

DETERMINATION OF ERYTHROSCORBIC ACID IN BAKER'S YEAST  
AND THE EFFECT OF D-ERYTHROSCORBIC ACID  
ON FLOW-PROPERTIES OF WHEAT FLOUR DOUGH

by

HEE SUN KIM

B.S., Seoul National University, Korea, 1985

---

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Food Science

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1988

Approved by:



Major Professor

LD  
21062  
174  
CIPSC  
108  
KSS  
62

TABLE OF CONTENTS

111208 129914

	PAGE
LIST OF TABLES -----	i
LIST OF FIGURES -----	ii
LIST OF ABBREVIATIONS -----	iii
ACKNOWLEDGEMENTS -----	iv
INTRODUCTION -----	1
EXPERIMENTAL -----	3
Materials -----	3
High performance liquid chromatography -----	3
Determination of erythroascorbic acid and ascorbic acid in baker's yeast -----	4
Stability of erythroascorbic acid and ascorbic acid in 0.05 M perchloric acid (pH 1.55) ----	7
Determination of erythroascorbic acid and ascorbic acid in fermented dough -----	7
Spread test -----	8
Effect of erythroascorbic acid on mixing time -----	9
Measurement of gluten strength by a stretching method -----	9
RESULTS AND DISCUSSION -----	11
Stability of erythroascorbic acid and ascorbic acid in 3% metaphosphoric acid and 0.05 M perchloric acid -----	11
Assay of yeast for ascorbic acid and erythroascorbic acid -----	12
Erythroascorbic acid in fermenting bread dough -----	13
Effect of erythroascorbic acid on mixing of wheat dough and on the spreading of dough -----	14

	PAGE
Effect of erythroascorbic acid on wheat gluten strength -----	15
CONCLUSIONS -----	17
LITERATURE CITED -----	18
TABLES -----	21
FIGURES -----	23

LIST OF TABLES

TABLE	PAGE
1. Concentrations of erythroascorbic acid (EAA) and ascorbic acid (AA) in various yeasts -----	21
2. Effect of D-erythroascorbic acid (D-EAA) on bread -----	22

## LIST OF FIGURES

FIGURES	PAGE
1. Structures of D-erythroascorbic acid (D-EAA), L-erythroascorbic acid (L-EAA) and L-ascorbic acid (L-AA) -----	23
2. HPLC-EC chromatograms of extracts of compressed yeast containing added D-EAA -----	25
3. HPLC-EC chromatograms of extracts of compressed yeast spiked with L-AA -----	27
4. Recoveries of D-EAA and L-AA, added to extracts of compressed yeast -----	29
5. Recoveries of D-EAA and L-AA stirred in 0.05 M perchloric acid at 25°C -----	31
6. Flow chart for the analytical determination of EAA and AA in yeasts -----	33
7. HPLC-EC chromatograms of 3% metaphosphoric acid extracts of compressed yeast -----	35
8. HPLC-EC chromatograms of extracts from yeasted wheat doughs spiked with D-EAA and L-AA or fermented for 0 to 3 h -----	37
9. Recoveries of D-EAA, and L-AA added to wheat doughs at 20 ppm and 24 ppm, respectively, based on wheat flour -----	39
10. Mixograms of flour-water dough -----	41
11. Effects of chemicals on the spread of flour-water dough -----	43
12. Stretching time (min) <u>vs</u> distance (cm) of malted flour, and unmalted flour -----	45

LIST OF ABBREVIATIONS

AA	Ascorbic acid
DKGA	2,3-diketo-L-gulonic acid
EAA	Erythroascorbic acid
EC	Electrochemical
HPLC	High performance liquid chromatography

### ACKNOWLEDGEMENTS

The author expresses her sincere appreciation to her major professor, Dr. Paul A. Seib for his guidance, encouragement and advice in the completion of this work. Deep appreciation is extended to Dr. Okkyung Kim Chung of U.S. Grain Marketing Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture for providing invaluable help, advice and facilities for the research, and for being on the author's graduate committee. The author also extends her great appreciation to Dr. Hosenev for being on the graduate committee and for reviewing this thesis.

Grateful acknowledgement is expressed to Dr. Charles W. Deyoe, Head, Department of Grain Science and Industry for providing funds needed to carry out this work.

Gratitude is due to all the people in the Department of Grain Science and Industry and also at the USGMRL for their kind help and cooperation, especially Miss Wan Soo Kim for her precious time, Dr. Doreen Liang, Dr. Deborah Rogers, Mrs. Margo Caley, Mrs. Bernadine Eichman and Mr. YangSheng Wu.

The author would like to express her deep thanks to her parents and family for their boundless support, encouragement, understanding and love through these years of study.

## INTRODUCTION

D-Erythroascorbic acid (D-EAA, Fig. 1A) occurs in yeast and fungi (Murakawa et al 1977; Nick et al 1986) where it is thought to be synthesized from a D-pentose. EAA was also reported as an indirect product in the fermentation of D-xylose by Serratia marcescens (Takahashi et al 1976). The bacterium released D-threo-2-pentulosonic acid, and the acidity of the broth catalyzed formation of D-EAA from the 2-keto acid.

L-Erythroascorbic acid (L-EAA, Fig. 1B), the enantiomer of D-EAA, has been found in purple sulfur bacteria where it is postulated to occur as a metabolic breakdown product of L-ascorbic acid (L-AA, Fig. 1C, Bast et al 1978). In this catabolic pathway for AA which was first reported by Ashwell et al (1961) in rat liver microsomes, AA is oxidized to dehydroascorbic acid, which then is hydrolyzed to 2,3-diketo-L-gulonic acid (DKGA). DKGA then is decarboxylated to give L-xylosone (Kang et al 1982), which is oxidized inside the bacterium to L-EAA. A non-enzymatic oxidation to L-EAA and L-AA was also demonstrated by Kang et al (1982). Besides the oxidative decarboxylation pathway for the catabolism of AA, the other well-known degradative pathway involves splitting AA into oxalic acid and a four-carbon fragment (Loewus 1987).

The absolute configuration of the D-EAA in yeast and fungi is not known with certainty. However, the D-configuration is implied for C-4 since D-arabinose, D-arabinate and especially D-arabino- $\gamma$ -lactone stimulate production of EAA in Candida utilis (Murakawa et al 1977). Furthermore, Lipomyces starkeyi does not accumulate AA, so its EAA is probably not the L-form from the catabolism of L-AA (Nick et al 1986).

Recently, D-EAA was synthesized at Kansas State University (Seib et al 1987). The synthetic material and the natural product from Lipomyces starkeyi gave, after trimethylsilylation, identical ion fragments in the mass spectrometer (Loewus 1987). Due to the availability of pure crystalline D-EAA, one objective of this investigation was to determine the level of EAA in baker's yeast. A second objective was to determine whether yeast released EAA into the liquid phase of fermenting wheat doughs. A third objective was to determine the effects of D-EAA on the mixing and rheological properties of dough and gluten.

