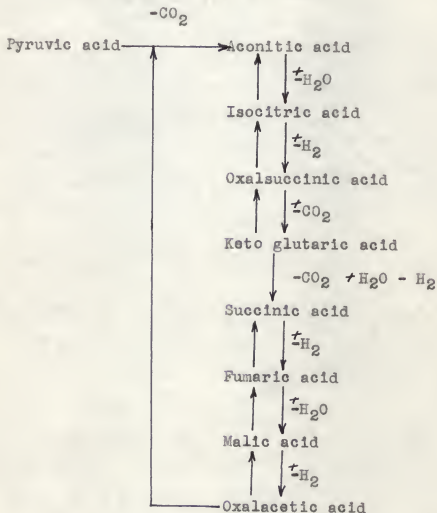


Fig. 1. Embden-Meyerhof-Parnas Scheme and Cori Scheme for Alcoholic Fermentation (From Porter, 1947).

and may be expected to be found in fermentation cultures.

In the above scheme acetaldehyde is formed by the action of the specific enzyme carboxylase which initiates the decarboxylation of the pyruvic acid to form acetaldehyde and carbon dioxide.

The Krebs Scheme is as follows:



Krebs tricarboxylic acid cycle theory (Gortner and Gortner, 9) indicates that the breakdown of pyruvic acid is a complicated series of reactions. These reactions take place predominately in aerobic fermentation.

This scheme, along with the Embden-Meyerhof-Parnas scheme, accounts for almost all of the products of yeast fermentation. Joslyn (14) reported that acetic acid 0.05-0.25 per cent, lactic acid 0.0-0.2 per cent, and succinic acid 0.5-0.7 per cent are produced as intermediate or by-products during industrial fermentation. Formic acid also is produced (Porter, 22). Neuberger (Porter, 22) suggests that formic and acetic acids may be produced by yeast action in the dismutation of acetaldehyde or by the breakdown of pyruvic acid.

Several organic constituents may be produced during dough fermentation as the result of the action of bacteria and mold which are present in flour. Lactic acid bacteria are capable of forming lactic acid by the oxidation of pyruvic acid. Bacteria, also, are capable of forming lactic and acetic acids during acetic fermentation by the dismutation of pyruvic acid. Propionic acid is produced by the metabolism of propionic acid bacteria. Coliform and aerogenes types of bacteria produce lactic, acetic, and formic acids during their metabolism. The conditions of dough fermentation are ideal for bacterial metabolism.

It should be recognized that lack of adequate methods of separation and analysis has hampered additional study of the acids present in dough and bread. The development of partition chromatography by Martin and Synge (17) has opened the way for the separation of structurally similar compounds that previously had not been separated. The theory was developed in an effort

to use a system analogous to fractional distillation for separating similar compounds by distributing them between two immiscible liquid phases. One phase is stationary by being adsorbed onto a support material. The other phase is the mobile phase and passes through the support material. The support material or adsorbent is packed into a glass tube. The sample to be separated is placed on the top of the column and carried through the column by the mobile phase or developing solvent. The difference in partition coefficients of the compounds in the sample permits separation and elution from the column in zones or bands.

The method of continual solvent flow and the separation of compounds into zones is known as elution analysis. A continuous series of small fractions are collected and analyzed for concentration of solute and a graph made of the quantity of solute versus the fraction number. The plot appears as peaks showing rise and fall in the concentration of the solutes as the zones pass out of the column. Under ideal conditions the peak will have a sharp rise and fall but usually in practice adsorptive properties of the support material prevents a sharp line of separation.

The adsorptive properties of the support materials are related to surface area, surface activity, and functional groups. The surface area is related to the size of the particle and to the porosity. The surface activity may be altered by the presence of other substances and the functional groups may determine

specific substances that will be adsorbed or exchanged on the surface. Frequently, two or three types of action will occur simultaneously in the same system. This makes it necessary to establish conditions for separation of substances by experimentation.

Care in the choice of a suitable solvent for development and elution is necessary. It is essential to select one which will give complete liberation of the adsorbed compounds. The eluant usually is made more polar as development progresses so it will elute the more strongly held compounds.

