

## EFFECT OF PROCESSING VARIABLES ON THE METHIONINE CONTENT OF FRANKFURTERS

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### ABSTRACT

The effects of processing and use of pro- and antioxidant additives on the methionine content of frankfurters were examined. Emulsification and cooking-smoking had no significant effect on the methionine content, but high peroxide fat or sodium ascorbate lowered the methionine content of the raw emulsions, presumably by oxidation. Cooking-smoking acted to restore the methionine level. Interaction of spice and nitrite on methionine levels was significant. Cooking and/or spices reduced the peroxide numbers of the frankfurters.

### INTRODUCTION

THE NUTRITIONAL QUALITY of meat proteins may be affected by the treatment they receive during processing. The methionine of intact proteins is particularly sensitive to changes caused by heat degradation (Shemer and Perkins, 1975; Bender and Husaini, 1976; Waibel et al., 1977), oxidation by hydrogen peroxide (Lipton and Bodwell, 1977; Sjorberg and Bostrom, 1977; Ellinger, 1978), and oxidation by lipid peroxides (Tannenbaum et al., 1969).

Under relatively mild conditions methionine can be oxidized to methionine sulfoxide, but this reaction is reversible both in vitro (Olah et al., 1977; Snow et al., 1976) and in vivo (Ellinger, 1978). Methionine can be partially replaced in the diet by methionine sulfoxide (Ellinger, 1978; Cuq et al., 1978; Miller and Samuel, 1970). Further oxidation of the sulfoxide to the sulfone is not reversible in vivo and can reduce the net protein utilization (Slump and Schreuder, 1973; Miller and Samuel, 1970).

The manufacture of emulsion-type meat products exposes meat proteins to air oxidation. In addition, after emulsification the protein is in intimate contact with peroxidizable lipids and various pro- and antioxidant additives. During the cooking and smoking process the methionyl residues in the protein of this type product may be subjected to oxidation or reduction.

The purpose of this study was to investigate the changes in the methionine content of protein in an emulsion-type product induced by lipid peroxides, additives, and exposure to heat-smoke components.

### MATERIALS & METHODS

BEEF (ungraded chuck) and pork back fat were purchased from commercial sources. The beef and pork fat were ground separately, once through a 3/4-in. plate and once through a 3/16-in. plate, and proximate analyses for fat, protein, and moisture were carried out (AOAC, 1965). All of the ground beef and half of the ground pork fat were divided into 20-lb blocks, packaged in polyethylene bags, and frozen at  $-34^{\circ}\text{C}$ . The remaining pork fat was exposed to air and light at  $13^{\circ}\text{C}$  until the peroxide value of the fat was  $>22$  meq peroxide/kg fat. It was then divided, packaged, and frozen as above.

Four different frankfurter emulsions were prepared each day. Each experimental emulsion contained beef, ice, salt, and sugar. The variables of high or low peroxide fat, spice (Baltimore Spice Mixture FF-3118), sodium nitrite, and/or sodium ascorbate were added in

the amounts shown in Table 1 according to the experimental design.

The frankfurter emulsions were prepared in a model 84145 Hobart Food Chopper. The beef, salt, sugar, most of the ice, and the variables (other than fat) were chopped for 2 min, the pork fat and remaining ice were added, and chopping was continued until the emulsion temperature reached  $15.6^{\circ}\text{C}$  (approx. 10–11 min total time). Samples of the raw emulsions were taken for methionine, peroxide, and Kjeldahl nitrogen determinations. The remaining emulsion was stuffed into #23 NO-JAX casings, linked, cooked, and

Table 1—Formulations used in making raw emulsions and frankfurters in replicates 1 and 2<sup>a</sup>

