White paper

The role of vitamin E in the antioxidant system and in enhancing meat quality

G. Litta, T.K. Chung and G.M. Weber, DSM Nutritional Products

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When selecting meat and meat products, the criteria buyers typically look for are color, flavor and texture. As the most immediate sign of quality, the visual aspect is usually the first parameter evaluated by consumers.

The origin of meat quality deterioration may come from different factors including genetics, as well as improper practices in either pre-slaughtering or in post-slaughtering handling, storage or cooking. However, lipid oxidation is probably the most important factor impacting the above mentioned parameters. Vitamin E can play an important role in reducing meat deterioration and color changes by reducing the negative effects of oxidation. This whitepaper discusses the unique antioxidant role of vitamin E and how this supports the stability of meat, with a review of key studies looking at the vitamin's mode of action, supplementation requirements and efficacy.

Understanding lipid oxidation

Lipid oxidation is a commonly occurring process which causes foods, including meat, to deteriorate and / or change in color. In meat, the process starts immediately after slaughter when blood circulation stops and anaerobic metabolism starts.

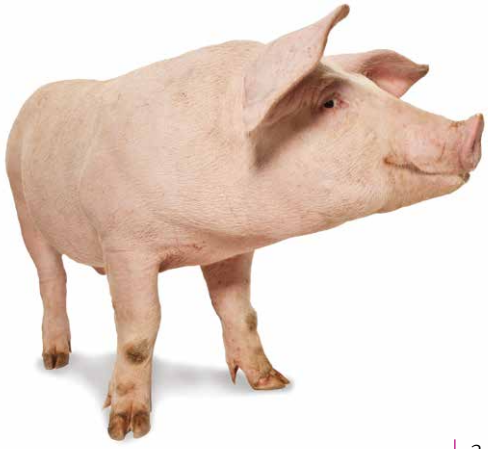
Lipid oxidation is catalyzed by the presence of free radicals. These are chemical entities with one or more free electrons which are derived from oxygen, nitrogen, sulfur and chloride. Hydroxide and nitrogen dioxide are among the most abundant free radicals and are often referred as 'Reactive Oxygen Species' (ROS) or 'Reactive Nitrogen Species' (RNS). Hydrogen peroxide is not a free radical but is also very detrimental to DNA.

Reactive species are physiological products of the metabolism, displaying fundamental cellular level functions when found in low concentration (e.g. in the immune system for the activation of phagocytes). When their concentration exceeds a certain threshold, these species become harmful to macromolecules such as lipids, proteins and DNA. Factors other than free radicals, which can also promote lipid oxidation, include the presence of metals like iron and copper, as well as compounds such as myoglobin and hemoglobin.

Importantly, lipid oxidation is an autocatalytic process. The products of oxidation catalyze further oxidations, resulting in a chain reaction. Once this cyclical process starts, the reaction rate rapidly increases.

Unsaturated fats – in particular those with a large number of double bonds – are extremely prone to oxidation. Cell membranes which contain phospholipids high in polyunsaturated fatty acids (PUFAs) are also particularly sensitive.

In meat preparation, mincing destroys the cell membranes and increases the surface exposed to oxygen therefore increasing the risk of oxidation. A further detrimental factor is the addition of sodium chloride during preparation, which is common in products such as sausages.



Consequences of lipid oxidation on meat quality

- **Off-flavor formation**

  Lipid oxidation degrades polyunsaturated fatty acids to volatile short-chain compounds such as aldehydes, ketones, alcohols, esters and acids.

  These compounds cause undesirable odors and flavors which strongly reduce the acceptability of the product by consumers. During cooking and subsequent storage, lipid oxidation in meat products is enhanced. This phenomenon, referred to as 'Warmed over Flavor', is a major concern for pre-cooked meats.

  Cholesterol oxidation

  Cholesterol is a component of cell membranes. 'Cholesterol Oxidation Products' (COPs) are detected at trace levels in raw meat while in storage. During cooking and irradiation the occurrence of COPs is enhanced due to an increase in the generation of free radicals, which arises due to the oxidation of PUFA's.

  COPs can cause adverse biological effects such as atherosclerosis, cytotoxicity, mutagenesis and carcinogenesis.

- **Drip loss**

  At the onset of rigor mortis, pH levels fall, myosin becomes denatured and actomyosin is formed. This causes a post-mortem shortening of myofibrils and promotes exudative loss from meat.

  Oxidative processes can affect the ability of the membranes to function as a semi-permeable barrier and may contribute to fluid leakage or drip loss (Jensen, 1998). For example, in fresh pork meat drip loss tends to increase during refrigerated storage and may cause product weight losses of 8-12 per cent (Dirinck et al., 1996, Monahan et al., 1994).

- **Muscle pigment oxidation**

  Color is the main factor affecting meat appearance at the time of purchase. The quality of this color is dependent on the chemical state of the muscle pigment, myoglobin.

