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Abstract

Various amounts of sodium D-erythrobate (D-ErA-Na, sodium D-*erythro*-ascorbic acid) were added to a basic baking formula, and effects of the additive on the bread quality of the wheat flour were investigated by measuring the loaf volume of the bread, the gelatinization temperature of starch in the dough, change of SH content in gluten, viscoelastic properties of the dough, and by scanning electron microscopic observations. The addition of L-ascorbic acid (L-AsA) (8ppm) or D-ErA-Na (20ppm) increased the loaf volume of the bread baked. With the addition of 50 ppm of the additives, the gelatinization temperature of starch was decreased, to 80°C by the addition of L-AsA from the 82°C of the control, and to 81°C by D-ErA-Na. By addition of 50 ppm of D-ErA-Na, the mechanical stability of the dough was distinctly improved, compared with the case where D-ErA was added. The computerized image analysis of the distribution of the gas cells showed some good relationships between the size of gas cells and the loaf volume of the bread baked. The improvement in the physical properties of the dough and the resulting bread would be caused by the formation of SS cross-linkages as a result of mild oxidation of SH groups by addition of D-ErA-Na. The relation between structural changes in the gluten and starch, and the improvement in the quality of the breads are discussed.

Introduction

L-Ascorbic acid (L-AsA), which is known as vitamin C, has anti-scorbutic effect and many other prominent properties, and is also widely used as an additive for foods and beverages in the food industry.[1] D-Erythrobic acid (D-*erythro*-ascorbic acid, D-ErA), an isomer of the hydroxyl group at the carbon 5 of L-AsA, also has a strong reducing activity like that of L-AsA[2]. D-ErA in an aqueous solution is known to be easily oxidized to the dehydro form by atmospheric oxygen, though D-ErA seems to be stable as a powder[3]. Also, D-ErA was tested as a dough improver for breadmaking in the 1960's, but it was considered ineffective[2]. In these circumstances, some more works are expected[4].

In this paper, we tested the effects of L-AsA, D-ErA, and sodium D-ErA (D-ErA-Na) in a home baker for domestic use. Addition of D-ErA-Na was found to improve the loaf volume of the bread baked, and also the behavior of the gelatinization of starch, and the physical properties of dough were tested with it and related additives.

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Materials and methods

Materials

Wheat flour "Hermes", a strong type of flour that contains 11.8% protein and 0.38% ash with 11.8% moisture, was donated by the Okumoto Flour Milling Co., Ltd. (Osaka). Sugar was obtained from the Mitsui Sugar Producing Co., Ltd. (Tokyo). Dry yeast "Fermi", made in England, and D-ErA-Na were donated by Asahi Kasei Co., Ltd. (Shizuoka) and Fujisawa Pharmaceutical Co., Ltd. (Tokyo), respectively. Dotaito, a kind of conductive silver paste, was obtained from Fujikura Kasei Co., Ltd. (Sano, Tochigi). L-AsA and D-ErA were purchased from Wako Pure Chemical Industries, Ltd. (Osaka). Other reagents used were of analytical grade.

Methods for breadmaking

The breadmaking formula was 280g of flour, 5g of sodium chloride, 17g of sucrose, 3g of dry baker's yeast, and 210g of water containing 14mg (50ppm) of AsA or D-ErA-Na, unless otherwise stated. Bread was made in an automatic breadmaker for domestic use (SD-BT-3, Matsushita Electric Co., Ltd., Osaka)[5,6]. The breadmaking process took 4 hours including mixing, proofing, and baking. After the baking ended, the bread was taken out and weighed immediately, and the loaf volume was measured by the method of rapeseed displacement. The specific volume of bread baked was obtained from the volume (ml) divided by the weight of the bread (g).

Assay of amount of D-ErA or L-AsA consumed

The solution of L-AsA or D-ErA (100μM) in 0.1M phosphate buffer (pH 6.8) was incubated at 25°C in the presence of cucumber AsA oxidase (3.3mU/ml, from Amano Pharmaceutical Co., Ltd), and the amount of AsA that remained was estimated by measuring the absorbance at 265 nm. The remaining amount of L-AsA and D-ErA (100ppm) in the dough during mixing or after baking was measured as reported[7].