	Replicate 1 (g)		Replicate 2 (g)	
Beef	881		1042	
Pork fat (high or low peroxide)	625		402	
Ice	368		429	
NaCl	50.2		50.2	
Sucrose	39.6		39.6	
	Levels		Levels	
	0	+	0	+
Spice mixture	0	30.0g	0	30.0g
NaNO <sub>2</sub>	0	.3g	0	.3g
Na ascorbate	0	1.06g	0	1.06g
Peroxide <sup>b</sup>	9.5 <sup>c</sup>	58 <sup>c</sup>	1.1 <sup>c</sup>	28.5 <sup>c</sup>

<sup>a</sup> Based on proximate analysis of beef and pork fat; Final composition to be 11% protein, 29% fat, and 54% moisture

<sup>b</sup> Peroxide contained in pork fat

<sup>c</sup> Milliequivalents peroxide/kg lipid

Table 2—Analysis of variance: Interaction of effects of additives on % methionine in raw emulsions

Source	Error	DF	SS	F
Data from both replicates	Error	48	1.31	
	Total	63	2.70	
	Peroxide (A)	1	0.34	12.64**
	Spice (B)	1	0.08	3.07
	Nitrite (C)	1	0.00	0.00
	Ascorbate (D)	1	0.28	10.38**
	AXC	1	0.01	0.23
	BXC	1	0.20	7.24**
	BXD	1	0.00	0.16
	CXD	1	0.00	0.07
	AXBXD	1	0.02	0.59
	AXBXCD	1	0.00	0.10
	Partial information on the following interactions			
Data from first replicate	Error	16	0.17	
	Total	31	1.39	
	AXD	1	0.01	1.01
	AXBXC	1	0.03	2.66
Data from second replicate	Error	16	0.30	
	Total	31	1.30	
	AXB	1	0.05	2.84
	AXCXD	1	0.05	2.42
	BXCXD—no information—confounded in both replicates			

\*\*  $p \leq 0.01$ .

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smoked to 71.1°C. After cooling, the cooked frankfurters were sampled for chemical analysis as above.

#### Peroxide determination

Total lipids in 5g of meat, pork fat, raw emulsion, or cooked and smoked frankfurter were extracted by the procedure of Maxwell et al. (1978). Peroxide values were determined on the total lipid extract (AOAC, 1965).

#### Methionine determination

Methionine was determined in meat, raw emulsions, and cooked and smoked frankfurters by the method of Lipton and Bodwell (1976, 1977). This procedure utilizes the oxidation-reduction reaction, catalyzed by a halogen hydride, of dimethyl sulfoxide (DMSO) with the thiomethyl residue of intact methionine to release dimethyl sulfide (DMS). This reaction is specific for methionine and does not measure methionine sulfoxide or methionine sulfone.

Five grams of sample were dispersed in 20 ml water with a Brinkman Polytron homogenizer. One ml of the suspension was mixed with 1 ml of 12N HCl and placed in a Wheaton serum bottle (125 or 155 ml total volume). This mixture was frozen on one side of the bottle, and 2 ml of DMSO were frozen on the other side. After being flushed with nitrogen, the bottle was sealed with a rubber septum and crimped locking collar. The samples were thawed and shaken vigorously to insure reagent mixing and were incubated for 30 min with shaking in a 50°C water bath. In contrast to Lipton and Bodwell's (1976, 1977) method, a flame ionization detector was used instead of a flame photometric detector. Only one peak was eluted from the head space of the reaction bottles and this peak corresponded to known DMS. Analysis of DMS in the head space of the sample reaction bottles was carried out on a Nuclear Chicago Selectra System Series 5000 gas chromatograph equipped with a flame ionization detector. A 6 ft × 1/8 in. stainless steel column packed with 10% SE-30 on 80-100 mesh Gas Chrom S was used. Gas flow rates (ml/min) were: helium, 20; hydrogen, 30; and air, 300. Temperatures were 20°C (column), 170°C (injection port), and 260°C (detector). A 500 µl gas-tight Hamilton syringe was used to make 100 µl injections. The average peak height of three injections was converted to picoamperes and then to moles of methionine by reference to methionine standards run the same day. Because this method measures both sulfhydryl groups and methionine residues, the methionine values were corrected for the sulfhydryls present (Lipton and Bodwell, 1976) by repetition of each reaction with 1N HCl in place of the 12N HCl. Peak heights of the DMS generated in the 1N HCl reaction bottles, which measure only sulfhydryls, were subtracted from those generated in the 12N HCl reaction bottles. The spice mixture and the sodium ascorbate used in our study also produced some interference with the methionine determinations. Corrections for these interferences were made where needed by determining the response of aqueous solutions of these two additives singly in the concentrations present in the assay in terms of µmoles methionine. The sodium ascorbate gave a response equivalent to 0.25 µmole methionine in the raw emulsion only, and the spice mixture gave a response equivalent to 0.175 µmole methionine in both the raw emulsions and the cooked and smoked frankfurters. All samples were run in duplicate. The methionine values are reported as % methionine in the protein. Protein concentration of the suspension was measured by the macro-Kjeldahl method (AOAC, 1965).

