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 met-heme 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

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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 α 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 oxidiation 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

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Table I. Means of Percent Methionine Sulfoxide Formed for Various Combinations of pH, H_2O_2 Concentration, and Reaction Times. Expressed as Percentage of Total Ninhydrin Positive Spots[a]

H_2O_2 concentration, M	reaction time, h								
	2 h			4 h			24 h		
	mean	SD	N	mean	SD	N	mean	SD	N
0.01	$39.9_D{}^A$	12	32^b	$48.4_D{}^B$	11.9	26^c	$53.1_D{}^B$	8.6	32
0.02	$58.2_E{}^A$	11.6	32	$67.8_E{}^B$	9.4	26	$83.3_E{}^C$	7.5	32
0.03	$70.0_F{}^A$	9.9	32	$81.6_F{}^B$	8.0	26	$98.6_F{}^C$	4.3	32
0.04	$78.4_G{}^A$	9.1	32	$91.5_F{}^B$	5.6	32	$100_F{}^C$	0	32

reaction time, h	H_2O_2 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^A	9.6	8^d	36.0^{AB}	12.2	8	46.0^{BC}	9.9	8	47.5^C	8.1	8
	0.02	52.3^A	12.2	8	52.9^A	10.6	8	62.7^{AB}	9.3	8	65.0^B	10.2	8
	0.03	62.9^A	8.5	8	66.9^{AB}	6.5	8	75.9^B	8.5	8	74.4^B	10.9	8
	0.04	77.4^A	5.1	8	75.6^A	15.1	8	79.0^A	5.9	8	81.4^A	7.8	8

[a] 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 subscripts in common within the same reaction time block (read down) are statistically different at $p < 0.05$ (Duncan's new multiple range test). [b] Results of oxidation reactions with the eight combinations of additives and four pH levels pooled. [c] 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. [d] Results of oxidation reactions with eight combinations of additives pooled.

Table II. Means of Percent Methionine Sulfoxide Fomred after a 2-h Reaction Time with 0.01 M H_2O_2 and 0.02 M Methionine[a] **for Various Combinations of Additives and pHs**

additives	pH											
	5.8			6.2			6.8			7.2		
	mean	SD	N	mean	SD	N	mean	SD	N	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[b]	38.0	4.7	4	35.8	8.6	4	39.8	14.9	4	31.8	16.0	4
DABCO[c]	39.0	9.9	4	34.0	14.6	4	40.2	8.2	4	40.5	12.8	4
sodium formate[d]	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

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

$(R_f = 0.74)$, 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_2O_2 does. The absorbance of H_2O_2 in reaction mixtures without methionine did not change during 240 min of spectrophotometric monitoring.

Reaction mixtures were prepared except that no H_2O_2 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_2O_2) 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⁻¹). 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. Pseuso-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_2O_2 concentration only.

Second-order rate constants were also calculated. Changes in H_2O_2 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_2O_2 at time t [(Abs – $H_2O_2/22.3$ cm⁻¹) × 1 cm], a_0 = initial concentration of methionine (0.02 M), and b_0 = initial concentration of H_2O_2 (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_2O_2 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_2O_2 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_2O_2 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_2O_2 concentration) and second-order (in terms of both H_2O_2 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_2O_2 from 4 to 24 h. The oxidation was complete after 4 h for 0.01 M H_2O_2 because all the H_2O_2 present had been used.

Increasing concentrations of H_2O_2 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_2O_2 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_2O_2 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_2O_2 were used. The mean percent methionine sulfoxide formed after 2 h for 0.01, 0.02, and 0.03 M H_2O_2 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

Simple Effects

pH				NaCl-peroxidase[a]		DABCO		sodium formate	
5.8	6.2	6.8	7.2	0[b]	+[c]	0	+	0	+
38.1 (N = 32)	38.0 (N = 31)	41.2 (N = 32)	39.8 (N = 32)	41.5 (N = 63)	37.0 (N = 64)	38.7 (N = 63)	39.8 (N = 64)	37.6 (N = 63)	40.9 (N = 64)
			+		+		0		+

Double Interactions

NaCl-peroxidase × DABCO:

	NaCl-peroxidase 0	NaCl-peroxidase +
DABCO 0	41.3 (N = 31)	36.2 (N = 32)
DABCO +	41.7 (N = 32)	37.9 (N = 32)

NaCl-peroxidase × sodium formate:

	NaCl-peroxidase 0	NaCl-peroxidase +
sodium formate 0	38.8 (N = 31)	36.4 (N = 32)
sodium formate +	44.2 (N = 32)	37.7 (N = 32)

[a] Significant effect or interaction, $p \leqslant 0.05$. [b] Additive not present. [c] Additive present.

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

		pH							
		5.8		6.2		6.8		7.2	
additives	time, h	mean	SD[a]	mean	SD[a]	mean	SD[a]	mean	SD[a]
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

[a] $N = 3$.

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 scavanger, is a nonsignificant increase of 9% (calculated from data in Table III) in percent methionine sulfoxide. The simple effect of DABCO, the single oxygen quencher, was a nonsignificant 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 scavanger) 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 peroxidasse 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.

