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The use of natural antioxidants in food products of animal origin

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12.1 Introduction

In this introduction, I will break my own cardinal rule of no first person referencing. However, when first asked to prepare the following review, I thought ‘why not?’ After all, lipid oxidation in meats had provided me with a doctorate dissertation and it was an area with which I had lost contact, other than at surface level. I needed to reacquaint myself with the literature and this was a perfect opportunity. What I found were some very exciting approaches to the problem that will have greater ramifications than just stabilising the meat lipids in the food system; not that this is not a good and noble goal. The greatest problem I faced was preparing this document, without exceeding the designated page limit, while presenting as broad a picture as possible. Therefore, in the meat area I stayed primarily with the most recent findings, i.e. since 1990, except for a historical perspective. This research has been divided into two categories. The first looks at how altering the meat’s concentration of inherent/endogenous antioxidants (tocopherol and the carotenoids) can affect the meat’s stability. In the second place, I wanted to update the role of exogenous natural antioxidants in preventing lipid oxidation in meat systems. In each of these two sections there is overlap and some work has been done to define any possible synergies between natural antioxidants in the meat system. Mechanisms/interactions as they have been defined have been presented.

Relative to the remainder of this document, i.e. dairy products, eggs and cholesterol, there was much less literature with which to work, since the addition of antioxidants is neither traditionally accepted nor needed in many of these foods. Again, I tried to stay with the most recent findings.
while still presenting a view of what has been done with natural antioxidants in preventing lipid oxidation. Again, there has been some work done in defining the role of inherent compounds and on ways to increase their levels as a means of reducing the level of oxidative rancidity.

12.2 Control of lipid oxidation in meat products

12.2.1 Background
Since its naming by Timms and Watts in 1958, warmed over flavor (WOF), now also called meat flavour deterioration (MFD), has been a primary research issue for food scientists and the meat industry. There are many endogenous and exogenous factors that have been shown to affect WOF. Early research showed that this was a phenomenon related to the meat’s phospholipids as opposed to its triacylglycerides. In addition it also found that a primary catalyst to WOF was iron and that during cooking there was a release of (free) iron from the heme compounds.

Since these early findings, researchers have begun to establish the role of endogenous factors in the development of WOF. Some endogenous factors help to control oxidation and include the presence of compounds that are active antioxidants, i.e. dipeptides, tocopherols, etc. while others are enzymes capable of deactivating active oxygen species. In contrast, pro-oxidants are also present in the cell; these can include free iron, ascorbic acid and active oxygen species. The final stability of the system is therefore dependent on the balance between these factors. The balance or lack of balance translates into the fact that there are differences in oxidative stability between animal species and between muscle types within a species. For example, generally between species differences are that beef is the most stable, followed by pork, chicken, turkey and finally fish, and within a species such as poultry, the dark (thigh) meat is more susceptible to oxidation than the white (breast) meat.

Exogenous factors affecting the oxidative stability of meats include the level of processing, cooking technique/time, pre- and post-cooking storage time and temperature, packaging system, and/or use of antioxidants. When meat is processed the balance of the system becomes altered. Grinding disrupts the muscle tissue and allows mixing of the cell contents with oxygen; it also allows pro-oxidants access to the more unsaturated fatty acids in the membranal phospholipids. Increases in free iron have been found to result from cooking and during storage. During storage reducing agents (ascorbate and superoxide anion) can act to release the iron from its chelated state. Myoglobin is a major source of this released iron while ferritin has been shown to be less susceptible to releasing iron when denatured. The released iron is very active and in close proximity to the lipid substrate. Finally cooking also acts to destabilise the unsaturated fatty acids in the membranal phospholipids.
Control of oxidation in meat systems can occur in the raw or cooked meat system. In the raw meat system, factors affecting the levels of endogenous antioxidants have been receiving a great deal of attention in the literature in the 1990s. Prior to this research, most of the activity was focused on the application of exogenous antioxidants during processing. The remainder of this section will be divided to discuss most recent findings on the role of endogenous and exogenous antioxidants and combinations of both in controlling lipid oxidation in meat systems. Antioxidants are either lipid soluble (tocopherols and carotenoids) or water-soluble (ascorbic acid, dipeptides, and plant phenolics or polyphenolics; raw meat will also contain antioxidant enzymes).

### 12.2.2 Endogenous antioxidants

Since about 1990, increasing the levels of tocopherols and carotenoids in muscle tissue via dietary supplementation has shown strong promise for increasing the oxidative stability of muscle foods.

#### 12.2.2.1 Beef

Because cattle are ruminants, it is difficult to alter their body composition through diet unless the ingredient is encapsulated to protect it from metabolism in the rumen. However, it is possible to increase the tocopherol content of beef muscle by feeding higher levels of tocopheryl acetate. Researchers\(^6\)–\(^9\) have shown that feeding 2000–3000 mg per day tocopheryl acetate to cattle for up to 135 days increased the tocopherol content of the resultant meat. Liu et al.\(^6\) reported that after 126 days there was a six-fold increase in the tocopherol content of *gluteus medius* from supplemented vs non-supplemented animals. Galvin et al.\(^7\) showed that muscle type responded differently relative to level of tocopherol content. In the *longissimus dorsi* (LM) muscle, the level went from (basal diet) 0.84 to 2.45 \(\mu\)g g\(^{-1}\) muscle, while in the *psoas major* (PM) muscle, the level went from 2.45 to 5.78 \(\mu\)g g\(^{-1}\) muscle. Higher levels fed longer times gave the greatest increase in muscle tocopherol levels. Lynch et al.\(^8\) noted the threshold level of tocopherol needed in beef tissue to increase its stability was 3.5 \(\mu\)g g\(^{-1}\) muscle.

When tocopherol-supplemented meat was cooked and evaluated for WOF, as reflected by TBARS (thiobarbituric reactive substances) values, studies\(^6,8\) have shown there was a significant increase in oxidative stability. O’Grady et al.\(^9\) compared fed tocopherol to tocopherol added during processing and found that dietary supplementation was more effective in controlling the stability of meat. Faustman et al.\(^10\) showed that during oxidation in beef tissue microsomes, there was a decrease of \(\alpha\)-tocopherol with an increase of its oxidation products, i.e. \(\alpha\)-tocopherolquinone (TQ) and 2,3-epoxy-\(\alpha\)-tocopherolquinone (TQE\(_2\)). This conversion of \(\alpha\)-tocopherol to its respective oxidation products was consistent with it acting as a peroxyl-radical scavenging compound.
Galvin et al.\textsuperscript{7} also found that although tocopherol supplementation significantly reduced lipid oxidation, stabilising cholesterol against oxidation appeared to be influenced by muscle type. There was a supplementation-attributable reduction of cholesterol oxidation, i.e. 7-ketocholesterol production, in the PM but not in the LM samples. In vacuum packaged meat, dietary tocopherol-treated meat showed less colour change, i.e. there was less metmyoglobin formation. However, O’Grady et al.\textsuperscript{9} also found that in meat stored at different levels of oxygen, tocopherol-supplemented meat showed no differences in colour stability.