Analytical methods

Differential scanning calorimetry (DSC) was done with a Shimadzu DSC apparatus model 50 using an aluminum pan with a capacity of 40μl. About 20mg of a dough sample mixed for 25 min in the home baker was used, as reported previously[8]. DSC measurements were started as soon as the instrument preparation and the sample weighing were completed. For the reference pan of DSC measurement, about 15mg of liquid paraffin was used.

Scanning electron microscopy (SEM) was done with a small portion of dough after mixing for 25 min[9,10]. After the dough sample was frozen with liquid nitrogen, the frozen sample was fractured into a size of about 1cm×1cm×0.5cm. The sample was lyophilized and defatted with *n*-hexane. After removal of the solvent, the sample was fixed with aqueous solution of OsO_4 (2%). The sample thus fixed was washed thoroughly with deionized water, and then lyophilized after the freezing of the sample. The lyophilized sample thus prepared was put on the surface of the silver paste on SEM metal stubs. The samples were coated with a thin layer (about 150μm) of palladium/platinum, and they were viewed at 6 kV and photographed at a speed of 100 sec/picture at a 17-mm working distance in a Hitachi scanning electron microscopic apparatus model S-800.

For farinograph operation with a Brabender farinograph apparatus, each additive, dissolved in deionized water, was added to the wheat flour (300g) and mixed at 30°C.

The viscoelastic properties of bread were tested with Fudoh rheometer equipped with

a recorder from Rikadenki. The plunger used was the type for viscoelastic measurement, having a 1-cm diameter. The dough sample mixed for 25 min in the home baker was used to fill a plastic vessel (2.5cm i. d. x 5cm). The speed of the plunger penetration into the sample was 30cm/min and the penetration depth was controlled at 2cm. The measurement was made at 30°C.

The apparatus for image analysis of cross-sectional views of bread was a Pias Computer Image Analyzer PIAS-III NS, equipped with a CCD camera PX390A, an RGB color monitor PVM-1440CS, and an image input stage IS-500[11,12]. The Zerox-copied cross-sectional views of bread crumb were placed under a non-reflective handling mask VS-S31, and the views were incorporated into the computer through the CCD camera. Pixels more than 0.24mm×0.24mm were counted as one gas cell and the data were processed as the Heywood diameter.

Results and Discussion

Degradation of additives in the solution or dough samples

The amount of L-AsA and D-ErA (100μM) remaining in the phosphate buffer (pH 6.8) after incubation of the reaction mixture containing AsA oxidase was tested photometrically at 265 nm, due to the endiol group of the materials. Ten percent of L-AsA and 47% of D-ErA remained after 30 min of incubation (Table 1).

The remaining amounts of L-AsA and D-ErA in the dough during breadmaking process were estimated by stopping the process several times after the additive (100 ppm) was mixed with the dough. After mixing for 10 min, 17% of the D-ErA remained; 10% after 30 min; and 8% after 90 min, as shown in Table 2. On the other hand, for L-AsA, the amount remaining was 10% after mixing for 10 min; 4% after 30 min; and less than 2% after 90 min. Johannson and Cooke reported that D-ErA was not oxidized to the dehydro-form during mixing, therefore D-ErA was not effective as an improver for breadmaking[13]. However, from this experiment, D-ErA can be said to be effective as an

Table 1 Oxidation of L-AsA and D-ErA by ascorbate oxidase in 0.1 M phosphate buffer, pH 6.8 at 25°C

	Time (min)								
	0	1	3	5	10	15	20	25	30
L-AsA remaining (%)	100	93.9	75.8	65.3	45.0	31.7	21.6	15.0	10.1
D-ErA remaining (%)	100	97.3	92.1	87.6	78.8	69.0	60.5	53.4	47.0

Each value is the average of duplicate measurements.

Each value shows the remaining amount (%) of L-AsA or D-ErA.

Table 2 Amount of D-ErA or L-AsA remaining in the dough during breadmaking

	Time (min)			
	10	20	30	90
D-ErA remaining (%)	17	10.7	9.6	7.8
L-AsA remaining (%)	9	7.5	4.2	1.7

Each value is the average of duplicate measurements.

improver for breadmaking.

The remaining amounts of D-ErA-Na (addition of additive: 30 or 120 ppm) were studied by using bread crumb after baking. No trace amount or 37% of D-ErA-Na remained in the crumbs after addition of 30 or 120 ppm of the additive, respectively.

