paper be moistened with the sample to be tested. This can be done in two ways: A drop of an extract can be added to the paper or the paper can be touched with the moist surface of a solid piece of the test material. The choice of method depends on the information desired. The result obtained with a drop of extract reflects the average peroxidase activity of all the samples used in preparing the extract, but by touching the paper with the moist surface of a piece of solid material, variations in the peroxidase activity of different areas of the piece can be observed. For example, a blue circle appeared on paper that was touched by a freshly cut cross section of blanched alfalfa root. The blue circle coincided with the phloem of the root, which showed that this part of the root still contained active enzymes whereas the center (xylem) was peroxidase-free. If a significant amount of peroxidase activity is present, a positive reaction occurs in less than a minute.

The use of the paper to indicate peroxidase-active portions of food material may be valuable to food processors. The method is unique as an enzyme method in being able to measure activity in situ.

The specificity of prepared papers for peroxidase is the same as using the particular substrate in conventional procedures. For example, papers prepared with o-tolidine will give a slight positive reaction with active iron preparations such as hemoglobin. Catalase will not give a positive reaction.

Some of the variables that have been studied are different substrates, ratio of substrate to urea peroxide, optimum amounts of these substances on the paper, quality of filter paper, and different solvents for the reagents.

Test papers have been prepared with benzidine, p-phenylenediamine, pyrogallic acid, and o-tolidine as substrates. o-Tolidine is preferred because of the vivid blue color of the peroxidase-oxidized product and the better stability of the prepared paper.

Papers were prepared in which the ratio of urea peroxide to substrate varied from 10:1 to 1:10. Within the range studied a ratio of 1 to 1 is satisfactory. Too large an excess of peroxide caused the papers to turn blue on drying, whereas an inadequate amount decreased the sensitivity of the paper.

With urea peroxide and o-tolidine in the ratio of 1 to 1, the concentrations of these substances in the solution used to prepare the paper were varied from 0.1 to 1.0%. The highest concentrations were unsatisfactory, in that the prepared papers showed undesirable browning on short storage. The lowest concentrations gave paper of low sensitivity. Papers prepared from a solution containing 0.5% of each reagent were satisfactory.

Several kinds of filter paper, from 7 to 11 cm., were used and found satisfactory—i.e., Whatman's Nos. 1 and 50, Carl Schleicher and Schuell Co. Nos. 576, 595, and 597. Heavy paper, such as Whatman's No. 3, was less satisfactory because of discoloration on drying.

Absolute ethyl alcohol, 95% ethyl alcohol, and 99% isopropyl alcohol have been used successfully as solvents for urea peroxide and the substrate. Solvents such as ethyl ether and acetone were not used because of the hazardous nature of peroxides of these compounds.

A detailed comparison of the sensitivity of the test paper procedure with other published methods has been made

on a series of vegetables. The results of this study (4) showed that the test paper is as sensitive as the Masure and Campbell (3) qualitative test (often referred to as the U.S.D.A. method), which is the method of choice of many food processors.

The effects of light and humidity on paper stability have not been studied in detail but are known to be important. Papers exposed to ordinary room light and uncontrolled relative humidity lost much of their sensitivity to peroxidase in a few hours. Desiccated papers stored in the dark at room temperature retain good quality for a week or more, and, if refrigerated, they remain serviceable for several months.

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PRE-FERMENTS IN BREADMAKING

Organic Acids and Esters Produced in Pre-ferments

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THE FLAVOR of fresh bread (7), originating largely through fermentation (3) and baking (2–4), has universal appeal. The usual baking procedure is known as the sponge process, which consists of allowing the yeast to ferment sugar in the presence of approximately 60% of the total water and flour. After 3 to 5 hours, the sponge is mixed with the remaining ingredients to produce a dough which is then divided, molded, proofed, and baked in the customary manner.

