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Effects of Types I, II and III Antioxidants on Phospholipid Oxidation in a Meat Model for Warmed Over Flavour

J. P. Roozen*

USDA–ARS–SRRC, PO Box 19687, 1100 Robert E. Lee Blvd,
New Orleans, LA 70179, USA

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ABSTRACT

Freeze-dried, washed ground meat was mixed with lecithin and antioxidants dissolved in water, heated and then stored in the refrigerator in order to study phospholipid oxidation as related to warmed over flavour. Type I and II antioxidants decreased the amount of thiobarbituric acid reactive substances, while most Type III's gave an increase, depending on their concentration. Similar effects on phospholipid oxidation were detected with capillary gas chromatography for representatives of each type of antioxidant, i.e. butylated hydroxy anisole, ethylenediaminetetraacetic acid, sodium bisulfite, cysteine and water activity regulators.

INTRODUCTION

Flavour becomes a critical property of food when it differs from that expected by consumers; e.g. they will not accept the warmed over meat flavour. This presents a challenge for the producers of precooked meat dishes in comparison to freshly cooked products. Warmed over flavour (WOF) was first studied by Timms & Watts (1958) who defined it as the rapid onset of rancidity in cooked meat during refrigerated storage. It has become clear that peroxidation of phospholipids is the major process for generating WOF (Pearson & Gray, 1983). Heme and non-heme iron are involved as transition metals (complexes), which abstract electrons and so produce radicals for initiation or propagation of peroxidation reactions of

* Present address: Agricultural University, Department of Food Science, Biotechnion, De Dreyen 12, 6703 BC Wageningen, The Netherlands.

unsaturated lipids (Pryor, 1976). Cooking of meat changes the status of iron by denaturation of muscle proteins and increases the amount of available phospholipids by disrupting the membranes (Erickson *et al.*, 1971; Sato & Hegarty, 1971). These processes are difficult to stop, so prevention of WOF seems best achieved only by using antioxidants.

Labuza (1971) divided the antioxidants into three categories: Type I: free radical stoppers, primarily phenolic type compounds, which can donate an electron to a radical, Type II: free radical production preventers, mostly chelating agents, which can tie up transition metals and Type III: environmental factors such as redox compounds and water activity regulators.

The present study uses a meat model system plus lecithin in which representatives from the three types of antioxidant are tested for their effectiveness in preventing peroxidation as analysed by the thiobarbituric acid reaction and direct capillary gas chromatography.

MATERIALS AND METHODS

Materials

Two batches of commercial beef top round roast were purchased: 70 g on November 21, 1985, and 350 g on January 27, 1986 (after 16 days' storage at 1°C) and ground in a kitchen grinder through a plate with holes 3 mm in diameter. To each batch an equivalent volume of deionised water was added, the slurry mixed thoroughly and, after settling, decanted. This washing was repeated 40 times at room temperature, then the batch was left overnight in the refrigerator and the next day the washing procedure was continued 60 more times to facilitate complete removal of water-soluble material and floating particles (mainly tallow). The washed residue was frozen in glass containers and freeze dried during 48 h (FTS Systems, Multidry). The yields from the 70 g and 350 g batches of meat were 7·3 g freeze-dried, washed ground meat (FWGM) and 35·7 g new-FWGM (NFWGM), respectively. Total iron contents of both batches were determined with the X-ray fluorescence method using 300 ppm Fe NBS 'Orchard leaves' as a standard. FWGM contains 14·2 ppm Fe and NFWGM 43·7 ppm Fe. The lecithin (LEC) used was vegetable lecithin from Nutritional Biochem. Co (12-803).

The Type I antioxidants used were: butylated hydroxy toluene (BHT) from Sigma (B-1378), butylated hydroxy anisole (BHA) from Sigma (B-1253), DL-α-tocopheryl acetate (TOCO) from Gibco (850-3600), *N*-propylgallate (PGAL) from Sigma (P-3130) and tert-butylhydroquinone (TBHQ) from Kodak (Tenox). The Type II antioxidants were: disodium salt

of ethylenediaminetetraacetic acid (EDTA) from Sigma (ED2SS), trisodium salt of citric acid, dihydrate (Citrate) from Sigma (C-7254) and pentasodium tripolyphosphate (TPP) from FMC (food grade granular). The Type III antioxidants used were: sodium salt of L-ascorbic acid (Ascorbate) from Sigma (A-7631), sodium bisulfite ($NaHSO_3$) from Mallinckrodt (7448), DL-cysteine (CYS) from Sigma (C-4022), Maillard compounds (Mail) formed during heating (30 min at 120°C) of 5000 ppm glycine (MCB) and 5000 ppm α-D(+)-glucose (Sigma, G-5000), L-histidine (HIS) from Sigma (H-8125), L-ascorbyl-palmitate (ASCPAL) from ICN, K&K Labs, magnesium L-ascorbate-2-phosphate (ASC2PP) and tetrasodium L-ascorbate-2-triphosphate (ASC2TPP) by courtesy of Dr P. A. Seib, Department of Grain Science and Industry, Kansas State University.

For distillation and thiobarbituric acid (TBA) reaction a Kontes microdistillation apparatus, boileezers (Fisher Scientific B-365) and antifoam B silicone emulsion (Baker B531-5) were used. The TBA reagent was 0·02M 4,6-dihydroxy-2-mercaptopyrimidine (Aldrich 11,350-6) in 90% acetic acid (Fisher Scientific A-38). The 'TBARS' standard was malonaldehyde bis(dimethyl acetal), 98% from Aldrich (10,838-3). Spectra were obtained with an HP 8450A Diode Array Spectrophotometer.