  Myoglobin can exist in a ferrous oxygenated form, oxymyoglobin, which produces a cherry-red color. Alternatively, it can be found in a ferric oxidized metmyoglobin form, which is brownish-red in color (Faustman and Cassens, 1990). During storage and retail display, oxymyoglobin is oxidized to metmyoglobin, with the meat turning an undesirable brown color.

The antioxidant system

Living organisms have developed specific antioxidant mechanisms which protect against reactive species. It is only through the presence of antioxidants that living organisms can survive in an oxygen-rich environment. These mechanisms are described by the general term 'antioxidant system' (Halliwell and Gutteridge, 2007; Surai, 2002).

Antioxidants can be defined as "any substance that delays, prevents or removes oxidative damage to a target molecule" (Halliwell and Gutteridge, 2007). They are divided into two categories, based on specific key characteristics:

- **Enzymes:**
  - Superoxide Dismutase (SOD)
  - Catalase
  - Glutathione Reductase
  - Glutathione Peroxidase (GPX)
- **Non-enzymatic:**
  - Glutathione
  - Uric Acid
  - Vitamin C
  - β-carotene
  - Vitamin E

Based on their physical properties, antioxidants can also be categorized as:

- **Water-soluble antioxidants:**
  - Vitamin C
  - Glutathione
  - Uric acid
- **Lipid-soluble antioxidants:**
  - Vitamin E
  - β-carotene

Vitamin E: chemistry and metabolism

Vitamin E is a generic term used to describe eight lipid-soluble compounds found in nature. Four of these compounds are called tocotrienols and the other four tocopherols. These compounds are isomers and differ from each other by their chemical structure. Tocopherols have higher efficacy than tocotrienols. In particular α-tocopherol is the most common and, biologically, most active form.

Vitamin E is supplemented in the diet as the ester of all-rac α-tocopherol. This ester, all-rac α-tocopheryl acetate, is characterized by greater storage, feed processing and passage stability through the digestive tract of animals. Pancreatic esterases rapidly release the native α-tocopherol for absorption from the small intestine (Faustman et al., 1998).

Studies conducted on the role and requirements of vitamin E are typically performed with commercially available all-rac α-tocopheryl acetate.

An extensive body of research has provided detailed information about vitamin E's exact metabolic function:

1. Vitamin E is absorbed by epithelial cells in the small intestine, where it is incorporated into chylomicrons
2. It is then transported via the intestinal lymph to the liver
3. From the liver cells it is secreted by an α-tocopherol transfer protein into general circulation
4. Finally, it is deposited into the membranes and sub-cellular structures, where it exerts its protective effects against phospholipids peroxidation

The role of vitamin E in the antioxidant system

An antioxidant system is made of various compounds, located in different cellular, sub-cellular and extra-cellular spaces and providing different levels of defense. Each antioxidant plays a specific role in the system and interacts with other compounds in a unique way. It is therefore important that all these compounds are present in the system; one cannot replace another.

The antioxidant system basically works at three different levels of defense:

- **First level**
  Responsible for the prevention of free radicals formation by antioxidant enzymes such as SOD and GPX
- **Second level**
  Combats the production of free radicals and is made of chain-breaking antioxidants such as vitamin E, β-carotene, vitamin A, vitamin C and uric acid
- **Third level**
  Activated for eliminating or repairing the molecules damaged by free radicals. It primarily consists of enzymes such as lipases, proteases, nucleases and various transferases.

  The location of vitamin E in the membrane allows it to function very efficiently in protecting highly oxidisable polyunsaturated fatty acids from peroxidation by reactive oxygen species (Liu et al., 1995). The molar concentration of vitamin E in the membranes is lower compared to phospholipids present. However, it is particularly efficient due to the fact that oxidized vitamin E can be converted back into the active form. It does this by reacting with other antioxidants like vitamin C and / or carotenoids.

  The chain-breaking antioxidant function of vitamin E is very strong and specific: it stops the self-perpetrating production of lipid peroxides (Benzie, 1996). However, for optimum efficacy in preventing lipid oxidation, vitamin E needs to be supplemented in feed at higher levels than usually given as the vitamin E which is not used for metabolic purposes is going to be deposited in the cellular membranes. When added to meat post-mortem, the vitamin will not be physiologically and naturally incorporated in cellular membranes (Liu et al., 1995).

Vitamin E and oxidative stability of meat

Several research papers have demonstrated the mode of action and effect of vitamin E in reducing lipid oxidation and improving meat quality. It has also been demonstrated vitamin E improves the fluidity of membranes and therefore reduces drip loss. The biggest effect in this area can be measured in frozen meat, where crystals penetrate the cell membrane and facilitate the leakage of the cytosol.