Recently, two breadmaking processes have attracted widespread interest: the pre-ferment process (9, 17–20) and the continuous process (1, 5, 12). Neither requires the mixing of a sponge and both depend on a liquid ferment consisting mainly of yeast, water, sugar, and an inorganic or milk buffer to produce the flavor constituents normally originating in the sponge. Bacteria present in the ingredients may also contribute to the flavor through fermentation. Although the industrial application of the pre-

ferment and continuous baking processes is not widespread, probably some type of pre-ferment (6) will eventually be used in the baking industry in conjunction with a continuous mixing process. Possibly, the pre-ferment may be supplanted by a synthetic mixture of the chemical components which normally are produced in the sponge or in pre-ferment solutions.

The effect of fermentation time on the chemical constituents of pre-ferments used in breadmaking has been reported

Quantitative analysis of organic acids and esters in liquid pre-ferments used in new methods of breadmaking was studied using chromatographic techniques. Three organic acids—acetic, lactic, and an unidentified acid—increased significantly during fermentation of the pre-ferment. The concentration of acids after 24 hours of fermentation caused the development of slight stickiness of the dough associated with the swelling of the gluten proteins. Other physical properties of the dough were not affected. Ethyl lactate was not detected but ethyl acetate reached a maximum concentration after 8 hours of fermentation. The acids or esters produced in the liquid pre-ferment in 8 to 24 hours had little detectable effect on the bread flavor.

by Johnson *et al.* (*14*). The kinds and concentrations of the various organic acids produced during fermentation were not investigated. Johnson (*13*) found that the increase in acidity of a fermenting dough was due mainly to lactic acid and, to a minor extent, to acetic acid. Choi *et al.* (*9*) reported on the increase in lactic acid during fermentation. Pence (*21*) found acids and esters among the compounds of a condensate from baking oven vapors. No inorganic acids were detected but formic and acetic acids were separated and identified. Baker, Parker, and Fortmann (*4*) identified ethyl alcohol and acetic acid and esters from condensates of baking oven vapors. Wiseblatt (*26*) analyzed the organic acids and esters in bread crumb. Relatively high concentrations of hydrocinnamic acid, relatively low concentration of benzilic, itaconic, lactic, and succinic acids, and ethyl esters of these acids were present. As the acids were extracted from the bread crumb, some may have been formed during the baking process and others during dough fermentation.

A host of compounds, including several organic acids, are formed by microbial fermentation of sugar. The Embden-Myerhof-Parnas scheme (*25*) shows the mechanism whereby this is accomplished. The tricarboxylic acid cycle indicates how pyruvic acid is transformed into many other organic acids. This latter cycle is believed to be unimportant in breadmaking because yeast fermentation in dough is primarily anaerobic. However, microbial fermentation by organisms other than yeast may contribute to acid production.

The problem of relating flavor to chemical components is complex, not only because of the large number of compounds involved but also because of their small concentration. One of the major limitations to research on flavor has been the lack of adequate methods of separation, particularly of compounds representing a homologous series. The development of chromatographic techniques has paved the way for such separation on a micro scale.

The objectives of this study were to separate organic acids and esters of pre-ferments by chromatographic techniques and to determine the concentra-

Table I. Pre-ferment Formulas

Ingredients	Quantity of Ingredients	
	ADMI (9), G.	Fleischmann, G. (20)
Water	320	320
Glucose	21	21
Sodium chloride	7	7
Nonfat dry milk	42	..
Brew improver[a]	..	2.1
Yeast food[b]	3.5	..
Compressed yeast[b]	14	14

[a] Arkady type, Standard Brands, Inc., New York 22, N. Y.
[b] Standard Brands, Inc., New York 22, N. Y.

tion of the individual acid and ester components.

Materials and Methods

Pre-ferments. Two different pre-ferments were used (Table I). These were modifications of the American Dry Milk Institute (ADMI) (*9*) and the Fleischmann (*20*) pre-ferments. The ingredients of each pre-ferment were mixed in a Waring Blendor for 1 minute, and allowed to ferment at 30° C. in Erlenmeyer flasks which were shaken gently and continuously. Samples were taken at intervals ranging up to 48 hours, centrifuged, and used to determine the acids and esters present. The pH of each pre-ferment was measured with a Beckman Model H-2 pH meter each time a sample was taken.