12.2.2.2 Pork

The stability of meat from monogastric animals is more easily affected by diet. Researchers have shown that increasing dietary tocopherol levels results in increased tocopherols in the meat tissue. Onibi et al.\textsuperscript{11} fed pigs 35 day-diets with two levels of tocopheryl acetate (basal and basal +200 mg kg\textsuperscript{-1} diet). Two basal diets were used, i.e. soy-based vs full-fat rapeseed meal. The authors\textsuperscript{11} found that there was a two–three-fold increase in muscle tocopherol content in the longissimus dorsi muscle depending on the diet fed, i.e. larger differences were found in the soy-fed pigs. This was partially explained by the fact that there was about twice as much (40 vs 16 mg kg\textsuperscript{-1} diet) natural tocopherol in the unsupplemented rapeseed diet than in the unsupplemented soy diet.

Buckley et al.\textsuperscript{12} investigated the effect of dietary vitamin E either short term (4 weeks prior to slaughter) or long term (10 weeks prior to slaughter) for its effects on membrane stability and meat quality. In addition, long term feeding of $\alpha$-tocopherol alone vs mixed tocopherols was investigated. $\alpha$-Tocopherol alone was much more effective than was feeding the mixed tocopherols, which essentially had no effect. It was felt that this was because the body absorbs the tocopherol isomers at different levels with $\alpha > \beta > \gamma > \delta$, resulting in less tocopherol in the meat when the mixture was fed. Tocopherol supplementation long term was more effective than short term in stabilising the lipids in pork microsomes and mitochondria to metmyoglobin/hydrogen peroxide mediated oxidation. In pork patties processed with and without salt and held at 4°C in dark or in light, tocopherol supplementation, either short term or long term, had no effect on the stability of meat patties made without salt. However, when salt was used tocopherol supplementation acted to increase the oxidative stability of the meat patties.\textsuperscript{12}

Monahan et al.\textsuperscript{13} found a reduction in microsomal free radical production and lipid oxidation in tissue from pigs fed $\alpha$-tocopherol (200 mg kg\textsuperscript{-1}) vs control pigs (10 mg kg\textsuperscript{-1}). Pork chops from the supplemented animals had lower susceptibility to oxidation during refrigerated storage. It was shown that tocopherol supplementation resulted in higher membrane levels of the vitamin and that there was suppression in the production of free radicals capable of initiating and/or propagating lipid oxidation.
Kingston et al.\textsuperscript{14} evaluated the individual and combined effects of muscle vitamin E levels, cooking rates and final temperature and packaging on lipid oxidation in refrigerated cooked pork from pigs fed extra dietary vitamin E. Individual effects showed that higher muscle tocopherol levels (4.24 $\mu$g g\textsuperscript{-1} vs 0.97 $\mu$g g\textsuperscript{-1}) increased pork oxidative stability. Cooking at a faster rate (2 °C min\textsuperscript{-1} vs 0.3 °C min\textsuperscript{-1}) and to a lower final temperature (72 vs 82 °C) both reduced oxidation and vacuum packaging reduced oxidation. When tested for interactions it was found that any combination of these factors were more effective than any single factor.

Feeding oxidised corn oil (peroxide value PV = 300) at 2% of the diet for 10 weeks resulted in increased susceptibility of cellular microsomes and mitochondria to lipid oxidation as evidenced by TBARS being higher than the control.\textsuperscript{14} Consuming oxidised oil might have provided a source of free radicals capable of destabilising subcellular membranal lipids. In contrast, Monahan et al.\textsuperscript{13} fed oxidised lipids to pigs and evaluated their effect on iron-induced free radical production in the meat. In this case, including oxidised (150 meq kg\textsuperscript{-1} PV) corn oil had no effect on the lipid stability of the muscle lipids.

\subsection*{12.2.2.3 Poultry}
The effect(s) of diet has been widely investigated in poultry; feeding different fats, tocopherols and/or carotenoids has been shown to have benefit in the final consumer-ready product. Feeding more saturated fats, such as coconut and olive oil, increases the oxidative stability of both thigh and breast meat from broilers.\textsuperscript{15} Ruiz et al.\textsuperscript{16} showed feeding broilers more unsaturated fat resulted in less stability in the meat. Lin et al.\textsuperscript{15} noted that feeding different levels of saturated/unsaturated fat affected the meat’s triglyceride more than phospholipid composition.

Renerre et al.\textsuperscript{17} reported that feeding highly unsaturated fats (tallow vs soybean vs linseed oils) to turkeys increased the levels of antioxidant enzymes (catalase, superoxide dismutase, and glutathione reductase) in the thigh and breast meat, with the higher levels found in the thigh meat. Maraschiello et al.\textsuperscript{18} fed broilers different fat sources (lard, sunflower and olive oil) and analysed for glutathione peroxidase (GSHPx) activity. Dietary fat affected the GSHPx activity, in that between sunflower and olive oil the more unsaturated sunflower oil resulted in the higher GSHPx activity. However, feeding lard produced the highest GSHPx activity.

Other dietary treatments, i.e. tocopherol and/or carotenoid supplementation have been studied for increasing the oxidative stability of poultry meat. When tocopherol is added via the diet it becomes incorporated in the subcellular membrane and is in position to be more effective in intercepting free radicals as they are formed. Lin et al.\textsuperscript{15} showed that tocopherol supplementation increased the level of tocopherol in the meat and improved the lipid stability of both the dark and white broiler meat. Ahn et al.\textsuperscript{19} found up to a three-fold increase in tocopherol content in turkey meat when the
dietary tocopherol content increased from 25 to 200 IU kg\(^{-1}\) diet. The tocopherol content of the thigh meat was about 50% greater than the breast meat.

Ahn et al.\(^{20}\) fed \(\alpha\)-linolenic acid and mixed tocopherols alone or in combination to broilers. It was found that the \(\alpha\)-linolenic acid supplementation increased the level of fatty acid unsaturation in the phospholipid fraction. Birds fed \(\alpha\)-linolenic acid and tocopherol had the highest meat tocopherol levels with the greatest amount in the thigh tissue. \(\alpha\)-Linolenic acid fed alone reduced tissue tocopherol content and this meat was most susceptible to oxidation. The authors\(^{20}\) noted that it was possible to increase the \(\omega\)-3 fatty acids content of poultry meat, but it may be hard to incorporate sufficient tocopherol to control the oxidation of the meat.

Ahn et al.\(^{19}\) showed that supplementing turkey diets with vitamin E, above basal levels of 25 IU kg\(^{-1}\), to 200, 400 or 600 IU kg\(^{-1}\) diet was effective in reducing oxidative stability in patties made from the thigh or breast meat. The patties were stored at 4°C for up to 14 days, under vacuum or open to air, and in each case the tocopherol-supplemented meat was more stable. These authors also showed tocopherol supplementation reduced oxidation when the patties were irradiated with 0 or 2.5 kGy prior to cold storage. The best combination for longer shelf-life was tocopherol supplementation and vacuum packaging.