- **Poultry**

  In a recent trial (Barroeta 2007) carried out on broilers, 200mg of vitamin E per kg of feed was found to reduce the development of thiobarbituric acid reactive substances (TBARS) – a byproduct of lipid peroxidation – by between 84 and 88 per cent. The production of oxidized cholesterol compounds in chicken meat was also found to lower by 50 per cent. In addition, it has also been established that higher levels of vitamin E in feed reduces the concentration of secondary oxidation products, such as aldehydes and ketones, by around 50 per cent.

- **Swine**

  The same levels of improvement have been found in the oxidative stability of pig meat (Buckley et al., 1995; Morrissey et al., 1996; Morrissey et al., 1998). A positive vitamin E antioxidant effect has been observed when supplemented at 200 mg per kg of feed in pig rations fortified with 3 per cent soy oil and tallow (Monahan et al., 1992).

  A comprehensive review published in 2001 analyzed and summarized 10 studies conducted between 1991 and 1998 which assessed nutritional impacts on pork quality (Pettigrew and Esnaola). The review found a consistent improvement of pork meat oxidative stability when pigs received 100 to 200 mg per kg feed of vitamin E.

- **Cattle**

  An analysis of 13 studies in cattle and 10 studies in pigs by Sales and Koukolová V. (2011), assessing the effects of vitamin E supplementation on muscle a-tocopherol levels, lipid oxidation and meat color. The rate of accumulation was slower in cattle than in pork meat. Data from swine indicated that tissue saturation in α-tocopherol was reached when feeding 200 to 500 mg per kg of diet. Reinforcing previous studies (Arnold et al., 1993), beef muscle α-tocopherol concentration could be increased from 1.4 to 3.3 µg/g of meat by feeding at least 1,300 mg/d of vitamin E for 44 days.

  Data also indicated that a muscle α-tocopherol value ›3 µg/g of meat was a threshold value for maximally retard metmyoglobin formation, and related color change, in beef muscle.

- **Meta-analysis studies**

  More recently, strong confirmation of the positive effect of dietary vitamin E levels on lipid oxidation has been provided by a meta-analysis (Trefan L. et al., 2011). The authors analyzed 10 published experiments where lipid oxidation was measured and 13 where vitamin E accumulation was evaluated, in both cases using *M. longissimus dorsi* muscle. The scope of the analysis was to determine the relationship between dose and duration of vitamin E supplementation and evolution of oxidative processes in post-slaughter muscle.

This meta-analysis established a quantitative relationship between dietary vitamin E and its effects on pork meat quality. A maximum accumulation in muscle tissue was achieved at ca. 6.4mg α-tocopherol per gram of tissue. At least 100 mg vitamin E per kg of feed was required to gain a significant decrease in lipid oxidation and every 1 mg of α-tocopherol per gram of tissue decreased lipid oxidation by ca. 0.05 TBARS units in *M. longissimus dorsi*. The meta-analysis also indicated that lipid oxidation gradually increased over three to four days storage post-slaughter and then stabilized up to 10 days post-slaughter.

- **Improving color stability in pork**
  The same authors carried out a meta-analysis on the effects of dietary vitamin E and storage conditions on the color stability of pork meat (Trefan L. et al., 2010). The analysis found a linear relationship between redness and α-tocopherol concentration. The model revealed that an increase of 1mg of α-tocopherol in the muscle led to an expected increase of 0.11 in redness across all storage times. Overall, the meta-analysis suggested that vitamin E supplementation affected redness of pork, but only when supplementation exceeds 100 mg per kg of feed and after six days post-slaughter.

- **Reducing drip loss in swine**
  A meta-analysis of the impact of vitamin E on enhancing pork meat's water holding capacity (Apple, 2007) recorded the following results:

Dietary vitamin E supplementation (mg/kg feed)	Water-holding capacity improvement (per cent)
100	10.1
200	30.5
Above 400	25.9

The analysis' author recommended that pigs should be fed a level of 200 mg of vitamin E per kg of feed for 84 to 130 days before slaughter. Much higher levels (750 to 1.000 mg/kg feed) could be fed for a minimum of 45 days in order to achieve a minimum threshold concentration of 2.6mg α-tocopherol per gram of meat tissue (Asghar et al., 1991; Dunshea et al., 2005)

Conclusion

The susceptibility of meat to lipid oxidation is influenced by the α-tocopherol content of the meat and the PUFA content of the membrane phospholipids. The beneficial effects of vitamin E on enhancing both poultry and pork meat quality, which stem from its membrane antioxidant properties in the lipid environment are scientifically proven and commercially tested, validated and implemented.

This is attributed to the ability of dietary vitamin E, after ingestion and absorption, to deposit and accumulate in meat. Therefore, vitamin E being the nature's most effective lipid-soluble, chain breaking antioxidant, its unique function of protecting cellular membrane integrity cannot be replaced by other antioxidants or substances possessing antioxidant-like properties.

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