#### Experimental design and statistical analysis

Four factors (peroxide, spice, sodium nitrite, and sodium ascorbate) were present at two levels. A 2<sup>4</sup> factorial experiment was performed in four blocks of four units. Blocking was by days, so that certain effects were confounded with days. Two replicates of the experiment were performed. The designs (Cochran and Cox, 1957) were chosen for the two replicates so that partial information was obtained on the interactions between peroxide and spices; peroxide and ascorbate; peroxide, spices, and nitrite; and peroxide, nitrite, and ascorbate. The interaction among spices, nitrite, and ascorbate was completely confounded with days. Simple effects and interaction effects were estimated by an analysis of variance and were tested for significance at the p = 0.05 significance level.

## RESULTS & DISCUSSION

EMULSIFICATION of the meat did not cause a significant decrease in the methionine content of either the raw emulsion or the frankfurters (Tables 3 and 5). A decrease was

Table 3—Mean % methionine of raw meat, raw emulsions, and significant interactions of additives on % methionine in raw emulsions

Raw emulsions		Raw meat	
1.20 ± 0.16 <sup>b</sup>		1.24 ± 0.27 <sup>a</sup>	
Simple effects			
Peroxide		Ascorbate	
0 <sup>c</sup>	+ <sup>d</sup>	0 <sup>c</sup>	+ <sup>d</sup>
1.27 <sup>e</sup>	1.12 <sup>e</sup>	1.26 <sup>e</sup>	1.12 <sup>e</sup>
Double Interaction			
Nitrite			
0 +			
Spice 0			
+ +	1.29 <sup>a</sup>	1.18 <sup>a</sup>	
	1.10 <sup>a</sup>	1.22 <sup>a</sup>	

a N = 16

b N = 64

c 0—variable not present or present at low levels

d +—variable present at high level

e N = 32

Table 4—Analysis of variance: Interaction of effects of additives on % methionine in frankfurters

	Source	DF	SS	F
Data from both replicates	Error	48	2.74	
	Total	63	4.89	
	Peroxide (A)	1	0.22	3.94
	Spice (B)	1	0.13	2.20
	Nitrite (C)	1	0.00	0.03
	Ascorbate (D)	1	0.08	1.36
	AXC	1	0.01	0.17
	BXC	1	0.35	6.08*
	BXD	1	0.04	0.63
	CXD	1	0.01	0.25
	AXBXD	1	0.10	1.76
	AXBXCXD	1	0.02	0.38
	Partial information on the following interactions			
Data from first replicate	Error	16	0.23	
	Total	31	3.00	
	AXD	1	0.00	0.09
	AXBXC	1	0.02	1.47
Data from second replicate	Error	16	0.27	
	Total	31	1.90	
	AXB	1	0.00	0.25
	AXCXD	1	0.04	2.51
	BXCXD — no information — confounded in both replicates			

\* p ≤ 0.05.

