

## Oxidation of Methionine in Model Systems

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Oxidation of methionine by  $H_2O_2$  was studied in model systems analogous to a meat emulsion product. Formation of methionine sulfoxide (Met-SO), the only oxidation product found, was monitored by thin-layer chromatography. The disappearance of  $H_2O_2$  from reaction mixtures, monitored spectrophotometrically, was used to calculate pseudo-first-order rate constants ( $k_1$ ). Effects of pH, a singlet oxygen generator (SOG), a singlet oxygen quencher (SOQ), and a hydroxyl radical scavenger (HRS) on the formation of Met-SO and the  $k_1$  were evaluated by analysis of variance. Increasing the pH from 5.8 to 7.2 significantly increased the amount of Met-SO formed and the  $|k_1|$ . The presence of SOG decreased amount of Met-SO formed but increased  $|k_1|$ . The SOQ had no consistent effect but HRS decreased  $|k_1|$  in the presence of peroxidase, a component of SOG.

Oxidation of the essential amino acid methionine can affect the nutritive value of protein food products. Rasekh et al. (1972) and Sjoberg and Bostrom (1977) reported significantly ( $p < 0.05$ ) lower protein efficiency ratios (PER) and Slump and Schreuder (1973) reported a lower biological value (BV) and net protein utilization (NPU) when fish protein was treated with hydrogen peroxide. When casein was treated similarly, PER values were slightly lower (Cuq et al., 1978), BV values were lower (Slump and Schreuder, 1973), and NPU's were lowered (Slump and Schreuder, 1973; Ellinger, 1978).

Cuq et al. (1973, 1978) and Ellinger (1978) reported that only methionine sulfoxide was formed from methionine residues in hydrogen peroxide oxidized proteins. Slump and Schreuder (1973), Sjoberg and Bostrom (1977), and Chang et al. (1982), however, found evidence for the formation of methionine sulfone.

In other studies of the methionine content of oxidized protein products (Strange et al., 1980; Tannenbaum et al., 1969), lowered methionine content was noted but the oxidized products were not identified.

Toennies and Callan (1939) studied the oxidation of free methionine with hydrogen peroxide. They noted the rapid disappearance of hydrogen peroxide with presumptive formation of the sulfoxide. Methionine sulfone was presumed to have been formed only if molybdate ion was present. Other investigators (Matsuo, 1953; Njaa, 1962; Hiller et al., 1981) reported formation of an assortment of oxidation products including decarboxylated and deaminated products.

The products and rate of the oxidation of free methionine were investigated in this study. Model systems were designed to approximate the methionine content and possible oxidizing conditions present during the production of a processed meat product. Sodium chloride and metheme proteins are present in meat emulsions, and the combination of a halide and an oxidized heme, which can act as a peroxidase, is a singlet oxygen generator (Korycha-Dahl and Richardson, 1978). A singlet oxygen generating system and two oxidation inhibitors, a hydroxyl radical scavenger and a singlet oxygen quencher, were studied for their effects and interactions on the methionine oxidation reaction.

### EXPERIMENTAL SECTION

**Model System.** The concentration of methionine used in all experiments was 0.02 M (0.2 mmol in 10 mL of

reaction mixture]. Comminuted meat emulsions contain approximately 11% protein with the lean beef protein ingredient containing about 2.4% methionine (Happich et al., 1975). All reactants were prepared in 0.1 M phosphate buffer of appropriate pH because the dihydrogen phosphate anion prevents oxidation of the  $\alpha$  amino nitrogen of methionine (Hiller et al., 1981).

The pHs of the 0.1 M phosphate buffers were adjusted to pH 5.8, pH 6.2 [the extremes of pH found in raw post-rigor meat used to make hot dog emulsions (Miller et al., 1980)], pH 6.8, [pH of prerigor meat (Elgasim et al., 1981; Whiting et al., 1981)], and pH 7.2 [pH of living tissue (Ganong, 1975)].