## MATERIALS AND METHODS

### Materials

D-Erythroascorbic acid (D-EAA, m.p. 132° to 135°C) was kindly supplied by Dr. Doreen Liang, Kansas State University. L-Ascorbic acid (AA), HPLC-grade methanol and sodium acetate were from Fisher Scientific, St. Louis, MO. All other chemicals were reagent grade. Red Star brand compressed yeast was obtained from Universal Foods Corporation, Milwaukee, WI. Three commercial instant yeasts were obtained from Saf Products Corporation, Minneapolis, MN, Universal Foods Corporation, Milwaukee, WI, and Gist-Brocades, Charlotte, NC. Straight grade flour was milled from a composite of hard red winter wheat cultivars grown in the Central Plains: it was malted by manufacturer (Ross Milling Co., Wichita, KS) and contained 11.9% protein on a 14% moisture basis (m.b.). For the gluten stretching test, both malted and unmalted flour were used. Unmalted flour contained 11.4% protein on a 14% m.b.

### High Performance Liquid Chromatography (HPLC)

The HPLC system consisted of a 6000A solvent delivery system (Waters Associates, Inc., Milford, MA), an electrochemical (EC) detector (Model LC-4, Bioanalytical System, West Lafayette, IN) with a glassy carbon working

electrode and a type TL-5A flow cell, a Rheodyne loop injector (0.02 ml, Alltech Associates, Inc., Deerfield, IL), and an integrating recorder, which was either an HP 3392A Integrator (Hewlett Packard, Avondale, PA) or a Chromatopac, Model C-R3A (Shimadzu Co., Tokyo, Japan). EAA and AA in yeast and dough were determined on a reverse-phase column (Spherisorb ODS-2, Rainin Instrument Co. Inc., Woburn, MA., or Alltech C-18, Alltech Associates Inc., Deerfield, IL., 5  $\mu$ m particle size, 250 x 4.6 mm). The analytical column was protected by a precolumn with a 40 x 4.6 mm ODS-10 cartridge (Bio-Rad Laboratories, Richmond, CA). The mobile phase contained 0.08 M acetate buffer (pH 4.2), 5% methanol, 0.1 mM ethylenediaminetetraacetic acid, and 1.0 mM tetrabutylammonium phosphate. Eluting solvents were degassed by an ultrasonic generator (Fisher Sonic Dismembrator, Model 300, Fisher Scientific, St. Louis. MO), and the flow rate was set at 0.8 ml/min. The column was maintained at 35°C and the potential of the detector was set at + 0.72V vs a Ag/AgCl reference electrode. Quantitative determinations of EAA and AA were made by comparing intergrated areas of known amounts of the standard compounds.

#### Determination of EAA and AA in baker's yeast

A standard curve of D-EAA was constructed as follows.

D-EAA stock solutions were prepared by dissolving D-EAA (3.12 - 12.48 mg) in 3% aqueous metaphosphoric acid (500 ml) that had been freshly made and degassed. Immediately prior to injection, an aliquot (1 ml) of a stock solution was diluted 25-fold with cold degassed 0.05 M perchloric acid, and 0.02 ml of the mixture containing 5 - 20 ng of D-EAA was injected.

The recovery of D-EAA added to yeast was done as follows. An aliquot of a D-EAA stock solution was added to freeze-dried yeast (1 g) or compressed yeast (4 g, "as is") in aqueous metaphosphoric acid. Then, 3% metaphosphoric acid (10 ml) was added and the mixture stirred on a magnetic stir plate at 25°C for 15 min to 2 hr. After different extraction periods, the extract was centrifuged (15,000 rpm, Eppendorf Model 5412 Microcentrifuge, Brinkmann Instruments Inc. Westbury, NY), and an aliquot (1 ml) of clear supernatant was pipetted into a volumetric flask (25 ml for freeze-dried and 10 ml for compressed yeast) and made to volume with cold degassed 0.05 M perchloric acid. The resulting solution was filtered through a 0.5  $\mu$ m micromembrane filter (Millipore Corporation, Bedford, MA) and injected into the chromatograph. The same methods were used for AA standard curve and recovery.

Yeast was assayed "as is" for EAA and AA or the yeast was assayed after it was freeze-dried. Compressed yeast (4 g "as is") was stirred with 10 ml of 3% metaphosphoric acid for 5 min at 25°C. After centrifugation, an aliquot (1 ml) of the clear supernatant was made to volume (10 ml) with cold 0.05 M perchloric acid, and 0.02 ml of the resulting solution was injected into the chromatograph. Freeze-dried yeast was ground with a mortar and pestle, and 1 g of ground dried yeast was extracted with 10 ml of 3% metaphosphoric acid for 5 min at 25°C. After centrifugation, an aliquot (1 ml) of the clear supernatant was made to volume (25 ml) with cold 0.05 M perchloric acid and 0.02 ml of the resulting solution was injected.

Each of the three samples of instant yeast (0.1 g each) was extracted with 10 ml of 3% metaphosphoric acid. After centrifugation, an aliquot (1 ml) of clear supernatant was diluted to 100 ml volume with cold degassed 0.05 M perchloric acid, and the diluted solution (0.02 ml) was injected into the chromatograph. Instant yeast samples were also assayed for EAA and AA after rehydration and freeze-drying. Instant yeast (10 g) was rehydrated with 25 ml of deoxygenized water for 5 min at 25°C. After freeze-drying the instant yeast samples were assayed as described in the previous paragraph.

#### Stability of EAA and AA in 0.05 M Perchloric Acid (pH 1.55)

D-EAA (12.5 mg) was dissolved in 3% metaphosphoric acid (250 ml), and a 1 ml aliquot was made to volume (50 ml) with 0.05 M cold perchloric acid. The mixture was shaken gently, and 0.02 ml of mixture was injected into the chromatograph at various times. AA (15.2 mg) was used for checking its stability in the extraction medium.

#### Determination of EAA and AA in Fermented Dough

Doughs were mixed to optimum at 25°C using the following ingredients ; flour, 100 g (14% m.b.) ; water, 66 g ; sucrose, 6 g ; sodium chloride, 1.5 g ; and compressed yeast, 2 g.

The recovery of D-EAA added to the dough extraction system was determined as follows. A D-EAA stock solution was prepared by dissolving 20 mg of D-EAA in 3% metaphosphoric acid (1 L). Dough (1 g, "as is") was mixed with 3% metaphosphoric acid (9 ml), and an aliquot (1 ml) of the standard D-EAA solution was added. The mixture contained 20 µg of EAA per gram of dough on an "as is" moisture basis. The mixture was stirred on a magnetic stir plate at 25°C for 0.5 - 2 hr. At different extraction periods, an aliquot (1 ml) was pipetted into a volumetric flask (5 ml), and made to volume with cold 0.05 M perchloric acid. After centrifugation and filtration,

clear supernatant (0.02 ml) was injected into the chromatograph. The same method was used for recovery of AA added to the dough extraction system.

To assay fermenting doughs for EAA, doughs were mixed to optimum development in a vertical pin mixer, and immediately after fermenting (0, 1, 2 and 3 h at 30°C and 90% RH), fresh dough (1 g, on "as is" basis) was extracted with 3% metaphosphoric acid for 30 sec using a Waring blender. After centrifugation, an aliquot (1 ml) of the clear supernatant was diluted to 10 ml volume with cold degassed 0.05 M perchloric acid, and the clear solution (0.02 ml) was injected into the chromatograph.