The chemical structure of the material being separated also has an effect on the separation that will be obtained. In the instance of carboxylic acids, Traube (Cassidy, 5) found that the surface activity increased with the number of methylene groups in the chain. He found that adsorption of fatty acids from aqueous solutions onto charcoal was correlated positively with the molecular weight of the acids. The opposite was true for adsorption of fatty acids from an organic solvent. Strain (24) also found that, in general, adsorption increased with molecular size within the same class. Thus, a knowledge of the structure of the compound studied is valuable and may help determine the order of adsorption in a given system. Since theory has not kept abreast of practice, experimental conditions must be established for a given system of adsorbent, solvent, and compounds to be separated.

Silica gel or commercial silicic acid have been used by many workers (10) (12) (16) (23) (25) as the adsorbents for separating organic acid mixtures. The application of silica gel to certain types of separations is limited, however, since its resolving power decreases with an increase in solubility of the solute. Many difficulties have been experienced in the preparation of silica gel and in variations in gel properties between batches.

Brimley and Barrett (2) pointed out that diatomaceous earth used as the support material has certain advantages. It has low adsorptive power. Using celite as the adsorbent, Phares et al. (21) obtained separation of many aliphatic organic acids including acetic, formic, fumaric, pyruvic, succinic, and lactic acids. Bulen et al. (4) used a celite column to separate acetic, pyruvic, fumaric, formic, lactic, and succinic acids from plant tissue.

Buffer solutions, water, indicators, and various acids have been used as the non-mobile phase. Isherwood (12) found it necessary to use dilute sulfuric acid as the stationary phase to reduce ionization and trailing of the zones when working with organic acids. Peterson and Johnson (20), however, separated formic, acetic, and propionic acids on celite columns with water as the stationary phase.

It is necessary, in some separations, to use a solvent that progressively increases in polarity. Donaldson et al. (7) de-

vised an apparatus that would automatically increase the solvent polarity as development of the column progressed.

Samples, usually, were introduced onto the column in non-aqueous solutions. Zbinovsky and Burris (27) developed methods for adding aqueous samples of sodium salts of organic acids directly to the column. These methods overcame water-logging of the adsorbent at the top of the column, which disrupts the equilibrium between the two phases and prevents separation of the compounds. This work by Zbinovsky and Burris also demonstrated that the best separations were obtained when the column was prepared with a maximum amount of water evenly distributed over the adsorbent and when a minimum of sample was used.

Paper chromatography was developed by Consden et al. (6) by replacing the column of adsorbent with a strip of filter paper. It has useful applications in separating sugars and amino acids. The partition coefficient values of the different organic acids, usually, are not sufficiently different, however, to effect separation. A few spray methods (Buch et al., 3) have been developed that will produce color reactions useful in identifying certain groups of acids in paper chromatography.

The developments in methods for the separation of organic acids by column partition chromatography lend themselves to a study of these constituents in dough and bread. This investigation was undertaken to separate the organic acids present in dough, oven vapors, and bread by chromatographic means and thereby gain a knowledge of the acid components of bread flavor.

MATERIALS AND METHODS

Organic acids were extracted from doughs and bread produced using both the straight dough and sponge dough methods. The quantities of ingredients were as follows:

<u>Ingredient</u>	<u>Amount</u>
Flour	700 g.
Sugar	42
Dry milk solids	28
Shortening	21
Yeast	21
Salt	14
Arkady	1.8
Malt	1.8
Water	490 ml

The baking procedure was that outlined by Miller and Johnson (18).

A 25 g sample of dough was extracted in a Waring Blender for 1 minute with a mixture of 75 ml of water and 5 ml of 10 per cent sulfuric acid at 50°-60° C and filtered. The acids contained in the filtrate were converted to the sodium salt by neutralization with 0.1 N sodium hydroxide followed by evaporation.

A 25 g sample of crumb from the center of the loaf was extracted with a solution of 75 ml of water and 5 ml of 10 per cent sulfuric acid. After blending and filtering, the filtrate was neutralized with 0.1 N sodium hydroxide and evaporated to dryness.

The oven vapors were collected by condensing the exhaust gases in a series of cold water condensers and flasks surrounded by ice water. The oven fan forced the vapors from the oven

through the cooling system. The aqueous solution of oven vapors was neutralized with 0.1 N sodium hydroxide and evaporated to dryness.