Effects of additives on the improvement of bread baked

The effects of D-ErA and L-AsA on the specific volume of bread were tested (Table 3). Addition of 8 ppm L-AsA to the ingredients increased the loaf volume to 4.54cm³/g from the control value of 4.49; at higher concentrations of L-AsA, the loaf volume decreased distinctly. The decrease in the loaf volume at higher concentrations of L-AsA may be caused by the overoxidation of SH groups in the gluten molecules. Addition of 20 ppm D-ErA-Na increased the loaf volume of bread significantly ($p<0.05$) compared with the control, then decreased it at higher concentrations.

Figure 1 shows a typical cross-sectional view of bread baked. The decrease in the loaf volume at the higher concentration of AsA derivatives may be caused by the formation of SS-cross linkages, as reported[2,14]. That is, the aeration or the presence of metal ions in the dough, or both factors, might oxidize the SH groups in the gluten molecules.

Table 3 Effects of L-AsA, D-ErA, and D-ErA-Na on specific volume of bread baked with wheat flour

	Amount of additive					
	0 ppm	10 ppm	20 ppm	30 ppm	40 ppm	50 ppm
L-AsA	4.49±0.07	4.54±0.03 [a]	4.27±0.04	4.21±0.03	4.08±0.04	4.02±0.03
D-ErA	4.41±0.07	4.31±0.13	4.47±0.07	4.29±0.03	4.25±0.05	4.05±0.07
D-ErA-Na	4.41±0.07	4.19±0.13	4.59±0.07 [b]	4.49±0.08	4.47±0.07	4.27±0.08

Each value shows the specific volume (cm³/g) of bread baked.

[a] Amount of additive: 8 ppm.

[b] Significantly different from the control value ($p<0.05$).

Number of experiments: 5.

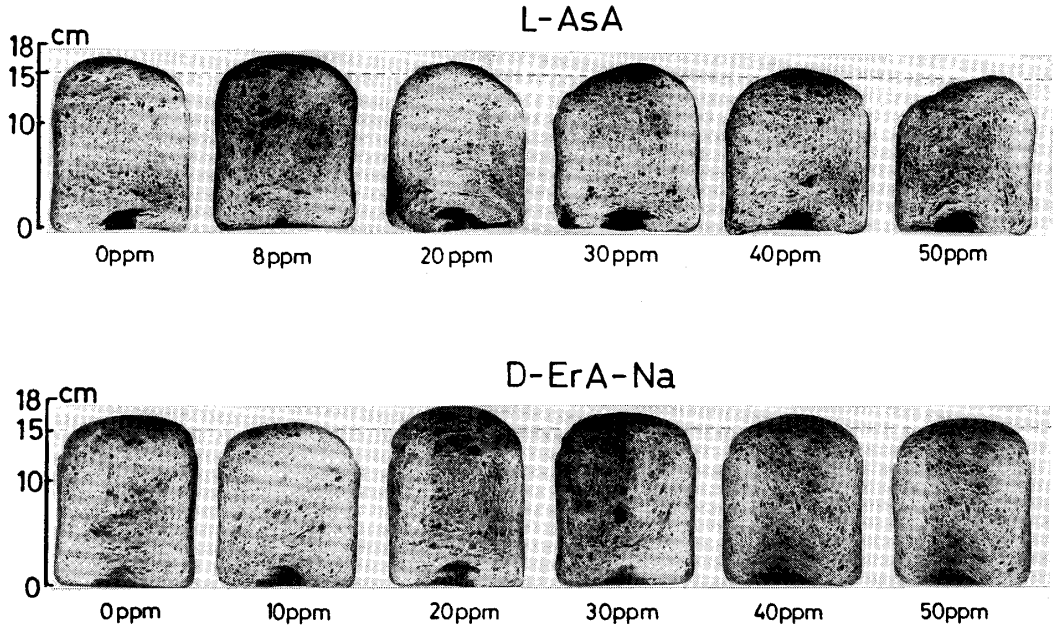

Fig. 1 Cross-sectional views of bread baked with D-ErA-Na and L-AsA.