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Table V. Mean Pseudo-First Order Rate Constants of 2- and 4-h Reaction Time for the Simple Effects of pH and the Additives NaCl-Peroxidase, DABCO, and Sodium Formate and for Interactions of the Additives

Simple Effects

2-h reaction time — additives (N = 48)

	NaCl-peroxidase[a]		DABCO[a]		sodium formate[a]	
	0[b]	+[c]	0	+	0	+
	-0.014	-0.017	-0.015	-0.016	-0.016	-0.015

pH[a] (N = 24):

	5.8	6.2	6.8	7.2
6.2	-0.015			
6.8	-0.016	-0.015		
7.2	-0.016	-0.016	-0.015	

4-h reaction time — additives (N = 48)

	NaCl-peroxidase[a]		DABCO		sodium formate	
	0	+	0	+	0	+
	-0.019	-0.022	-0.020	-0.020	-0.021	-0.019

pH (N = 24):

	5.8	6.2	6.8	7.2
6.2	-0.020			
6.8	-0.020	-0.020		
7.2	-0.020	-0.019	-0.022	

Double Interactions (N = 24)

2-h reaction time

NaCl-peroxidase[a]:

	0	+
DABCO: 0	-0.014	-0.017
DABCO: +	-0.014	-0.018
sodium formate: 0	-0.014	-0.017
sodium formate: +	-0.014	-0.016

4-h reaction time

NaCl-peroxidase[a]:

	0	+
DABCO: 0	-0.018	-0.022
DABCO: +	-0.019	-0.022
sodium formate: 0	-0.018	-0.023
sodium formate: +	-0.019	-0.020

Triple Interactions (N = 12)

2-h reaction time, NaCl-peroxidase[a]

	sodium formate 0	sodium formate +
DABCO: 0	-0.013	-0.014
DABCO: +	-0.015	-0.014
	-0.017	-0.017
	-0.020	-0.015

4-h reaction time, NaCl-peroxidase

	sodium formate 0	sodium formate +
DABCO: 0	-0.018	-0.018
DABCO: +	-0.019	-0.019
	-0.022	-0.020
	-0.024	-0.019

[a] Simple effect or interaction significant, p < 0.05. [b] Additive not present. [c] Additive present.

DISCUSSION

The only oxidation product of methionine found in this study was methionine sulfoxide.

Hydrogen peroxide at temperatures below 40 °C is not a sufficiently strong oxidizing agent to oxidize the sulfoxide to the sulfone (Chang et al., 1982) except at extremely high concentration of hydrogen peroxide. Chang et al. found traces of methionine sulfone formed in egg white solids and casein treated with H_2O_2 at 40 °C for 2 h. However, in the mixtures in which sulfone was formed, H_2O_2 was present at approximately 20 times the concentration of methionine originally present in egg white solids and at approximately 60 times the concentration of methionine originally present in casein. In the experiments reported here the highest H_2O_2 concentration was twice the methionine concentration. Formation of the sulfone at temperatures below 40 °C indicates catalysis, possibly from trace metal contamination (Cuq et al., 1978).

Control of processing temperature and trace metal contamination are more important than concentration of oxidant and pH under normal meat-processing conditions for the prevention of methionine sulfone formation.

Preventing or controlling the oxidation of methionine to its sulfoxide during oxidation is more difficult. Marshall et al. (1982) demonstrated that methionine was stable (no sulfoxide was formed) during vegetable protein processing and that the trace amounts of methionine sulfoxide found in these proteins were present originally in the raw vegetable samples. Strange et al. (1980) showed that methionine is stable during emulsification and heating procedures used in meat processing if the protein is not exposed to oxidizing agents. Cuq et al. (1973, 1978) and Strange et al. (1980) showed that mild treatment with oxidizing agents can initiate oxidation of methionine.

Results from experiment 1 indicate that methionine reacts quantitatively with hydrogen peroxide to form the sulfoxide. Toennies and Callan (1939) showed that methionine reacted quantitatively with hydrogen peroxide after about 2 h at pH 1, 3, 4.5, and 5 and even more rapidly at higher acid concentrations (reaction complete in less than 25 min) with a 20% molar excess of hydrogen peroxide as the oxidizing agent. They measured the disappearance of hydrogen peroxide. Their reaction solutions did not contain dihydrogen phosphate ion and the amino nitrogen of the methionine was susceptible to oxidation. In our study, oxidation of methionine took place at a slower rate. After 2 h, the oxidation of methionine was 70%-80% complete in terms of percentage of methionine sulfoxide formed for all levels of hydrogen peroxide used. In the Toennies and Callan (1939) study, this level of peroxide utilization was reached after less than 25 min; however, it may have reflected oxidation of the amino nitrogen in addition to that of the sulfur.

The rate of oxidation of methionine is also affected by pH. Increasing pH increases the amount of methionine sulfoxide formed (Table I) and the rate of the oxidation reaction (Table V). The pH does not interact with any of the additives examined.

Sodium chloride-peroxidase, the singlet oxygen generator, has a significant effect on both the formation of methionine sulfoxide (Table III) and the reaction rate constants (Table V). This effect was large but in opposing directions. Sodium chloride-peroxidase decreased the amount of methionine sulfoxide formed and increased the absolute value of the reaction rate constants. Sodium chloride-peroxidase had no effect on the eventual stoichiometry of the reaction (experiment 1). The sodium chloride-peroxidase combination is not acting as a single

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 scavanger, 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.

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