Relative humidities were controlled by Drierite (Hammond Drierite Company) and saturated salt solutions of $MgCl_2$ (J. T. Baker 1-2444) and NaCl (Sigma S-9625). Volatiles were analysed by gas chromatography on a HP Ultra2 fused silica capillary column (50 m × 0·32 mm inside diameter) crosslinked with 0·52 μm film of 5% Ph Me silicone, using an HP 5880A instrument equipped with a heated FID and an External Closed Inlet Device from Scientific Instrument Systems, River Ridge, LA, (ECID, 185°C and valve temperature, 210°C; Legendre *et al.*, 1979). Gases used were obtained from Union Carbide: nitrogen as the carrier gas (1 ml/min), make-up gas (32·5 ml/min), as well as sample drying gas and ECID injection gas; flow rates for hydrogen and air were 31 and 430 ml/min, respectively. The glass ECID cartridges (9 mm × 85 mm) were plugged with sample (mixed with sand: purified, Baker 3382), glass wool and Tenax-GC 60/80 mesh (2,6-diphenylene oxide, TekLab, T-0999).

Sample preparation

For each model system 100 mg FWGM (or NFWGM) was weighed into a 12 × 75 mm culture tube (RTU 7813) and 0·4 ml solution added, in which 3·5 mg LEC and the additive (amounts given in Tables 2 and 3—see below) were dissolved. Each tube was closed with Parafilm (PM-992), mixed on a Vortex-Genie (Scientific Products), left for 1 h at room temperature, incubated at 70°C for 5 min and afterwards at 5°C for 40 h or 64 h.

Analysis of thiobarbituric acid reactive substances (TBARS)

This was based on the method of Tarladgis *et al.* (1960).

Distillation
0·5 g of sample was quantitatively transferred into a 25-ml roundbottom flask by rinsing with 15 ml of deionised water. Twenty-five drops of 5N HCl were added to lower pH below 2 and eight drops of antifoam and a few boiling chips were added for smooth distillation. The roundbottom flask and a graduated centrifuge tube were connected to the still and 10·0 ml distillate were obtained in about 20 min.

TBA-reaction
The distillate was mixed well and 5 ml pipetted in a 15 ml KIMAX tube, to which 5 ml TBA-reagent were added. The tube was closed with a Teflon lined screw cap, well mixed and immersed in a boiling water bath for 30 min together with a reagent blank. Then the sample was cooled in tap water for 10 min and its absorption spectrum recorded from 420 to 600 nm. Means and standard deviations of triplicates of absorbances at 532 nm were calculated and subjected to statistical analysis (Snedecor, 1946).

Gas chromatography

After incubation, the sample was mixed with 1 g of sand and transferred into a glass cartridge between two glass wool plugs. Then about 50 mg Tenax were added on top of one of the plugs and held in place by another glass wool plug. Afterwards the cartridge with the Tenax at the far end was dried for 2 h in a slow stream of nitrogen gas (about 380 mg weight loss). The cartridge was placed in the ECID with the Tenax facing the incoming nitrogen stream. The ECID was then turned in its injection mode for 3 min, during which the column oven temperature was held at 35°C (initial hold period). The injector was run in the split mode (ratio 1:5) at 210°C. After 3 min initial holding the oven temperature was programmed at 5°C/min to 210°C and 10 min post time at 250°C. The FID was set at an attenuation of 10^{-4} AFS and a temperature of 260°C. Peak integration was performed on an HP Lab Automation System 3356 and chromatograms were plotted on an HP 7221 T Plotter.

RESULTS

Table 1 shows that TBARS are present in freeze-dried, extensively washed ground meat samples, which have been rehydrated, heated and stored in the

TABLE 1

Thiobarbituric Acid Reactive Substances Formed in a Rehydrated and Mixed Model Systems[a] after Heating (5 min at 70°C) and Storage (40 h at 5°C).

System[b]	*pH*	*Absorbance (532 nm)*[c]	*TBARS (ppm)*
FWGM	6·5	0·070 ± 0·014	2·9
LEC	6·4	0·014 ± 0·002	0·6
FWGM–LEC	6·5	0·182 ± 0·016	7·6

[a] 100 mg FWGM + 3·5 mg LEC dissolved in 0·4 ml water.
[b] Explanation of codes in 'Materials'.
[c] Mean and standard deviation of triplicates.

refrigerator. It also demonstrates that the addition of dissolved lecithin contributes much more to the amount of TBARS detected than just its original content. This makes it an interesting model system for studying the effects of potential oxidation-retarding additives ('antioxidants') on the formation of TBARS. The various treatments had only a minor influence on the pH of the models: max. 0·6 increase (Table 2), except for TPP which affected pH.

Types I and II antioxidants lowered, and type III increased, the

TABLE 2

Effect of Antioxidants[a] on the Formation of Thiobarbituric Acid-Reactive Substances in Meat Model Systems, Which Have Been Heated (5 min at 70°C) and Stored (40 h at 5°C).

Antioxidant	*Type*	*Amount (ppm)*	*pH*	*ABS. (532 nm)*[b]	*D.ABS.*[c]
Control[d]	—	—	6·5	0·236 ± 0·018	—
TOCO	I	200	6·5	0·215 ± 0·016	−0·021
BHA	I	200	6·5	0·143 ± 0·007	−0·093*
BHT	I	200	6·5	0·176 ± 0·005	−0·060*
TBHQ	I	200	6·5	0·081 ± 0·021	−0·155*
PGAL	I	200	6·5	0·152 ± 0·010	−0·084*
EDTA	II	2400	6·5	0·181 ± 0·001	−0·055*
Citrate	II	5000	6·5	0·173 ± 0·013	−0·063*
TPP	II	5000	7·1	0·185 ± 0·016	−0·051*
$NaHSO_3$	III	2000	6·5	0·298 ± 0·025	+0·062*
Mail	—	—	6·5	0·239 ± 0·003	+0·003
HIS	—	5000	6·5	0·242 ± 0·006	+0·006

[a] Explanation of codes in 'Materials'.
[b] Mean and standard deviation of triplicates.
[c] D.ABS. = Absorbance of sample − Absorbance of control; * significant difference ($p < 0·05$).
[d] Mixture of 100 mg NFWGM + 3·5 mg LEC dissolved in 0·4 ml water; significantly different ($p < 0·05$) from the FWGM (Table 1) and 64 h storage (Table 3) controls.

