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Vitamin E and meat quality

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Lipid oxidation is a major cause of deterioration in the quality of muscle foods. Oxidation leads to the production of off-flavours and odours, reduction of polyunsaturated fatty acids, fat-soluble vitamins and pigments, lower consumer acceptability, and the production of compounds such as peroxides and aldehydes which may be toxic. Lipid oxidation is a free-radical-mediated process which occurs in raw muscle, and especially in cooked muscle. The process is believed to be initiated at the membrane level owing to the oxidation of the highly unsaturated membrane lipids. Modern trends towards convenience foods have resulted in an increase in the production of precooked and restructured meat products which are very susceptible to lipid oxidation. In addition, dietary recommendations favouring the consumption of less saturated fat have led to an increase in demand for foods containing higher levels of unsaturated fatty acids. However, such foods are very susceptible to peroxidation, and present the food technologist with new challenges.

LIPID OXIDATION IN MUSCLE SYSTEMS

It is generally believed that lipid oxidation in muscle foods is initiated in the highly unsaturated phospholipid fraction in subcellular membranes (Gray & Pearson, 1987). The autocatalytic peroxidation process probably begins immediately after slaughter. The biochemical changes that accompany the conversion of muscle to meat give rise to conditions where the process of lipid oxidation is no longer tightly controlled and the balance between pro-oxidative factors and antioxidative capacity favours oxidation. Orderly metabolic activity continues during the early post-slaughter period, but because of the cessation of blood flow, the product of glycogen breakdown becomes lactic acid. This accumulates in the tissue, gradually lowering the pH from near neutrality to a more or less mildly acid (approximately pH 5·5) value. In the post-slaughter phase, it is highly unlikely that the armoury of defensive mechanisms available to the cell in the live animal still function because of quantitative changes in several metabolites and physical properties (Table 1). The rate and extent of oxidation of muscle foods are also likely to be influenced by preslaughter events such as stress, and post-slaughter events such as: early postmortem pH, carcass temperature and techniques such as electrical stimulation. In addition, any disruption of the integrity of muscle membranes by mechanical deboning, mincing, restructuring or cooking alters cellular compartmentalization. This facilitates the interaction of pro-oxidants with unsaturated fatty acids, resulting in the generation of free radicals and propagation of the oxidative reaction (Asghar *et al.* 1988).

Lipid oxidation in muscle foods is catalysed by myoglobin, haemoglobin, cytochromes

Table 1. *Post-slaughter changes which predispose muscle foods to oxidation*

Circulation of blood ceases
pH declines to approximately 5·5
Circulation of nutrients rapidly ceases
Preventative antioxidant enzymes are unlikely to function
Acute-phase proteins (ceruloplasmin (*EC* 1.16.3.1), transferrin, haptoglobulin) which scavenge Fe are unlikely to be activated
Sarcoplasmic reticulum loses its Ca-accumulating ability
Ca-dependent proteinases (μ-calpain and m-calpain) degrade muscle proteins
Some destruction of cell compartmentalization
Free or low-molecular-weight chelatable Fe is released
Membrane lipid peroxidation is initiated

and non-haem-Fe (Tichivangana & Morrissey, 1985). However, the relative catalytic effects of the various fractions on lipid oxidation in muscle foods has not been clearly defined (Decker *et al.* 1993; Monahan *et al.* 1993*a*).

Lipid oxidation is inhibited by nitrite (Morrissey & Tichivangana, 1985), metal-chelating agents (Sato & Hegarty, 1971) and synthetic antioxidants (Crackel *et al.* 1988). In recent years, however, resistance to the use of synthetic antioxidants in foods has increased. There is now considerable interest in the antioxidant properties of naturally occurring substances, including vitamin E, which is usually incorporated in the diet as α-tocopheryl acetate.

DIETARY VITAMIN E SUPPLEMENTATION AND TISSUE LEVELS

Recent studies in our laboratories have evaluated the influence of dietary vitamin E on tocopherol concentration in chick and pig tissues. In poultry, tissue α-tocopherol concentrations respond to dietary intake in the order: heart lung > liver > thigh muscle > brain (Sheehy *et al.* 1991). These findings are in agreement with those reported from experiments using pigs (Monahan *et al.* 1990*b*).

