The Maillard Reaction in Foods and Nutrition

George R. Waller, EDITOR
Oklahoma State University

Milton S. Feather, EDITOR
University of Missouri

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Mechanism Responsible for Warmed-Over Flavor in Cooked Meat

A. M. PEARSON and J. I. GRAY
Michigan State University, Department of Food Science and Human Nutrition, East Lansing, MI 48824

Studies using a model meat system have demonstrated that phospholipids are the primary contributors to warmed-over flavor (WOF) in cooked meat. Although phosphatidylcholine has little effect upon WOF, phosphatidylethanolamine plays an important role which is related to the propensity of its constituent polyunsaturated fatty acids to autoxidation. Both nitrite and EDTA inhibit WOF as does removal of heme pigments, which suggests that myoglobin may catalyze WOF development. However, purified myoglobin in the model system does not cause autoxidation. It has been demonstrated that WOF is catalyzed by the non-heme iron released from meat pigments during cooking. Evidence has indicated that overheating of meat protects against WOF by producing Maillard reaction products possessing antioxidant activity.

The term "warmed-over flavor" (WOF) was coined by Tims and Watts (1) to describe the rapid development of oxidized flavor in cooked meat upon subsequent holding. The rancid or stale flavor becomes readily apparent within 48 h in contrast to the more slowly developing rancidity that becomes evident only after freezer storage for a period of months. Although WOF was first recognized as occurring in cooked meat, hence the name WOF, it also develops in raw meat that is ground and exposed to air (2, 3). With increased consumption of precooked convenience meat entrees, such as quick-frozen meat dishes and TV dinners, preventing the development of WOF has become of great economic importance (4).

The present review will cover evidence on the mechanism involved in WOF development, with description of a model system that has been utilized to clarify some of the pathways. The role of meat pigments and various lipid components will be reviewed. Finally some procedures for inhibiting WOF in cooked

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meats will be discussed. Although an earlier review covers some of these points (5), considerable new information is now available and will be presented herein.

Description of Model Meat System

Love and Pearson (6) have described a model meat system in which bovine muscle was ground and extracted with distilled, deionized water at 4°C until it was devoid of color, indicating the removal of all meat pigments, i.e., myoglobin and hemoglobin. Other water-soluble components would also be partially or completely extracted by this procedure. The remaining extracted muscle was then used as a model system to which purified myoglobin, ferrous iron and ferric iron were added back to ascertain their role in WOF.

Essentially the same system has since been used in our laboratory to study the role of phospholipids and of triglycerides in development of WOF using beef and chicken dark and white meat (7). Figure 1 shows not only how the system was prepared for studying the effect of adding back myoglobin but also how chloroform-methanol was utilized to extract the total lipids (8) and then how triglycerides and phospholipids could be separated by silicic acid column chromatography with elution by chloroform and methanol (9).

Mechanism of WOF Development

Role of Myoglobin and Non-Heme Iron. The model meat system containing the indigenous lipids was used to obtain the data presented in Table I. Oxidation was followed by 2-thiobarbituric acid (TBA) numbers obtained using the distillation procedure of Tarladgis *et al.* (10). The data show the effects of different concentrations of metmyoglobin (MetMb) and ferrous iron (Fe^{2+}) upon TBA numbers in the model beef muscle system (6). MetMb concentration had no effect upon autoxidation, whereas Fe^{2+} increased oxidation of the model system with the extent being directly related to the concentration. Thus, it was shown that MetMb does not catalyze oxidation of the model system, but Fe^{2+} causes rapid autoxidation of the residual lipids. These results are in agreement with an earlier report (3), in which both Fe^{2+} and ascorbate were shown to catalyze development of WOF. Thus, it has been clearly shown that myoglobin is not directly responsible for development of WOF, although this concept has been widely accepted (11-14).