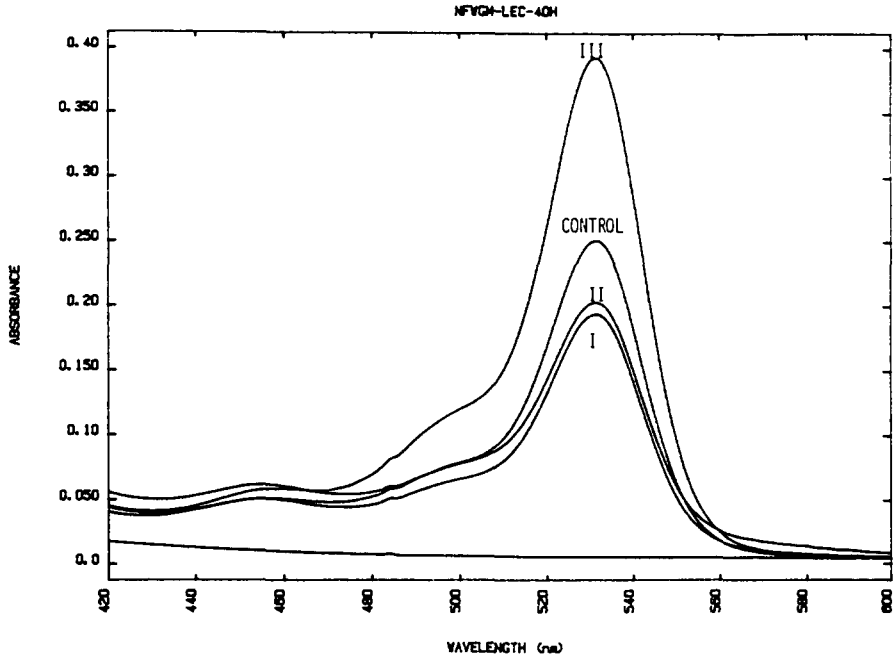

Fig. 1. Absorption spectra of compounds formed during the TBA-reaction with distillates from the 40 h NFWGM-LEC model system (control), and from control + antioxidants Types I, II and III. The examples shown are: I = +200 ppm BHT, II = +5000 ppm TPP, and III = +200 ppm ascorbate.

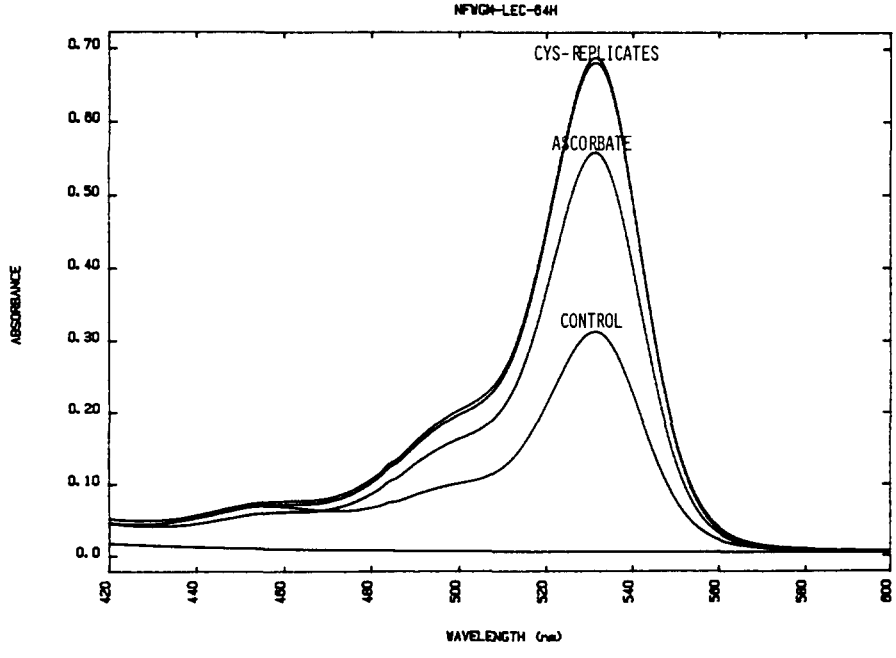

Fig. 2. Absorption spectra of 'TBARS' from 64 h NFWGM-LEC model system (control), and from control + 200 ppm ascorbate; control + 5000 ppm CYS.

TABLE 3

Effect of Cysteine, Ascorbate and its Derivatives on the Formation of Thiobarbituric Acid-Reactive Substances in Meat Model Systems, Which Have Been Heated (5 min at 70°C) and Stored (64 h at 5°C).

Addition[a]	*Amount (ppm)*	*pH*	*ABS. (532 nm)*[b]	*D.ABS.*[c]
Control[d]	—	6·5	0·284 ± 0·015	—
Ascorbate	200	6·5	0·565 ± 0·010	+0·281*
Ascorbate	5000	6·7	0·269 ± 0·016	−0·015
ASC2PP	200	6·6	0·534 ± 0·024	+0·250*
ASC2PP	5000	7·0	0·232 ± 0·029	−0·052*
ASCPAL	200	6·6	0·265 ± 0·010	−0·019
ASCPAL	5000	6·5	0·166 ± 0·020	−0·118*
ASCTPP	200	6·7	0·353 ± 0·028	+0·069*
ASCTPP	5000	7·0	0·201 ± 0·015	−0·083*
CYS	5000	6·5	0·670 ± 0·009	+0·386*

[a] Explanation of codes in 'Materials'.