#### Spread Test

Flour-water doughs were mixed with a pin mixer from 100 g flour to optimum consistency and development. Doughs were left standing at 30°C and 90% RH, and after 0, 1, 2 and 3 h standing, doughs were mechanically rounded and permitted to rest 60 min at 30°C and 90% RH. Spread ratios were then determined by dividing the width of a molded dough piece by its height (Hoseney et al 1979). Reported spread ratios are the average of two determinations.

The compounds tested for their effects on dough flow were AA, D-EAA, and L-cysteine hydrochloride. The level of a compound in a dough was expressed in parts per million

based on flour weight. AA and D-EAA were tested in equivalent amounts on a molar basis, and their solutions were freshly prepared and added immediately before mixing.

#### Effect of D-EAA on Mixing Time

A 10-g mixograph, described by Finney and Shogren (1972), was used to study the effect of D-EAA on mixing time. Flour (10 g on 14% m.b.) was placed in the mixograph bowl, and optimum water absorption for the flour was determined to be 62% from the shape of the mixogram. Then, various concentrations of D-EAA solutions were used so as to maintain absorption at 62% while EAA level was varied. In the same manner, L-AA and L-cysteine hydrochloride were tested at levels equivalent to EAA on a molar basis. The level of reagents used was expressed in part per million based on flour weight. All solutions were freshly prepared before use. Each dough was mixed a total of 8 min.

#### Measurement of Gluten Strength by a Stretching Method

The gluten stretching apparatus consisted of four 1-L tubes (50 mm x 390 mm) filled with distilled water at 32°C. The top cover of each tube contained a small hook from which gluten balls could be hung. The filled tubes were maintained at 32°C using a water bath. Gluten samples were tested in duplicate. Gluten was washed from a dough

containing 12.5 g (on 14% m.b.) of flour and optimum water as described by Al-Obaidy (1986) and Chung et al (1987), except for the change in water temperature from 30°C to 32°C. After mixing to optimum development, the dough was kneaded under a stream of distilled water until the wash water appeared free of starch. The wet gluten was rolled between the hands to remove excess water and weighed. Two 2.5-g portions were cut off from the same gluten sample, moulded into a ball with the fingers, and then rested 10 min in distilled water at 32°C. The wet gluten ball was hooked through its center by a simple fishing hook to be hung on the top cover of the tube and by another hook containing a 3-g weight. Two fishing hooks were hooked along a radius of the sphere, each at the center of the sphere. The distance of gluten stretching was recorded every 20 min, and the time for the gluten to reach the bottom of the tube (35 cm distance) was recorded.

## RESULTS AND DISCUSSION

### Stability of EAA and AA in 3% Metaphosphoric acid and 0.05 M Perchloric acid

To determine L-ascorbic acid in a biological system, the classical extraction medium is aqueous metaphosphoric acid (Pachla et al 1976). Extracts are often diluted with 0.05 M perchloric acid when AA is to be quantitated by HPLC-EC. In this work, it was important to compare the stabilities of AA and EAA in aqueous metaphosphoric acid and 0.05 M perchloric acid.

Compressed yeast in 3% metaphosphoric acid was spiked with D-EAA or L-AA, and each mixture was stirred at 25°C for up to 2 h. The chromatograms in Figs. 2 and 3 show that D-EAA and L-AA were practically stable in 3% metaphosphoric acid (pH 1.5); recoveries of added D-EAA or L-AA were quantitative after 30 min stirring at 25°C (Fig. 4). However, D-EAA was markedly less stable than L-AA in 0.05 M perchloric acid (pH 1.55). Fig. 5 shows 40% recovery of EAA after stirring 2 h in 0.05 M perchloric acid at 25°C vs 80% for AA. Thus, in assaying for EAA, it is important to use cold perchloric acid solution, and to inject immediately those extracts that have been diluted with 0.05 M perchloric acid.

### Assay of Yeast for AA and EAA

The assay methods to determine EAA and AA in yeast are outlined in Fig. 6. The recovery of D-EAA and L-AA added to compressed yeast in 3% metaphosphoric acid was linear over a range of 0 - 60 µg per gram of yeast (dry basis); the coefficients of variation were  $\pm 2.3\%$  and  $\pm 1.2\%$ , respectively for D-EAA and L-AA. The EC detector had a sensitivity of 1 ppm of EAA or AA in yeast.

Extraction of three samples of fresh compressed yeast with 3% metaphosphoric acid gave 1 - 4 ppm EAA on a dry solid basis, and 1.5 - 4.5 ppm AA (Table 1). However, when the compressed yeast was freeze-dried prior to extraction, the samples gave 35 - 51 ppm EAA and 2.4 - 4.9 ppm AA. Obviously, the dry compressed yeast cells were lysed either during freeze-drying or during the first stages of extraction. Typical chromatograms are shown in Fig. 7. The concentration of EAA in the three samples of compressed yeast averaged at least one order of magnitude higher than AA (Table 1). Nick et al (1986) found only EAA and no AA in two samples of Saccharomyces cerevisiae.

Three samples of instant yeasts also gave a higher release of EAA (21 - 69 ppm) after hydration and freeze-drying than with no pretreatment (12 - 27 ppm). The instant active dry yeast contained the highest level of EAA

(69 ppm) and AA (6 ppm). Sample 4 (Table 1) showed a very high level (2720 ppm) of AA, although addition of AA to the dry yeast was not included on the label. However, the manufacturer admitted during the poster session of the AACC meeting in Nashville, TN, October, 1987, that AA had been added.

#### EAA in Fermenting Bread Dough

L-AA is widely used in breadmaking because it improves loaf volume and crumb grain. Elkassabany et al (1980) reported that about 15 ppm L-AA was sufficient to realize its maximum improving effect. Most bakeries use large excesses of L-AA in dough since overages cause no detrimental effects. If yeast releases EAA during fermentation, and if EAA improves dough, then the dough strengthening effect of yeast fermentation might be explained.

Bread dough was assayed for EAA and AA by the same approach used to assay yeast in Fig. 6, except doughs were extracted in metaphosphoric acid by homogenizing 30 sec in a blender. Typical chromatograms are shown in Fig. 8. Recoveries of EAA and AA from spiked dough (20 ppm, 24 ppm for D-EAA and L-AA, respectively) were 96 - 99% (Fig. 9). EAA was not detected in the fermenting dough, so its concentration in the dough was less than 1 ppm based on dry

solids. Thus, either the yeast releases little or no EAA into dough, or else EAA is lost from the dough during fermentation. If the same quantity of EAA was released into 3% metaphosphoric acid from the compressed yeast in dough (1 - 4 ppm), the dough would be expected to give 0.1 - 0.4 ppm EAA. On the other hand, if the yeast in the dough unexpectedly lysed during extraction, we would expect the dough to contain about 1 - 2 ppm EAA per gram of dry dough solids.

Effect of EAA on Mixing of Wheat Dough  
and on the Spreading of Dough

Mixograms showed D-EAA, like L-AA, had little effect on mixing time at levels of up to about 1000 ppm in a typical dough (Fig. 10). Equivalent level of L-cysteine showed the expected reduced mixing time and mixing tolerance.

The spread test (Hoseney et al 1979) was used to compare the effects of D-EAA, L-AA and L-cysteine on the flow of a flour-water dough. Fig. 11 shows that, compared to the control dough, L-AA (100 ppm) strengthened dough and reduced its flow, while cysteine (100 ppm, equimolar to AA) greatly increased dough flow. On the other hand, an equivalent level of D-EAA (82 ppm) gave intermediate results, that is, D-EAA gave a slight increase in dough

flow at 0 standing time, but no further change with additional standing.