A subsequent study (15) using the model meat system showed that removal of the heme pigments or addition of 156 ppm of nitrite significantly inhibited lipid oxidation in cooked meat, which supports the earlier concept that myoglobin (Mb) is involved in WOF. However, it should be borne in mind that removal of heme pigments by leaching will also remove a number

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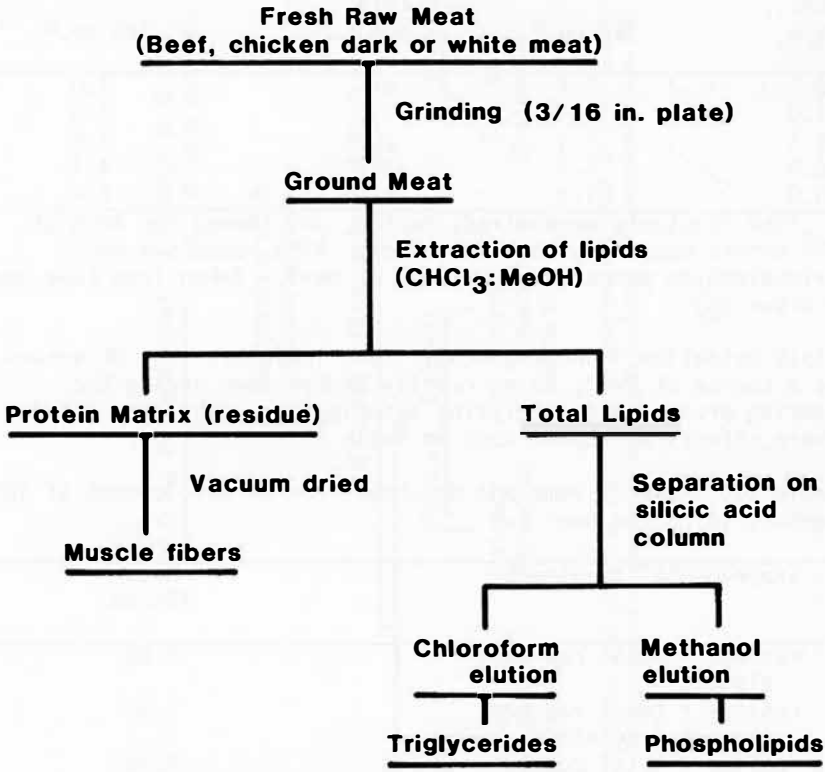

Figure 1. Preparation of model meat system. (Reproduced from Ref. 7. Copyright 1979, American Chemical Society.)

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of other compounds including Fe^{2+}. By using the scheme shown in Figure 2, Igene et al. (15) demonstrated that the level of free Fe^{2+} greatly increased during cooking, and accelerated

Table I. Effects of Various Concentrations of MetMb and Fe^{2+} on TBA Numbers of Beef Muscle Residue[a]

MetMb		Fe^{2+}	
Conc., mg/g	TBA no.[b]	Conc., ppm	TBA no.[b]
0	1.4	0	1.0
1.0	1.4	1.0	1.5
2.5	1.4	2.0	3.6
5.0	1.4	3.0	4.1
10.0	1.4	4.0	5.4

[a]The reactants were mixed, heated, and stored for 48 h at 4°C before measuring the TBA values. [b]TBA numbers = mg of malonaldehyde produced per 1000 g of meat. Taken from Love and Pearson (6).

lipid oxidation in cooked meat. This indicates that Mb serves as a source of Fe^{2+}, being readily broken down during the cooking process and catalyzing autoxidation. The data showing these effects are summarized in Table II.

Table II. Role of Heme and Non-heme Iron on Development of TBA Numbers in Cooked Beef.[a,b]

Experimental treatment	Mean TBA no.
residue + total raw meat pigment	5.00
residue + total raw meat pigment (chelated)	1.55
residue + total cooked free meat pigment	4.35
residue + total cooked free meat pigment (chelated)	1.46
residue + H_2O_2-treated total meat pigment	6.02
residue + H_2O_2-treated meat pigment (chelated)	1.54

[a]Each experimental treatment used 100 g of beef residue in addition to 50 mL of the concentrated extract. [b]EDTA was used to chelate the inorganic or free iron at a concentration of 2%. Taken from Igene et al. (15).