The support material used for the chromatographic columns was a diatomaceous earth, celite 545¹. It was thoroughly washed with water to remove small particles and dried prior to use. The celite supported the non-mobile phase 0.5 N sulfuric acid. Glass tubes 1.0 cm x 80 cm were used to hold the support material.

The eluting solvents were reagent grade chloroform and n-butanol. They were equilibrated with sulfuric acid by shaking 400 ml of solvent with 80 ml of 0.5 N acid.

Standard 0.1 M solutions of formic, acetic, propionic, pyruvic, lactic, succinic, and fumaric acids were used to establish the pattern of elution from the celite columns. These acids were added to the column as the sodium salts in aqueous solutions.

The packing of the column required great care. The celite was moistened with the proper amount of acid, (0.6 ml of 0.5 N sulfuric acid per 1.0 g celite) slurried with pure chloroform, and packed in small increments into the glass tube by means of a glass rod. Enough celite was used to make a column 50 cm high. The column was kept covered with solvent until used.

¹ Johns-Manville Co.

The sample to be chromatographed was acidified with 0.1 ml 9 N sulfuric acid and placed on top of the celite column after the excess chloroform had been removed. After the sample had passed into the column, two 1 ml increments of chloroform were added and allowed to progress downward to the column surface. After adding an additional 20 ml of chloroform, the solvent flow was started using an automatic mixing apparatus of the type designed by Donaldson et al. (7). This gave a mixture of chloroform — n-butanol (1-1) flowing through chloroform. Thus, the column was developed with a solvent progressively increasing in percent butanol. The development continued until no more acids passed from the column. Equal fractions of eluant were collected with an automatic fraction collector.

The unknown acid peaks were tentatively identified by several tests. The initial identifications were made by comparing the peaks of known acids with the unknown. The peaks were established by titrating 160 μ l of each fraction with 0.1 N sodium hydroxide from a microburette. Other tests included the microscopic examination of the sodium salts of the unknown acids as compared with the crystals of the salts of known acids. Unsuccessful attempts also were made to compare the infrared absorption patterns and the paper chromatograms of known and unknown acids.

Several spot tests specific for certain acids were used (Feigl, 8). The ammoniacal silver nitrate and chromotropic acid tests were used for formic acid; lanthanum nitrate test for

acetic acid; ammonium vanadate and o-hydroxydiphenyl tests for pyruvic acid and the phenol-ferric chloride color test and p-hydroxydiphenyl test for lactic acid.

EXPERIMENTAL RESULTS AND DISCUSSION

The organic acids present in fermenting dough, baking oven vapors, and bread have been separated using a celite partition column. Plate I shows the separations of acids from straight dough samples. Curve A is a sample taken immediately after mixing. Curves B and C are samples taken at the end of 2 and 4 hours of fermentation, respectively. It is evident that separation of the acids was not complete and that overlapping occurred in the fractions. This appears particularly true for dough extracts in which the production of acids were slight. Specific qualitative tests also indicated that separation was not complete, nevertheless, certain general trends could be observed. The amount of acid increased as the fermentation time was extended to two hours. After 4 hours of fermentation, the amount of acid represented by peaks 1 and 4 decreased, 2 and 3 remained essentially unchanged and peak 5, lactic and succinic acid, increased. This decrease in the other acids between the second and fourth hours of fermentation is not readily explainable. Pyruvic acid, lactic acid and lactates are utilized by yeast during metabolism. Hoffman et al. (11) found the greatest amount of yeast growth occurs between the second and fourth hours of

EXPLANATION OF PLATE I

Separation of organic acids from straight doughs.