The spread test results indicate D-EAA does not act to oxidize dough as does L-AA. No structural analogue of L-AA has yet been found to give the oxidant effect in wheat doughs. Since the stereochemical requirement for L-AA to improve dough can not be met by D-EAA, this shows that the C-5 methyl group is essential for bread-improving activity. The preliminary bread baking data in Table 2 confirm that D-EAA does not oxidize dough.

#### Effect of EAA on Wheat Gluten Strength

The gluten washed out from an optimally mixed dough containing 83 ppm D-EAA shortened the stretching time from 406 min for the control gluten (obtained from the optimally mixed control dough) to 84 min (Fig. 12A) to reach 35 cm, indicating that 82 ppm D-EAA showed increased gluten extensibility. Adding L-cysteine was more effective in increasing the gluten extensibility and in reducing the stretching time. L-Cysteine (100 ppm) shortened the stretching time to 42 min. However, an equivalent amount of L-AA affected only slightly the stretching behavior of gluten when compared to the control gluten. It should be mentioned that the flour used was malted by the manufacturer.

The gluten stretching times were also checked with the gluten washed from doughs of unmalted flour. When gluten was washed from unmalted flour, the gluten containing L-AA and control gluten showed significantly reduced stretching time (Fig. 12B) compared to glutens from malted flour (Fig. 12A). L-Cysteine and D-EAA showed the same effectiveness in increasing gluten extensibility and in reducing the stretching time of gluten washed from malted flour.

It is not known whether an oxidant may have been inadvertently present in the malted flour. As in the spread ratio test, D-EAA showed no oxidizing effect. On the other hand, it gave a reducing effect which confirmed the C-5, C-6 stereochemical requirement of L-AA to oxidize dough.

## CONCLUSIONS

- (1) D-EAA was less stable than L-AA in 0.05 M perchloric acid at 25°C.
- (2) Baker's compressed yeast contained approximately 35 - 51 µg EAA per gram of dry solids and approximately 2 - 5 µg AA per gram of dry solids, while instant yeasts contained 21 - 69 µg EAA and 4 - 8 µg L-AA per gram of dry solids.
- (3) EAA was not detected in fermenting wheat dough (less than 1 ppm EAA in dry dough solids).
- (4) D-EAA added to wheat dough at up to 1000 ppm (based on flour) gave no effect on mixing time and no improving (oxidant) effect on loaf volume in baked bread.
- (5) D-EAA substantially improved the gluten extensibility, i.e. reducing the stretching time of gluten to reach 35 cm.

### LITERATURE CITED

- Al-Obaidy, K.A. 1986. Dough and gluten characteristics of good and poor quality flours: Lipid-protein bindings affected by mixing time, water absorption, chemicals and heat. Ph. D. Dissertation. Kansas State University. Manhattan, Kansas.
- Ashwell, G., Kanfer, J., Smiley, J.D. and Burns, J.J. 1961. Metabolism of ascorbic acid and related uronic acids, aldonic acids and pentoses. Ann. N.Y. Acad. Sci., 92 : 105.
- Bast, E., Marx, F. and Pfeilstcker, K. 1978. Formation of erythroascorbic acid and 2,3-enediol pentonic acid from L-ascorbic acid by purple sulfur bacteria. Z. Naturforsch. 33C : 789.
- Chung, O.K., Al-Obaidy, K.A. and Whetzel, S.Z. 1987. A modified gluten stretcher. Cereal Foods World. 32 : 669.
- Elkassabany, M. and Hosney, R.C. 1980. Ascorbic acid as an oxidant in wheat flour dough II. Rheological Effects. Cereal Chem. 57(2) : 88-91.
- Finney, K.F., and Shogren, M.D. 1972. A 10-g mixograph for

- determining and predicting functional properties of wheat flour. Baker's Dig. 46(2) : 32.
- Hoseney, R.C., Hsu, K.H. and Junge, R.C. 1979. A simple spread test to measure the rheological properties of fermenting dough. Cereal Chem. 56(3) : 141.
- Kang, S.-O., Sapper, H. and Lohmann, W. 1982. The oxidative degradation of L-ascorbic acid via an  $\alpha$ -ketoaldehyde. Z. Naturforsch. 37C : 1064.
- Loewus, F.A. and Loewus, M.W. 1987. Biosynthesis and metabolism of ascorbic acid in plants. CRC Crit. Rev. Plant Sci. 5(1) : 101.
- Loewus, F.A. 1987. Private Communication.
- Murakawa, S., Sano, S., Yamashita, H. and Takahashi, T. 1977. Biosynthesis of D-erythroascorbic acid by Candida. Agric. Biol. Chem. 41(9) : 1799.
- Nick, J.A., Leung, C.T. and Loewus, F.A. 1986. Isolation and identification of erythroascorbic acid on Saccharomyces cerevisiae and Lypomyces starkei. Plant Sci. Letters. 46 : 181.
- Pachla, L.A. and Kissinger, P.T. 1976. Determination of ascorbic acid in foodstuffs, phamaceuticals, and

body fluids by liquid chromatography with  
electrochemical detection. Anal. Chem. 48 : 364.

Seib, P.A., Liang, D. and Lung. Private communication.

Seib, P.A. Private communication.

Takahashi, T. and Murakawa, S. and Barada, M.  
1976. Ascorbic acid analogs as indirect products  
of Serratia marcescens. Agric. Biol. Chem. 40 :  
1255.

Table 1. Concentrations of EAA and AA in various yeasts

Yeast	Sample No.	Concentration <sup>a</sup> of EAA (ppm)	Concentration of AA (ppm)
Fresh	1	1.0	1.5
Compressed	2	4.0	4.5
Yeast	3	1.4	2.0
Freeze-dried	1A	50.9	2.4
Compressed	2A	52.0	4.9
Yeast	3A	35.2	5.4
Instant	4 <sup>b</sup>	18.1	2720.1
Yeast	5 <sup>c</sup>	26.9	8.2
	6 <sup>d</sup>	11.7	4.3
Hydrated and	4A	37.3	-
Freeze-dried	5A	20.9	3.8
Instant	6A	68.7	5.5
Yeast			

- (a) Dry basis.  
 (b) Contains other ingredients ; polyglycerol esters of vegetable fatty acids, potato starch, soybean oil. Manufacturer reported in a private communication that L-AA was added to yeast.  
 (c) Contains other ingredient ; sorbitan monostearate.  
 (d) Instant Active Dry Yeast.

Table 2. Effect of D-EAA on Bread<sup>a,b</sup>

Oxidant	Level (ppm)	Loaf Volume (cc)
None	-	835
KBrO <sub>3</sub>	10	885
L-AA	100	865
D-EAA	80	835
D-EAA	166	818 <sup>c</sup>

- (a) Data kindly provided by Y. S. Wu, Kansas State University.
- (b) Pup-loaves baked with various oxidants using a hard wheat flour (100 g, 11.8% protein).
- (c) Dough felt weak and sticky.

