

## Note

## Effect of Metal Salts and Fructose on the Autoxidation of Methyl Linoleate in Emulsions

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Sugars such as pentose, hexose and reducing disaccharide have been demonstrated to be strong prooxidants of methyl linoleate or linoleic acid in aqueous emulsion systems.<sup>1~4)</sup> In the previous papers,<sup>4,5)</sup> we have reported that the prooxidant effect of reducing sugars on the autoxidation of methyl linoleate depends on trace amounts of metal ions in the reaction system; these sugars reduce metal ions in the reaction system, and the reduced metal ions accelerate the peroxidation process. Such prooxidant effects of certain transition metal ions, primarily copper and iron, on edible oils is well known and is a constant cause of practical problems.<sup>6)</sup> This paper deals with the prooxidant effect of some transition metal ions on the autoxidation of methyl linoleate emulsions in the presence and absence of a reducing sugar, fructose. The peroxidation was carried out at two pH conditions, pH 7.0 and 3.0. Especially, the pH 3.0 condition was a model of some acidic emulsifying foods such as salad dressings.

Autoxidation of methyl linoleate occurred under the following conditions: 10 mM methyl linoleate emulsion, 10 mM fructose and 10  $\mu$ M metal salt in 50 mM sodium phosphate buffer at pH 7.0, or in 50 mM sodium acetate/HCl buffer at pH 3.0, containing 0.5% Triton X-100. The buffer solutions used in the experiments were treated with Dowex A-1 resin to remove the trace amounts of metal ions.<sup>7)</sup> This autoxidation was carried out at 37°C. The peroxide concentrations were directly determined in each 100  $\mu$ l of the sample by an iodometric method as previously described.<sup>5)</sup> For all experiments, the average values of triplicate runs are presented, which did not vary by more than  $\pm 10\%$ .

Reducing sugars such as fructose have been reported to accelerate the autoxidation of methyl linoleate in the aqueous emulsion system.<sup>4,5)</sup> On the other hand, the addition of fructose did not accelerate the lipid peroxidation when endogenous metal ions in the reaction system were removed by treating with a chelating resin (Table I). The addition of metal salts alone showed little effect on the autoxidation of methyl linoleate, lipid per-

oxidation being accelerated by the combined effect of a metal salt and fructose. CuSO<sub>4</sub> plus fructose showed the most marked effect on the acceleration of lipid peroxidation. FeSO<sub>4</sub> or FeCl<sub>3</sub> plus fructose also proved to be a powerful prooxidant. The results substantiated our previous study<sup>5)</sup> that the reducing sugar reduces a transition metal ion, and that this reduced metal ion accelerates the peroxidation. From this idea, FeSO<sub>4</sub>, a reduced form of the iron ion, should be a powerful prooxidant, but this salt did not accelerate the lipid peroxidation. This might have been due to spontaneous oxidation of the ferrous ion at pH 7.0.<sup>5)</sup> On the basis of our results, it seems likely that the reducing form of a metal ion, especially copper and iron ions, is one of the important elements controlling the lipid peroxidation reaction.

Table II shows the effect of metal salts and fructose on the autoxidation of methyl linoleate at pH 3.0. Lipid peroxidation was hardly accelerated by the combined effect of a metal salt and fructose. Reducing sugars could reduce the ferric ion to ferrous ion at the neutral pH condition.<sup>5)</sup> On the other hand, fructose at pH 3.0 hardly reduced the ferric ion (data are not shown here). Therefore, the combined effect of a metal salt and fructose did not appear at this condition. Among the various species of metal ion tested, FeSO<sub>4</sub> was the most powerful prooxidant on the autoxidation of methyl linoleate, and FeCl<sub>3</sub> also had a prooxidant effect at pH 3.0, whereas these iron salts did not accelerate the peroxidation process at pH 7.0. According to O'Brien,<sup>8)</sup> the decomposition of linoleic acid hydroperoxide by transition metal salts was markedly pH dependent. Fe<sup>2+</sup> and Fe<sup>3+</sup> had a maximal activity at pH values below 5.5. Cu<sup>2+</sup>, on the other hand, had a maximal activity in the pH range of 5.5~6.0. These results indicate that the prooxidant effect of metal salts on the lipid peroxidation was pH dependent.

TABLE I. EFFECT OF METAL SALTS ON THE AUTOXIDATION OF A METHYL LINOLEATE EMULSION AT pH 7.0 IN THE PRESENCE AND ABSENCE OF FRUCTOSE

Metal salt	Peroxide (mmol/mol of methyl linoleate)			
	Without fructose		With fructose	
	24 hr	48 hr	24 hr	48 hr
None	0	3	0	6
CdCl <sub>2</sub>	0	9	97	168
CoCl <sub>2</sub>	0	3	62	126
Cr(NO <sub>3</sub> ) <sub>3</sub>	8	20	99	213
CuSO <sub>4</sub>	3	46	568	803
FeSO <sub>4</sub>	3	12	310	514
FeCl <sub>3</sub>	4	8	230	555
HgCl <sub>2</sub>	2	13	14	41
MnSO <sub>4</sub>	2	14	20	63
ZnSO <sub>4</sub>	0	24	136	335

TABLE II. EFFECT OF METAL SALTS ON THE AUTOXIDATION OF A METHYL LINOLEATE EMULSION AT pH 3.0 IN THE PRESENCE AND ABSENCE OF FRUCTOSE

Metal salt	Peroxide (mmol/mol of methyl linoleate)			
	Without fructose		With fructose	
	24 hr	48 hr	24 hr	48 hr
None	7	19	7	12
CdCl <sub>2</sub>	19	20	11	12
CoCl <sub>2</sub>	4	12	15	26
Cr(NO <sub>3</sub> ) <sub>3</sub>	17	19	13	20
CuSO <sub>4</sub>	20	21	36	91
FeSO <sub>4</sub>	152	499	194	484
FeCl <sub>3</sub>	39	155	55	280
HgCl <sub>2</sub>	12	16	24	26
MnSO <sub>4</sub>	24	41	5	14
ZnSO <sub>4</sub>	19	23	14	23

The iron salts was an important factor for the acceleration of methyl linoleate peroxidation at pH 3.0. The free radical scavengers, butylated hydroxytoluene and  $\alpha$ -tocopherol, could inhibit the iron salt-catalyzed peroxidation, which implies the involvement of free radicals, possibly the peroxy and alkoxy radicals, in the peroxidation process. Such free radicals formed by the reaction of iron salts with lipid hydroperoxide cause the initiation of lipid peroxidation.<sup>9)</sup>

There are a wide variety of emulsifying foods showing

low pH values such as salad dressings. These products contain oil, water, acetic acid and many additives such as sugars, proteins and salts. Acetic acid has been reported to have a prooxidant effect on the autoxidation of methyl linoleate.<sup>10)</sup> In addition, the present study indicates that a trace amount of iron salts was an important factor on the oxidative deterioration of acidic oil products, and that a reducing sugar did not affect the acceleration of lipid peroxidation in the low pH condition.

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