Note on the Antioxidant Effect of Ascorbic Acid on Flour Free Fatty Acids

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The work needed to develop a dough mechanically can be greatly reduced by incorporating small amounts of ascorbic acid and cysteine (Johannson and Cooke 1971). Dahle and Murthy (1970) observed that a number of lipid antioxidants affected dough properties in a way similar to that of ascorbic acid. They suggested that certain flour lipids play a vital role in the development of dough and that ascorbic acid protects these lipids from oxidation. Other reports relating flour lipid oxidation to dough properties were cited in support of this hypothesis (Smith and Andrews 1957, Smith et al 1957, Sullivan et al 1936). The effects of flour lipids on bread-baking properties have been reviewed recently by Chung et al (1978) and by Pomeranz (1978).

Mann and Morrison (1974) demonstrated that unsaturated fatty acids in the free form or as monoglycerides are substantially oxidized during dough mixing, with negligible oxidation of the other lipid components in flour. These authors subsequently reported that the addition of ascorbic acid had an insignificant effect on the oxidation of free linoleic acid in doughs (Mann and Morrison 1975). We have further examined the effect of ascorbic acid on the oxidation of unsaturated free fatty acids in a flour/water system and have found that the additive does provide a short term protective effect.

MATERIALS AND METHODS

A commercial first clear flour, milled from Canadian HRS wheat and containing no additives (moisture 13.6%, ash 0.87%, nitrogen 2.72%), was used throughout. All chemicals were reagent grade and solvents were freshly distilled. 1-Butanol and chloroform were purified as described previously (Grant 1974). Sixty-five ppm of butylated hydroxytoluene were dissolved in 1-butanol whenever that solvent was used in a lipid extraction procedure.

Ascorbic acid oxidase activity was determined as described previously (Grant 1974).

Flour/water slurries (1:3, w/v) were stirred magnetically at room temperature for various periods of time. Added ascorbic acid was included in some of the slurries in amounts of either 120 or 240 γ/g of flour. At the end of the mixing period, the lipid components were extracted overnight with 15 volumes of 1-butanol per gram of flour. These quantities resulted in a water-saturated system. The insoluble residue was recovered by centrifugation and extracted a second time with an additional 15 volumes of water-saturated 1-butanol per gram of flour. As a control, the lipids in the flour were extracted directly, using 15 volumes of water-saturated 1-butanol per gram of flour, in each of two successive extractions without any preliminary mixing with water. Extracts were evaporated and partitioned in a biphasic chloroform-methanol system as described by Clayton et al (1970).

The free fatty acids were separated from other lipid components by preparative thin-layer chromatography (TLC) on 250-μ layers of activated silica gel G using solvent system B (Clayton et al 1970). They were recovered from the TLC plates by extraction with chloroform/methanol (Clayton and Morrison 1972).

Methyl esters were prepared (Castell and Mallard 1974) and separated on an Aerograph series 1200 gas chromatograph at 195°C using a 0.32 × 244-cm stainless steel column packed with 15% ethylene glycolsuccinate on 80–100 mesh Chromosorb W, with nitrogen as the carrier gas and a flame ionization detector. Peak areas and percentage composition for the methyl esters of palmitic, stearic, oleic, linoleic, and linolenic acids were recorded automatically by a Hewlett Packard model 3380A integrator.

RESULTS AND DISCUSSION

The extractable lipids made up 2.8 ± 0.2% of the weight of the flour. This is within the range reported by Mecham (1978). The free fatty acids recovered from the TLC plates accounted for 1.0 ± 0.5% of the extracted lipids. This is a substantially smaller proportion than the 5.8% reported by Mann and Morrison (1974), even after making allowance for the fact that we did not attempt a completely quantitative recovery of this lipid fraction.

The effects of added ascorbic acid on the free fatty acid composition of flour/water slurries are presented in Table I. Assuming that the content of free palmitic acid remains constant (Mann and Morrison 1974), the decline in the ratios of the unsaturated free fatty acids to palmitic acid with longer mixing time is an indication of the extent to which oxidation has occurred. From the data in Table I, we have calculated that in the absence of added ascorbic acid, approximately one half of the polyunsaturated acids were oxidized in 15 min. Insignificant further oxidation occurred during the next 15 min. At 120 ppm of added ascorbic acid, some measure of protection was provided for linoleic acid during the first 15 min, but none thereafter. At 240 ppm, significant protection for both linoleic and linolenic acids persisted for 30 min, but was not significant at 60 min.

The ascorbic acid oxidase activity of the flour was 8.4 ± 0.5 γ/min/g of flour at 22°C. At 120 ppm of added ascorbic acid, virtually all of the additive would be oxidized after 15 min of mixing. Therefore, we were not surprised that the protective effect did not persist beyond 15 min. When the amount of additive was doubled, the time required for enzymatic oxidation would also be doubled (Grant 1974), thus extending the duration of the antioxidant effect. Mann and Morrison (1975) did not detect any antioxidant effects with 50 ppm of added ascorbic acid and a 10-min dough mixing time. In dough, where enzymatic oxidation of ascorbic acid is probably faster than it is in flour/water slurries, that amount of additive may not be sufficient to significantly protect the polyunsaturated free fatty acids over that period of time.

Our results do not conflict with the antioxidant hypothesis of Dahle and Murthy (1970). In that hypothesis, a negative role for ascorbic acid oxidase was implied, in that high levels of the enzyme reduced the effectiveness of the antioxidant.

The proportions of free fatty acids that we observed in flour differed somewhat from those reported by Mann and Morrison (1974). The relative amount of oleic acid was found to be higher whereas that of linoleic acid was lower. The gas chromatograph data indicated that the five fatty acids listed in Table I made up at least 90% of the free fatty acid fraction.

A part of the polyunsaturated free fatty acids in a flour/water system appear to be protected from rapid oxidation in some way. In our studies this part was approximately one half of the total. Mann and Morrison (1974, 1975) observed in their investigation with doughs that 85% of the polyunsaturated acids were oxidized within 10 min, but the remaining 15% were not further oxidized after 60 min. The difference between their results and ours may be

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TABLE I
Effect of Ascorbic Acid on the Free Fatty Acid Composition of Flour/Water Slurries

Mixing Time (min)	Ascorbic Acid Added (γ/g of flour)	Fatty Acid Composition[a]				
		Palmitic	Stearic	Oleic	Linoleic	Linolenic
0[b]	0	20.6 + 0.7[c]	1.4 + 0.2	17.8 + 0.1	56.0 + 0.3	3.6 + 0.2
15	0	30.3	1.6	19.8	44.9	2.3
15	120	24.9	0.8	21.2	50.2	2.7
30	120	30.1	1.8	20.9	43.6	2.8
30	0	30.0	1.2	24.2	41.7	2.3
30	240	16.7	1.9	21.4	54.7	3.3
60	240	27.9	3.5	20.2	42.4	2.6

[a] As a percentage of the sum of the gas chromatographic peak areas for the five fatty acids listed.
[b] Lipids were extracted directly from the flour without mixing.
[c] Probable error of the mean for three replicate analyses.

related to the higher levels of free fatty acids in the flour they were using. Further study is needed to determine why this oxidation reaction stops short of completion.

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