Addition of carotenoids to poultry diets has become popular since they give the finished carcass a ‘golden’ tone that is considered desirable by the consumer. This practice can have some stability implications. Work by Ruiz et al.\(^{16}\) showed that adding \(\beta\)-carotene alone to broiler diet resulted in pro-oxidant activity in the meat. They further showed that some level of tocopherol was necessary for \(\beta\)-carotene to act as an antioxidant in the raw and cooked meat. The authors felt that work is needed to determine the optimum ratio of tocopherol to \(\beta\)-carotene for controlling lipid oxidation in poultry meats.

Canthaxanthin is another carotenoid that has been studied for its effect on poultry meat stability. Chickens were fed diets containing full-fat flaxseed and supplemented with mixed tocopherol and canthaxanthin.\(^{21}\) Both tocopherol- and canthaxanthin-supplemented cooked meat was more stable during refrigerated storage. The best system was the combination of the two compounds. Tocopherol is known to act as a hydrogen donor; however, canthaxanthin can stop peroxyl free radical chain propagation by trapping the radical in its conjugated polyene system.\(^{16}\)

12.2.2.4 Fish

As indicated earlier, fish muscle is probably the most susceptible to oxidation, primarily because of the high level of unsaturation found in its lipids. Feeding sources of antioxidant compounds have also been studied as a means of increasing the oxidative stability of fish muscle. Canthaxanthins are naturally occurring carotenoids found in red fish tissue, such as salmon.
and rainbow trout, and in cultivated fish achieving a colour similar to that found in the wild is important to consumer acceptance of the cultivated product. Sigurgisladottir et al.\textsuperscript{22} fed salmon mixed tocopherols and found that there was an equilibrium in muscle tocopherol levels after 15 weeks of supplementation. It was also found that the fish incorporated more of the \( \delta \) - and \( \beta \)-tocopherol isomers than they did the \( \alpha \)- and \( \gamma \)-isomers. Akhtar et al.\textsuperscript{23} reported that feeding 500 mg kg\(^{-1}\) \( \alpha \)-tocopherol in the diet resulted in a five-fold increase of tocopherol in rainbow trout muscle, and the muscle with the higher levels of tocopherol were more stable to oxidation.

Akhtar et al.\textsuperscript{23} also reported that it was possible by using a surface application of a rosemary oleoresin on muscle from fish supplemented with tocopherol to enhance further the stability of rainbow trout muscle. Sant’Ana and Mancini-Filho\textsuperscript{24} fed 100 ppm \( \alpha \)-tocopheryl acetate, 100 ppm butylated hydroxytoluene (BHT) or 1.4 g rosemary extract (Herbalox\textsuperscript{25})/kg diet to freshwater fish and found differences in the lipid stability of the fish muscle in the order of rosemary > tocopherol > BHT > control. When the muscle was irradiated, the order of efficacy changed to BHT > tocopherol = rosemary > control.

Akhtar et al.\textsuperscript{25} also fed tocopherol, canthaxanthin or oleoresin paprika alone or in combination. When vitamin E and canthaxanthin were both included in the diet, a strong antioxidant effect was found and this was attributed to the concept that these two compounds use different mechanisms to control lipid oxidation. \( \alpha \)-Tocopherol is a hydrogen donor and can donate the hydrogen from its C-6 carbon while canthaxanthin acts to trap the peroxyl free radical in its conjugated polyene system. The oleoresin of paprika would also contain cartenoids and this treatment appeared to give equal protection as found with the canthaxanthin.\textsuperscript{25} These authors also noted that canthaxanthin’s effect could be due to its being converted to \( \beta \)-carotene, which has been shown to occur metabolically in rainbow trout.

Clark et al.\textsuperscript{26} supplemented rainbow trout with canthaxanthin prior to processing into patties and showed that the amount of canthaxanthin deposited was critical if lipid stability was to be achieved. In a liposome system canthaxanthin delayed the formation of TBARS in a concentration-dependent manner.\textsuperscript{23}

\textbf{12.2.3 Exogenous antioxidants}

Adding antioxidants during processing has been the more traditional technique used to control lipid oxidation in meats. Some of the added compounds are found at low levels in meats, i.e. ascorbic acid and carnosine, while others are derived from plants, i.e. phenolics/polyphenolics.

\textbf{12.2.3.1 Carnosine}

Meat tissue contains several dipeptides, i.e. carnosine, anserine and opheline, that have been shown to have antioxidant activity.\textsuperscript{27} Carnosine has
received the most attention relative to its ability to control lipid oxidation in meats. Carnosine (β-alanyl-l-histidine) was first identified in 1900 by Gulewitsch and Amiradzibi.\textsuperscript{28} However, its antioxidant activity was not heavily investigated until the early 1990s. Since then carnosine has been shown to have many mechanisms by which it can affect the rate of lipid oxidation in meats.

Carnosine, added to salted and unsalted frozen and cooked ground pork, at high levels (1.5%) was more effective in inhibiting WOF than 0.5% sodium tripolyphosphate (STPP), α-tocopherol and BHT; the last two were added at 0.02% of total fat. Its superiority to α-tocopherol and BHT indicated that carnosine was a better hydrogen donor than these two known donors and its superiority to STPP indicated that it could chelate metals.\textsuperscript{29,30} However, a lower level (0.5%) of carnosine added to these same systems was ineffective in the salted meats, indicating that salt interfered with its ability to act as an antioxidant and that carnosine’s activity as a metal chelator was concentration dependent. Salt has been shown to be a powerful pro-oxidant in meat systems; however, exactly how salt acts to increase oxidation in these systems has not been totally defined. Studies have shown that salt can stimulate lipid oxidation through iron activation.\textsuperscript{31} Chloride ions were reported to be involved in iron activation in mackerel muscle, while the sodium ions may act to displace the iron from the myoglobin creating free iron, which can catalyse lipid oxidation.\textsuperscript{32}

The role of transition metal ions as lipid oxidation accelerators is well documented in the literature. Carnosine can act to inhibit iron and copper accelerated oxidation.\textsuperscript{32} One of carnosine’s roles in controlling iron-catalysed oxidation is by scavenging hydroxyl groups generated by the Fenton reaction. In addition, iron has been shown to catalyse lipid oxidation in muscle in the presence of ADP and NADPH and it has been theorised that this occurs via a reduction of the ferric into the ferrous ions. Ferrous ions are more powerful than ferric ions in catalysing the decomposition of peroxides into free radicals.\textsuperscript{33}