α-Tocopherol levels in tissues also depend on the supplementation time. Brandon *et al.* (1993) investigated the time-course of α-tocopherol uptake by broiler leg and breast muscle in order to determine the time necessary for saturation to occur. One group of chicks was fed on a basal diet containing 30 mg α-tocopheryl acetate/kg feed continuously up to slaughter at 6 weeks, while other groups were given a supplemented diet containing 200 mg α-tocopheryl acetate for 1, 2, 3, 4 or 5 weeks immediately before slaughter. The α-tocopherol content of leg and breast muscle increased as the pre-slaughter supplementation period increased from 0 to 5 weeks.

α-Tocopherol levels in porcine tissues also depend on the supplementation time. When pigs were fed on a diet supplemented with 200 mg α-tocopheryl acetate/kg, the α-tocopherol levels increased with increasing supplementation time up to 13 weeks in all tissues examined (Sisk *et al.* 1994). Kidney fat and subcutaneous fat demonstrated a further significant increase between week 13 and week 18. α-Tocopherol was found in highest concentrations in kidney fat, followed by subcutaneous fat lower layer > subcutaneous fat upper layer > muscle.

DIETARY VITAMIN E SUPPLEMENTATION AND LIPID OXIDATION

The rate and extent of lipid oxidation in meats are dependent on a number of factors including the α-tocopherol concentration and the degree of unsaturation of the fatty acids present in the muscle system (Buckley & Morrissey, 1992). Monahan *et al.* (1990*a*,*b*) showed that dietary supplementation (200 mg α-tocopheryl acetate/kg feed) significantly improved the oxidative stability of both raw and cooked pork muscle during storage at 4° for up to 8 d. The oxidative stability of rendered fat was also significantly improved. In similar studies, Asghar *et al.* (1991) showed that pork chops from pigs receiving α-tocopheryl acetate at 200 mg/kg diet exhibited the smallest increase in thiobarbituric acid-reacting substances (TBARS) numbers when stored at 4° under fluorescent light for up to 10 d.

Sheehy *et al.* (1990) observed a significant reduction in the rate of Fe–ascorbate-induced peroxidation in dark muscle from chicks fed on a diet containing 64 or 180 mg α-tocopheryl acetate/kg, compared with those fed on diets containing 5 or 25 mg/kg. The oxidative stability during frozen storage (−20°) of raw and cooked leg muscle from chicks fed on high levels of α-tocopheryl acetate (65 or 180 mg/kg) was greater than that of the chicks given lower levels (5 or 25 mg/kg) (Sheehy *et al.* 1993*a*). Dietary vitamin E also protected ground leg and breast muscle against the pro-oxidant effect of NaCl (Brandon *et al.* 1992).

The effect of dietary vitamin E supplementation on the sensory properties of broiler meat during storage have been studied by several authors. Recently, Blum *et al.* (1992) reported that the flavour of meat from broilers fed on a control diet containing 20 mg α-tocopheryl acetate/kg feed deteriorated significantly during 12 d storage at 4°, whereas flavour remained unchanged in meat from broilers fed on a supplemented diet (160 mg/kg) up to the end of the storage period.

EFFECT OF DIETARY FAT AND VITAMIN E ON LIPID OXIDATION

Fat composition

There is now considerable emphasis on modification of the fatty acid composition of animal tissues in an attempt to produce new 'designer' or 'functional' foods. However, increasing the degree of unsaturation of the muscle membranes by dietary manipulation reduces oxidative stability in pig meat (Monahan *et al.* 1992*a*) and poultry meat (Lin *et al.* 1989*b*). Monahan *et al.* (1992*a*) observed that muscle from pigs fed on a soya-bean-oil diet (50 g oil/kg) had significantly higher $C_{18:2}/C_{18:1}$ values in the neutral and polar lipid fractions of muscle tissue and in the total fraction of adipose tissue, when compared with pigs fed on a tallow diet (50 g/kg). In addition, muscle from pigs fed on the soya-bean-oil diet was significantly more susceptible to lipid oxidation than muscle from pigs fed on the tallow diet. α-Tocopheryl acetate supplementation (200 mg/kg) significantly increased the oxidative stability of muscle from pigs fed on both the tallow and soya-bean-oil diets. Lin *et al.* (1989*b*) also reported that meat from broilers fed on olive oil or coconut oil was consistently more stable than meat from broilers fed on linseed oil. Again, oxidative stability was significantly improved by dietary supplementation with α-tocopheryl acetate (100 mg/kg).