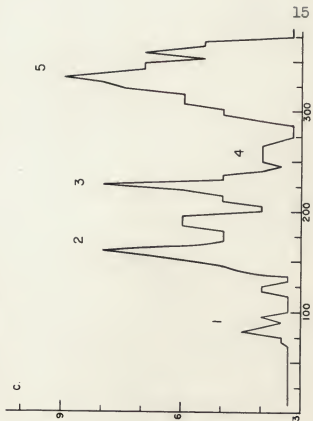
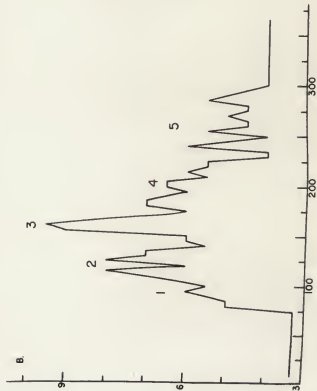
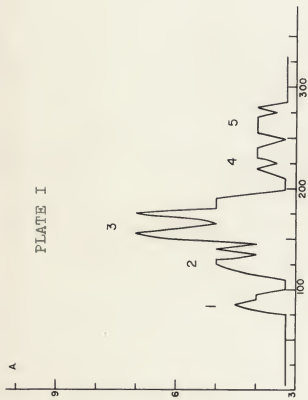
Curve A. 0 hours of fermentation.

Curve B. 2 hours of fermentation.

Curve C. 4 hours of fermentation.

1. Formic-Acetic acids
2. Fumaric acid
3. Pyruvic acid
4. Propionic acid
5. Lactic-Succinic acids

0.10 N SODIUM HYDROXIDE (UL)



FRACTION

NUMBER

fermentation in a straight dough. This may account for the decrease in the pyruvic acid. The continued increase in the lactic acid peak, however, seems to point to the possible growth of lactic acid bacteria which are naturally present in flour. These results are in agreement with Johnson (13) concerning the lactic acid production during fermentation. These results, however, do not confirm the fact that acetic acid is produced in relatively large amounts.

The curves in Plate II show the results from samples of a sponge dough series. Curves A, B, C, and D are samples from sponge doughs fermented 0, 2, 4, and 6 hours, respectively. Again it is evident that separation of the acids is not complete. The small quantities of certain acids produced leave uncertainties in their separations and identifications.

Table 1 shows the pH and total acidity acquired in various doughs. The total acidity values correspond well with the quantities of acids separated by chromatography.

The analysis of the oven vapors for organic acids in represented in Plate III. The quantities of acids present represent the content in vapors collected from 130 loaves of bread. The small amount of acid present in the vapors may indicate that the acids do not escape rapidly during baking.

Bread samples baked from the various sponge doughs were analyzed. The curves in Plate IV show the separations of the acids present in bread made from 2, 4, and 6 hour sponge doughs. A small but measurable amount of acids remain in the dough

EXPLANATION OF PLATE II

Separation of organic acids from sponge doughs.

Curve A. 0 hour sponge dough.

Curve B. 2 hour sponge dough.

Curve C. 4 hour sponge dough.

Curve D. 6 hour sponge dough.

1. Formic-Acetic acids.

2. Fumaric acid.

3. Pyruvic acid.

4. Propionic acid.

5. Lactic-Succinic acids.

PLATE II

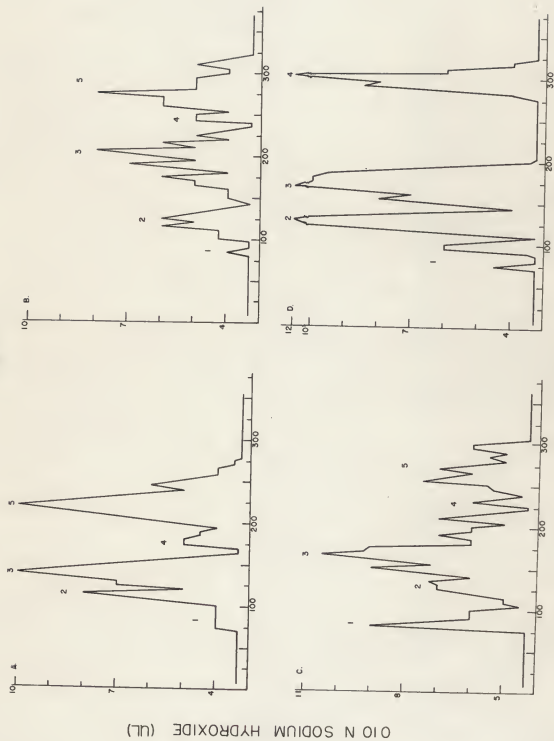


Table 1. Relationship of time of fermentation to pH and acidity in dough and bread.