Other components in the reaction mixtures were hydrogen peroxide ( $H_2O_2$ ), sodium chloride, horseradish peroxidase, sodium formate, and 1,4-diazobicyclo[2.2.2]-octane (DABCO).

Hydrogen peroxide was used to initiate oxidation; it acts as an oxidizing species itself, and it can generate other oxidizing species depending on the reaction conditions (Korycha-Dahl and Richardson, 1978). The combination of NaCl (2.5%) and peroxidase (0.01%) along with  $H_2O_2$  acts as a singlet oxygen generator. Sodium formate (0.5 M) is an effective hydroxyl radical scavenger (Pederson and Aust, 1975) and DABCO (0.02 M) is a singlet oxygen quencher (Aurand et al., 1976). Different combinations of sodium chloride-peroxidase, sodium formate, DABCO, pHs, and  $H_2O_2$  concentrations (0.01, 0.02, 0.03, and 0.04 M) were evaluated for their effects and interactions on the methionine oxidation reaction.

### METHODS OF ANALYSIS

**Thin-Layer Chromatography.** The relative content of methionine and its oxidation products in reaction mixtures were determined by thin-layer chromatography (TLC). A 6.5 cm high column of 2 g of ion retardation resin (Bio-Rad AG-11-A8 (50–100 mesh)) was prepared in disposable Pasteur pipets by using a small glass wool plug to support the resin column. The resin was added as a water slurry, packed with vacuum, and plugged with additional glass wool. A 2-mL aliquot of the reaction mixture was desalted on a minicolumn. Seven microliters taken from the first 2 drops through the column was used for spotting the silica gel plates (Whatman LK-50). Plates were dried for 10 min under nitrogen and were chromatographed in a 1:1:1 1-butanol-pyridine-water mixture for 3.5 h. Pyridine was redistilled over ninhydrin to remove ninhydrin positive contaminants. Methionine and its oxidation products were visualized by spraying the plate with 0.2% ninhydrin in acetone followed by heating in a 100 °C oven for 4 min. Relative contents of the methionine

Table I. Means of Percent Methionine Sulfoxide Formed for Various Combinations of pH, H<sub>2</sub>O<sub>2</sub> Concentration, and Reaction Times. Expressed as Percentage of Total Ninhydrin Positive Spots<sup>a</sup>

H <sub>2</sub> O <sub>2</sub> concentration, M	reaction time, h								
	2 h			4 h			24 h		
	mean	SD	N	mean	SD	N	mean	SD	N
0.01	39.9 <sup>DA</sup>	12	32 <sup>b</sup>	48.4 <sup>DB</sup>	11.9	26 <sup>c</sup>	53.1 <sup>DB</sup>	8.6	32
0.02	58.2 <sup>EA</sup>	11.6	32	67.8 <sup>EB</sup>	9.4	26	83.3 <sup>EC</sup>	7.5	32
0.03	70.0 <sup>FA</sup>	9.9	32	81.6 <sup>FB</sup>	8.0	26	98.6 <sup>FC</sup>	4.3	32
0.04	78.4 <sup>GA</sup>	9.1	32	91.5 <sup>GB</sup>	5.6	32	100 <sup>F</sup>	0	32

  

reaction time, h	H <sub>2</sub> O <sub>2</sub> concentration, M	pH											
		5.8			6.2			6.8			7.2		
		mean	SD	N	mean	SD	N	mean	SD	N	mean	SD	N
2	0.01	30.0 <sup>A</sup>	9.6	8 <sup>d</sup>	36.0 <sup>AB</sup>	12.2	8	46.0 <sup>BC</sup>	9.9	8	47.5 <sup>C</sup>	8.1	8
	0.02	52.3 <sup>A</sup>	12.2	8	52.9 <sup>A</sup>	10.6	8	62.7 <sup>AB</sup>	9.3	8	65.0 <sup>B</sup>	10.2	8
	0.03	62.9 <sup>A</sup>	8.5	8	66.9 <sup>AB</sup>	6.5	8	75.9 <sup>B</sup>	8.5	8	74.4 <sup>B</sup>	10.9	8
	0.04	77.4 <sup>A</sup>	5.1	8	75.6 <sup>A</sup>	15.1	8	79.0 <sup>A</sup>	5.9	8	81.4 <sup>A</sup>	7.8	8