Table 5—Mean % methionine of cooked and smoked frankfurters and significant interactions of additives on % methionine in frankfurters

Frankfurters	
1.25 ± 0.24 <sup>a</sup>	
Double interaction	
Nitrite	
0 <sup>b</sup> + <sup>c</sup>	
Spice 0	
+ +	1.23 <sup>d</sup>
	1.37 <sup>d</sup>
	1.29 <sup>d</sup>
	1.13 <sup>d</sup>

a N = 64

b 0—variable not present or present at low level

c +—variable present at high level

d N = 16

**Table 6—Analysis of variance: Interaction of effects of additives and heat-smoke component on % methionine**

Source	DF	SS	F
Error	96	4.04	
Total	127	7.69	
Peroxide (A)	1	0.01	0.15
Spice (B)	1	0.21	4.90*
Nitrite (C)	1	0.00	0.02
Ascorbate (D)	1	0.03	0.76
Heat-smoke (E)	1	0.11	2.60
AXC	1	0.00	0.00
AXE	1	0.56	13.35**
BXC	1	0.01	0.25
BXD	1	0.03	0.77
BXE	1	0.00	0.05
CXD	1	0.00	0.07
CXE	1	0.00	0.02
DXE	1	0.33	7.79**
AXBXD	1	0.10	2.33
AXBXE	1	0.05	1.20
AXCXE	1	0.02	0.37
AXDXE	1	0.09	2.17
BXCXE	1	0.53	12.66**
BXDXE	1	0.01	0.18
CXDXE	1	0.01	0.31
AXBXCXD	1	0.01	0.11
AXBXCXE	1	0.00	0.01
AXBXDXE	1	0.02	0.43
AXCXDXE	1	0.01	0.22
BXCXDXE	1	0.02	0.55
AXBXCXDXE	1	0.02	0.46

The following interactions were confounded in either one or both replicates: AXB, AXD, AXBXC, AXCXD, BXCXD.

\*  $p \leq 0.05$   
 \*\*  $p \leq 0.01$

**Table 7—Significant interactions of additives on % methionine. Heat-smoke components cooking additional factor**

Double Interactions					
Peroxide			Ascorbate		
	0 <sup>a</sup>	+ <sup>b</sup>		0	+
Raw	1.27 <sup>c</sup>	1.12 <sup>c</sup>	Raw	1.26 <sup>c</sup>	1.12 <sup>c</sup>
Cooked	1.20 <sup>c</sup>	1.31 <sup>c</sup>	Cooked	1.22 <sup>c</sup>	1.29 <sup>c</sup>
Triple Interaction					
Raw Nitrite			Cooked Nitrite		
	0	+		0	+
Spice 0	1.29 <sup>d</sup>	1.18 <sup>d</sup>	Spice 0	1.23 <sup>d</sup>	1.37 <sup>d</sup>
+	1.10 <sup>d</sup>	1.22 <sup>d</sup>	+	1.29 <sup>d</sup>	1.13 <sup>d</sup>

<sup>a</sup> 0—variable not present or present at low levels  
<sup>b</sup> +—variable present at high level  
<sup>c</sup> N = 32  
<sup>d</sup> N = 16

**Table 8—Effect of additives on peroxide numbers of emulsions and cooked and smoked frankfurters<sup>a</sup>**

	Emulsion		Frankfurter	
	0 <sup>b</sup>	+ <sup>c</sup>	0	+
Peroxide	3.9 <sup>d</sup>	25.3*	3.2	9.8*
Spice	18.3	11.0*	8.1	5.2
Nitrite	13.8	15.4	6.2	6.8
Ascorbate	14.1	15.1	7.6	5.5

\*  $p < 0.01$ —simple effect of additive changes the peroxide number of emulsion or frankfurter  
<sup>a</sup> Data from replicate 2 only  
<sup>b</sup> 0—variable not present or present at low level  
<sup>c</sup> +—variable present at high level  
<sup>d</sup> Mean milliequivalent of peroxide/kilogram of lipid; N = 8

expected because air is incorporated during emulsification; however, the conditions were not sufficiently severe to cause significant oxidation of methionine.