Sample	pH	Total acidity ¹
0 Hr. sponge:		
dough	5.6	.40
bread	5.35	.45
2 Hr. sponge:		
dough	5.3	.5
bread	5.19	.5
4 Hr. sponge:		
dough	4.90	1.20
bread	5.5	.625
6 Hr. sponge:		
dough	4.55	3.65
bread	5.5	.65

¹ 25 ml of sample titrated with 0.1 N sodium hydroxide.

after baking. The quantities are directly related to the increase in length of fermentation time and the amount of acid present in the dough.

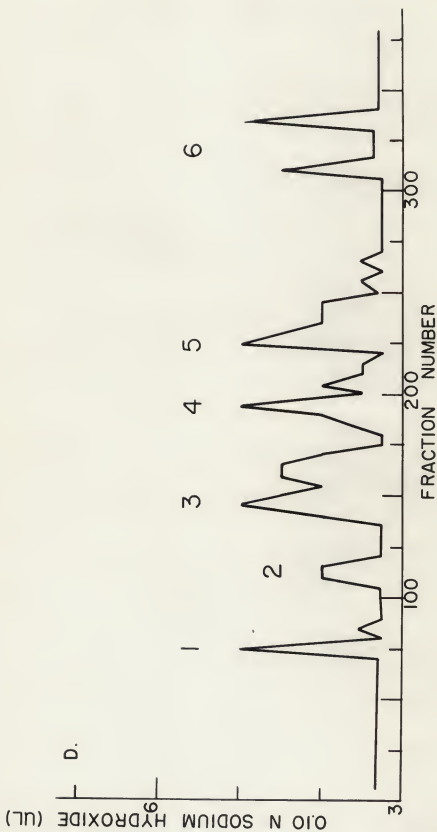
The separation of the organic acids has indicated the presence of formic, acetic, propionic, fumaric, succinic, pyruvic, and lactic acids in fermenting dough, oven vapors, and baked bread. Separations while not complete or as satisfactory as desired, provides evidence of changes in the concentration of organic acids as bread dough ferments. Some, although relatively small amounts of acids, is lost during the baking of bread. Longer fermentation develops greater quantities of organic acids and this is reflected in a general way in the baked bread. It

EXPLANATION OF PLATE III

Separation of organic acids in baking oven vapors.

1. Formic-Acetic acid.
2. Fumaric acid.
3. Pyruvic acid.
4. Propionic acid.
5. Lactic-Succinic acids.
6. Unidentified.

PLATE III



EXPLANATION OF PLATE IV

Separation of organic acids from bread crumb.

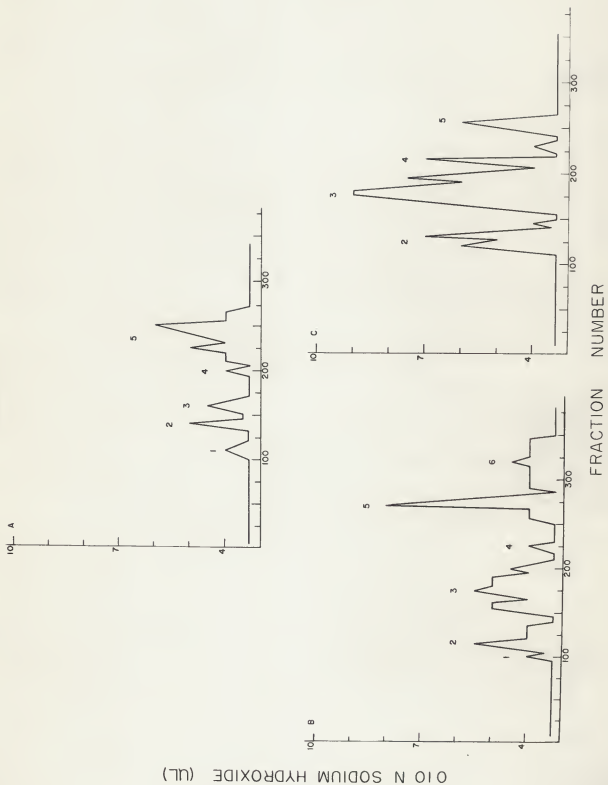
Curve A. 2 hour sponge dough bread.

Curve B. 4 hour sponge dough bread.

Curve C. 6 hour sponge dough bread.