Relative to copper-catalysed oxidation, carnosine can chelate copper but cannot chelate iron. Carnosine forms a tetramer with copper when its concentration is 100–1000 times more than that of the metal ion.\textsuperscript{33} The formed tetramer involves binding of four molecules of carnosine to copper through the N-3 of the imidazole ring.\textsuperscript{33} Although carnosine does form a complex with copper that appears to be unreactive, the complex may in fact still be catalytically active and able to form peroxy radicals.\textsuperscript{33} The ability or lack of ability of carnosine to form a complex with copper and iron, respectively, is not affected by the oxidation state of the metal.\textsuperscript{33} Lee et al. showed that carnosine was able to inhibit copper (II)-catalysed ascorbate oxidation.\textsuperscript{34} In addition carnosine has been shown to inhibit lipid peroxidation catalysts including hydrogen peroxide activated haemoglobin, photoactivated riboflavin and lipoxygenase.\textsuperscript{34} Lee and Hendricks\textsuperscript{35} evaluated the antioxidant activity of carnosine in model systems. In the presence of ascorbic acid,
carnosine inhibited the iron-catalysed deoxyribose degradation in a dose-dependent manner. This effect indicates that carnosine can scavenge the hydroxyl radical.

Carnosine has been tested for its ability in the presence of other antioxidants. In a salted and unsalted ground turkey dark muscle, Calvert and Decker\textsuperscript{36} tested carnosine (0.5\%) alone and in combination with \(\alpha\)-tocopherol (0.05\%), ascorbyl palmitate (0.05\%), sodium tripolyphosphate (0.5\%), or citrate (0.01 or 0.05\%). An additive effect was found with the combination of tocopherol and carnosine in both salted and unsalted meat systems. The combination of carnosine and ascorbyl palmitate was effective, as evidenced by reduced TBARS, in the salted meat but not in the unsalted meat. When carnosine was combined with either the STPP or citrate, they did not enhance its activity.

A series of studies with phytic acid with and without carnosine have been reported. Lee et al.\textsuperscript{37} evaluated carnosine and phytic acid for their effect on pH, meat colour stability, glycogen and oxidation levels in a fresh prerigor meat model system held at 4 °C for 30 days. They found that during the first 7.5 h of storage, carnosine- and phytic-treated systems had a more rapid decline in muscle pH and glycogen contents, with phytic acid having the greatest effect. During storage, the authors found that carnosine and phytic acid inhibited metmyoglobin production; carnosine kept the meat bright red while phytic acid maintained a purple-red color.\textsuperscript{37} Both carnosine and phytic acid inhibited TBARS in the test systems in a dose-dependent manner with phytic acid having the greatest effect. Lee et al.\textsuperscript{37} felt both of these compounds were chelating transition metals and preventing free radical production, which prevented myoglobin oxidation and kept TBARS low.

In another work, Lee and Hendricks\textsuperscript{38} added phytic acid (0 to 2 mM) to beef homogenate and tested for lipid oxidation. They also evaluated the effect of pH on the activity of phytic acid and found that as the pH increased so did the antioxidant activity with the best being at neutral or higher pH. Phytic acid complexes metals in a dose-dependent manner and these complexes are insoluble over a wide pH range. Phytic acid can bind with six divalent molecules and the metal can bridge between two or more phytate molecules, creating a complex that is unable to participate in the Fenton reaction and thus reducing the production of hydroxyl radicals. A molar ratio of 0.25 phytate to 1 of iron is minimal for controlling the superoxide generation of hydroxyl radicals. Lee and Hendricks\textsuperscript{39} also reported that phytate acted in a dose-dependent manner in controlling TBARS production in metal-catalysed model systems. It facilitated the conversion of \(\text{Fe}^{2+}\) to \(\text{Fe}^{3+}\) and prevented the reverse conversion of \(\text{Fe}^{3+}\) to \(\text{Fe}^{2+}\) by ascorbic acid.

Lee et al.\textsuperscript{34} showed that carnosine was more effective than ascorbic acid in controlling lipid oxidation, as measured by TBARS, in refrigerated beef patties. When the patties were cooked, carnosine increased cook yield and
salt-soluble proteins more than ascorbic acid. This was attributed to the fact that carnosine increased the pH of the meat which subsequently increased the water-holding capacity and solubility of the salt-soluble proteins. Ascorbic acid levels (low vs high) have different effects on lipid peroxidation. Low levels accelerate oxidation while high levels do not. At low levels ascorbic acid could be facilitating the formation of a Fe$^{2+}$-$\text{O}_2$-Fe$^{3+}$ complex which could initiate oxidation, while at high levels of ascorbic acid, it can reduce the ferric ions to ferrous ions and reduce lipid oxidation. Muscle foods do not contain appreciable amounts of ascorbic acid; therefore, its addition to meat could be beneficial. However, ascorbic acid can be readily oxidised in the presence of copper and/or iron with copper (II) being about 80 times more reactive than iron (III). With carnosine being able to inhibit the copper (III)-catalysed ascorbate oxidation, the application of these two compounds to ground beef could add some strong benefits in maintaining the quality of fresh ground beef.

Lipid stability and cholesterol oxidation were monitored in salted chicken thigh meat from chickens fed tocopherol 200 mg kg$^{-1}$ $\alpha$-tocopheryl acetate and then treated with carnosine. The meat was processed into patties to which carnosine (1.5%), salt (1%) or a mixture of both was added. The patties were held raw or cooked and then held refrigerated under fluorescent light. Including tocopherol into the diet or adding carnosine to the meat did reduce the oxidation of both the lipids and cholesterol. The combination showed the greatest level of protection.

**12.2.3.2 Plant phenolics**

Man has always relied on plant materials as part of his diet. Some plants such as herbs and spices were traditionally used to increase the palatability of the food. That these plant materials were also contributing to the safety and shelf-life of the food was not recognised to any great extent in the scientific literature until the late 1950s. The findings reported by Chipault and co-workers in 1957 and 1958 were hallmarks in what has now become the basis for the development of a more natural, healthier food supply. Plants from the family Labiatae, which includes rosemary, sage, thyme, oregano and the mints, to name a few, have shown strong promise for providing compounds capable of inhibiting lipid oxidation. Rosemary and sage have received the greatest amount of attention as potential inhibitors of WOF in meats and are usually found to be more effective than plants such as savory, marjoram and oregano.

Rosemary in the 1970s was originally processed as an oleoresin that was sold to provide flavour to foods and ‘by the way’ it had antioxidant activity. The problem with this product was that it contained strong colour and strong flavour that was only acceptable in a limited number of foods, one of which was meats. However, since that time, new rosemary-based systems have been developed which are colourless and odourless; this expands their potential use in a wider variety of foods. But since the focus of this chapter...
is animal-based foods, the following will only discuss findings in meat systems. Rosemary oleoresin has been shown to be as effective as polyphosphate and a combination of BHT/BHT/citric acid in mechanically deboned poultry meat\textsuperscript{44} and in a breakfast sausage made from mechanically deboned poultry meat.\textsuperscript{45} When combined with sodium tripolyphosphate (STPP), rosemary oleoresin (RO) was effective in controlling oxidation in restructured chicken nuggets.\textsuperscript{46} However, Stoick et al.\textsuperscript{47} reported that the combination of STPP/OR was not effective in restructured beef steaks. Liu et al.\textsuperscript{48} also found that neither an oil-soluble RO nor a water-soluble RO was as effective as STP in controlling oxidation in restructured pork steaks. In cooked frozen fish flakes, Boyd et al.\textsuperscript{49} found a rosemary extract to be as effective as TBHQ/ascorbic acid treatment in controlling lipid oxidation.