Sources of Standard Acids and Esters. Formic (c.p.), acetic (c.p.), propionic, *n*-butyric, and isobutyric acids were obtained from Distillation Products Industries, Rochester 3, N. Y. Lactic acid (c.p.), pyruvic acid (reagent grade), ethyl acetate (N.F.), and ethyl lactate (highest purity) were obtained from Fisher Scientific Co., St. Louis, Mo. All acids and esters were redistilled under vacuum.

Preparation of Samples for Analysis. For analysis of lactic and the unknown acids by paper chromatography, 100-gram aliquots of the centrifuged pre-ferments were neutralized to pH 7.2 and evaporated under vacuum at 40 ° C. to less than 20 ml. The weight of the residue was adjusted to 20 grams with

water and stored in the refrigerator until chromatographed. For analyses of the ethyl lactate, the pre-ferments were adjusted to pH 10.0 prior to evaporation. According to previous analyses, concentrating zero-hour pre-ferments containing ethyl lactate and acetate solutions in vacuum at pH 7.2, 7.9, 8.6, 9.0, and 10.0 resulted in 0, 0, 30, 60, and 100% hydrolysis of the ester, respectively. The difference in quantities of lactic acid in solutions adjusted at pH 7.2 and 10.0 before concentration indicated the amount of lactic acid in the ester form.

For the analysis of acetic acid using column chromatography, samples of pre-ferments were adjusted to approximately pH 10.0 before storing at 5° C. The difference in quantities of acetic acid in solutions adjusted to pH 7.2 and 10.0 indicated the amount of acetic acid in the ester form.

Paper Chromatography of Lactic and an Unknown Acid. Lactic acid was analyzed using the chromatographic techniques of Denison and Phares (*11*). Ten-microliter samples of the known acid and the concentrated pre-ferments were spotted on the paper. The developing solvent was a solution of 13 parts of diethyl ether, 3 parts of glacial acetic acid, and 1 part of water. The development time was 3 hours and the temperature was 30° C. The 12 × 24 inch borosilicate glass jar, serving as a chromatography chamber, was covered with a cardboard box to minimize temperature and light effects.

The known acid solutions and the pre-ferments were adjusted to pH 10.0 before being placed on the paper. A standard curve for sodium lactate was prepared by plotting the area of the spots covered by chromatographed quantities of the salts equivalent to 16, 32, 48, 64, and 80 γ of lactic acid.

The method of Magasanik and Umbarger (*15*), using 95 parts of water-saturated 1-butanol and 5 parts of 90% formic acid, also was used for the separation of lactic from an unidentified acid. These acids were also resolved using some of the solvents of Stark, Goodban, and Owens (*24*). Those effective included: chloroform, 95% ethyl alcohol, and 90% formic acid (2:1:0.06); and phenol, water, and 90% formic acid (3:1:0.04).
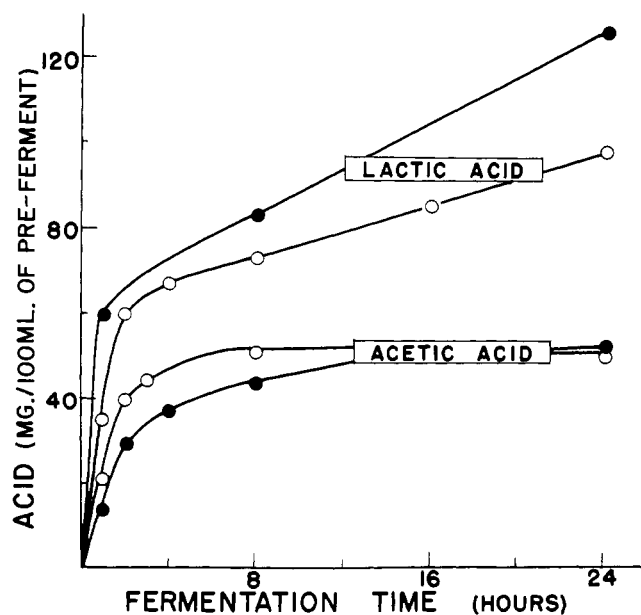
Figure 1. Effect of fermentation time on amount of acetic and lactic acid produced in different pre-ferments