1. Formic-Acetic acids.
2. Fumaric acid.
3. Pyruvic acid.
4. Propionic acid.
5. Lactic-Succinic acids.
6. Unidentified.

PLATE IV



is not evident why the concentration of certain acids should decrease as fermentation progresses.

SUMMARY

The organic acids present in fermenting dough, oven vapors, and bread have been partially separated and have provided evidence for the presence of formic, acetic, propionic, pyruvic, succinic, lactic, and fumaric acids. Partition chromatography using a diatomaceous earth (Celite 545) with 0.5 N sulfuric acid as the stationary phase has been used for the separation of the acids.

Samples of dough produced, both by straight dough and sponge dough methods, were analyzed. In general, the amounts of acids produced increased with increased fermentation time.

Oven vapors were collected and analyzed for organic acids, also. There appeared to be less acid loss in the vapors than expected. They may be retained in the crumb and the crust rather than being driven off during baking.

Analysis of the crumb of bread showed appreciable amounts of acids present. These amounts were related to the concentrations found in the doughs. Extended fermentation time produced increased amounts of acids in the bread.

The relationship of acid content to flavor is uncertain. Too much acid production during fermentation gives the bread an acidic flavor. Likewise, lack of production of acids leaves

the bread devoid of flavor. The results of this study show the content of acids to be small in all cases. However, these small amounts might be sufficient to add to the flavor and odor of bread.

ACKNOWLEDGMENTS

The author would like to express his thanks and acknowledge his indebtedness to Dr. John A. Johnson for his assistance in supervising this research problem and in the preparation of the manuscript; to Dr. Byron S. Miller for the many helpful suggestions and encouragement; and to other members of the staff of the Department of Flour and Feed Milling Industries who were so generous with their assistance.

Acknowledgment and thanks are given to Corn Products Sales Company, Corn Products Building, 201 North Wells Street, Chicago 6, Illinois, for their cooperation in making this project possible.

LITERATURE CITED

1. Baker, J. C., E. K. Parker, and K. L. Fortmann.
Flavor of bread. *Cereal Chem.* 30: 22-30. 1953.
2. Brimley, R. C., F. C. Barrett.
Practical chromatography. New York: Reinhold, 1953.
3. Buch, M. L., Rex Montgomery, and W. L. Porter.
Identification of organic acids on paper chromatograms.
Anal. Chem. 24: 489-491. 1952.
4. Bulen, W. H., J. F. Varner, and P. C. Burrell.
Separation of organic acids from plant tissue. *Anal. Chem.* 24: 187-190. 1952.
5. Cassidy, H. G.
Adsorption and chromatography. New York: Interscience, 1951.
6. Consden, R., A. H. Gordon, and A. J. P. Martin.
Qualitative analysis of proteins: a partition chromatographic method using paper. *Biochem. J.* 38: 224-232. 1944.
7. Donaldson, K. O., V. J. Tulane, and L. M. Marshall.
Automatically increasing solvent polarity in chromatography. *Anal. Chem.* 24: 185-187. 1952.
8. Feigl, F.
Qualitative analysis by spot tests. New York: Norde-mann, 1939.
9. Gortner, R. A. Jr., and W. A. Gortner.
Outlines of biochemistry. New York: John Wiley & Sons, 1949.
10. Higuchi, T., N. C. Hill, and Geraldine B. Corcoran.
Chromatographic separation and determination of dicarboxylic acids, C_4-C_{10} . *Anal. Chem.* 24: 491-493. 1952.
11. Hoffman, C., T. R. Schweitzer, and G. Dalby.
Factors affecting the growth of yeast in fermenting doughs. *Cereal Chem.* 18: 342-349. 1941.
12. Isherwood, F. A.
The determination and isolation of the organic acids in fruit. *Biochem. J.* 40: 688-695. 1946.