Lee et al.\textsuperscript{50} used mechanically deboned Leghorn spent hen meat to make breakfast sausages and evaluated the effect of two rosemary oleoresins (Herbalox\textsuperscript{®} Type O extract and Colorlife\textsuperscript{®} powdered concentrate) on the products' oxidative stability during refrigerated (10 days) and frozen (6 months) storage. In the refrigerated samples there was no difference in TBARS relative to the control; all levels were in the range 0.24–0.25 mg malondialdehyde (MDA)/kg sample. In the frozen samples, by 6 months the Colorlife\textsuperscript{®} treated samples had the lowest TBARS values (3.92 mg MDA/kg sample); the Herbalox\textsuperscript{®} did not exhibit antioxidant activity.

Vareltzis et al.\textsuperscript{51} studied the stability of filleted and minced horse mackerel (\textit{Trachurus trachurus}) and Mediterranean hake (\textit{Merluccius mediterraneus}) that had been treated with rosemary extract prior to being held frozen (\textdegree{}C) for 120 days. Mackerel is a high fat fish while the hake is a low fat fish. In both systems, rosemary-treated samples had lower rates of lipid oxidation during the study. In addition, there was reduced loss of polyunsaturated fatty acids (PUFA).

Wada and Fang\textsuperscript{52} showed that the mixture of \alpha-tocopherol (AT) (0.05 \%) and rosemary extract (RE) (0.02 \%) had the strongest activity (as measured by TBA and PV) among the antioxidants tested (rosemary, AT and BHA) in a sardine oil model system and in a frozen-crushed fish meat. Fang and Wada\textsuperscript{53} tested the activity of a combination of tocopherol and rosemary in a sardine oil model system and in the dark muscle of bonito fish. It was found that in the sardine oil system catalysed by \textit{Fe}^{2+} or haemoprotein, the mixture showed a stronger antioxidant activity than the individual antioxidants. They theorised that the synergistic mechanism between RE and AT was that AT acts as a primary antioxidant/hydrogen donor and RE acts to regenerate the AT by H donation. Once RE was depleted AT began to oxidise and was lost from the system. It was also believed that RE was able to chelate metals which contributed to the synergy in the \textit{Fe}^{2+} catalysed system.

More recently, Wong et al.\textsuperscript{54} studied lipid oxidation and its inhibition by rosemary and sage extracts and tocopherol in a cooked beef homogenate
held 5 days at 5°C. The addition of vitamin E at increasing levels (25–100 μg g⁻¹) showed a reciprocal reduction in TBARS. Addition of the rosemary and sage extracts (30 μg g⁻¹) reduced TBARS by 53 and 62%, respectively. When equal parts (15 μg g⁻¹) of tocopherol and each plant extract were tested in combination, no synergism was found. This could have been due to using a more purified rosemary system than that used by Fang and Wada;⁵³ the earlier extracts carried many other compounds (flavonoids) which could have contributed to the synergy observed in the earlier work. Rosemary has been shown to contain diterpenes, which have antioxidant activity, including carnosic acid (most active), carnosol, rosmarinic acid, and rosmarinic acid.

Many other natural antioxidant systems and pure compounds have been evaluated for activity in animal-based systems. Jurdi-Haldemann et al.⁵⁵ studied the antioxidant activity of onion (20%) and garlic (4.8%) juice vs controls containing salt (1%) or water in ground lamb patties that were cooked, cooled, vacuum packaged and stored in the light at 5°C for 3–7 days or stored at -20°C for 15–30 days. Onion juice was more effective than garlic juice on the TBARS and sensory analysis. Some of the effect, as measured by sensory analysis, could have been due to the flavour effect of the treatment as in masking WOF. Karastogiannidou⁵⁶ treated chicken thigh meat with onion or quercetin prior to cooking, packaging and storing at 5°C for 6 days. The addition of dried onion reduced lipid oxidation by 84% while equivalent levels of quercetin reduced TBARS by 59%. This indicated that quercetin was an important onion antioxidant but it did not account for the total activity of the onion. Onion was tested at two levels in the thigh meat, i.e. 1.6 and 3.0%, both levels reduced the TBARS levels, i.e. control at 5 days was at 19.9 mg MDA/kg vs 3.2 and 0.5, respectively for the 1.6 and 3% onion. Sensory analysis showed the higher level of onion to be offensive while the lower level was pleasant.

Ramanathan and Das⁵⁷ evaluated the antioxidant activity of some polyphenolics, including rutin, quercetin, morin, myricetin, kaemferol, tannic acid, ellagic acid, ascorbic acid and α-tocopherol in raw and cooked fish samples. It was found that quercetin, myricetin, tannic acid and ellagic acid were excellent antioxidants, but ascorbic acid was the pro-oxidant in the cooked fish. Ramanathan and Das⁵⁸ also tested seven natural systems, including fresh spices – ginger, turmeric, onion, and garlic; dry spices – cloves, cinnamon, cumin, black pepper, fennel, and fenugreek; and polyphenols – ellagic acid, tannic acid, myricetin and quercetin. The polyphenols were most active followed by the dry spices and then the fresh spices. Within each group it was found that ellagic acid > tannic acid > myricetin > quercetin; cloves > cinnamon > cumin = black pepper > fennel = fenugreek; ginger and turmeric were more potent that the onion and garlic.

Ahn et al.⁵⁹ treated ground turkey meat patties with a variety of natural antioxidants alone or in combination with tocopherol or ascorbate. The natural systems included tripolyphosphate (TPP), citrate, EDTA, cysteine,
histidine, ascorbate, BHA, or egg white. The meat was either hot or cold vacuum packaged before refrigerated storage. Hot packaged meat had lower TBARS values and by day 7 of storage all treatments had lower TBARS than the untreated control. When vacuum packaging was used in combination with tocopherol or ascorbate there was a reduction in TBARS with no difference between those treated with tocopherol or ascorbate. When the system was challenged with salt, ferrous chloride or both, it was found that all treatments reduced TBARS.

He and Shahidi\textsuperscript{60} using a model system made from blended white mackerel tissue tested the antioxidant effectiveness of green tea, extracts of green tea and individual catechins found in green tea. After addition of each treatment the fish was cooked and held at 4 °C for 7 days. All treatments had good activity and within the catechins the activity was decreasing in the order of EGCG (epigallocatechin gallate) ≈ ECG (epicatechin gallate) > EGC (epigallocatechin) >> EC (epicatechin). Catechins, flavonoids found in many plants, have been shown to have excellent antioxidant activity in many systems and act by scavenging free radicals and chelating metals. Green tea has been receiving wide attention for its potential health benefits, most of which has been attributed to the catechins.