<sup>a</sup> Only spots corresponding to methionine and/or methionine sulfoxide were detected. All means that do not have letter superscripts in common within the same row (read across) are statistically different at  $p < 0.05$ . All means that do not have letter superscripts in common within the same reaction time block (read down) are statistically different at  $p < 0.05$  (Duncan's new multiple range test). <sup>b</sup> Results of oxidation reactions with the eight combinations of additives and four pH levels pooled. <sup>c</sup> Results of oxidation reactions with eight combinations of additives for pHs 6.2, 6.8, and 7.2 and results of oxidation reactions with no additives and both sodium formate and DABCO for pH 5.8 were pooled. <sup>d</sup> Results of oxidation reactions with eight combinations of additives pooled.

Table II. Means of Percent Methionine Sulfoxide Formed after a 2-h Reaction Time with 0.01 M H<sub>2</sub>O<sub>2</sub> and 0.02 M Methionine<sup>a</sup> for Various Combinations of Additives and pHs

additives	pH											
	5.8			6.2			6.8			7.2		
	mean	SD	N									
none	33.8	13.6	4	38.7	6.8	3	40.2	3.1	4	45.2	8.8	4
NaCl-peroxidase <sup>b</sup>	38.0	4.7	4	35.8	8.6	4	39.8	14.9	4	31.8	16.0	4
DABCO <sup>c</sup>	39.0	9.9	4	34.0	14.6	4	40.2	8.2	4	40.5	12.8	4
sodium formate <sup>d</sup>	42.8	4.3	4	37.8	15.6	4	45.5	6.8	4	47.8	14.8	4
DABCO + formate	43.0	13.9	4	46.2	8.3	4	47.5	14.2	4	43.2	12.4	4
NaCl-peroxidase + DABCO	35.5	8.3	4	34.2	6.0	4	37.8	15.4	4	38.5	7.9	4
NaCl-peroxidase + sodium formate	31.8	11.2	4	36.2	10.8	4	40.2	15.9	4	36.0	11.8	4
NaCl-peroxidase + sodium formate + DABCO	41.0	11.4	4	42.2	9.8	4	38.2	5.9	4	35.5	10.0	4

<sup>a</sup> Expressed as percent of total ninhydrin positive spots; only methionine and/or methionine sulfoxide were found. <sup>b</sup> NaCl (2.5%)–peroxidase (0.01 mg/10 mL), singlet oxygen generator. <sup>c</sup> DABCO (0.02 M), singlet oxygen quencher. <sup>d</sup> Sodium formate (0.5 M), hydroxyl radical scavenger.

( $R_f = 0.74$ ), methionine sulfoxide ( $R_f = 0.33$ ), and methionine sulfone ( $R_f = 0.50$ ) were determined by measuring the intensity of the various spots on a densitometer (Gelman ACD-18) and determining the relative peak size with a planimeter.

A series of equimolar mixtures of L-methionine, L-methionine DL-sulfoxide, and L-methionine sulfone were chromatographed before and after minicolumn treatment. Relative percents measured for methionine–methionine sulfoxide–methionine sulfone before minicolumn treatment were 32 (4)%–40 (4)%–29 (4)% ( $N = 4$ ) and after minicolumn treatment were 32(5)%–38(5)%–30(5)% ( $N = 4$ ). All three compounds separated cleanly and were recovered in approximately the same relative concentrations before and after minicolumn treatment. Any differences in intensity of color development were obscured by the variability inherent in the TLC–densitometer technique.