Changes in the farinograms of dough containing additives

Effects of additives on the changes of farinograms after addition of D-ErA and L-AsA (50 ppm) to wheat flour were studied. The arrival times for all tested samples that reached the standard consistency (500 B. U.), were about 2.5 min from the start of mixing

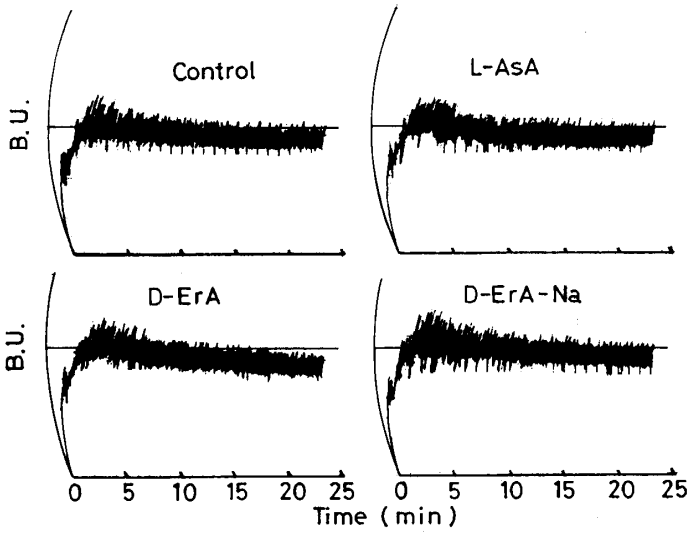

Fig. 2 Farinograms of wheat flour dough with L-AsA, D-ErA, or D-ErA-Na. Bars in the figure show 500 B. U.

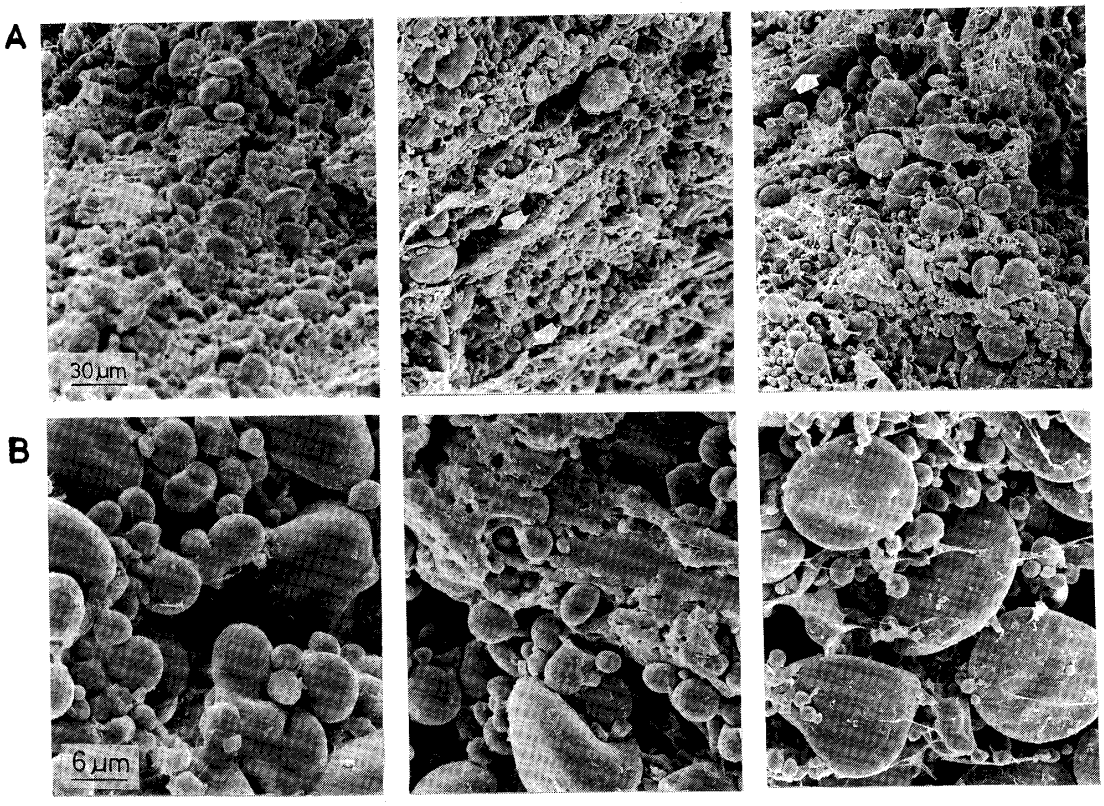

Fig. 3 Scanning electron micrographs of dough containing 50 ppm L-AsA or D-ErA-Na in breadmaking for 25 min. The arrows in the photos show a portion of the rod-like state.