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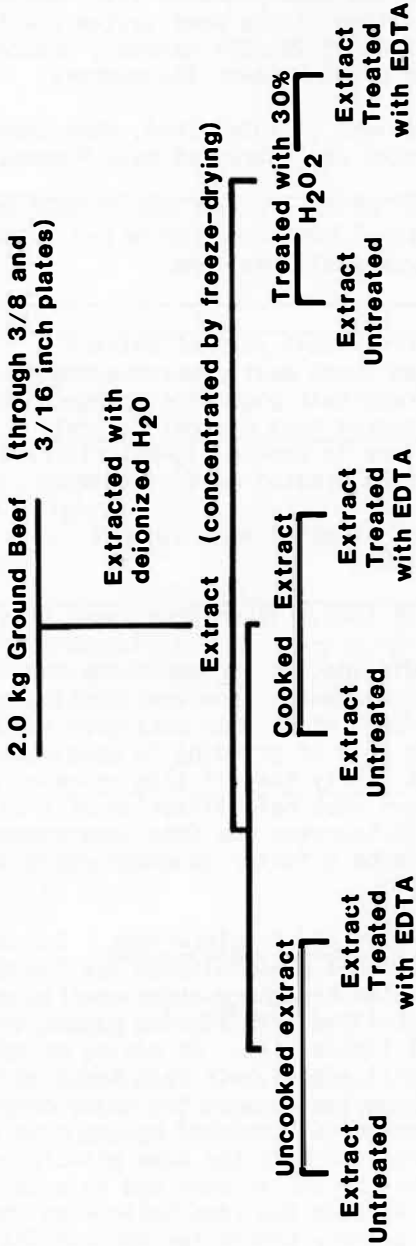

Figure 2. Preparation and design of experiment to compare the effect of heme and non-heme iron on the development of warmed-over flavor. (Reproduced with permission from Ref. 16.)

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It is interesting to also note that treatment with H_2O_2 destroys even more of the heme pigments than heating, resulting in still greater oxidation of the meat system (Table III). On the other hand, addition of 2% EDTA markedly reduced autoxidation as shown by much lower TBA numbers.

Table III. Concentrations of Total Iron, Heme Iron, and Free Non-heme Iron in Treated and Untreated Meat Pigment Extract

Experimental treatment	Amount of free Fe^{2+}, μg/g of meat
total iron in fresh meat pigment extract	20.64
non-heme iron in fresh meat pigment extract	1.80
heme iron in fresh meat pigment extract	18.84
total iron in cooked meat pigment filtrate	5.51
free non-heme iron in cooked pigment filtrate	4.18
total iron in H_2O_2-treated meat pigment extract	13.59
free iron in H_2O_2-treated meat pigment extract (chelated[a])	12.33

[a]Chelated by adding 2% EDTA. Taken from Igene et al. (15).

Results demonstrate that Mb per se is not the catalyst of lipid oxidation in cooked meat. However, cooking destroys part of the Mb, releasing Fe^{2+} which then catalyzes the development of WOF. Although the role of grinding in development of WOF was not studied, it seems likely that it also releases Fe^{2+}. It has recently been shown that solubilization of iron from grinding equipment can increase the free iron content of fish meal, which could also be a factor in autoxidation of fresh ground meat (16).

Role of Phospholipids and Triglycerides Circumstantial evidence has suggested that phospholipids are the major contributors to WOF, with the correlation coefficients between TBA numbers and phospholipid levels being higher than those for TBA numbers and total lipids (17). On adding phospholipids, triglycerides, and total lipids back to a model meat system, it was verified that phospholipids were the major contributors to WOF (7). This conclusion is supported by the data given in Table IV, which demonstrate that the same principles are involved in development of WOF in beef and in both chicken dark and light meat. The data in the same table also show a relationship between sensory scores for WOF and TBA numbers. Although the addition of triglycerides resulted in some increase in TBA numbers, the effect was always considerably lower than

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Table IV. TBA Numbers and Sensory Scores for Cooked Beef, Chicken Dark Meat and White Meat Model Systems[a,b]

Meat type	Composition of treatments	Mean TBA no.	Mean sensory scores[c]
beef	0.8% phospholipids	5.76[b]	2.69[a]
	9.2% triglycerides	1.88[a]	3.84[b]
	10% total lipids	6.81[b]	2.64[a]
	control	1.16[a]	3.75[b]
chicken dark meat	0.74% phospholipids	8.48[b]	2.97[a]
	4.3% triglycerides	6.30[a,b]	3.65[a,b]
	5% total lipids	11.74[c]	3.25[a]
	control	5.78[a]	3.88[a,b]
chicken white meat	0.7% phospholipids	5.03[b]	2.80[a]
	4.3% triglycerides	2.99[a]	3.35[a]
	5% total lipids	5.53[b]	3.09[a]
	control	2.18[a]	4.02[b]

[a]There were four replicates for each treatment.
[b]Taste panel score were from 1-5, with 1 very pronounced WOF and 5 no WOF.
[c]All numbers in same column within a meat type followed by the same superscript are not significant at the 5% level. Taken from Igene and Pearson (7).