**Rate Determinations.** *Spectrophotometric.* Methionine sulfoxide does not absorb between 248 and 252 nm but H<sub>2</sub>O<sub>2</sub> does. The absorbance of H<sub>2</sub>O<sub>2</sub> in reaction mixtures without methionine did not change during 240 min of spectrophotometric monitoring.

Reaction mixtures were prepared except that no H<sub>2</sub>O<sub>2</sub> was added. The mixtures were placed in a constant-temperature bath (33 °C) and pumped through flow cells mounted in a Hewlett-Packard 8450A UV/vis spectro-

photometer. Mean absorbance of the reaction mixture (without H<sub>2</sub>O<sub>2</sub>) was measured at 248–252 nm and stored in the instrument memory as a reagent blank. Following addition of hydrogen peroxide, the spectrophotometer again measured the mean absorbance at 248–252 nm minus the reagent blank values at various specified time intervals. Concentration of hydrogen peroxide is directly related to corrected absorbance measurements ( $Abs - H_2O_2$ ) (molar extinction coefficient for hydrogen peroxide at 248–252 nm is 22.3 cm<sup>-1</sup>). Measurements were made at 5-min intervals for a total of 240 min.

**Rate Calculations.** Semilog plots of  $Abs - H_2O_2$  (log scale) vs. time were calculated and linear regressions determined. Goodness of fit was measured by linear correlation coefficients. Pseudo-first-order rate constants are equal to the slope of the linear regression line for  $\ln(Abs - H_2O_2)$  vs. time (min). This rate constant is calculated by using changes in H<sub>2</sub>O<sub>2</sub> concentration only.

Second-order rate constants were also calculated. Changes in H<sub>2</sub>O<sub>2</sub> concentration was assumed to be indicative of changes in methionine concentration. The slope of the regression line for  $(\ln a_t - \ln b_t)/(a_0 - b_0)$  vs. time (min) where  $a_t$  = concentration of methionine at time  $t$ ,  $b_t$  = concentration of H<sub>2</sub>O<sub>2</sub> at time  $t$  [ $(Abs - H_2O_2)/22.3$  cm<sup>-1</sup> × 1 cm],  $a_0$  = initial concentration of methionine (0.02 M), and  $b_0$  = initial concentration of H<sub>2</sub>O<sub>2</sub> (0.01 M)

is equal to the second-order rate constant.

**Experimental Design.** *Experiment 1.* The identity and relative quantity of reaction products were measured by TLC. The approximate stoichiometry of the oxidation reaction as affected by the different additives, pHs, H<sub>2</sub>O<sub>2</sub> quantity, and reaction times was determined from the results of this experiment. All eight combinations of additives, pHs of 5.8, 6.2, 6.8, and 7.2, hydrogen peroxide concentrations of 0.01, 0.02, 0.03, and 0.04 M, and reaction times of 2, 4, and 24 h were examined in a single survey.

*Experiment 2.* The effects of additives on the amount of methionine sulfoxide formed by hydrogen peroxide oxidation were studied in this experiment by using TLC with densitometry. All eight combinations of additives, pHs of 5.8, 6.2, 6.8, and 7.2, one concentration of hydrogen peroxide (0.01 M), and a reaction time of 2 h were examined. This series was done 4 times.

*Experiment 3.* The reaction rates for the oxidation of methionine as determined by the disappearance of H<sub>2</sub>O<sub>2</sub> from the reaction mixture and the effects of the additives and pHs on these reaction rates were measured in these experiments. All eight combinations of additives, pHs of 5.8, 6.2, 6.8, and 7.2, and 0.01 M H<sub>2</sub>O<sub>2</sub> were examined. This series of reactions was repeated 3 times. Each reaction was monitored by UV/vis spectrophotometry for 4 h, and pseudo-first order (in terms of H<sub>2</sub>O<sub>2</sub> concentration) and second-order (in terms of both H<sub>2</sub>O<sub>2</sub> and methionine concentrations) reaction rates constants were calculated for both 2- and 4-h reaction times.