(Fig. 2). The development time with D-ErA was about 5 min and was the longest, and those with the other additives used were about 4 min after mixing. Therefore, no distinct difference was obtained in the farinograms. For D-ErA,the stability of the consistency of the dough during mixing also had a narrow farinogram, and somewhat weak consistency, followed by a gradual decrease in the consistency. This suggests that D-ErA has an effect of lowering the pH of the dough, since D-ErAwas not easily oxidized in the presence of AsA oxidase. On the other hand, D-ErA-Na was stable for 20 min during mixing and had a relatively high consistency like that of L-AsA.

SEM changes in the dough containing additives

SEM observation of dough containing L-AsA or D-ErA-Na (50ppm) was done with dough after mixing for 25 min (Fig. 3). The control dough without additives seemed to have gluten that covered the surface of primary or secondary starch granules almost uniformly at all magnifications of 500, 1500, and 2500 tested, though the figure shows those of 500 and 2500. Therefore, the boundaries of starch granules could not be seen. This may be caused by the uniform wrapping of gluten mixed with glutenin and gliadin during mixing for 25 min in the home baker[13]. With L-AsA, the dough seemed to cover the secondary starch granules uniformly, but the large primary granules were not covered similarly. Most of the gluten parts became filamentous or rod-like, so the starch granules could not be covered uniformly with gluten[14]. Hence, we could easily identify the boundaries of the starch granules.

These observations suggest that the SH groups in the gluten were oxidized to SS-cross linkages, followed by the formation of rigid structures[8]. With the addition of D-ErA-Na a somewhat filamentous part of the dough can be seen, but as a whole, primary starch granules were covered with gluten. This suggests that the addition of D-ErA-Na to the dough causes an intermediary change of dough properties between those of L-AsA and the control, and also that a part of gluten was oxidized to form a three-dimensional network of gluten.

Gelatinization temperature of wheat starch by DSC measurement

The gelatinization temperature of starch granules in the dough containing 50 ppm D-ErA-Na, and the changes in enthalpy were tested by DSC (Table 4). The onset temperatures of gelatinization of starch granules in the dough with L-AsA or the control were both almost 59.0°C, but the peak temperature differed clearly: 79.8°C for L-AsA and 82.0°C for the control. But, for the case of D-ErA-Na, the peak temperature of gelatinization of starch was 80.7°C, and this temperature was almost halfway between that of L-AsA and the control. The final temperature of gelatinization upon the addition of L-AsA and in the control were 103°C and 106°C, respectively. The enthalpy for the

Table 4 Summary of gelatinization temperatures of starch in wheat flour dough with D-ErA-Na, D-ErA, or L-AsA (50 ppm) as an additive in breadmaking for 25 min

	Onset temp.	Peak temp.	Final temp.	Δh
	°C	°C	°C	(J/g)
Control	59.0	82.0	106.0	3.00±0.11 [a]
L-AsA	59.0	79.8	103.0	3.34±0.24
D-ErA-Na	60.0	80.7	104.9	3.18±0.30
D-ErA	58.9	82.2	106.1	3.17±0.19

[a] Mean±S. D. (three samples)

gelatinization of starch showed the highest value of 3.3J/g for the addition of L-AsA, decreasing in the order of D-ErA-Na (3.2 J/g), D-ErA (3.2 J/g), and the control (3.0 J/g). The value of D-ErA-Na was somewhat closer to that of L-AsA than that of control, but these differences may not be significant.