[b] Mean and standard deviation of triplicates.

[c] D.ABS. = Absorbance of sample − Absorbance of control; * significant difference ($p < 0·05$).

[d] Mixture of 100 mg NFWGM + 3·5 mg LEC dissolved in 0·4 ml water.

absorbance spectra in comparison to the control (Fig. 1). In most cases the differences are significant for the maximum absorbances at 532 nm (Table 2). A longer time in the refrigerator (64 h) enhances the maximum absorbance of the control significantly. This condition (64 h at 5°C) has been used for comparison of low (200 ppm) and high (5000 ppm) amounts of ascorbate and its derivatives (Table 3). The differences in absorbances between control and treated samples were positive, or not significant, for the lower amounts of antioxidant and they were negative, or not significant, for the high amounts. In contrast, the equivalent high amounts of CYS gives a substantial increase (Table 3), even bigger than the low amount of ascorbate (Fig. 2). Addition of the antioxidants to the distillation mixture of the control had no influence on the absorbance measured.

The model systems were made suitable for direct capillary gas chromatography by mixing with sand and drying in a stream of nitrogen. Tenax prevented the escape of volatiles related to lipid oxidation (Suzuki & Bailey, 1985). Preliminary experiments showed that, for the ECID, 185°C is an acceptable compromise for getting the volatiles released from sample, Tenax, and labile lipid oxidation products, without generating them extensively from other degradation reactions.

The profile of the control is represented in Fig. 3, and the areas of the 30 main peaks are given in Table 4. During the first 15 min the peaks consist of

TABLE 4

Effect of Antioxidants on Areas of Major Peaks in Gas Chromatograms from Meat Model Systems, Which Have Been Heated (5 min at 70°C) and Stored (64 h at 5°C). Means of Replicates

Retention time (range in min)	*Area (Computer counts)*						
	Control[a]			*BHA*[a]	*EDTA*[a]	*$NaHSO_3$*[a]	*CYS*[a]
	Mixed	*Heated*	*Stored*[b]				
3·86– 4·41	1 511	2 916	4 477 ± 908	2 650	2 247	1 129	3 857
4·57– 4·69	62	2 514	2 093 ± 532	1 061	2 061	1 900	4 578
4·95– 5·06	2 091	4 538	4 220 ± 145	4 063	3 797	3 106	6 612
5·27	—	—	—	—	—	1 169	—
5·60– 5·69	660	2 314	2415 ± 72	2 070	2 347	2 267	4 488
6·50– 6·62	3 236	5 442	5 002 ± 572	2 369	4 815	2 640	2 738
6·74– 6·76	—	—	—	—	—	1 443	4 825
7·46– 7·59	5 976	6 941	6 609 ± 301	6 298	6 254	5 515	8 519
8·30– 8·61	—	—	8 164 ± 716	—	—	523	1 096
9·13	—	—	—	—	—	3 459	—
9·38– 9·58	—	5 298	—	—	6 225	1 766	15 040
9·83–10·00	3 450	5 222	5 080 ± 315	3 412	3 023	4 185	7 076
10·95–11·03	5 142	6 251	6 667 ± 353	4 805	5 771	4 852	7 446
12·37–12·48	223	401	209 ± 92	331	243	232	1 092
13·38–13·51	197	506	511 ± 81	526	531	496	944
13·67–13·82	961	986	1 076 ± 155	1 130	1 082	1 007	4 795
14·56–14·63	8 136	7 863	9 633 ± 793	7 579	8 667	7 348	20 692
15·76–15·88	161	305	433 ± 79	150	276	124	—
16·17–16·42	133	174	—	199	154	82	46 523
17·38–17·48	—	—	—	—	—	44	667
17·69–17·88	420	742	717 ± 79	616	814	474	2369
18·20–18·34	1 393	1472	1 719 ± 294	1 701	1 759	1 032	2 811
18·60–18·80	104	220	296 ± 65	317	213	167	1 237
19·02	—	—	—	—	—	—	414
19·10–19·22	128	202	—	177	186	115	334
19·51–19·68	336	444	493 ± 54	342	427	535	975
19·71–19·79	—	—	—	179	204	163	1 096

19·95–20·05	—	—	—	—	—	83	1 038
20·90–21·08	373	537	391 ± 12	510	385	230	1 471
21·42	—	—	—	—	—	—	500
21·59	—	—	—	—	—	—	484
21·76–21·91	558	559	653 ± 92	417	605	522	2 152
22·19–22·26	306	320	1 135 ± 145	972	624	114	—
23·22	—	—	—	—	—	—	370
24·39–24·50	—	—	—	—	—	322	613
24·91–25·01	195	190	179 ± 24	215	201	146	—
25·12–25·27	1 209	1 017	1 200 ± 136	1 061	1 011	864	2 767
25·40–25·42	—	—	—	—	—	210	236
25·70–25·80	—	—	—	—	—	34	239
26·08	—	—	—	—	—	—	215
26·58–26·68	—	—	—	—	—	64	1 139
27·01–27·14	395	171	424 ± 164	305	412	463	647
27·53–27·61	—	—	—	—	—	848	680
28·37–28·52	143	171	231 ± 17	164	155	108	420
29·98	—	—	—	—	—	—	5 168
30·37–30·52	638	236	595 ± 96	381	397	408	577
30·90–31·05	595	181	478 ± 99	342	345	392	519
31·29–31·43	123	96	190 ± 61	125	148	148	217
31·57–31·72	—	—	—	—	—	33	388
32·99–33·10	—	—	—	—	—	33	370
33·28	—	—	—	—	—	256	—
34·04–34·18	72	205	274 ± 57	44 343[c]	456	123	355
35·87	—	—	—	—	—	—	271
36·87–37·02	103	126	291 ± 76	168	197	177	224
Total area (TA)	39 030	58 560	65 855 ± 3 186	95 160	49 807	46 351	171 284
TA of matching peaks	38 035	51 866	55 670	42 743	47 706	35 197	89 223
TA of $NaHSO_3$/CYS matching peaks						41 083	163 862

— Not present (area < 25 computer counts).

