# Reaction of $^{14}$C-Cysteine with Wheat Flour Water Solubles Under Ultraviolet Light[1]

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ABSTRACT

Cysteine and glutathione reacted with $^{14}$C-fumaric acid when exposed to ultraviolet (UV) irradiation. The UV irradiation presumably initiated a free radical on the sulfhydryl group, which then added to the activated double bond of fumaric acid. Ferulic acid and *trans*-cinnamic acid, both of which contain activated double bonds, affected dough mixing tolerance in a manner similar to that of fumaric acid. UV irradiation of wheat flour water solubles, in the presence of $^{14}$C-cysteine, followed by fractionation of the water-soluble fraction on Sepharose 4B showed that most of the bound radioactivity was eluted at the void volume of the column. This result was expected for cysteine reacting with ferulic acid esterified to the water-soluble arabinoxylan. This finding suggests that ferulic acid is an indigenous activated double bond compound that affects mixing tolerance and also provides a mechanism by which pentosans are covalently bound to proteins during dough mixing.

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Activated double bond compounds decrease the mixing time and, particularly, the mixing tolerance of wheat flour dough (Schroeder and Hoseney 1978). Recent work (Sidhu et al 1980) has shown that cysteine residues (apparently formed by disulfide bonds breaking during dough mixing) add to double bonds of fumaric acid, presumably by a free radical type of reaction. Irradiation of cysteine and glutathione with ultraviolet (UV) radiation initiates a free radical type of addition of these mercaptans to compounds that contain activated double bonds (Jocelyn 1972). We report here similar types of free radical reactions with activated double bond compounds in flour water solubles.

## MATERIALS AND METHODS

A composite hard winter wheat flour with a protein content of 12.2% and an ash content of 0.39% was used. {1-$^{14}$C}-Fumaric acid, 3.03 mCi/mM, was obtained from ICN Pharmaceuticals, Inc., and DL-{3-$^{14}$C}-cysteine hydrochloride (15 mCi/mM) from Research Products International Corp. All other chemicals used were reagent grade.

### Irradiation of Amino Acids and $^{14}$C-Fumaric Acid with UV Light

$^{14}$C-Fumaric acid (0.025 $\mu$Ci) was added to 50 mg of cysteine, lysine, tryptophan, histidine, and the peptide glutathione, separately, each in 5 ml of water. The solutions were then irradiated with short wave length UV light (Model C-3, Chromato-Vu, Ultra-Violet Products, Inc.) for 24 hr. A 2-ml aliquot of each solution was placed on an IR-120 (H+) cation exchange column (1.5 × 10 cm) and eluted first with distilled water to remove unreacted fumaric acid, then with 2*N* ammonium hydroxide to elute the addition product of fumaric acid and amino acid. (Free $^{14}$C-fumaric acid is not retained and eluted with distilled water, but the addition product of fumaric acid and an amino acid requires the base for elution because it then carries a positive charge.)

### Mixograph

A 10-g mixograph was used according to the procedure of Finney and Shogren (1972). Additives were dispersed in water and neutralized to pH 7.0 with dilute sodium hydroxide.

### Reaction of $^{14}$-Cysteine with Flour Solubles Under UV Irradiation

Flour (10 g) was slurried for 1 hr with 100 ml of water and the water-soluble fraction separated by centrifugation (650 × *g*). $^{14}$C-Cysteine (2.5 $\mu$Ci) was added to the supernatant and the solution irradiated with UV light at room temperature for 24 hr. Control samples were prepared in the same manner but without the added cysteine or without irradiation.

After being irradiated, the samples were dialyzed against water until no further radioactivity was lost from the dialysis bag. The dialyzed sample (5 ml) was loaded on a Sepharose 4B column (2.9 × 62 cm) and eluted (in 50-ml fractions) with 0.3% NaCl containing 0.05% sodium azide. The fractions were analyzed for total carbohydrate (as xylose) by the phenol-sulfuric acid procedure (Dubois et al 1956), for protein by the Lowry procedure (Lowry et al 1951), for ferulic acid by absorption at 320 nm, and for radioactivity by the procedure of Turner (1968).