- Fig. 1
- (A) D-erythroascobic acid (D-EAA)  
(D-glycero-pent-2-enono-1,4-lactone)
  - (B) L-erythroascorbic acid (L-EAA)
  - (C) L-ascorbic acid (L-AA)  
(L-threo-hex-2-enono-1,4-lactone)

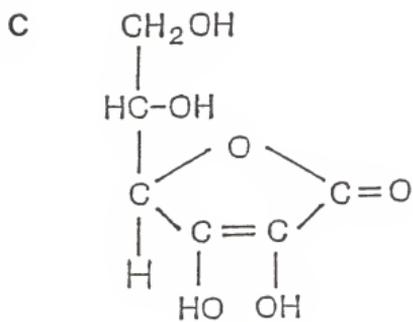
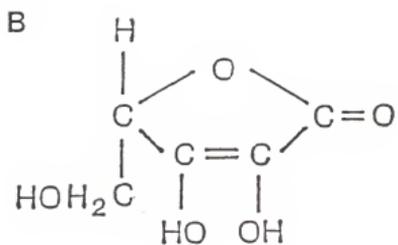
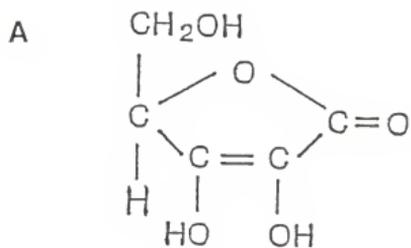


Fig. 2 HPLC-EC chromatograms of extracts (3% metaphosphoric acid) of compressed yeast containing added D-EAA.

The extracts were diluted 10-fold with cold 0.05M perchloric acid and immediately injected into the chromatograph as described in Materials and Methods. The HPLC column was the Spherisorb ODS-2, and the integrator was the Hewlett Packard model HP 3392A.

- (A) Compressed yeast, "as is".
- (B) D-EAA added, 20 ppm based on "as is" weight of yeast, 0 min stirring.
- (C) Same as (B), 30 min stirring.
- (D) Same as (B), 60 min stirring.
- (E) Same as (B), 120 min stirring.

A

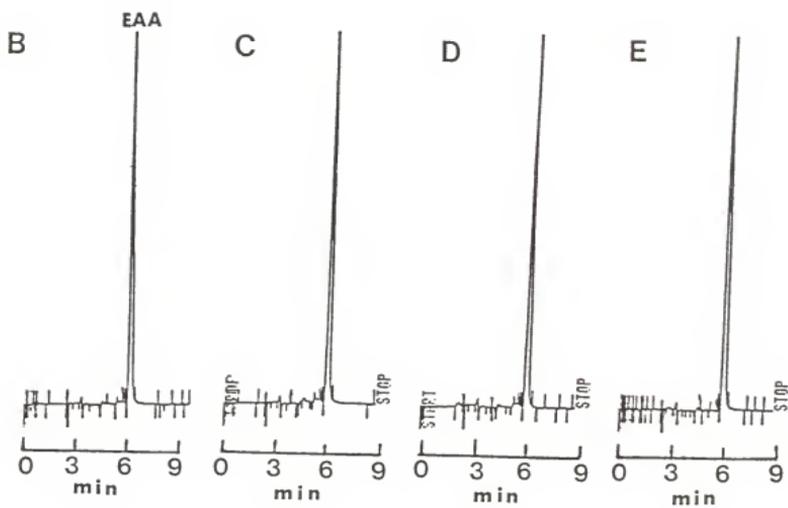


Fig. 3 HPLC-EC chromatograms of extracts (3% metaphosphoric acid) of compressed yeast spiked with L-AA. HPLC-EC assay was done as described in caption to Fig. 2.

- (A) compressed yeast, "as is".
- (B) AA added, 20 ppm based on "as is" compressed yeast, 0 min stirring.
- (C) Same as (B), 30 min stirring.
- (D) Same as (B), 60 min stirring.
- (E) Same as (B), 120 min stirring.

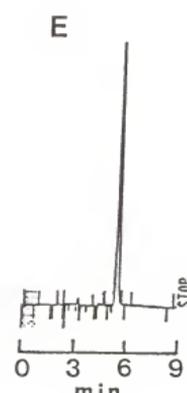
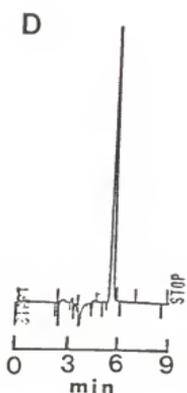
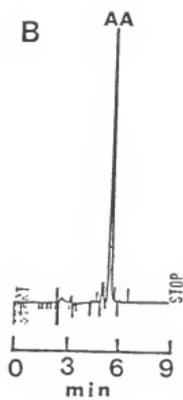
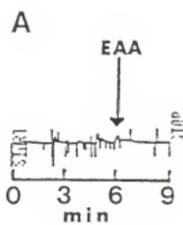


Fig. 4 Recoveries of (A) D-EAA, (B) L-AA, added to extracts (3% metaphosphoric acid) of compressed yeast.

HPLC-EC was done as described in caption to Fig. 2.

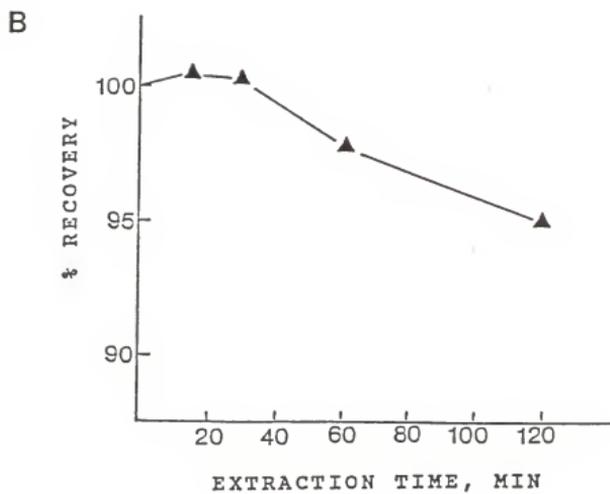
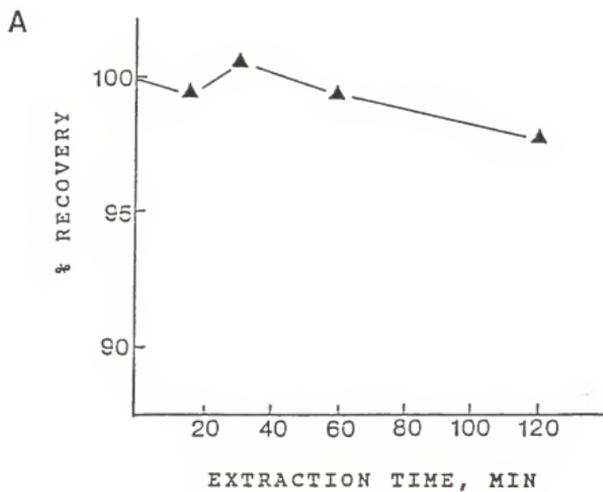
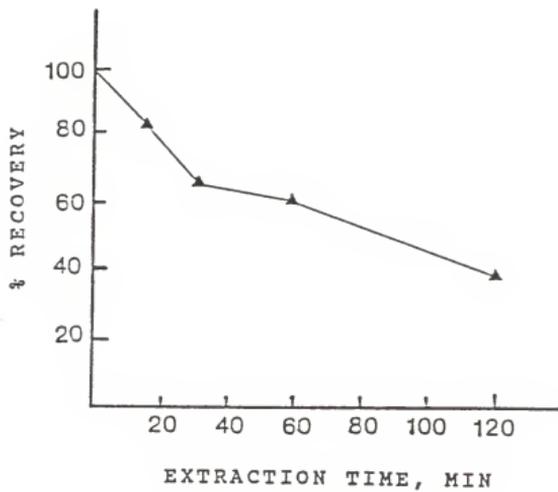


Fig. 5 Recoveries of (A) D-EAA, and (B) L-AA stirred in 0.05 M perchloric acid at 25°C.