Fat quality

Feeding oxidized lipids to different species can result in reduced appetite, growth depression, diarrhoea and even death. However, the general consensus has been that consumption of abused fats at realistic levels is not harmful, since the dietary intake of lipid oxidation products is likely to be low. Recent studies (Lin *et al.* 1989*a*; Sheehy *et al.* 1993*b*, 1994) suggest that caution should be exercised in the use of oxidized oils in the feeding of poultry if undesirable changes in composition and oxidative stability of carcass lipids are to be avoided. One of the consequences of feeding chicks oxidized oil is a reduction in the concentration of α-tocopherol in the muscle tissue (Sheehy *et al.* 1993*b*, 1994). This effect occurs even when the α-tocopheryl acetate concentrations of the fresh and oxidized oil diets are the same. For example, chicks fed on diets containing heated sunflower or linseed oil (supplemented to concentrations of 50 mg α-tocopheryl acetate/kg feed) had significantly lower α-tocopherol concentrations in thigh and breast muscle, compared with those fed on diets containing fresh oil and similar concentrations of α-tocopheryl acetate (Sheehy *et al.* 1993*b*). As a consequence, the susceptibility of the muscle tissues to Fe–ascorbate-induced lipid oxidation was increased. The results suggest that chronic ingestion of oxidized lipids may compromise free-radical-scavenging ability *in vivo* by depleting α-tocopherol in the gastrointestinal tract, or possibly in plasma and other tissues. If inferior-quality lipids are to be fed to poultry or pigs (Murphy *et al.* 1991), the dietary α-tocopheryl acetate concentrations must be increased to compensate for the increased oxidative stress imposed on the tissues and the meat products.

VITAMIN E AND CHOLESTEROL OXIDATION

Many researchers have been slow to recognize that cholesterol also undergoes autoxidation by a free radical mechanism involving the abstraction of a labile H from the molecule by peroxy or oxyradicals of polyunsaturated fatty acids (Smith, 1981). Cholesterol oxidation products (COPS) have been identified in a variety of processed foods (Addis & Warner, 1991; Pie *et al.* 1991). The latter group observed that COPS increased significantly on cooking of meats and during subsequent refrigerated storage. In a recent study, we investigated the effectiveness of dietary α-tocopherol in controlling cholesterol oxidation (Monahan *et al.* 1992*c*). Three COPS, namely 5β,6β-epoxycholestan-3β-ol (β-epoxide), cholest-5-ene-3β,7β-diol (7β-OH) and 7-oxocholest-5-en-3β-ol (7-keto) were consistently identified and present in detectable amounts in all cooked samples. After 2 d of storage at 4°, cooked pork from pigs fed on the supplemented diet (100 or 200 mg α-tocopheryl acetate/kg) had significantly lower levels of β-epoxide, 7β-OH, 7-keto and total COPS, and lower TBARS numbers, than pork from pigs fed on the basal diet (10 mg/kg). In addition, TBARS numbers and cholesterol oxide product formation were positively correlated in cooked pork.

VITAMIN E AND COLOUR STABILITY

In red meats, a bright red colour is perceived by consumers as being indicative of freshness, while consumers discriminate against meat which has turned brown in colour. The rate of discolouration of meat is believed to be related to the effectiveness of oxidative processes and enzymic reducing systems in controlling metmyoglobin levels in beef (Faustman *et al.* 1989). Dietary supplementation with α-tocopheryl acetate

effectively controlled loss of desirable colour, lipid oxidation and accumulation of metmyoglobin in beef (Faustman *et al.* 1989; Arnold *et al.* 1993). Metmyoglobin formation and the accumulation of lipid oxidation products were linearly related and positively correlated (Faustman *et al.* 1989). Analysis of data showed that an α-tocopherol concentration in excess of 3 mg/kg meat is necessary to delay metmyoglobin accumulation or increases in TBARS values in ground beef.