Black pepper extract from supercritical carbon dioxide extractions was effective in inhibiting lipid oxidation in ground pork. Black pepper antioxidant activity has been attributed to its piperine and piperine isomers and some of its monoterpenes. Piperine isomers include chavicine, isopiperine and isochavicine.\textsuperscript{61}

12.3 Dairy products

12.3.1 Fluid milk

Milk is a very interesting and very perishable food system with a shelf-life affected by many factors including microbial load, processing factors (such as agitation, temperature of processing and/or storage prior to processing, exposure to light). In addition, milk contains components that affect its lipid stability, i.e. ascorbic acid, copper ions, tocopherols, etc. The inherent compounds in milk will be the focus of the following discussion.

Riboflavin in milk has positive and negative effects; it contributes to the high nutritional quality of milk but it also aggravates the light oxidation of milk fat. Under light, as found in dairy cases, riboflavin produces superoxide anions and singlet oxygen which are responsible for accelerated lipid oxidation. Allen and Joseph\textsuperscript{62} reported that riboflavin acts to initiate lipid oxidation in milk by two mechanisms; these can be shown as follows:

\[
\text{Riboflavin} + h \nu \rightarrow ^3\text{Riboflavin} \\
^3\text{Riboflavin} + ^3\text{O}_2 \rightarrow \text{Riboflavin} + ^1\text{O}_2 \\
^1\text{O}_2 + \text{LH} \rightarrow \text{LOO}^\cdot
\]
and

\[ ^3\text{Riboflavin} + \text{LH} \rightarrow \text{L}^* \]

\[ \text{L}^* + ^3\text{O}_2 \rightarrow \text{LOO}^* \]

In both cases the lipid peroxy radical (LOO*) is available for the propagation of lipid oxidation.

The actual mechanism producing ‘light oxidised’ flavour in milk starts with the oxidation of methionine and what happens next has been debated in the literature. Allen and Parks in 1975 reported finding methional in light-oxidised milk and postulated it was formed through a Strecker-like degradation mechanism.\(^{63}\) However, other researchers found evidence that methional decomposed to methyl mercaptan and dimethyl sulphide and these were the active flavour components.\(^{63}\) In addition, Dimick\(^{64}\) stated that three components were needed for generation of the light-oxidised flavour in milk, i.e. riboflavin, oxygen and protein-containing sulphur amino acids. Jung et al.\(^{63}\) conducted a study on the flavour impact in model milk systems exposed to light. Milk was prepared with and without riboflavin and one of three amino acids (methionine, cysteine or valine). Results showed that riboflavin alone did not contribute to off-flavour production nor did valine. However, in the cysteine/riboflavin sample a strong hydrogen sulphide-like odour was found and in the methionine/riboflavin sample a dimethyl disulphide-like odour was found.

As noted earlier, milk is an important source of riboflavin and its degradation represents a major dietary loss of an essential vitamin. The light-induced loss of riboflavin has been shown to follow first-order kinetics and is affected by many factors including the intensity and wavelength of the light, exposure time, and package system used; in addition the surface area relative to volume plays a role.\(^{65}\)

Ascorbic acid is another compound that plays a major role in the oxidative stability of milk lipids. It has strong singlet oxygen and superoxide anion quenching ability and has been shown to protect riboflavin loss in milk and in a dose-dependent manner. The type of milk also plays a role; 0.1 % ascorbic acid treatment reduced riboflavin loss by 25.5 and 50 % in skim and whole milk, respectively.\(^{65}\) Jung et al.\(^{63}\) reported similar results when they evaluated the effect of ascorbic acid on the development of light-oxidised flavour. They showed that the addition of ascorbic acid (200, 500 and 1000 ppm) reduced the formation of dimethyl disulphide by acting as a singlet oxygen quencher.

However, ascorbic acid can also act as a strong pro-oxidant and its effect has been related to concentration and to the presence of copper ions. Allen and Joseph\(^{62}\) noted that at low concentrations (20–100mg l\(^{-1}\)) ascorbic acid was a pro-oxidant but at higher levels (>500mg l\(^{-1}\)) it acted as an antioxidant; milk generally contains between 12 and 25mg l\(^{-1}\) ascorbic acid.
low level of ascorbic acid in combination with the presence of Cu²⁺ ions has been shown to play a major role in milk lipid oxidation. Copper ions are known to be strong promoters of the propagation stage of oxidation. Their presence in milk has been attributable to the fact that during processing the fat globule membrane is disrupted resulting in the release of protein bound copper. Aurand et al.⁶⁶ presented a mechanism to explain the interaction between ascorbic acid, copper and milk lipid oxidation, i.e.

\[
\text{Cu}^{2+} + \text{ascorbate} + {^{3}\text{O}_2} \rightarrow \text{O}_2^{\cdot \, \cdot} \rightarrow {^{1}\text{O}_2} \\
\text{Superoxide anion} \quad \text{Singlet oxygen}
\]

Both of these oxygen products are known to be promoters/initiators of lipid oxidation.

At present, the addition of antioxidants (synthetic) is not allowed in dairy products and so their stability must rely on components present in the milk. Milk does contain tocopherol and β-carotene that are known to act as antioxidants. Tocopherol has been shown to act as a hydrogen donor and to serve as a singlet oxygen quencher, while β-carotene acts as a singlet oxygen quencher. Several studies have looked at the effect of diet on increasing the stability of milk lipids. In 1991 Nicholson and St. Laurent⁶⁷ reported that supplementing Holstein cows with 7000 IU day⁻¹ of α-tocopherol acetate totally eliminated oxidised milk flavour. In 1998, Focant et al.⁶⁸ found that with dietary tocopherol supplementation, they were able to increase significantly the level of tocopherol in the milk and subsequently increase the stability of the milk fat. Fearon et al.⁶⁹ fed dairy cattle diets supplemented with naked oats and found oat supplementation increased the oxidative stability of the milk. The milk lipid was found to contain higher levels of monounsaturated fatty acids which contributed to this effect, but it was felt that other factors might also be pertinent. Oats have been shown to contain many antioxidant phenolic compounds.

The role of amino acids in stabilising milk lipids has also been investigated. Chen and Nawar,⁷⁰ using oxygen uptake, studied the effect of a series of amino acids, their non-amino acid counterparts, or the amino acid with its amine group blocked on the rate of oxidation in milk fat held at 95 °C. Of the amino acids tested, cysteine, tryptophan and lysine were most protective. Using the acid counterpart it was determined that the primary amino group was important in the antioxidant activity of amino acids. Blocking the epsilon amino group showed it plays a role but was not as important as the primary amino group. In addition it was found that the indole group was not important in the antioxidant activity of amino acids. Chen and Nawar⁷⁰ also showed that dissolution of alanine in water or 0.1 N HCl reduced its antioxidant activity. It was thought that in water, activity was lost due to possible hydrogen bonding of the amino acid with the water. The effect of the acid on activity was thought to be due to the
state of the —COOH group. In acid the —COOH would exist which is less effective than the —COO⁻ group.