D-EAA (12.5 mg) or L-AA (15.2 mg) was dissolved in 3 % metaphosphoric acid (250 ml), and an aliquot (1 ml) was made to volume (50 ml) with 0.05 M perchloric acid. After various times of stirring, the mixture (0.02 ml) was injected into the chromatograph.

A



B

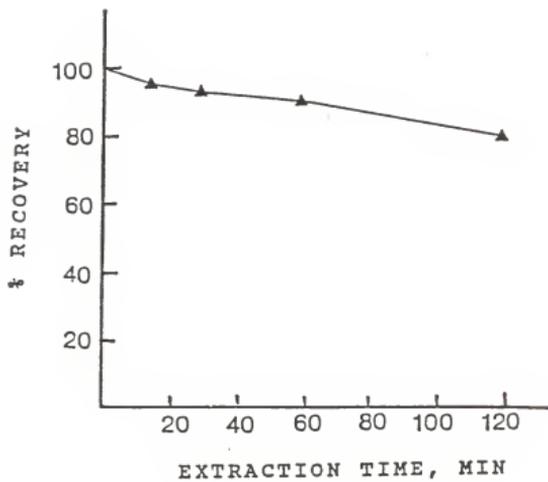


Fig. 6 Flow chart for the analytical determination of EAA and AA in yeasts.

Compressed Yeast

Instant Yeast

4 g Compressed yeast or  
1 g freeze-dried,  
compressed yeast.  
Extract 5 min at 25°C  
with 10 ml of fresh  
3 % metaphosphoric acid

0.1 g Instant yeast  
or 1 g freeze dried,  
instant yeast.  
Extract 5 min at 25°C  
with 10 ml of fresh  
3 % metaphosphoric acid

Centrifuge

Centrifuge

1 ml Aliquot of  
supernatant  
Make to 10 ml (fresh)  
or 25 ml (freeze-dried)  
volume with  
cold 0.05 M  
perchloric acid

1 ml Aliquot of  
supernatant  
Make to 10 ml (fresh)  
or 25 ml (freeze-dried)  
volume with  
cold 0.05 M  
perchloric acid

Immediately filter thru  
0.5  $\mu$ m micromembrane filter.  
Inject 20  $\mu$ l into  
chromatograph.

Reverse phase C-18 column, 35°C,  
EC detector +0.72 V.  
Mobile phase 0.08 M Acetate buffer (pH 4.2)  
0.1 mM EDTA, 1.0 mM TBA, 5% methanol by volume.

Fig. 7 HPLC-EC chromatograms of 3% metaphosphoric acid extracts of compressed yeast. The extracts were diluted 10 - 25 fold prior to injection into the chromatograph.

- (A) Compressed yeast, "as is".
- (B) Compressed yeast, "as is", plus 20 ppm L-AA.
- (C) Compressed yeast, "as is", plus 20 ppm D-EAA.
- (D) Freeze-dried compressed yeast.
- (E) Instant yeast, "as is".
- (F) Freeze-dried instant yeast.

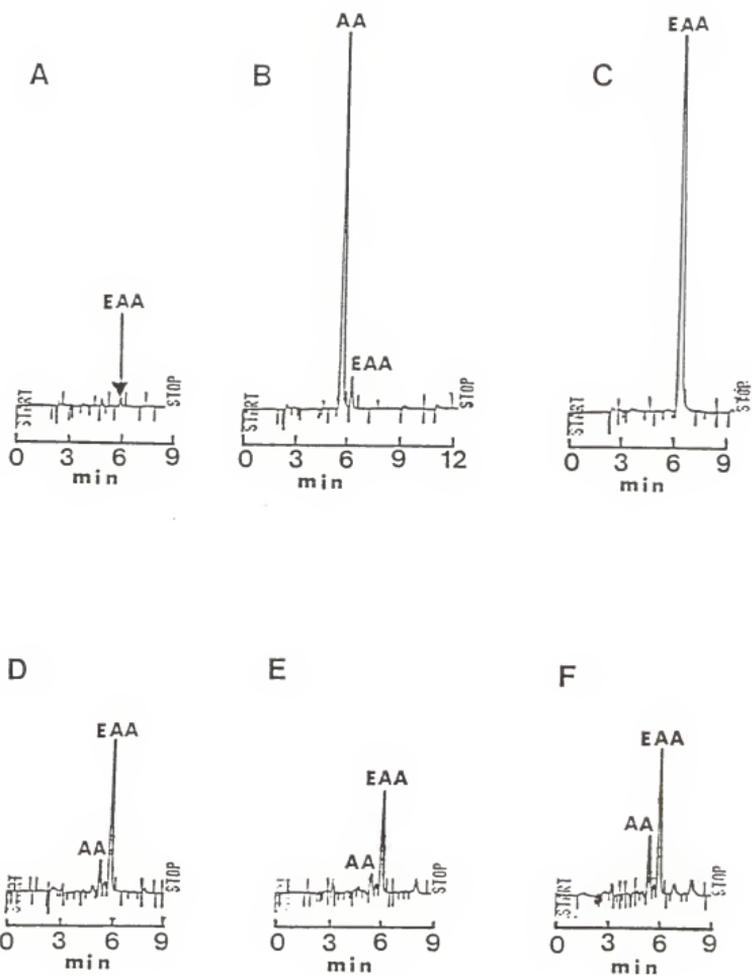


Fig. 8 HPLC-EC chromatograms of extracts from yeasted wheat doughs spiked with D-EAA and L-AA or fermented for 0 to 3 h.

- (A) Blank dough.
- (B) Blank dough, L-AA spike (24 ppm).
- (C) Blank dough, D-EAA spike (20 ppm).
- (D) Dough, 0 h fermentation.
- (E) Dough, 3 h fermentation.

Dough was extracted with 3% metaphosphoric acid for 30 sec using a Waring blender, centrifuged, and an aliquot (1 ml) was diluted to 10 ml volume with cold 0.05 M perchloric acid. The clear supernatant (0.02 ml) was injected into the chromatograph. HPLC-EC was done as described in caption to Fig. 2. A reverse-phase column (Alltech C-18, 5  $\mu$ m particle size, 250 x 4.6 mm) was used. Integrating recorder used was Model C - R3A Chromatopac, Shimadzu Co., Tokyo, Japan.