13. Johnson, A. H.
Identification and estimation of the organic acids produced during bread dough and cracker dough fermentation. *Cereal Chem.* 2: 345-364. 1925.
14. Joslyn, M. A.
Wallerstein Lab. Commun. 3: 30-43. 1940.
15. Krebs, H. A.
The intermediary stages in the biological oxidation of carbohydrates. *Advan. in Enzy.* 3: 191-252. 1943.
16. Marshall, L. M., K. O. Donaldson, and F. Friedberg.
Chromatographic resolution of organic acids with progressively changing solvents. *Anal. Chem.* 24: 773-775. 1952.
17. Martin, A. J. P., and R. L. M. Synge.
A new form of chromatogram employing two liquid phases. *Biochem. J.* 35: 1358-1368. 1941.
18. Miller, B. S. and J. A. Johnson.
High levels of alpha amylase in baking. I. Evaluation of the effect of alpha amylase from various sources. *Cereal Chem.* 25: 168-177. 1948.
19. Pence, E. A.
A study of baking oven vapors. Unpublished Masters of Science thesis, Kansas State College, Manhattan, Kansas. 1952.
20. Peterson, M. H., and M. Johnson.
The estimation of fatty acids of intermediate chain length by partition chromatography. *J. Biol. Chem.* 174: 775-789. 1948.
21. Phares, E. F., E. H. Mosbach, F. W. Denison, and S. F. Carson.
Separation of biosynthetic organic acids by partition chromatography. *Anal. Chem.* 24: 660-662. 1952.
22. Porter, J. R.
Bacterial chemistry and physiology. New York: John Wiley and Sons, 1947.
23. Ramsey, L. L. and W. I. Patterson.
Separation and identification of the volatile saturated fatty acids (C_1 - C_4). *J. A. O. A. C.* 28: 644-656. 1945.
24. Strain, H. H.
Chromatographic adsorption analysis. New York: Interscience, 1947.

25. Vandenheuvel, F. A. and E. R. Hayes.
Partition chromatography of aliphatic acids. Anal.
Chem. 24: 960-965. 1952.
26. Visser't Hooft, F., and F. J. G. de Leeuw.
The occurrence of acetylmethylcarbinol in bread and
its relation to bread flavor. Cereal Chem. 12: 213-
229. 1935.
27. Zbinovsky, Vladimir, and R. H. Burris.
New techniques for adding organic acids to silicic
acid columns. Anal. Chem. 26: 208-210. 1954.



APPENDIX

SUGGESTIONS FOR FURTHER STUDY

The full relationship of acid content of a dough to bread flavor can not be understood until further investigations are made. A study relating bread sample flavor to acid content is necessary.

The source of production of the acids should be studied. A study of the bacteria of dough might yield important information as to the possible source of production of numerous acids.

Improvements in the chromatographic technique for the separation of organic acids would be of value in determining quantitatively the acids present.

Methods of identification of these acids need to be improved. Most tests are not specific enough for the small quantities present.

A study of the organic acids remaining in the bread after storage and staling would be of value. Their loss or presence may be a factor in causing stale odor and flavor.

THE DETERMINATION OF THE ORGANIC ACIDS IN
FERMENTING DOUGH, OVEN VAPORS AND BREAD

by

FRED FUNSTON BARRETT, JR.

B. S., Kansas State College
of Agriculture and Applied Science, 1951

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Flour and Feed Milling Industries

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1955

The constituents of bread flavor are unknown. This investigation was undertaken to separate and identify the organic acids that are present in fermenting dough, oven vapors, and bread. These acids may be important components of bread flavor.

Partition chromatography was used for the separation of the organic acids known or expected to be present in dough and bread. A column of a diatomaceous earth with 0.5 N sulfuric acid as the stationary phase was used with solvents that progressively increased in polarity during the course of separation. The organic acids found present in fermenting dough, oven vapors, and bread were tentatively identified as formic, acetic, propionic, pyruvic, lactic, succinic, and fumaric.

Samples of both straight doughs and sponge doughs were analyzed for acids. The amounts of acids increased, in general, with the extension of fermentation time.

Oven vapor samples were collected and analyzed. The content of acids in the vapors appeared to be less than expected. The results suggest that relatively small amounts of acids are found in the oven vapor and that relatively larger amounts are retained in the crumb and crust of the bread.

Analysis of the bread crumb showed measurable amounts of acids present. These quantities were generally related to the concentration found in the doughs. Extended fermentation time produced greater quantities of acids in the bread.

The results of this study show the content of acids in dough, oven vapors, and bread, to be relatively small in all

cases. The exact extent of the contribution of organic acids to bread flavor is not known. It is believed that small amounts of acids found present in bread are sufficient to add to the flavor and odor of such products.