Chen and Nawar also treated milk with the phospholipids dipalmitoylphosphatidyl-ethanolamine (DPE) or dipalmitoylphosphotidyl-choline (DPC) in a dry or wet (dissolved in water) system and stored it at 50 and 95 °C. Samples were monitored for oxidation using oxygen uptake. In the dry system it was found that DPE and DPC both exhibited antioxidant activity, with DPE being the best. The authors felt DPE’s antioxidant activity was due to the free amino group of the PE. The protonated amino group (NH₃⁺) accelerates oxidation while the non-protonated (NH₂) group inhibits lipid oxidation. This was thought to be due to the free amino group possibly reacting with free radicals from lipid oxidation forming Schiff’s base reaction products, which could also have antioxidant activity. However, in the wet system both DPC and DPE were pro-oxidant with DPE having the greatest impact. It is theorised that DPE could form a more dispersed system, which could allow more oxygen accessibility and enhance oxidation.

12.3.2 Milk protein stability

Although antioxidants are not used in milk, two studies were found involving the addition of phenolic compounds and evaluating their effects on the heat stability of the milk proteins. The stability of milk proteins to denaturation by heat, known as heat coagulation time (HCT), is affected by various factors found in the milk. A primary factor is pH and in typical milk heated to 140 °C, HCT increases as pH increases from 6.4 to 6.7 after which there is a sharp decrease and it is at its lowest at pH 6.9. After 6.9 there is a gradual increase in HCT. Typical milk is referred to as ‘type A’ milk. However, some milk, referred to a ‘type B’, is not typical and exhibits a HCT curve less affected by pH; the HCT increases with pH. Sweetsur and White reported that the type A milk coagulates in two steps, while the type B milk coagulates in one.

O’Connell and Fox evaluated the effect of polyphenolic compounds on milk protein heat stability. Chlorogenic acid, guaiacol, thymol, vanillin, BHT, PG, BHT and 2,5-dimethoxycinnamic acid and 3,4-dimethoxycinnamic did not affect the heat stability of the milk while quinic acid reduced it. Pyrogallol, catechol, tannic acid, ellagic acid, phloroglucinol, and gallate caused the heat coagulation time-pH profile to change from an A to a B type. Ferulic and vanillic acid increased heat stability in the region of the maximum, which did not recover with increased pH; however they had little effect on the minimum heat stability of the milk. Caffeic acid increased the heat stability of the milk protein.

These authors went on to study how caffeic acid increases the heat stability of milk. They found that several factors affected the ability of caffeic acid to alter the heat stability of milk:
Caffeic acid had to be converted to a more active quinone form and this conversion required the presence of oxygen and when in oxygen it was temperature dependent.

Caffeic acid was capable of chelating Ca\(^{2+}\) which is important since Ca\(^{2+}\) ions reduce the heat stability of milk proteins.

Caffeic acid can block the \(\varepsilon\)-amino groups of lysine and inhibit the production of hydroxymethylfuraldehyde (HMF), which is indicative of the Maillard reaction. The authors felt that blocking lysine also prevented the dissociation of \(\kappa\)-casein-rich proteins from the casein micelles.

Caffeic acid reduced the level of sulphhydryl in the heated milk by being able to interact with the cysteine group of the \(\beta\)-lactoglobulin and other whey proteins.

The final concept elucidated by O’Connell and Fox\(^75\) postulated the nature of the structural components responsible for the heat stabilisation effect of caffeic acid. Using a series of caffeic acid derivatives, they found that the hydroxyl groups played a role and they had to be in either the ortho- or para-position on the benzene ring; the chain on C-1 was important, i.e. replacing the C-1 chain with an aldehyde group increased the heat stabilising effect; but the double bond in the C-1 chain had no effect; saturation of the ring eliminated the activity; and it appeared that the carboxylic group was important in the calcium-chelating role.

### 12.3.3 Butter

The susceptibility of butter to oxidative reactions has been investigated. Emmons et al.\(^76\) showed that butter held frozen (\(-18^\circ\text{C}\)) and in the dark showed no evidence of oxidation after 1 year of storage, but there was some loss of butter quality after 14 weeks storage in the dark at 5\(^\circ\text{C}\). However, when Luby et al.\(^77,78\) stored butter in light, either in fluorescent in the cold (5\(^\circ\text{C}\)) or daylight at 22\(^\circ\text{C}\), evidence of lipid oxidation (cholesterol oxide production) was found. Their data showed that both singlet and free radical oxidation was occurring.

Other researchers have looked at the potential for natural antioxidants to prevent lipid oxidation in butter. Zegarska et al.\(^79\) showed that an ethanolic extract of rosemary increased the stability of butter against oxidation and that the effect was concentration dependent. This study also evaluated the effectiveness of the rosemary extract in inhibition of copper-catalysed oxidation and found evidence that the extract was able to chelate metals. Farag et al.\(^80\) showed that thyme and cumin essential oils could prevent oxidation in butter stored at room temperature, and at 200 ppm the essential oils were more effective than BHT in inhibiting lipid oxidation in the butter. Farag et al.\(^80\) felt the preservative effect of the essential oils from thyme and cumin was due to the phenols found in the oils. The phenolic hydroxy group would be able to donate hydrogen to lipid.
12.4 Eggs and egg products

Eggs, like milk, contain many naturally occurring antioxidant compounds. Because of the number, concentrations and location of the antioxidant compounds in eggs, the in-shell system is very stable to oxidation. Eggs contain two proteins, phosvitin and conalbumin that have been reported to have antioxidant activity. Phosvitin, a yolk protein, has been shown to inhibit Fe$^{2+}$ and Cu$^{2+}$ catalysed oxidation; it was more effective against iron than copper catalysis. Lu and Baker also showed that pasteurisation did not affect phosvitin’s antioxidant activity. Conalbumin (ovotransferrin) found in the albumin, can also bind di- and trivalent ions, including Fe$^{3+}$ and Cu$^{2+}$. Work by Froning et al. showed that conalbumin was capable of reducing oxidation in cooked turkey thigh meat.

Egg yolk contains a wide range of fatty acids ranging in length from 8 carbons to 20 carbons; of these around 11% are saturated with palmitic (7%) and stearic (3%) predominant; 14–15% are monounsaturated with oleic (13%) predominant and the remaining 4–5% are polyunsaturated with linoleic (3.8%) predominant. The yolk contains relatively high levels (7%) of phosphatidylethanolamine (lecithin) which has been shown to have antioxidant activity. In addition, the yolk contains α-tocopherol, again a proven antioxidant, and xanthophylls, including lutein, zeaxanthin and cryptoxanthins; lutein has been shown to have antioxidant activity.