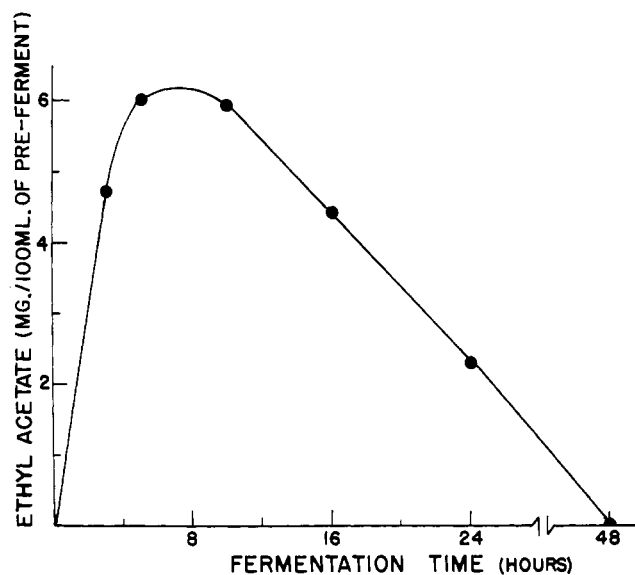● ADMI pre-ferment   ○ Fleischmann pre-ferment

Figure 2. Effect of fermentation time on amount of ethyl acetate in a Fleischmann pre-ferment

Column Chromatography of Other Organic Acids. Other organic acids were analyzed using the techniques of Corcoran (10) with a few modifications. In preparing the column, only 20 ml. of glycine buffer was mixed with 25 grams of the hydrochloric acid–treated silicic acid. Two-milliliter aliquots of the pre-ferment solution, the concentrated pre-ferment solution, or a known acid mixture in a pre-ferment solution made without yeast was placed on the column and eluted with chloroform-butanol mixtures. The concentrations of butanol were 0.75, 5, and 25% in chloroform. n-Butyric, isobutyric, and propionic acids were eluted by the first 200 ml. of the 0.75% butanol-chloroform solvent. n-Butyric and isobutyric acids were eluted in the same tubes and could not be distinguished by chromatographic means. Acetic acid was eluted by 150 ml. of 5% butanol-chloroform solvent. Lactic acid was eluted in the next 200 ml. of solvent increased to 25% butanol. In each case, it was necessary to subtract a blank determined by chromatographing a 2-ml. aliquot of the pre-ferment made without yeast. The blank was particularly high for lactic acid, as an unknown acidic material emerged in the first 80 ml. of 25% butanol-chloroform solvent.

Identification of Acids. The acids present in the pre-ferments were identified by formation of derivatives and by infrared spectra. The derivatives were formed using the procedure of Shriner and Fuson (23). The infrared spectra of the sodium salts were obtained using the potassium bromide pellet technique.

Results and Discussion

Identity of Organic Acids in Pre-ferments. An organic acid, which was eluted from a silicic acid column by a 5% butanol-95% chloroform solvent (10), behaved like acetic acid in chromatography and was identical in infrared spectrum to sodium acetate precipitated from methanol (22). The p-bromophenacyl derivative melted at 85° C., the same as that of authentic sodium acetate.

Another acid that was separated by paper chromatography (11) had an R_f value of 0.81, the same as a known sample of lactic acid. The sodium salt of this acid had an infrared spectrum identical to that of sodium lactate precipitated from methanol.