The effect of additives on the methionine contents of raw emulsions was varied. In raw emulsions (Tables 2 and 3) a significant interaction was discovered between spice and nitrite. When either nitrite or spice was added to the emulsion preparation, there was a decrease in the methionine content; however, when spice and nitrite were added in combination, the magnitude of the change decreased.

In raw emulsions high peroxide fat significantly decreased the methionine content. This was expected inasmuch as lipid peroxides oxidize the methionine to methionine sulfoxide (Tannenbaum et al., 1969). Sodium ascorbate also significantly lowered the methionine content. This would seem to indicate that sodium ascorbate acts as a prooxidant rather than as an antioxidant. The lowered methionine content could also be caused by an experimental artifact. Because ascorbate reduced DMSO to DMS, a correction of 0.25  $\mu$ mole for ascorbate was included in the calculations in determining the methionine content of raw emulsions containing sodium ascorbate. Lipton and Bodwell's (1976) data suggest that 0.1  $\mu$ mole of methionine is the correction needed. If Lipton and Bodwell's correction is used instead of the experimentally determined correction, the sodium ascorbate effect is not significant. The size of the correction has no effect on any of the other variables.

In cooked-smoked frankfurters (Tables 4 and 5) the only significant interaction was between spice and nitrite. The presence of both spice and nitrite tended to decrease the methionine content significantly.

The effect of heat-smoke components was investigated by imposing heat-smoke components on the experimental design described in the experimental section. This resulted in a 2<sup>5</sup> factorial experiment arranged in four blocks of eight units. The same interactions were confounded as both levels of the additional factor were present in each block an equal number of times. Significant interactions were found between peroxide and heat-smoke components; ascorbate and heat-smoke components; and spice, nitrite, and heat-smoke components (Table 6). When raw emulsions containing either spice or nitrite were cooked and smoked, the methionine content was increased, but when raw emulsions containing both spice and nitrite were cooked and smoked the methionine content was decreased (Table 7). The methionine content was decreased in emulsions made with high peroxide fat, but when these emulsions were cooked and smoked the methionine content was increased. High peroxide fat oxidizes methionine to methionine sulfoxide in an emulsion system. Heat plus smoke components, however, caused a reduction of the methionine sulfoxide to methionine. Sodium ascorbate seems to behave similarly to high peroxide fat, but an accurate interpretation of this phenomenon is complicated by ascorbate reducing DMSO to DMS.

Spices reduce the peroxide content of the raw emulsions significantly (Table 8). The peroxide-spice interaction for methionine content was not significant, but this interaction was confounded in one replicate and had a  $p = 0.11$  in the other replicate. Heat-smoke components significantly lowered the peroxide number. Raw emulsions contained a mean 14.6 milliequivalents of peroxide per kilogram lipid while the frankfurter contained a mean 6.5 milliequivalents of peroxide per kilogram lipid.

## CONCLUSION

THE METHIONINE CONTENT of raw emulsions and frankfurters is not affected by the emulsification procedure. The methionine content of raw emulsions is decreased by high peroxide fats due to oxidation of methionine to methionine sulfoxide. Sodium ascorbate may act as a pro-

oxidant towards methionine in the emulsions, but the results are complicated by experimental difficulties. Spice lowers the lipid peroxides in an emulsion, thus possibly decreasing the methionine loss. Nitrite alone has little or no effect on either the peroxides or methionine content. Spice and nitrite, however, interact with each other to lower the methionine content of frankfurters. The heat and smoke component does not affect the methionine content but does lower the peroxide number significantly.

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