As in the case of milk, addition of antioxidants to eggs is not allowed. However, several studies have reported the effects of dietary supplementation as a means of increasing the antioxidant content of eggs. Increasing the level of dietary tocopherol(s) has been shown to increase the lipid stability of eggs during storage. When menhaden oil was fed to hens, the eggs had higher levels of 20:5 n-3 and 22:6 n-3 fatty acids; dietary tocopherol supplementation was effective in reducing oxidation in these eggs during 40 days of storage at 4 °C. Tocopherol-supplemented eggs had higher (three–four-fold) levels of tocopherol. During storage there was a gradual loss of tocopherol across treatments, especially the δ-tocopherol, but the supplemented eggs retained the higher levels.

Two studies were found that had a novel twist; herbs known to contain antioxidant compounds were fed to hens. Laying hens were fed one of three diets, i.e. control, control +0.28 % rosemary extract or control +0.57 % rosemary extract. Eggs from the hens were collected approximately every 4 days for 28 days and analysed for carnosic acid content. It was found that the hen deposited carnosic acid into the yolk and deposition increased with increased dietary levels of rosemary extract; a maximum level of deposition occurred between day 12 and day 20. Botsoglou et al. showed that adding thyme to layer diets produced eggs which were more stable against oxidation. To explain some of their findings the authors also tested the effectiveness of added thyme, thymol (an active ingredient from thyme), a thyme
extract found to be 98% thymol, and ascorbic acid on the stability of the egg yolk. It was found that the thyme extract was a better inhibitor of oxidation. Ascorbic acid exhibited a pro-oxidant effect.

12.5 Cholesterol

Cholesterol oxidation has been of great interest due to its implication in heart-related diseases. Specifically, cholesterol oxidation products (COPS) have been shown to have potential as cytotoxic and mutagenic compounds. Cholesterol is found in the cell membrane and is associated with polyunsaturated fatty acids of the membranal phospholipids. Therefore, cholesterol oxidation is accelerated by the same factors affecting lipid oxidation and the level of cholesterol oxidation is closely related to the degree of unsaturation in the neighbouring fatty acids. Therefore, antioxidants effective in reducing lipid oxidation should have a strong impact on the oxidation of cholesterol.

Rankin and Pike\textsuperscript{88} used an aqueous meat model system to evaluate the effect of rosemary oleoresin (Herblox\textsuperscript{8}), quercetin, myricetin, tocopherols ($\alpha$-, $\gamma$-, and $\delta$-isomers) alone and mixed and BHA (control) on cholesterol oxidation at pH 5.5 and at 80 °C. Antioxidant activity was determined by measuring the induction period for the production of 7-ketocholesterol. It was found that rosemary oleoresin, quercetin, myricetin, or BHA were ineffective in reducing cholesterol oxidation. The tocopherols were able to inhibit cholesterol oxidation with the $\gamma$- and $\delta$-isomers being most active; no synergism was found with blends of the tocopherols. Dietary supplementation of tocopherol has also been shown to reduce the oxidation of cholesterol in chicken meat\textsuperscript{32,89} and tocopherol supplementation with or without surface application of a rosemary extract reduced cholesterol oxidation in rainbow trout muscle.\textsuperscript{90}

Dairy products are generally resistant to cholesterol oxidation, which is attributed to their low level of transition metals, low cholesterol content and a fat system that contains high levels of saturated fatty acids. However, drying milk can contribute to the production of COPS and since dry milk is usually stored at room temperature COPS production will occur. Angulo et al.\textsuperscript{91} studied the effect of factors including time, temperature and packaging atmosphere (air or nitrogen) on the production of cholesterol oxides (COPS) in whole and skim milk powders. They found a direct relationship between the level of COPS and storage time (1 year in the dark) at two different temperatures (32 °C and 55 °C). The primary COPS formed was 7-ketocholesterol. In samples stored in air, 7-ketocholesterol and 7$\beta$-hydroxycholesterol were found. These are formed via autoxidation involving triplet oxygen ($^3$O$_2$). When stored in nitrogen two additional COPS were found, i.e. cholestanetriol and $\alpha$-epoxide, indicating a double-oxidation which occurs through a ground-state dioxygen and hydroperoxy-
induced free radical mechanism. The samples held at 55 °C developed a brown colour, which indicated that the Maillard reaction initiated by free radicals was also occurring.

Although in-shell eggs are relatively stable against oxidation, dried eggs have been shown to be subject to lipid oxidation, specifically cholesterol oxidation. Several methods can be used to produce dried eggs, but the most common process is spray-drying. Many studies have reported the presence of COPS in spray-dried eggs and the data shows that COPS are formed at a higher level in direct fire spray-dryers as opposed to the indirect heat dryers. This difference in COPS production has been attributed to the formation of nitrogen oxides (NO and NO₂) that are formed in the natural gas flame.

Fontana et al. showed that egg powders stored at room temperature developed COPS, with production of the 7α and β-hydroxycholesterols, the β-cholesterol epoxide being predominant. Some work has been reported on the use of natural antioxidants in helping to control COPS production in spray-dried eggs. As in controlling lipid oxidation in meat, meat and dairy systems, increasing the inherent level of tocopherol in the egg via dietary supplementation has shown promise in reducing COPS in the final product. Li et al. also showed that the dried products from supplemented eggs had higher tocopherol levels and were therefore able to maintain their stability during four months of room temperature storage.

12.6 Summary and future trends

Natural antioxidants have great impact on the safety and acceptability of the food system and will continue to do so. Not only do they keep the food stable against oxidation but can also be effective in controlling microbial growth. By increasing the inherent levels of the antioxidants in animal products through dietary supplementation, we are providing a more consumer-acceptable product. This area of research is exploding in the literature. Since the first writing of this document, many articles have been published evidencing the viability of this practice in meat animal systems. That references, albeit lesser in number, were found for dairy produce and eggs shows that this avenue has just started being used to improve these food systems. However, as shown in this paper, the traditional practice of adding antioxidants during processing can still play a very important role since the added compounds have the potential for enhancing the activity of the inherent antioxidants systems. The literature cited shows that more work is needed to define the optimum dietary combinations and/or the minimum levels of the compound in the food necessary for obtaining the greatest stability in the resultant product. This may involve defining interactions of dietary components on the uptake on the desired compounds; this will eventually require more sophisticated feed formulations and a better understanding.
of the nutrient impact of the by-products that are traditionally used as animal feeds. One of the biggest problems for this approach is the cost of the feed, especially if plant phenolics are to be used. Sources need to be identified other than the traditional ones; for instance, rosemary may not always be the best source for its active compounds. Finding less valuable plants for feed formulations may be necessary.

12.7 Sources of further information

To learn more about the systems discussed the following books are recommended


12.8 References