Viscoelastic properties of dough

Viscoelastic properties of dough containing D-ErA-Na or D-ErA were tested (Table 5). For all additives tested, addition of L-AsA gave the largest value for the modulus of elasticity and viscosity coefficient, then the values decreased in the order of D-ErA-Na, control, and D-ErA. This agreed with the results obtained from the farinograph data,

Table 5 Effects of additives on the viscoelastic properties of dough, as measured by a rheometer

	Modulus of elasticity	Viscosity coefficient	Relaxation time
	(dyn/cm²) x10⁻⁵	(poise) x10⁻⁶	sec
Control	4.09±0.21	9.9±1.3	24.0±2.0
L-AsA	4.99±0.82	19.5±4.8	38.6±6.0
D-ErA-Na	4.49±0.17	10.2±0.1	22.7±0.8
D-ErA	3.59±0.35	5.9±1.1	16.6±2.2

Data are means of three trials.

Table 6 Effects of additives on the size of gas cells of bread, counted using an Image Analyzer.

Sample	Amount of additive (ppm)				
	Control	10	20	30	50
D-ErA-Na	1.53±1.49	1.24±0.95	1.59±1.34	1.46±1.19	1.41±1.20
	(262)	(404)	(204)	(243)	(398)
D-ErA	1.31±0.87	1.35±0.89	1.36±1.12	1.21±0.92	1.32±0.98
	(268)	(256)	(234)	(301)	(340)
L-AsA	1.44±1.08	1.41±0.99	1.38±1.02	1.38±1.08	1.42±1.13
	(336)	(279)	(315)	(329)	(294)

Each value shows the Heywood diameter±S.D. (mm) of the gas cells of bread. The value in parenthesis shows the number of gas cells counted in the tested area (65mm×57.3mm) which is in the middle part of a bread crumb. One pixel for the input to Image Analyzer is 0.0575mm².

Table 7 Effects of various additives on the distribution of gas cells of bread crumb, counted with an Image Analyzer.

Sample	Amount of additive added (ppm)				
	0	10	20	30	50
D-ErA-Na	48	79	44	57	65
D-ErA	53	50	63	51	62
L-AsA	73	43	65	85	65

The values show the number of gas cells counted at a distance of 6.5cm in a middle part of a bread crumb. One pixel for the input to Image Analyzer is 0.0575mm².

and also roughly coincided with the loaf volume of the bread baked.

Distribution of gas cell of bread crumb

Table 6 shows the preliminary data on relationships between the number of gas cells and amount of additives added to the bread ingredients. The more gas cells in the crumb, the bigger the specific volume of the bread became. The result coincided with the relationships between the specific volume of bread and amount of additives as shown in Table 3.

For the evaluation of the fine distribution of gas cells of bread crumb, Kitamura *et al*[11]. reported that the number of gas cells counted at a distance in the middle of the bread crumb can be substituted for the index of homegeneity of the distribution of gas cells. Therefore, we applied this method to the Zerox-copied cross-sectional views of bread.

Table 7 shows the effects of additives on the number of gas cells. After the addition of 20 ppm D-ErA-Na, the number of gas cells showed the smallest value of 44, suggesting the presence of the biggest gas cells in the bread crumb tested, considered with the results in Fig. 1. Likewise, addition of 8 ppm L-AsA showed the smallest value of 43, among the values for the L-AsA tested. These results suggest that the data processed by Image analysis of the gas cells by use of Zerox-copied bread crumb, is sufficient for the evaluation of the quality or fine cell distribution of bread crumb, without the use of photographs or printing methods with India ink as reported[11,12], because the latter methods are tedious and not convenient for the evaluation of the gas cell distribution. From these results, for the evaluation of fine distribution of gas cells of bread crumb, computerized image analysis is very convenient for the practical application and quality control of bread by use of Zerox-copied cross-sectional views of bread.

Improving effects on loaf volume were observed with around 20 ppm D-ErA-Na, but the amount of D-ErA-Na necessary to increase the loaf volume was large compared with that of L-AsA. This suggests that the oxidation rate of D-ErA is the rate determining-factor for the improvement of loaf volume. Therefore, the dehydro form of D-ErA-Na thus formed may oxidize SH groups and form a network of gluten molecules. As a matter of fact, the mode of wrapping of gluten molecules over the surface of starch granules can be changed by the addition of D-ErA-Na. Hence, it may be considered that the gelatinization temperature of starch upon the addition of D-ErA-Na is intermediate between those of L-AsA and the control. Thus, the addition of D-ErA-Na can make a good quality of bread both from internal and external points of view. Therefore, D-ErA-Na is expected to be used as a practical improver for breadmaking.

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