**Statistical Analysis.** Simple effects of pH, sodium chloride-peroxidase, sodium formate, and DABCO and their interactions on the amount of methionine sulfoxide formed (experiment 2) and on the reaction rate constants (experiment 3) were compared by using analysis of variance procedures for factorial experiments (Steel and Torrie, 1960). Differences in means (experiment 1) were evaluated by using Duncan's new multiple range test as described by Steel and Torrie (1960). All statistical tests were performed at the  $p = 0.05$  significance level.

## RESULTS AND DISCUSSION

The data collected in experiment 1 are shown in Table I. Methionine sulfone was not formed under any conditions studied. Methionine sulfone was not detected by TLC in 18 of the reaction mixtures even after an additional 24-h reaction time (48-h total). The apparent stoichiometry of the reaction (most clearly shown by the 0.01 M hydrogen peroxide data) is 1 mol of methionine oxidized by 1 mol of hydrogen peroxide to form 1 mol of methionine sulfoxide. The amount of methionine sulfoxide formed increased significantly ( $p < 0.05$ ) with increasing reaction time in all cases except for 0.01 M H<sub>2</sub>O<sub>2</sub> from 4 to 24 h. The oxidation was complete after 4 h for 0.01 M H<sub>2</sub>O<sub>2</sub> because all the H<sub>2</sub>O<sub>2</sub> present had been used.

Increasing concentrations of H<sub>2</sub>O<sub>2</sub> oxidize methionine to the sulfoxide more rapidly. The percent methionine sulfoxide present in the reaction mixture at 2, 4, and 24 h increased significantly [ $p < 0.05$  (Duncan's new multiple range test)] when the H<sub>2</sub>O<sub>2</sub> concentration was increased from 0.01 to 0.02 to 0.03 M (Table I). There was no significant change at 4- and 24-h reaction time when the H<sub>2</sub>O<sub>2</sub> concentration was increased from 0.03 to 0.04 M (Table I). Raising the pH of the reaction mixture from 5.8 through 7.2 significantly ( $p < 0.05$ ) increased the percent methionine sulfoxide formed after 2 h when 0.01, 0.02, and 0.03 M H<sub>2</sub>O<sub>2</sub> were used. The mean percent methionine sulfoxide formed after 2 h for 0.01, 0.02, and 0.03 M H<sub>2</sub>O<sub>2</sub> at pH 5.8 was 48.4% and at pH 7.2 was 62.3% or a 28% increase (calculated from data presented in Table I).

Table III. Mean Percentage of Methionine Sulfoxide Formed for the Simple Effects of pH and the Additives Sodium Chloride-Peroxidase, DABCO, and Sodium Formate and for the Interactions of the Additives

pH	Simple Effects			sodium formate
	NaCl-peroxidase <sup>a</sup>	DABCO		
5.8	0 <sup>b</sup>	0	+	+
38.1 (N = 32)	38.0 (N = 31)	41.2 (N = 32)	39.8 (N = 32)	37.0 (N = 64)
6.2	0	0	+	+
38.0 (N = 31)	41.5 (N = 63)	38.7 (N = 68)	39.8 (N = 64)	37.6 (N = 63)
6.8	0	0	+	+
41.2 (N = 32)	37.0 (N = 64)	38.7 (N = 64)	39.8 (N = 64)	40.9 (N = 64)
7.2	0	0	+	+
39.8 (N = 32)	36.2 (N = 32)	37.9 (N = 32)	38.8 (N = 31)	36.4 (N = 32)
39.8 (N = 32)	41.3 (N = 31)	41.7 (N = 32)	44.2 (N = 32)	37.7 (N = 32)
	Double Interactions			
	NaCl-peroxidase	NaCl-peroxidase		
	+	+		
	0	0		
	DABCO: 0			
	+			
	sodium formate: 0			
	+			

<sup>a</sup> Significant effect or interaction,  $p \leq 0.05$ . <sup>b</sup> Additive not present. <sup>c</sup> Additive present.