## RESULTS AND DISCUSSION

With histidine, tryptophan, and lysine, only small amounts (0.37–2.0%) of the $^{14}$C-fumaric acid were retained on the column (Table I). However, with cysteine and glutathione, 28.45 and 16.25%, respectively, of the $^{14}$C-fumaric acid eluted with 2*N* ammonium hydroxide. The amino acids, which contain a sulfhydryl group, apparently had formed free radicals and reacted with $^{14}$C-fumaric acid. On a sulfhydryl-equivalent basis, the two compounds reacted essentially equally.

### Reaction of Ferulic Acid and Related Compounds with Flour

Although fumaric acid decreases the mixing tolerance of dough (Schroeder and Hoseney 1978), significant amounts of fumaric acid are not indigenous to wheat flour. Schroeder and Hoseney (1978), however, reported that ferulic acid, which contains an activated double bond system like fumaric acid's, was active in decreasing mixing tolerance. They also showed that a water-soluble fraction of flour, presumed to contain ferulic acid (Fausch et al 1963, Fulcher et al 1972, Geissmann and Neukom 1973) was active in decreasing mixing tolerance.

Mixograms of flour containing added ferulic acid, *trans*-cinnamic acid, 4-hydroxy-3-methoxybenzoic acid, and 4-

TABLE I
Reaction of Certain Amino Acids with $^{14}$C-Fumaric Acid During Irradiation with UV Light (254 nm)

| Amino Acid and Peptide | Radioactivity (%) Eluted from IR-120 ($H^+$) with | |
|---|---|---|
| | Water | 2*N* $NH_4OH$ |
| Histidine | 99.63 | 0.37 |
| Tryptophan | 98.06 | 1.94 |
| Lysine | 99.34 | 0.66 |
| Cysteine | 71.55 | 28.45 |
| Glutathione | 83.75 | 16.25 |

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hydroxyphenyl-3-propanic acid are shown in Fig. 1. Ferulic acid is clearly effective in reducing mixing tolerance. Much less ferulic acid (250 ppm) than fumaric acid (2,000 ppm) was required to produce a similar decrease in mixing tolerance. *trans*-Cinnamic acid, which also contains the activated double bond, had an effect similar to that of ferulic acid. 4-Hydroxy-3-methoxybenzoic acid and 4-hydroxyphenyl-3-propanic acid had no effect on mixing tolerance, and neither contained an activated double bond. The activated double bond appears to be necessary for the effect on mixing tolerance. Why ferulic and *t*-cinnamic acids are more active than fumaric acid on a weight basis is not clear.

**Irradiation of Flour Water Solubles with UV Irradiation**

To study the possible reaction of cysteine with the ferulic acid present in the water-soluble pentosans, we irradiated all flour water solubles with UV irradiation in the presence of $^{14}$C-cysteine. After 24 hr of irradiation, the water solubles were dialyzed and fractionated on a Sepharose 4B column. Untreated samples of the total water solubles and an irradiated sample without added cysteine were also fractionated on the Sepharose 4B column.

The untreated water solubles (Fig. 2) gave two well-resolved carbohydrate peaks and a broad protein peak at about 270 ml. The only significant absorption at 320 nm (ferulic acid) was at the void volume (Vo) of the column. Yeh et al (1980) also reported that the ferulic acid is eluted at the Vo of Sepharose 4B fractionations of purified water-soluble pentosans. The carbohydrate profiles are similar to those reported by Fincher and Stone (1974) and by Yeh et al (1980) for purified pentosans.

Irradiation of the water solubles with UV light in the presence of $^{14}$C-cysteine materially changed the elution profiles from Sepharose 4B (Fig. 3). The most significant observation is that 67% of the radioactivity eluted at Vo, which coincides with the absorption of ferulic acid at 320 nm and suggests that cysteine adds to the ferulic acid, presumably the same way cysteine adds to fumaric acid under similar conditions. Other changes in the elution profile resulted from irradiation. Although they were significant (eg, the introduction of an intermediate molecular weight carbohydrate material), they did not alter the basic conclusion that cysteine adds to indigenous high molecular weight compounds. The second carbohydrate peak (arabinogalactan) appeared to be unaffected. A major increase in protein at the Vo of the column and an accompanying decrease in protein eluted later from the column were also found. Irradiation of the water solubles without adding $^{14}$C-cysteine gave materially the same elution profile as did irradiated water solubles with cysteine.