Monahan *et al.* (1992*b*) showed that TBARS values were lower, and surface redness (Hunter 'a' values) were higher in pork chops from pigs fed on 100 or 200 mg α-tocopheryl acetate/kg diet, compared with pigs fed on 10 mg/kg diet after 2, 4, 6 and 8 d of refrigerated storage.

VITAMIN E AND DRIP LOSS FROM MEAT

Recent evidence shows that drip losses from meat may be reduced by feeding high levels of vitamin E. Asghar *et al.* (1991) reported that drip losses from thawing pork steaks were lower in muscle from pigs fed on α-tocopheryl acetate-supplemented diets (100 and 200 mg/kg feed). In a recent study, Monahan *et al.* (1994) showed that dietary α-tocopherol supplementation (200 mg/kg feed) led to a reduction in both lipid oxidation and drip losses. However, the results suggest that the reduced exudation in pork chops from pigs fed on supplemental α-tocopherol may not be directly related to oxidative-induced changes in membrane fluidity.

FUNCTION OF VITAMIN E IN MUSCLE FOODS

Vitamin E is now well accepted as nature's most effective lipid-soluble, chain-breaking antioxidant, protecting cell membranes from oxidative damage. A recent paper by Monahan *et al.* (1993*b*) showed that in the muscle microsomal fraction, dietary α-tocopherol supplementation led to a suppression in the production of free radicals. When microsomal fractions were stressed with $FeCl_2$, the rate of formation of the free radical adduct of α-(4-pyridyl-1-oxide)-N-tert-butylnitrone (4-POBN) was higher in fractions from pigs fed on the control diet (10 mg α-tocopheryl acetate/kg feed) than in those from pigs fed on the supplemented diet (200 mg/kg).

The effect of vitamin E on the fluidity of porcine muscle microsomal fraction has also been investigated (Monahan *et al.* 1994). Fluorescence anisotropy (r_s) measurements, using the membrane probe diphenylhexatriene (DPH) showed that microsomal membranes from pigs fed on an α-tocopheryl acetate-supplemented diet (200 mg/kg) were significantly less susceptible to changes in r_s values than fractions from pigs fed on the control diet (10 mg/kg). The increase in r_s, indicative of a reduction in the mobility of DPH in the lipid bilayer and a decrease in membrane fluidity, is likely to result from an increase in the molecular order of fatty acyl chains in the bilayer. Crosslinking between acyl radicals, protein–acyl radicals or via the bifunctional malonaldehyde molecule may also be responsible for changes in membrane structure.

CONCLUSIONS

The present review focused primarily on the effect of vitamin E and lipids on α-tocopherol status, oxidative stability, safety and quality of muscle food systems. It is

tempting to speculate that under ideal conditions, all cellular and subcellular membranes have an 'optimum' lipid profile (almost certainly not highly saturated), which allows cell functions such as membrane transport, enzyme activity and eicosanoid production to proceed in an optimal fashion. In parallel, cells possess what is increasingly recognized as a highly organized defence system to prevent the spread of oxidative reactions, both within the membrane and between membranes and other cellular constituents. One of the challenges for biologists, nutritionists, biochemists and other workers in this field is to elucidate the optimal balance between membrane lipids and antioxidants, so that cells may escape the effects of harmful oxidative reactions, and function closer to their genetic potential. In addition to vitamin E, the function of dietary carotenoids and vitamin C in biological systems and muscle foods must be studied. Peptides such as carnosine, anserine and homocarnosine also have interesting antioxidant properties, and may possibly be modified by dietary intake. Retinol is likely to be significant as a preventative antioxidant, since it is necessary for tissue integrity and compartmentalization of Fe. Last, several nutritionally essential minerals such as Zn, Cu, Mn and Se, which are incorporated into protective antioxidant enzymes, may indirectly increase the inherent oxidative stability of muscle foods.

REFERENCES

Addis, P. B. & Warner, G. T. (1991). The potential health aspects of lipid oxidation products in foods. In *Free Radicals and Food Additives*, pp. 77–119 [O. I. Arouma and B. Halliwell, editors]. London: Taylor Francis.