Table IV. Mean Pseudo-First-Order Methionine Oxidation Reaction Rate Constants for 2- and 4-h Reaction Times

additives	time, h	pH							
		5.8		6.2		6.8		7.2	
		mean	SD <sup>a</sup>						
none	2	-0.013	0.001	-0.0133	0.0004	-0.0136	0.0004	-0.014	0.001
NaCl-peroxidase (B)	2	-0.016	0.004	-0.016	0.001	-0.018	0.001	-0.019	0.001
DABCO (C)	2	-0.015	0.001	-0.015	0.001	-0.0147	0.0003	-0.0147	0.0003
sodium formate (D)	2	-0.013	0.001	-0.014	0.001	-0.014	0.001	-0.0138	0.0004
C + D	2	-0.013	0.001	-0.014	0.001	-0.013	0.001	-0.014	0.002
B + C	2	-0.019	0.003	-0.019	0.004	-0.021	0.004	-0.023	0.004
B + D	2	-0.016	0.001	-0.016	0.001	-0.018	0.001	-0.018	0.001
B + C + D	2	-0.0152	0.0001	-0.015	0.001	-0.016	0.001	-0.016	0.001
none	4	-0.018	0.001	-0.0181	0.0003	-0.0183	0.0004	-0.0180	0.0003
NaCl-peroxidase (B)	4	-0.024	0.002	-0.021	0.002	-0.022	0.002	-0.023	0.001
DABCO (C)	4	-0.019	0.002	-0.0192	0.0001	-0.0192	0.0002	-0.0192	0.0003
sodium formate (D)	4	-0.0188	0.0004	-0.0182	0.0002	-0.0186	0.0002	-0.0183	0.0003
C + D	4	-0.018	0.001	-0.020	0.004	-0.018	0.001	-0.019	0.002
B + C	4	-0.022	0.002	-0.022	0.005	-0.025	0.003	-0.026	0.003
B + D	4	-0.020	0.001	-0.019	0.001	-0.022	0.002	-0.021	0.001
B + C + D	4	-0.018	0.001	-0.019	0.001	-0.0193	0.0002	-0.0199	0.0004

<sup>a</sup>  $N = 3$ .

Experiment 2 was designed to evaluate the effects of the additives and their interactions on the formation of methionine sulfoxide. Data are shown (Table II) and the percent methionine sulfoxide means for simple effects and some interaction of additives (Table III) are presented. The only significant effect found by analysis of variance was for sodium chloride-peroxidase, the singlet oxygen generator. When sodium chloride-peroxidase was present, the formation of methionine sulfoxide was decreased by 11% (calculated from data in Table III). This result was unexpected since singlet oxygen is considered to be a stronger oxidizer than hydrogen peroxide (Korycha-Dahl and Richardson, 1978). The interaction of sodium chloride-peroxidase and DABCO (the singlet oxygen quencher) was not significant, and examination of the means indicates DABCO had no effect on the percentage of methionine sulfoxide formed when sodium chloride-peroxidase was present (Table III).

Examination of the means for the sodium chloride-peroxidase and sodium formate interaction (Table III) shows that the addition of sodium formate to the reaction mixture containing sodium chloride-peroxidase increases the amount of methionine sulfoxide formed; however, this interaction was not significant. The simple effect of sodium formate, the hydroxyl radical scavenger, is a non-significant increase of 9% (calculated from data in Table III) in percent methionine sulfoxide. The simple effect of DABCO, the single oxygen quencher, was a non-significant increase of 3% (calculated from data in Table III) in percent methionine sulfoxide. The simple effect of pH, while not significant (Table III), is consistent with trends found in experiment 1. The percent methionine sulfoxide increased by 6% (calculated from data in Table III) when the reaction was carried out at the higher pHs. Because of large variabilities inherent in the method of analysis (TLC plus densitometry) (Table II), subtle changes in the amount of methionine sulfoxide formed would be undetected.