The finding that cysteine when irradiated with UV light reacts with an activated double-bond compound, such as fumaric acid, provides a mechanism to explain the mixing tolerance effect of flour water solubles (Schroeder and Hoseney 1978). We suggest that free radicals formed by the rupture of disulfide bonds during dough mixing (Sidhu et al 1980) combine with the ferulic acid, or similar compounds, esterified to the water-soluble pentosan and thus covalently bind some of the pentosans to the gluten protein during dough mixing. The introduction of such a large hydrophilic residue on the gluten protein would be expected to have a major effect on dough's rheological properties. For instance, Graveland et al (1979) claimed that carbohydrate was covalently bound to gluten protein.

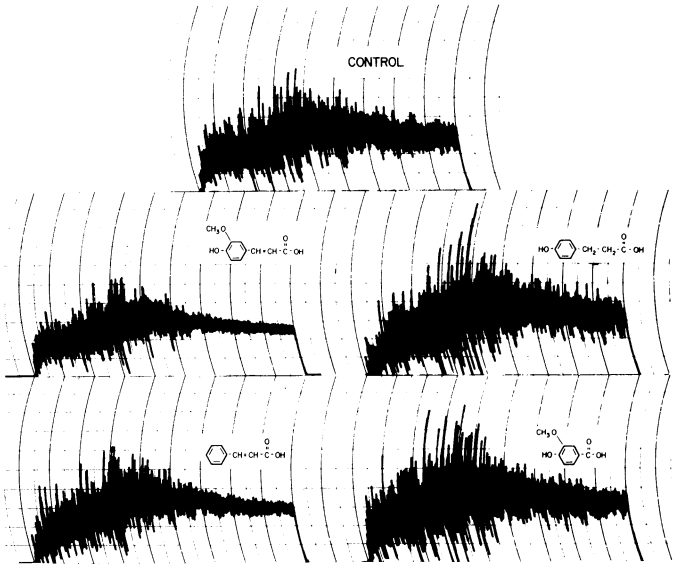


**Fig. 1.** Mixograms showing effects of certain additives on mixing tolerance. Additives were added at 250 ppm based on flour weight.

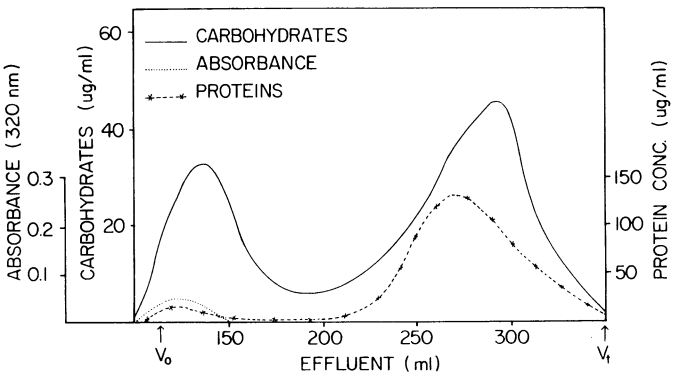


**Fig. 2** Elution profiles of the untreated wheat flour water-soluble fraction from Sepharose 4B.

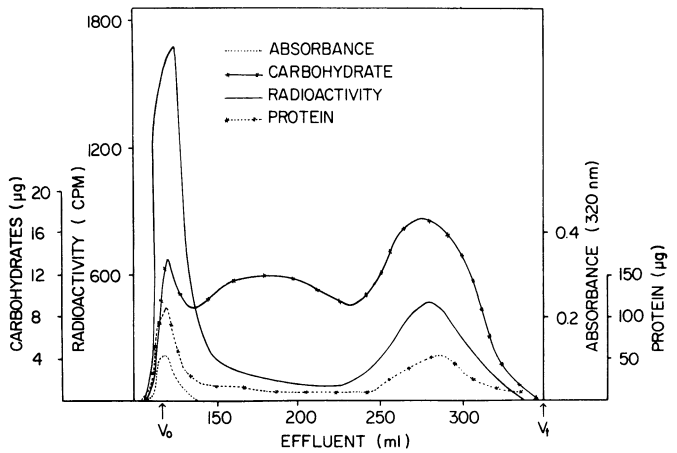


**Fig. 3.** Elution profile of the water-soluble fraction containing $^{14}$C-cysteine irradiated with ultraviolet light. The sample was dialyzed and separated on Sepharose 4B.

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