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that of the phospholipids. The additive effect of triglycerides was, however, greater in chicken dark meat than for chicken white meat or beef. This is probably due to the greater degree of unsaturation in the triglycerides from chicken dark meat.

Table V presents information on the relative effects of adding back phosphatidylcholine (PC), phosphatidylethanolamine (PE), and total blood serum phospholipids (TP) to the model meat system. Nitrite was also added but will be discussed later herein. The addition of both PE and TP increased TBA numbers, with the effect of PE being the greatest. Sensory scores also indicated that oxidation of PE, TP, and PC contributed to WOF, with PE again having the greatest effect.

Table V. TBA Numbers and Sensory Scores for Cooked Meat Model Systems Containing Added PC, PE, Serum Phospholipids, or Nitrite[a,b]

Experimental treatment	Mean TBA no.	Mean sensory score
model system only	0.36 ± 0.07[b]	3.33 ± 0.42[a,b]
model system + nitrite	0.26 ± 0.06[a]	4.11 ± 0.44[b,c]
model system + PC	0.34 ± 0.03[a,b]	3.36 ± 0.51[a,b]
model system + PC + nitrite	0.29 ± 0.07[a]	4.52 ± 0.27[c]
model system + PE	0.81 ± 0.13[d]	2.64 ± 0.58[a]
model system + PE + nitrite	0.36 ± 0.07[b]	3.61 ± 0.23[b]
model system + TP	0.62 ± 0.12[c]	3.29 ± 0.49[a,b]
model system + TP + nitrite	0.34 ± 0.05[a,b]	4.04 ± 0.43[b,c]

[a]Each treatment was replicated four times.
[b]A significant $P<0.01$) "r" value of -0.62 was found between TBA numbers and sensory score. Numbers in the same column bearing same letter are not significant at 5% level. Take from Igene and Pearson (7).

In summary, it has been shown that phospholipids are the major contributors to development of WOF, with triglycerides playing only a minor role. Studies on the individual phospholipids have demonstrated that PE is the main phospholipid involved in development of WOF.

Comparison of WOF and Normal Oxidation

It is well known that meat lipids are stable during freezer storage of fresh meats for periods of up to 12 months or more (5). Recent work has shown that α-tocopherol levels declined steadily during storage and had largely disappeared by the end of 6 months in freezer storage (18). In contrast to the involvement of phospholipids in development of WOF in cooked

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meats, Igene et al. (19) have recently shown that the major changes occurring during frozen storage were due to losses in the triglyceride fraction. The phospholipids remained relatively constant during frozen storage for up to 18 months. The extent of deterioration in the triglycerides has been shown to be related to the degree of unsaturation as well as to the length of storage (20).

Although there is some decline in the constituent phospholipids (PC and PE) during frozen storage of uncooked meat, the decline is much greater in cooked meat (21). Drippings collected upon cooking contain primarily triglycerides, while PE is essentially absent suggesting that it is membrane-bound (21). Since there is an increase in the proportion of PE in cooked meat, this along with its propensity to oxidize may account for the faster oxidation in cooked meat, even during freezer storage. Thus, the faster deterioration of cooked meat is evident and even occurs during freezer storage. These studies help in explaining the relative roles of triglycerides and phospholipids in autoxidation of cooked meat and have bearing on our understanding of WOF.

Inhibition of WOF

Phosphate, ascorbate, NaCl, nitrite, Maillard reaction products, and other antioxidants or prooxidants have been reported to influence development of WOF. Their roles have been reviewed (5), but newer evidence is now available and will be covered herein.