Experiment 3 was designed to furnish data of sufficient precision to evaluate the effects of additives on the rate of disappearance of hydrogen peroxide during the oxidation. This spectrophotometric method followed the decrease in concentration of hydrogen peroxide rather than the increase in oxidation products. The assumption was made that no side reactions existed to compete with methionine for hydrogen peroxide. Pseudo-first-order kinetics were used to calculate the reaction rate constants that were

used in the analysis of variance. Second-order reaction rate constants were also calculated for selected oxidation reactions. The linear correlation coefficients generated by these two types of calculation were all highly significant ( $p < 0.01$ ), but the pseudo-first-order fit is closer than the second-order fit, and no differences in fits were observed for oxidations carried out at various pH levels.

Table IV lists the mean pseudo-first-order reaction rate constants and their standard deviations. The average coefficient of variation for the replicates of the reaction rate constant determination is 7% (calculated from data shown in Table IV) while the average coefficient of variation for the TLC data is 27% (calculated from data shown in Table II). Reaction rate constants for the 2-h data indicate a slower reaction than the 4-h rate constants (Table IV).

Analysis of variance for the pseudo-first-order rate constant data was carried out, and the pseudo-first-order reaction rate constant means for simple effects and some interactions of additives are shown in Table V.

No interactions involving pH are significant; pH has a significant simple effect for the 2-h reaction time only. The mean reaction rate constants indicate a 10% faster oxidation as the pH increases (calculated from data in Table V). This effect, however, was not significant for the 4-h reaction time.

The triple interaction of sodium chloride-peroxidase (a singlet oxygen generator), DABCO, (single oxygen quencher), and sodium formate (a hydroxyl radical scavenger) was significant for 2-h data only. Sodium chloride-peroxidase increased the reaction rate significantly by 25% (calculated from data in Table V). The combination of sodium formate and DABCO had little effect on mean reaction rates if sodium chloride-peroxidase were not present; however, if sodium chloride-peroxidase were present, this combination slowed the oxidation rate by about 11%. Sodium formate alone slowed the oxidation rate by 14% in the presence of sodium chloride-peroxidase, but sodium formate had little effect if sodium chloride plus peroxidase were not present. DABCO, a singlet oxygen quencher, alone, increased the rate of oxidation by 5% with or without presence of sodium chloride-peroxidase, a single oxygen generator (calculated from data in Table V).

Similar effects were noted for the 4-h reaction time rate constants except that DABCO alone had little effect on the reaction rate constants.

oxygen generator in these reactions (a stronger oxidizing agent than hydrogen peroxide) because DABCO, the single oxygen quencher, does not significantly interact with sodium chloride-peroxidase (Table V). Peroxidase can complex with hydrogen peroxide at a more rapid rate than methionine reacts with either hydrogen peroxide or with the peroxidase-hydrogen peroxide complex, thereby lowering the amount of methionine sulfoxide formed after 2 h but not affecting the eventual completion of the reaction.

Sodium formate, the hydroxyl radical scavenger, decreases the rate of disappearance of hydrogen peroxide in reaction mixtures containing sodium chloride-peroxidase, indicating a hydroxyl radical involvement in the formation of the hydrogen peroxide-peroxidase complex.

The reactions involved in the sodium chloride-peroxidase-hydrogen peroxide complex decrease the rate of methionine oxidation by lowering the effective concentration (or activity) of the oxidizing species. These reactions are reversible because they do not affect the eventual completion of the oxidation, and they also involve a hydroxyl radical because sodium formate, the hydroxyl radical scavenger, partially reverses the effect of the addition of sodium chloride-peroxidase.

#### CONCLUSIONS

Methionine is oxidized only to methionine sulfoxide under conditions examined in this study. Formation of the sulfone derivative during processing of meat emulsion products is unlikely, and the disappearance of methionine during processing in an oxidizing system (Strange et al., 1980) results from formation of the sulfoxide.