Arnold, R. N., Scheller, K. K., Arp, S. C., Williams, S. N. & Schaefer, D. M. (1993). Dietary α-tocopheryl acetate enhances beef quality in Holstein and beef breed steers. *Journal of Food Science* **58,** 28–33.

Asghar, A., Gray, J. I., Booren, A. M., Gomaa, E. A., Abouzied, M. M. & Miller, E. F. (1991). Effects of supranutritional dietary vitamin E levels on subcellular deposition of α-tocopherol in the muscle and on pork quality. *Journal of the Science of Food and Agriculture* **57,** 31–41.

Asghar, A., Gray, J. I., Buckley, D. J., Pearson, A. M. & Booren, A. M. (1988). Perspectives on warmed-over flavour. *Food Technology* **42** (6), 102–108.

Blum, J. C., Tourraille, C., Salichon, M. R., Richard, F. H. & Frigg, M. (1992). Effect of dietary vitamin E supplies in broilers. 2. Male and female growth rate, viability, immune response, fat content and meat flavour variations during storage. *Archiv für Geflügelkunde* **56,** 37–42.

Brandon, S., Morrissey, P. A. & Buckley, D. J. (1992). Effect of dietary α-tocopherol and sunflower oil on the oxidative stability of chicken tissues. In *Proceedings of the 38th International Congress of Meat Science and Technology*, pp. 463–466. Clermont-Ferrand, France: ICoMST.

Brandon, S., Morrissey, P. A., Buckley, D. J. & Frigg, M. (1993). Influence of dietary α-tocopheryl acetate on the oxidative stability of chicken tissues. *Proceedings of 11th European Symposium on The Quality of Poultry Meat*, pp. 397–403. Tours, France: World Poultry Science Association.

Buckley, D. J. & Morrissey, P. A. (1992). *Vitamin E and Meat Quality*. Switzerland: F. Hoffmann-La Roche Ltd.

Crackel, R. L., Gray, J. I., Booren, A. M., Pearson, A. M. & Buckley, D. J. (1988). Effect of antioxidants on lipid stability in restructured beef steaks. *Journal of Food Science* **53,** 656–657.

Decker, E. A., Crum, A. D., Shantha, N. C. & Morrissey, P. A. (1993). Catalysis of lipid oxidation by iron from an insoluble fraction of beef diaphragm muscle. *Journal of Food Science* **58,** 233–236, 258.

Faustman, C., Cassens, R. G., Schaefer, D. M., Beuge, D. R., Williams, S. N. & Scheller, K. K. (1989). Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. *Journal of Food Science* **54,** 858–862.

Gray, J. I. & Pearson, A. M. (1987). Rancidity and warmed-over flavour. *Advances in Meat Research* **3,** 221–269.

Lin, C. F., Gray, J. I., Asghar, A., Buckley, D. J., Booren, A. M., Crackel, R. L. & Flegel, C. J. (1989*a*). Effect of oxidized dietary oil and antioxidant supplementation on broiler growth and meat stability. *British Poultry Science* **30,** 855–864.

Lin, C. F., Gray, J. I., Asghar, A., Buckley, D. J., Booren, A. M. & Flegel, C. J. (1989*b*). Effects of dietary oils and α-tocopherol supplementation on lipid composition and stability of broiler meats. *Journal of Food Science* **54,** 1457–1460.

Monahan, F. J., Buckley, D. J., Gray, J. I., Morrissey, P. A., Asghar, A., Hanrahan, T. J. & Lynch, P. B. (1990*a*). Effect of dietary vitamin E on the stability of raw and cooked pork. *Meat Science* **27,** 99–108.

Monahan, F. J., Buckley, D. J., Morrissey, P. A., Lynch, P. B. & Gray, J. I. (1990*b*). Effect of dietary α-tocopherol supplementation on α-tocopherol levels in porcine tissues and on susceptibility to lipid peroxidation. *Food Sciences and Nutrition* **42F**, 203–212.

Monahan, F. J., Buckley, D. J., Morrissey, P. A., Lynch, P. B. & Gray, J. I. (1992*a*). Influence of dietary fat and α-tocopherol supplementation on lipid oxidation in pork. *Meat Science* **31,** 229–241.