[a] Mixture of 100 mg NFWGM + 3·5 mg LEC dissolved in 0·4 ml water; explanation of codes in 'Materials'; amounts added as in Tables 2 and 3.

[b] Mean and standard deviation of triplicates.

[c] Mean of BHA-peaks: 44 200 and 44 487.

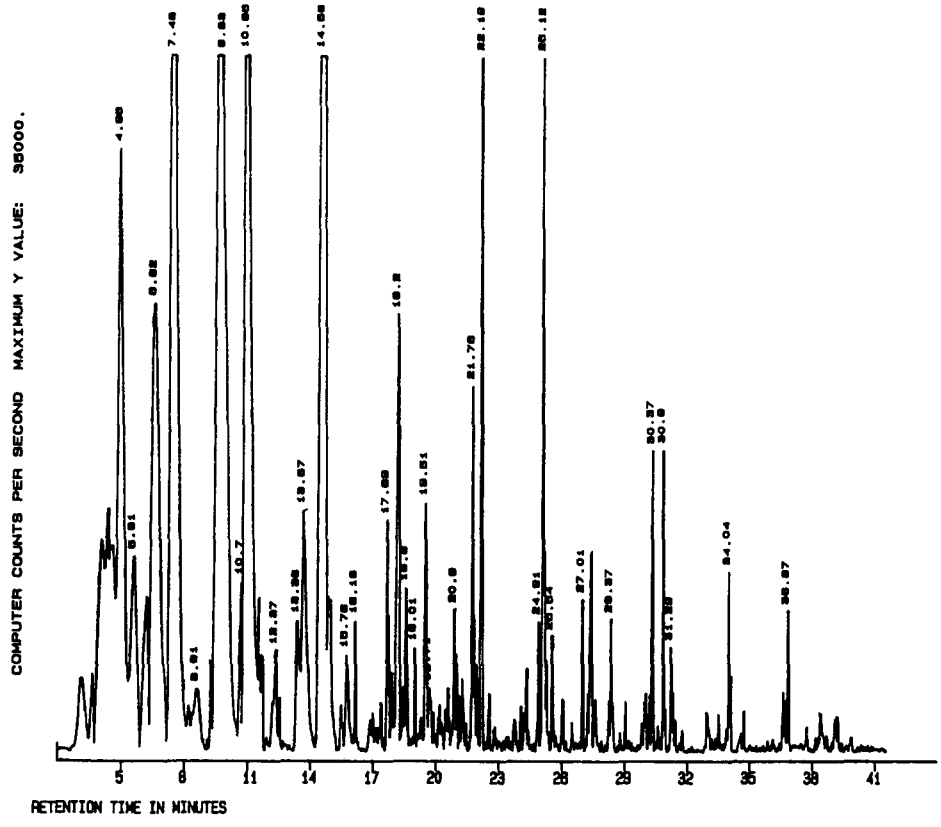

Fig. 3. Direct sampling capillary gas chromatography of volatiles from NFWGM-LEC model system (control), which has been heated (5 min at 70°C) and stored (64 h at 5°C).

TABLE 5

Formation of Volatiles in N_2-Dried Mixtures of Sand and NFWGM-LEC During Storage at Different Relative Humidities (rH) and 5°C for 8 Days. (Means of Replicates; ECID at 120°C during Gas Chromatography Injection)

rH Control	*rH*[a] (%)	*Moisture content*[b] (% *w/w NFWGM*)	*Total area* (*computer counts*)
Drierite	1	1	25 540
$MgCl_2$	33	6	17 304
NaCl	75	20	1 264

[a] Labuza *et al.* (1976).

[b] Calculated from $\frac{\text{(weight rH equilibrium} - \text{weight dry) sample}}{\text{weight NFWGM}} \times 100\%$.

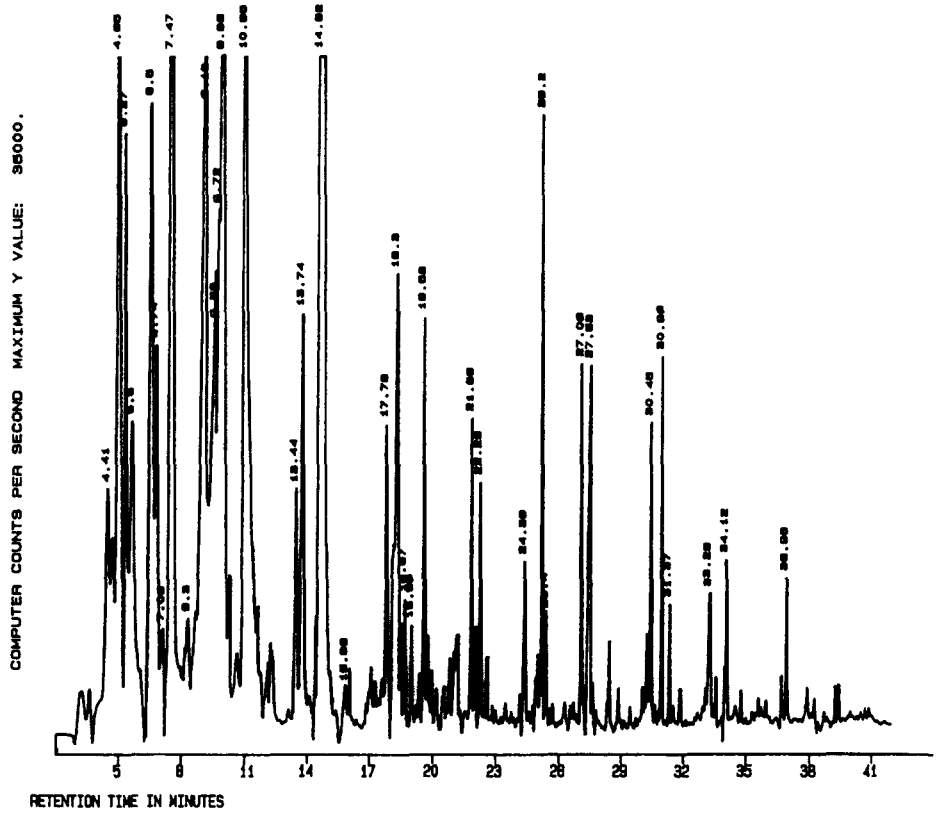

Fig. 4. Gas chromatogram of volatiles obtained from control + 2000 ppm $NaHSO_3$. Conditions as in Fig. 3.