The rate of reaction of the methionine in oxidizing systems is slower than previously assumed and is clearly influenced by certain additives, particularly the combination of sodium chloride-peroxidase. Sodium chloride-peroxidase, under the conditions employed in this study, does not appear to generate stronger oxidizing species (singlet oxygen) but actually retards the rate of formation of methionine sulfoxide.

**Registry No.** L-Methionine, 63-68-3; methionine sulfoxide, 454-41-1; hydrogen peroxide, 7722-84-1; hydroxyl radical, 3352-57-6; sodium chloride, 7647-14-5; peroxidase, 9003-99-0.

#### LITERATURE CITED

- Aurand, L. W.; Boone, N. H.; Giddings, G. G. *J. Dairy Sci.* **1976**, *60*, 363.
- Chang, K. C.; Marshall, H. F.; Satterlee, L. D. *J. Food Sci.* **1982**, *47*, 1181.
- Cuq, J. L.; Besancon, P.; Chartier, L.; Cheftel, C. *Food Chem.* **1978**, *3*, 85.
- Cuq, J. L.; Provansal, M.; Guilleux, F.; Cheftel, C. *J. Food Sci.* **1973**, *38*, 11.
- Elgasim, E. A.; Kennick, W. H.; McGill, L. A.; Rock, D. F.; Soldner, A. *J. Food Sci.* **1981**, *46*, 340.
- Ellinger, G. M. *Ann. Nutr. Aliment.* **1978**, *32*, 281.
- Ganong, W. F. "Review of Medical Physiology", 7th ed.; Lange Medical Publications: Los Altos, CA, 1975; p 489.
- Happich, M. L.; Whitmore, R. A.; Fairheller, S.; Taylor, M. M.; Swift, C. E.; Naghski, J.; Booth, A. N.; Alsmeyer, R. H. *J. Food Sci.* **1975**, *40*, 35.
- Hiller, K.-O.; Masloch, B.; Gobl, M.; Asmus, K.-D. *J. Am. Chem. Soc.* **1981**, *103*, 2734.
- Korycha-Dahl, M. B.; Richardson, T. *CRC Crit. Rev. Food Sci. Nutr.* **1978**, *11*, 209.
- Marshall, H. F.; Chang, K. C.; Miller, K. S.; Satterlee, L. D. *J. Food Sci.* **1982**, *47*, 1170.
- Matsuo, Y. *Nature (London)* **1953**, *171*, 1021.
- Miller, A. J.; Ackerman, S. A.; Palumbo, S. A. *J. Food Sci.* **1980**, *45*, 1466.
- Njaa, L. R. *Acta Chem. Scand.* **1962**, *16*, 1359.
- Pederson, T. C.; Aust, S. D. *Biochim. Biophys. Acta* **1975**, *385*, 232.
- Rasekh, J.; Stillings, B. R.; Sidwell, V. *J. Food Sci.* **1972**, *37*, 423.
- Sjoberg, L. B.; Bostrom, S. L. *Br. J. Nutr.* **1977**, *38*, 189.
- Slump, P.; Schreuder, H. A. W. *J. Sci. Food Agric.* **1973**, *24*, 657.
- Steel, R. G. D.; Torrie, J. H. "Principles and Procedures of Statistics"; McGraw-Hill: New York, NY, 1960.
- Strange, E. D.; Benedict, R. C.; Miller, A. J. *J. Food Sci.* **1980**, *45*, 632.
- Tannenbaum, S. R.; Barth, H.; LeRoux, J. P. *J. Agric. Food Chem.* **1969**, *17*, 1353.
- Toennies, G.; Callan, T. P. *J. Biol. Chem.* **1939**, *129*, 481.
- Whiting, R. C.; Strange, E. D.; Miller, A. J.; Benedict, R. C.; Mozersky, S. M.; Swift, C. E. *J. Food Sci.* **1981**, *46*, 484.

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