Monahan, F. J., Crackel, R. L., Gray, J. I., Buckley, D. J. & Morrissey, P. A. (1993*a*). Catalysis of lipid oxidation in muscle model systems by haem and inorganic iron. *Meat Science* **34,** 95–106.

Monahan, F. J., Gray, J. I., Asghar, A., Buckley, D. J. & Morrissey, P. A. (1992*b*). Influence of dietary vitamin E (α-tocopherol) on the colour stability of pork chops. In *Proceedings of the 38th International Congress of Meat Science and Technology*, Clermont-Ferrand, France, pp. 543–546.

Monahan, F. J., Gray, J. I., Asghar, A., Haug, A., Shi, B., Buckley, D. J. & Morrissey, P. A. (1993*b*). Effect of dietary lipid and vitamin E supplementation on free radical production and lipid oxidation in porcine muscle microsomes. *Food Chemistry* **46,** 1–6.

Monahan, F. J., Gray, J. I., Asghar, A., Haug, A., Strasburg, G. M., Buckley, D. J. & Morrissey, P. A. (1994). Influence of diet on lipid oxidation and membrane structure in porcine muscle microsomes. *Journal of Agriculture and Food Chemistry* **42,** 59–63.

Monahan, F. J., Gray, J. I., Booren, A. M., Miller, E. R., Buckley, D. J., Morrissey, P. A. & Gomaa, E. A. (1992*c*). Influence of dietary treatment on lipid and cholesterol oxidation in pork. *Journal of Agriculture and Food Chemistry* **40,** 1310–1315.

Morrissey, P. A. & Tichivangana, J. Z. (1985). The antioxidant activities of nitrite and nitrosylmyoglobin in cooked meat. *Meat Science* **14,** 175–190.

Murphy, T. K., Lynch, P. B., Buckley, D. J., Monahan, F. J. & Morrissey, P. A. (1991). Effect of dietary fat quality and α-tocopheryl acetate supplementation on the susceptibility of porcine tissue to lipid peroxidation. In *Proceedings of the 37th International Congress of Meat Science and Technology*, Kulmbach, Germany, pp. 1269–1273.

Pie, J. E., Spahis, K. & Seillan, C. (1991). Cholesterol oxidation in meat products during cooking and frozen storage. *Journal of Agriculture and Food Chemistry* **39,** 250–254.

Sato, K. & Hegarty, G. R. (1971). Warmed-over flavour in cooked meats. *Journal of Food Science* **38,** 1098–1102.

Sheehy, P. J. A., Morrissey, P. A. & Flynn, A. (1990). Effect of dietary α-tocopherol level on susceptibility of chicken tissues to lipid peroxidation. *Proceedings of the Nutrition Society* **49,** 28A.

Sheehy, P. J. A., Morrissey, P. A. & Flynn, A. (1991). Influence of dietary α-tocopherol on tocopherol concentration in chick tissues. *British Poultry Science* **32,** 391–397.

Sheehy, P. J. A., Morrissey, P. A. & Flynn, A. (1993*a*). Increased storage stability of chicken muscle by dietary α-tocopherol supplementation. *Irish Journal of Agriculture and Food Research* **32,** 67–73.

Sheehy, P. J. A., Morrissey, P. A. & Flynn, A. (1993*b*). Influence of heated vegetable oils and α-tocopheryl acetate supplementation on α-tocopherol, fatty acids and lipid peroxidation in chicken tissues. *British Poultry Science* **34,** 367–381.

Sheehy, P. J. A., Morrissey, P. A. & Flynn, A. (1994). Consumption of thermally oxidized sunflower oil by chicks reduces α-tocopherol status and increases susceptibility of tissues to lipid oxidation. *British Journal of Nutrition* **71,** 53–65.

Sisk, H., Molloy, M., Morrissey, P. A. & Buckley, D. J. (1994). Uptake of α-tocopherol in porcine plasma and adipose tissue. *Proceedings of the Nutrition Society* **53** (In the Press).

Smith, L. L. (1981). *Cholesterol Autoxidation*. New York: Plenum Press.

Tichivangana, J. Z. & Morrissey, P. A. (1985). Metmyoglobin and inorganic metals as prooxidants in raw and cooked muscle systems. *Meat Science* **15,** 107–116.

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