several compounds, mainly due to the relatively long duration of the injection. From the different steps involved in the production of the control sample, heating contributes predominantly to the areas of the more volatile compounds, while storage also contributes to the less volatile ones. Thus, each step increases the total area of the control. Additives such as $NaHSO_3$ (Fig. 4) have lower total areas than the control sample with CYS as an exception because it produced several additional peaks (Fig. 5). The influence of moisture content or water activity on the formation of the more volatile compounds is given in Table 5: the lower the moisture content, the higher the amount of volatiles.

DISCUSSION

The oxidation of phospholipids is strongly related to WOF development in meat (Wilson *et al.*, 1976). Cooking enhances the availability of

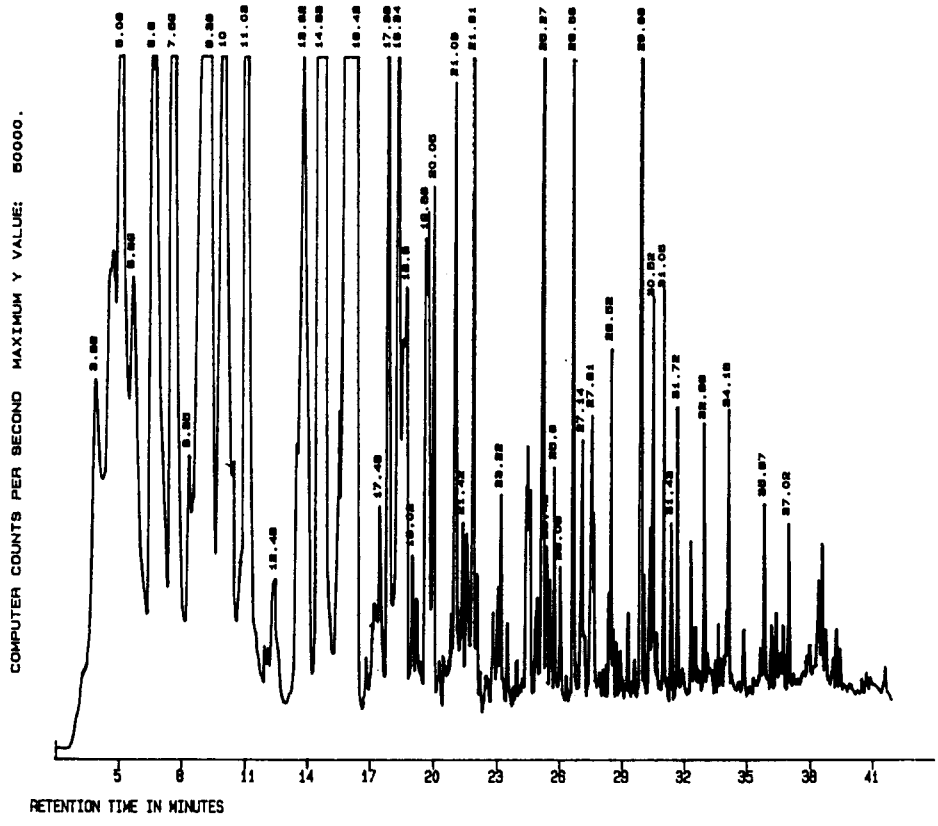

Fig. 5. Gas chromatogram of volatiles obtained from control + 5000 ppm CYS. Conditions as in Fig. 3.

phospholipids for oxidation by destroying cell membranes (Igene & Pearson, 1979). In the present study the availability of phospholipids is secured by addition of LEC at levels equivalent to that analysed in meat (0·7%; Wilson *et al.*, 1976). As shown in Table 1, LEC addition promotes TBARS formation and creates an interesting model system for analysing the effects of antioxidants on phospholipid oxidation in meat.

In the model systems, one can expect to have peroxides (including H_2O_2) from (N)FWGM as well as from LEC (Halliwell & Gutteridge, 1984). During mixing and heating, these peroxides oxidise the heme group of residual (met)myoglobin. The result is either an increase in non-heme iron (Pearson & Gray, 1983) or formation of activated metmyoglobin (MetMgB; Kanner & Harel, 1985). In both cases lipid peroxidation will be increased by propagation reactions. There is probably no major role for singlet oxygen since the data indicate that its scavenger HIS has no effect on TBARS formation (Table 2; Kanner & Harel, 1985). Thus, initiation reactions are only possible with activated MetMgB, the nature of which

remains unresolved. MetMgB is proposed as an intermediate with two equivalents oxidised above the ferric(Fe^{3+})heme resting state, giving a formal oxidation state of Fe^{5+} and a resonance structure $X^{\cdot d+}{=}Fe^{4+}{=}O$. The reaction scheme for autoxidative lipid peroxidation by MetMgB is as follows:

* MetMgB-activation

$$\underset{\text{ferric } (Fe^{3+})\text{heme}}{[\text{MetMgB}]^{3+}} + \text{HOOH} \rightarrow [\text{MetMgB—HOOH}]^{3+} \rightarrow \underset{\text{oxyferryl}(Fe^{5+})\text{heme}}{[\text{MetMgB}{=}\text{O}]^{3+}} + H_2O$$