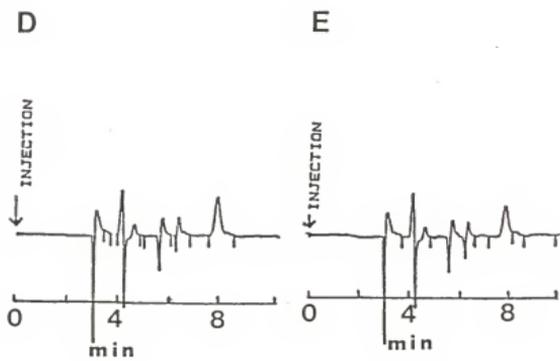
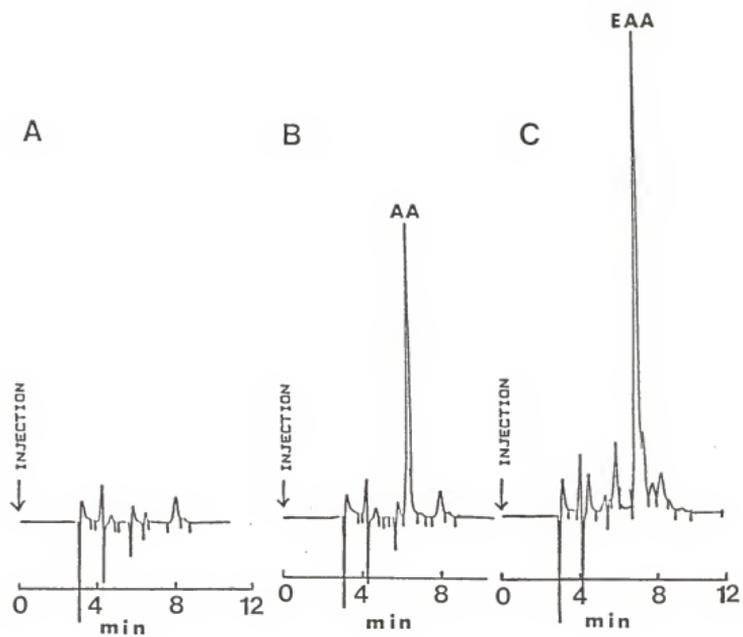


Fig. 9 Recoveries of (A) D-EAA, and (B) L-AA added to wheat doughs at 20 ppm and 24 ppm, respectively, based on wheat flour.

Doughs (1 g "as is") were mixed with 3% metaphosphoric acid (9 ml), and an aliquot (1 ml) of a standard D-EAA or L-AA solution was added. The mixture was stirred on a magnetic stir plate at 25°C for 0.5 - 2 h. After stirring, HPLC-EC assay was done as described in caption to Fig. 8.

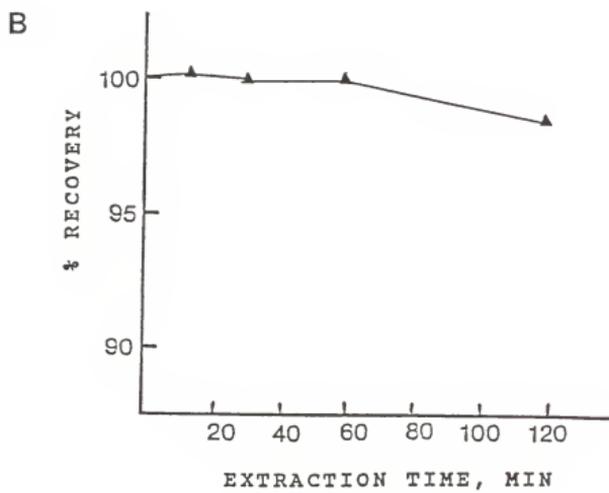
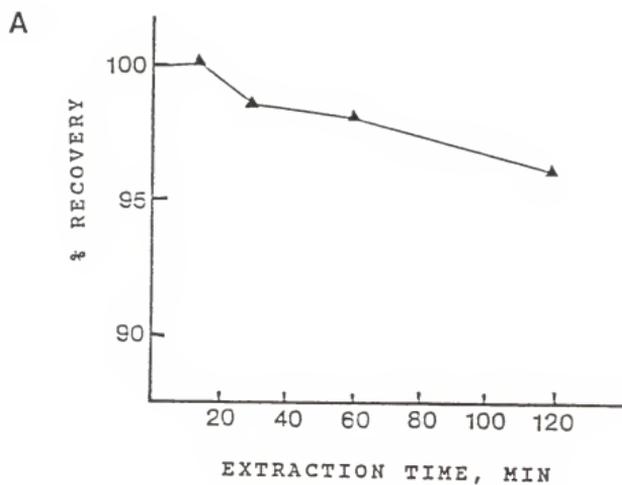


Fig. 10 Mixograms (10 g flour, 11.9% protein on 14% m.b.) of flour-water dough.

- (A) Control (no additive).
- (B) L-AA added.
- (C) D-EAA added.
- (D) L-Cysteine hydrochloride added.

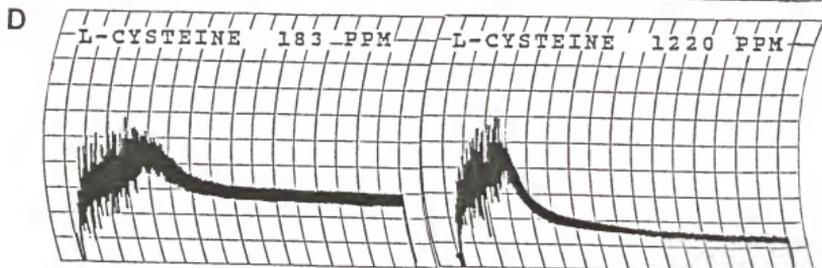
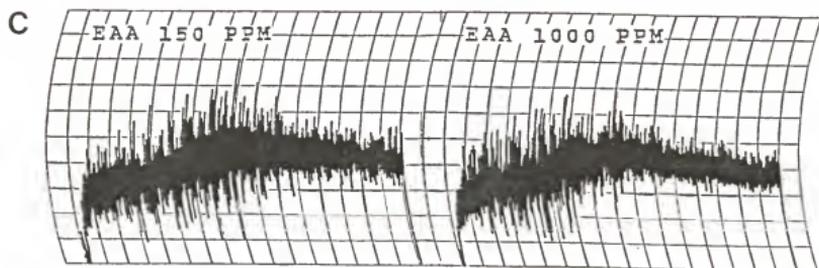
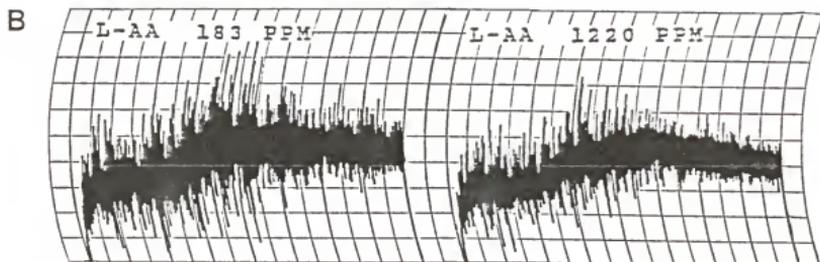
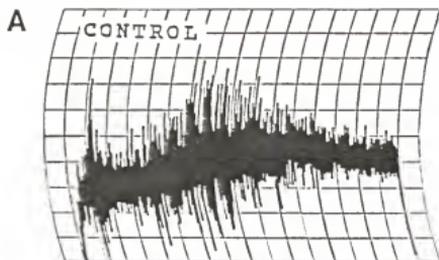


Fig. 11 Effects of chemicals on the spread of flour-water doughs. 100 ppm of L-AA, 82 ppm of D-EAA and 100 ppm of L-cysteine hydrochloride were used in equivalent amounts on a molar basis.