Role of Phosphates Tims and Watts (1) showed that addition of phosphates to cooked meat protects against autoxidation. This was found to be the case for pyro-, tripoly-, and hexametaphosphate, but orthophosphate had no antioxidant effect. Sato and Hegarty (3) verified the effects of phosphates in retarding the development of WOF by showing that pyro-, tripoly-, and hexametaphosphate protected cooked ground beef against WOF during storage at 2°C.

The phosphates appear to prevent autoxidation by chelating metal ions, although the exact mechanism is not known. Other work, however, has shown that metal chelators do protect meat against autoxidation (1, 22). EDTA has been shown to inhibit development of WOF by chelating the non-heme iron in cooked meat (15). Thus, evidence suggests that the phosphates probably chelate Fe^{2+} and thus inhibit WOF.

Role of Ascorbates At low levels (< 100 ppm) ascorbic acid has been shown to catalyze development of WOF as shown by increased TBA values (1, 3). At higher levels (>1,000 ppm), however, ascorbic acid retards oxidation (3). Although the mechanism of retardation is not known, it has been suggested that high levels of ascorbates may upset the balance between

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Fe^{2+} and Fe^{3+} or else could have its effect by acting as an oxygen scavenger (5).

Tims and Watts (1) showed that a combination of ascorbates and phosphates acted synergistically to retard development of rancidity. Sato and Hegarty (3) verified the antioxidant activity of the combination and suggested that ascorbic acid acts by keeping part of the iron in the Fe^{2+} state. It has been shown that ascorbic acid and phosphates act synergistically in preventing oxidation of cured meat (23), and probably help in explaining the virtual absence of WOF in cured meats.

Role of NaCl The activity of NaCl in initiating color and flavor changes in meat is well known but poorly understood. The effect is further complicated by the fact that NaCl is both a prooxidant and an antioxidant, depending upon the concentration dissolved (24). Lea (25) has discussed the fact that salt may be a prooxidant in some cases and either have no effect or be an antioxidant in other foods. Watts (26) concluded that salt may have antioxidant activity in dilute solutions but on crystallization has a prooxidant effect. On the other hand, Mabrouk and Dugan (24) found that salt inhibited autoxidation in aqueous emulsions of methyl linoleate, its effectiveness as an antioxidant being directly associated with increasing concentrations. They suggested that dissolved oxygen may be eliminated from the system as the NaCl concentration is increased.

Some of the studies on salt are, no doubt, complicated by the fact that salt may contain metal contaminants, which could serve as catalysts of lipid oxidation. Nevertheless, rancidity may still develop in the fat of dry cured hams, even though salt with a low metal content is used (27). The use of an antioxidant in combination with such salt, however, did inhibit rancidity and improve flavor scores. Further work to clarify the role of salt in lipid oxidation is needed before its mechanism is fully understood.

Role of Nitrite Sato and Hegarty (3) reported that 2000 ppm of nitrite completely eliminated WOF, while as little as 50 ppm greatly inhibited its development. Bailey and Swain (28) further confirmed the effectiveness of nitrite in preventing oxidation of fresh meat stored under refrigeration and verified its role in preventing WOF. A concentration of 156 ppm of nitrite has been shown to inhibit WOF development in cooked meat, with a twofold reduction of TBA values for beef and chicken and a fivefold reduction for pork (29). Table V also demonstrates that nitrite inhibits WOF development. Undoubtedly these results explain the higher flavor scores for nitrite as compared to NaCl-containing cured pork (30).

The mechanism by which nitrite prevents or inhibits WOF has been suggested to be related to stabilization of the lipids in

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the membrane (3, 5), which are normally disrupted and exposed to oxygen by cooking or grinding. Zipser et al. (31) proposed that nitrite forms a stable complex with the iron porphyrins of heat-denatured meat, thus inhibiting WOF. Kanner (32) has demonstrated that S-nitrosocysteine is a potent antioxidant and has suggested that it may be generated in the nitrite curing of meat. Thus, S-nitrosocysteine could also serve as an inhibitor of WOF in cured meat. Since non-heme iron has been shown to be the major lipid prooxidant in uncured, heated meat systems (3, 6, 15), it seems more probable that nitrite stabilizes the heme pigments so that they do not release Fe^{2+} and thus catalyze development of WOF. The inhibition of WOF by EDTA lends further credence to this theory, which as yet is unproven.