* initiation

$$\underset{\text{oxyferryl}(Fe^{5+})\text{heme}}{[\text{MetMgB} = \text{O}]^{3+}} + \text{RH} \rightarrow \underset{\text{hydroxyferryl}(Fe^{4+})\text{heme}}{[\text{MetMgB} - \text{OH}]^{3+}} + \text{R}^{\cdot}$$

* propagation

$$\text{R}^{\cdot} + O_2 \rightarrow \text{ROO}^{\cdot}$$

$$\text{ROO}^{\cdot} + \text{RH} \rightarrow \text{ROOH} + \text{R}^{\cdot}$$

$$\underset{\text{hydroxyferryl}(Fe^{4+})\text{heme}}{[\text{MetMgB} - \text{OH}]^{3+}} + \text{ROOH} \rightarrow \underset{\text{ferric}(Fe^{3+})\text{heme}}{[\text{MetMgB}]^{3+}} + \text{ROO}^{\cdot} + H_2O$$

Being a transition metal, non-heme iron decomposes peroxides according to the Haber–Weiss cycle (Mead, 1976):

$$Fe^{2+} + \text{ROOH} \rightarrow Fe^{3+} + OH^{-} + \text{RO}^{\cdot}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + H^{+} + HO_2^{\cdot}$$

$$\text{RO}^{\cdot} + \text{ROOH} \rightarrow \text{ROH} + \text{ROO}^{\cdot}$$

$$\text{ROO}^{\cdot} + \text{RH} \rightarrow \text{ROOH} + \text{R}^{\cdot}$$

$$\text{R}^{\cdot} + O_2 \rightarrow \text{ROO}^{\cdot}$$

Since there is no difference in the reaction products formed in both schemes, it makes it difficult to decide in favour of one of them. Furthermore, the presence of an initiation reaction does not help because radicals are readily formed by degradation of peroxides. Heating stimulates this by thermal homolysis of peroxides (Hiatt & Irwin, 1968):

$$\text{ROOH} \rightarrow \text{RO}^{\cdot} + \text{OH}^{\cdot},$$

or by bimolecular decomposition:

$$2\text{ROOH} \rightarrow \text{RO}^{\cdot} + \text{ROO}^{\cdot} + H_2O.$$

The problem of catalysis of lipid oxidation by heme iron versus non-heme iron is also not solved by the existing methods of analysis (Igene *et al.*, 1979; Schricker *et al.*, 1982; Chen *et al.*, 1984), which are based on differences in

solubility of iron in its different forms. It is not clear if the solvent qualities used are able to solubilize iron bound in oxyferryl(Fe^{5+})heme. Probably the conclusion of Liu & Watts (1970) is still valid; namely, that both heme and non-heme iron are important catalysts in cooked meat.

Different types of antioxidants have been used in the model systems to retard or prevent lipid oxidation (Table 2 and 3). Their effects have been analysed by TBARS analysis and gas chromatography. TBARS analysis involves acid decomposition of hydroperoxides during distillation, while, during ECID injection in the gas chromatograph, hydroperoxides are thermally decomposed (St. Angelo *et al.*, 1980). Frankel *et al.* (1984) point out that thermal decomposition products are the result of homolytic β-scission and the acid ones are produced by a heterolytic cleavage of lipid hydroperoxides. This should result in an absence of dialdehydes in the gas chromatograms and an abundance of malondialdehyde in the distillates of the TBARS analyses. The spectra in Figs 1 and 2 show absorbance maxima at 532 nm. However, this does not mean that only malondialdehyde was present in the distillates (Ward, 1985). The TBA-reaction lacks that specificity as is discussed by Fujimoto *et al.* (1984), who found especially 2,4-alkadienals as major lipid oxidation products responsible for the development of the red pigment. Sinnhuber & Yu (1958) crystallised the pigment consisting of 2 TBA's and 1 malonaldehyde and determined its conjugated double bound structure. This structure is considered to be a prerequisite for maximum absorbance at 532 nm. Yellow pigments are formed by reaction of 1 TBA with, for example, alkanals (maximum absorbance at 450 nm; Ward, 1985). These pigments have been used for indicating lipid oxidation, but absorbance at 532 nm is more relevant (Figs 1 and 2; Igene *et al.*, 1985).

The ECID gas chromatography technique offers a unique possibility to directly analyse secondary lipid oxidation products, e.g. alcohols, acids, ketones and aldehydes. However, the capillary gas chromatographic method does not allow for the presence of water in the sample. Galt & MacLeod (1984) have successfully used Tenax GC for separation of water vapour and volatiles in a headspace sampling method. Tenax is relatively hydrophobic, has a very high affinity for organic compounds and releases aroma volatiles almost quantitatively upon heating at 200°C (Barnes *et al.*, 1981). In the present study Tenax has been used for retaining volatiles from the nitrogen gas stream used for drying the sample and releasing them during heating in the ECID at 185°C. This temperature is the experimentally found compromise for a high recovery of volatiles and low amount of pyrolysis of the sample, i.e. a second run of the same cartridge gives less than 5% of the original total peak area. This aim has been accomplished for all cases except for the model systems with CYS added.