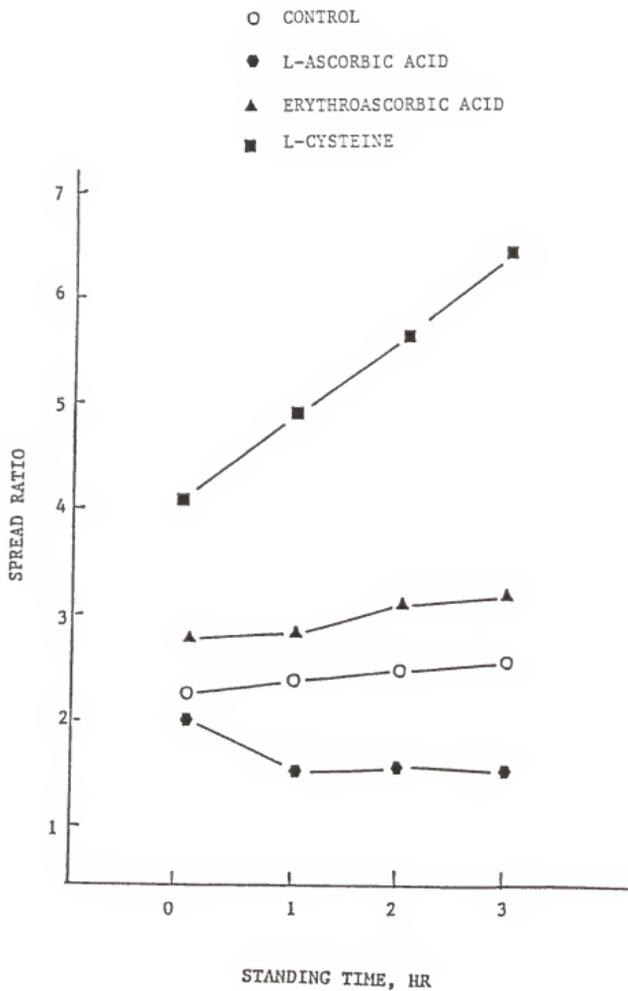
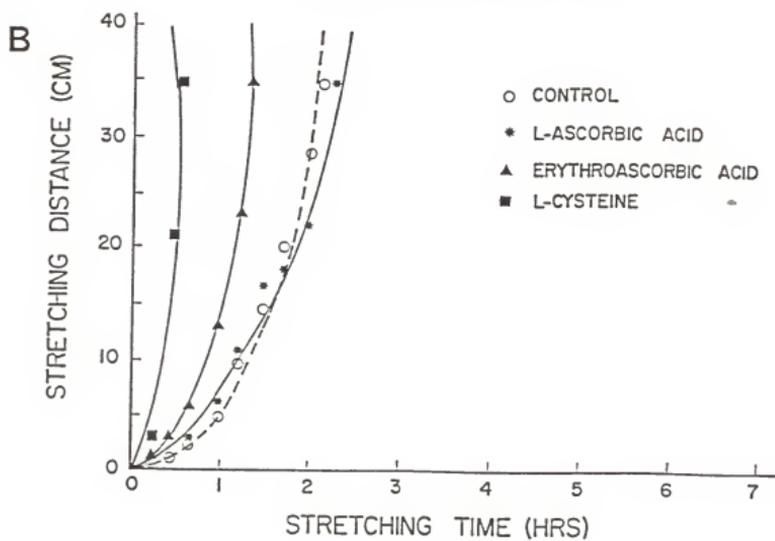
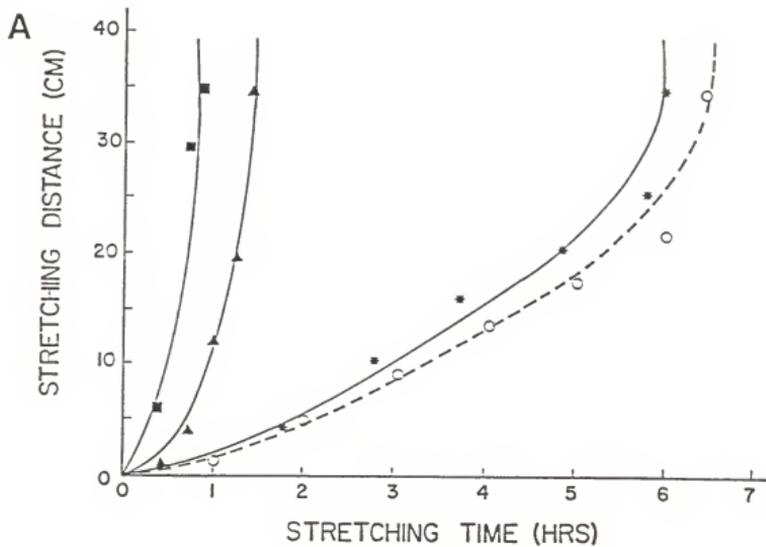


Fig. 12. Stretching time (min) vs distance (cm) of  
(A) Malted flour, and (B) Unmalted flour.

Glutens were obtained from optimally mixed doughs at optimum water absorption. The doughs contained none (control), 82 ppm D-EAA, 100 ppm L-AA and L-cysteine hydrochloride.

Data were the average of two replicates (each replicate was duplicated).



DETERMINATION OF ERYTHROASCORBIC ACID IN BAKER'S YEAST  
AND THE EFFECT OF D-ERYTHROASCORBIC ACID  
ON FLOW-PROPERTIES OF WHEAT FLOUR DOUGH

by

HEE SUN KIM

B.S., Seoul National University, Korea, 1985

AN ABSTRACT OF MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Food Science

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1988

## ABSTRACT

Erythroascorbic acid (EAA), a 5-carbon analog of L-ascorbic acid (AA), has been reported in Saccharomyces cerevisiae and other fungi. In this investigation the level of EAA in baker's instant and compressed yeast was determined using high-performance liquid chromatography with electrochemical detection (HPLC-EC). Baker's yeast was found to contain approximately 50 µg of EAA and 5 µg of AA per gram of dry yeast. Wheat-flour doughs were fermented and assayed to determine whether EAA was released in dough. Portions of dough were fermented 0 - 3 hr and extracted using 3% metaphosphoric acid. The doughs contained insignificant quantities of EAA (less than 1 ppm based on flour). The spread-ratio test was used to examine the effects of adding D-erythroascorbic acid (D-EAA) on the flow properties of dough. D-EAA (82 ppm based on flour) increased flow immediately out of the mixer, but with increased lay time, dough flow was not affected further. An equivalent amount (100 ppm) of cysteine caused about 40% more flow than EAA, immediately out of the mixer, and flow increased with lay time. D-EAA at 82 - 1000 ppm had little effect on mixing time as determined by the mixograph. Gluten washed out from a flour-water dough containing 82 ppm D-EAA or 100 ppm L-cysteine were tested for stretchability: gluten containing cysteine or D-EAA stretched at faster rates than did the control gluten.