A third acid had an R_f of 0.56 using water saturated 1-butanol–formic acid (15) as the developing solvent. Using chloroform-–ethyl alcohol–formic acid and phenol-water–formic acid (26) as developing solvents, the R_f values of the third acid were 0.72 and 0.50, respectively. These R_f values obtained with different solvents failed to identify the third acid. Experiments with known acids showed that this was not from the tricarboxylic acid cycle. Likewise, it was not malonic, tricarballylic, thioglycollic, glycollic, glycollyl glycollic, or lactylyl lactic acid. Further attempts to identify the third acid have failed. It is possible that this acid, like lactic acid, is produced by the natural bacteria present in the pre-ferments.

Amounts of Organic Acids Present in Pre-ferments. The pH of the pre-ferment decreased slightly with time,

because of the development of carbon dioxide and organic acids during fermentation. These data—not presented —corroborate the results of Carroll, Miller, and Johnson (8). The changes in pH did not reflect the full production of organic acids because of the presence of milk and inorganic buffer salts in the American Dry Milk Institute and Fleischmann pre-ferments, respectively. Acetic, lactic, and a third acid were the only organic acids present in any appreciable quantity in either Fleischmann or American Dry Milk Institute pre-ferments. The changes in acetic acid and lactic acid concentration with time are shown in Figure 1. The acetic acid reached its maximum concentration after 8 hours but the concentration of lactic acid continued to increase for as long as 24 hours—presumably, owing to the presence of Lactobacilli organisms in the pre-ferments. Only a trace of butyric acid could be detected. The third acid developed during fermentation. Although no quantitative data can be obtained until the acid is identified, the concentration of this acid in the pre-ferment is estimated to be less than that of lactic acid.

No effect of acetic or lactic acid on colloidal dough properties could be demonstrated by such devices as the alveograph, extensograph, or the farinograph (16). However, dough made with the levels of acetic and lactic acids produced in pre-ferments after 6 hours (Figure 1), tended to become sticky. This is a property of dough associated with the swelling of gluten proteins in the presence of acids.

Esters Present in Pre-ferments.
The only esters detected in the Fleisch-
mann pre-ferment were those of acetic
acid. The ethyl ester was presumably
produced because of the high concen-
tration of ethyl alcohol in the fermenta-
tion mixture. Wiseblatt (26) found
only ethyl esters in bread crumb.

The change in ethyl acetate concentra-
tion in the pre-ferment with time is
shown in Figure 2. The ester reached
its maximum concentration after 6 to 8
hours of fermentation and decreased to
zero after 48 hours. The concentration
of ethyl acetate in American Dry Milk
Institute pre-ferments was not deter-
mined because it was necessary to adjust
the pH of that pre-ferment to 10.0 to
precipitate a part of the nonfat dry milk.
The ester was hydrolyzed at this pH.

Acknowledgment

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WATER CONTENT OF MEATS

Determination of Water-Holding Capacity of Fresh Meats

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The water-holding capacity of nondisintegrated muscles and ground and comminuted fresh lean meats was determined on fresh and heated meat. By pressing a 400- to 600-mg. fresh muscle sample on No. 1 Whatman filter paper of constant humidity in a specially made press operating under 500 p.s.i., the area of the paper wetted in 1 minute by the expressed juice is directly proportional to the weight of water in the press juice. The method gives the reproducible results within 2 to 5%. The amount of free water in beef, pork, veal, and lamb varies from 30 to 50% of the total moisture content, depend-ing on the kind of meat and period of aging.

RECENT STUDIES on consumer quali-
ties of meats, such as tenderness,
texture, drip on freezing and thawing,
and shrinkage on cooking indicate that
these qualities depend on the degree of
hydration of muscle proteins (1, 17,
36-40). The highly polar water mole-
cules are attracted to the muscle proteins
by ionizable basic and acidic groups as in
arginine, histidine, lysine, glutamic acid,
and aspartic acid or by polar nonionic
groups such as in cystine, cysteine,
serine, methionine, threonine, tyrosine,
and tryptophan. The mechanism of the
protein hydration is not well understood.
Some pioneering work on hydration of
various proteins, other than muscle
proteins, and polypeptides has been done
by Bull (2), Pauling (33), Mellon and
Hoover (29), and others (4, 5, 26).
Lean meat contains about 3.5 grams