Low amounts of Type I antioxidants significantly decrease the formation of TBARS (Table 2). Tocopheryl acetate is less effective, probably because it is oxidised by Fe^{3+} (Cort *et al.*, 1978). As a radical it can undergo ring opening and become inactive; *in vivo* thiols act as second antioxidants to reconvert the radical into tocopherol (Pryor, 1976). It is difficult to mimic this in foodstuffs because of the danger of forming pro-oxidants (see Type III). The efficiencies of Type I antioxidants often do not parallel one another in complex, biological systems. The compounds differ in their ability to delocalise the unpaired electron produced in the reaction with the free radical. TBHQ scores the highest effect among the additions shown in Table 2, because it has the more effective resonance forms. The Type I antioxidants inactivate the catalyst and suppress the formation of lipid peroxy radicals in the propagation reaction (Labuza, 1971). As a consequence they do not interfere with the reactions following oxidation and leading to degradation products with an undesirable flavour, e.g. WOF. The model systems with PGAL added turned grey after heating, which could be the result of polymerisation of oxidised PGAL or complex formation with iron. In Table 4 the 'major peak' composition and the total amount of volatiles (minus BHA-peak) correspond very well between BHA and control-mixed and not between BHA and control-heated columns. This means that BHA plays an important role in keeping oxidation reactions under control during heating. It also suggests that users of this type of antioxidant should add them to a product before heating to obtain an optimal effect.

Type II antioxidants are effective in limiting oxidation reactions because of their ability to form a complex between pi acceptor ligands (e.g. heterocyclic bases) and the lower oxidation state of a transition metal (Labuza, 1971). However, these compounds can also stabilise peroxyl complex formation (e.g. *o*-phenanthroline; Wills, 1965) which increases the rate of radical formation. The results in Table 2 show that the model systems contain 12–25 times more in ppm's of Type II than of Type I while the effect in TBARS is somewhat lower. The relatively large amount of added TPP affected the pH of the model and can have an influence on the results by itself (Trout & Schmidt, 1984; Chen & Waimaleongora-ek, 1981). Most chelate complexes are less stable at higher temperatures (Labuza, 1971). Therefore, in Table 4 the results of EDTA are more like control-heated than control-mixed, as for BHA. Obviously, the effect of chelating agents is restricted to the cool storage oxidation reactions. For users of this type of antioxidant it means that there is no protection during heating.

The action of Type III antioxidants is much more complicated than that of the foregoing types. In the first place, there is, in many cases, a concentration-related pro- and antioxidant effect, e.g. 200 ppm ascorbate:

pro-oxidant; 5000 ppm ascorbate: antioxidant; 5000 ppm CYS: pro-oxidant (Fig. 2, Table 3). Secondly, the composition of the sample plays an important role: 2000 ppm ascorbate acts in beef as pro-oxidant (Schricker & Miller, 1983) and in a model system as antioxidant (Sato & Hegarty, 1971), 300 ppm ascorbate acts as pro-oxidant in the latter model and as antioxidant in the beef samples of Igene *et al.* (1985). In the examples given above iron and oxygen are present and the pH is below 7; this makes the formation of an intermediate ternary complex possible between ferric ion, oxygen and mono-anion of ascorbate (Seib, 1985). The intermediate ternary complex then undergoes a single- or two-step oxidation to give dehydroascorbic acid and H_2O_2 which enters the Haber-Weiss cycle (see above) or the Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$$

The generation of $OH^{\cdot}$ by these reactions is probably the reason for the pro-oxidant activity of ascorbate. At higher concentrations, being an antioxidant, ascorbate can neutralise the radicals formed by its own action. The composition of the sample can promote or suppress the formation of the ternary complex, which explains the seemingly contradictory nature of ascorbate mentioned previously. Table 3 shows that the 2-derivatisation in ASC2PP does not change the ascorbate effect whereas, in ASCTPP, it changes into a more antioxidant effect, i.e. the ternary intermediate is not, or is hardly, formed. The 6-derivatisation with a large hydrophobic group in ASCPAL seems to make the formation of the ternary intermediate quite unlikely, perhaps because of the drastic change in water solubility. Its compatibility with apolar foodstuffs is well illustrated by inhibition of thermal degradation of frying fats and oils (Gwo *et al.*, 1985).

The sulfur-containing antioxidants studied are both pro-oxidants, even at the high ppm levels ($NaHSO_3$ in Table 2 and CYS in Table 3). In this case Fe^{3+} can abstract an electron from the sulfur compound and the radicals formed strongly propagate the lipid peroxidation:

$$Fe^{3+} + HSO_3^- \rightarrow Fe^{2+} + HSO_3^{\cdot}$$
$$HSO_3^{\cdot} + O_2 \rightarrow HSO_3OO^{\cdot}$$
$$HSO_3OO^{\cdot} + RH \rightarrow HSO_3^{\cdot} + ROOH$$

Alternatively, cyclooxygenase can convert bisulfite to the sulfur trioxide radical anion, which can react with oxygen to yield the sulfur pentoxide radical anion, which makes it possible, in turn, to abstract one of the methylene hydrogens of unsaturated lipids (Motley *et al.*, 1982). The gas chromatograms show more similarity between the control (Fig. 3) and the bisulfite model systems (Fig. 4) than between the latter and CYS (Fig. 5).

The reason could be that CYS participates in reactions producing volatiles during ECID injection at 185°C (Kato *et al.*, 1973), giving large peaks at 9·38, 14·63 and 16·42 min retention times. It is also possible that bisulfite compounds formed in the model system are not degraded in the ECID and so lower the total amount of volatiles detected. These bisulfite compounds are perhaps unstable at the low pH of the distillation and so contribute to the enhanced TBARS of this model system (Table 2). Altogether, it makes it very difficult to decide on some conclusion by comparison of the profiles and total peak areas of the Type III antioxidants and the control model systems presented in Table 4. In his review, Labuza (1971) includes water activity (a_w) as a Type III antioxidant-environmental factor. Table 5 shows, indeed, a big influence of the rH ($\simeq a_w \times 100\%$) on the production of volatiles in the model systems. For the practice of cooking and handling of cooked meat, it means that the product should be prevented from drying out.

Pearson & Gray (1983) describe the possible role of Maillard reaction products as antioxidants in preventing WOF in meat. In the present study no effect in the TBARS formation has been detected (Table 2). Further study is under way with Maillard reaction products from a different origin.

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