Role of Maillard Reaction Products Certain products of the Maillard reaction are known to have antioxidant properties and are produced during retorting of meat. Zipser and Watts (33) first described the development of an antioxidative effect in overcooked meat and suggested that diluted slurries could be used to protect normally cooked meats from oxidation. Sato et al. (34) demonstrated that retorted meat, indeed, possessed strong antioxidant activity against development of WOF. They then demonstrated that the material retained by dialysis of extracts of the retorted meat had no antioxidant activity but that the diffusate possessed strong inhibitory activity against WOF. This suggested that the substances responsible for inhibiting WOF in retorted meat are water soluble and of low molecular weight. Huang and Greene (35) confirmed these findings and suggested that a temperature of about 90°C is required to produce the browning compounds responsible for the antioxidant activity in cooked meat. They further showed that there was a relationship between antioxidant activity and development of the brown color, which was accompanied by an increase in fluorescence.

Porter (36) has reviewed the role of Maillard reaction products as antioxidants in food systems, pointing out the importance of high temperatures (~100°C) to their development in contrast to lower temperatures (~70°C), which accelerate development of WOF. Eichner (37) has shown that browning intermediates -- primarily Amadori rearrangement products -- have strong antioxidant activity even though they are colorless. The mechanism by which these reductone and reductone-like compounds inhibit autoxidation seems to by decomposing hydroperoxides and inactivation of free radicals (37). Sato and Herring (38) have reported that reductic acid and maltol, which are browning products, possess strong antioxidant activity against WOF. Ascorbic acid, a related reductone, has been reported to accelerate WOF at concentrations of 100 ppm or less but to inhibit the reaction at levels of 1,000 ppm or over (3). Lingnert and Eriksson (39) have

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demonstrated that Maillard reaction products inhibit oxidation in emulsion-type sausages.

The studies reviewed demonstrate that browning products produced on retorting of meat inhibit development of WOF, so that canned meat products are not subject to this flavor defect. The flavor of canned meat is less desirable, however, than that of freshly cooked meat. Nevertheless, the strong inhibitory action of the Maillard reaction products against WOF suggests that they could be useful in preventing development of WOF, so further research in this area could be fruitful.

Role of Other Antioxidants A great deal has been written about the primary antioxidants, which may be used to inhibit development of WOF. Porter (36) has reviewed these compounds, their structures, and probable modes of action so they will not be discussed. The discussion will focus on the other natural antioxidants that may be useful in controlling WOF development.

Sato et al. (34) demonstrated that a variety of common meat additives, including cottonseed flour, nonfat dry milk, spray-dried whey, wheat germ, and textured soy flour, inhibited WOF in the meat system. These products may have exerted their inhibitory effect on WOF through the Maillard reaction, since most of them contain some reducing sugars. Pratt (40) reported soybeans and soy protein concentrate had an inhibitory effect upon development of WOF and was able to demonstrate that the active components are water soluble. Fractionation and analysis of the water-soluble fraction showed the antioxidant activity was due to the presence of isoflavones and hydroxylated cinnamic acids (40). This confirms earlier work showing that the flavonoids present in plant extracts inhibit oxidation in sliced roast beef (41).

The addition of smoke to meat also imparts antioxidant properties (42). This has also been shown on application of smoke to fatty fishes (43). Porter (36) has reviewed the antioxidant properties of smoke and concluded that a number of phenolic compounds are responsible. Several of these phenolic components, including phenol, guaiacol and catechol, are not only potent antioxidants but also have antibiotic activity. These components would also serve as inhibitors of WOF, but some of the components of wood smoke are known carcinogens (36, 42).

Summary

The use of model meat systems has been shown to be helpful in elucidating the role of different compounds in development of WOF. Results of these studies have shown that the major catalyst of WOF is Fe^{2+}, which is released from the heme pigments by heating, and presumably by grinding. The phospholipids are the major lipids involved in development of WOF, in contrast to oxidation during frozen storage, where the

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triglycerides are mainly involved. The role of phosphates, nitrite, salt, ascorbate, other additives, and of Maillard reaction products are discussed in light of their role as possible inhibitors of WOF. These active inhibitors of WOF could play an important role in protecting pre-cooked meat entrees against WOF.

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