Plants as natural antioxidants for meat products

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Abstract. The meat industry is demanding antioxidants from natural sources to replace synthetic antioxidants because of the negative health consequences or beliefs regarding some synthetic ones. Plants materials provide good alternatives. Spices and herbs, generally used for their flavouring characteristics, can be added to meat products in various forms: whole, ground, or as isolates from their extracts. These natural antioxidants contain some active compounds, which exert antioxidative potential in meat products. This antioxidant activity is most often due to phenolic acids, phenolic diterpenes, flavonoids and volatile oils. Each of these compounds often has strong H-donating activity, thus making them extremely effective antioxidants; some compounds can chelate metals and donate H to oxygen radicals, thus slowing oxidation via two mechanisms.

Numerous studies have demonstrated the efficacy of natural antioxidants when used in meat products. Based on this literature review, it can be concluded that natural antioxidants are added to fresh and processed meat and meat products to delay, retard, or prevent lipid oxidation, retard development of off-flavours (rancidity), improve colour stability, improve microbiological quality and extend shelf-life, without any damage to the sensory or nutritional properties.

1. Muscle lipids and lipids oxidation

Fresh and processed meats offer numerous nutritional and health benefits and provide unique eating satisfaction in the lifestyle of the modern society. However, consumption of red meat, including processed products, is subjected to increasing scrutiny due to the health risks associated with toxins that potentially could be generated during meat preparation and storage [1].

Lipids and their derivative fatty acids are present in muscles as structural components of muscle membranes, as storage droplets of triacylglycerol between muscle fibres and as adipose tissue [2]. The fatty acids in the lipids of muscle tissues can be saturated or unsaturated. Free fatty acids are electron-deficient at the oxygen atom of the carbonyl group (C=O); unsaturated fatty acids are also electron-deficient at points of carbon–carbon unsaturation (C=C). These electron deficient regions make fatty acids susceptible to attack by a variety of oxidizing and high-energy agents generating free radicals [3]. Triglycerides contain straight chains of primarily 16- to 18-carbon fatty acids and minimal amounts of unsaturated fatty acids. Phospholipids in tissue membranes contain up to 15 times the amount of unsaturated fatty acids (C18:4, C20:4, C20:5, C22:5 and C22:6) found in triglycerides. They are much more susceptible to oxidation because of the number of C=C they contain [4].
Lipid oxidation is described as an oxygen-dependent, oxidative deterioration of saturated and unsaturated fatty acids. This modification of fatty acid is principally carried out by an autocatalytic mechanism of free radicals, called auto-oxidation and consisting of three phases: initiation, propagation and termination. In the first reaction, the presence of prooxidants, or reactive oxygen species, or any other oxidation-favourable condition, results in the loss of a hydrogen radical from unsaturated fatty acids. In the absence of such oxidation-favourable conditions, the reaction between fatty acids and oxygen molecules cannot occur because of the unequal electronic state and spin barrier posed by these ground states. Thus, the reactive oxygen species or other prooxidants, after thermal, redox or light reaction, can produce free radicals and thus start the primary reaction of lipid oxidation. In the second stage, molecular oxygen reacts with the alkyl radical of an unsaturated fatty acid and results in peroxide radical formation. In a subsequent reaction, the formation of hydroperoxides occurs. These are primary products of lipid oxidation and are relatively stable at moderate reaction conditions (low temperature/absence of prooxidative metal ions). However, because of the adverse conditions present in the muscle foods, the hydroperoxides become susceptible to further free radical chain reactions, such as isomerization and decomposition. This produces secondary products, including pentanal, hexanal, 4-hydroxynonenal and malondialdehyde. The last stage is known as termination reaction, during which the free radicals react in various combinations to form stable products. Other unstable compounds are also formed during the termination reaction, which also affect the quality of meat products and give rise to an unpleasant flavour (taste and odour) [2]. Many compounds formed during lipid oxidation (aldehydes, ketones) contribute off-odours that are perceptible at very low concentrations. Odour detection thresholds for pentanal, hexanal and heptanal, compounds typically generated from the breakdown of oxidized linoleic acid have been reported to be <34, <38 and 62 ppb, respectively, in a gelatine model system [5].

The lipid oxidation rate is directly proportional to the unsaturation of fatty acids. Lipid oxidation also depends upon: the level of antioxidants (internal or external) and the presence of prooxidants, such as free iron [6].

When meat ages it turns brown as the myoglobin is converted to metmyoglobin (oxidized form). Lipid oxidation increases the rate of metmyoglobin formation; metmyoglobin acts as a catalyst for lipid oxidation, which further increases the rate of lipid oxidation and deterioration of product colour and flavour occurs [2].

Thus, lipid oxidation is responsible for development of primary and secondary oxidation products, reduction in nutritional quality, as well as changes in flavour and colour, which can precipitate health hazards and economic losses in terms of inferior product quality.

2. Natural antioxidants

Antioxidants are compounds or systems that delay autoxidation by inhibiting formation of free radicals or by interrupting propagation of the free radical by one (or more) of several mechanisms: (1) scavenging species that initiate peroxidation, (2) chelating metal ions such that they are unable to generate reactive species or decompose lipid peroxides, (3) quenching $\cdot$O$_2$-, so preventing formation of peroxides, (4) breaking the autoxidative chain reaction and/or (5) reducing localized O$_2$ concentrations [3]. Chain-breaking antioxidants differ in their antioxidative effectiveness depending on their chemical characteristics and physical location within a food (proximity to membrane phospholipids, emulsion interfaces, or in the aqueous phase). The chemical potency of an antioxidant and its solubility in oil influence its accessibility to peroxo radicals, especially in membrane, micellar and emulsion systems and its amphiphilic character required for effectiveness in these systems [7].

Based on their mode of action, antioxidants inhibit or prevent oxidation; they are again classified into two groups. The first group is primary antioxidants, which react directly with lipid radicals and convert them into relatively stable products; these are also called chain-breaking antioxidative compounds. The second group is secondary antioxidants, which can reduce the rate of oxidation by different mechanisms of action. Most primary antioxidants act by donating a hydrogen atom (H$\cdot$). Secondary antioxidants can act by binding metal ions (Fe$^{2+}$, Fe$^{3+}$ and Cu$^{2+}$) able to catalyse oxidative
processes, by scavenging oxygen, by absorbing UV radiation, by inhibiting enzymes, or by decomposing hydroperoxides [8].

Antioxidant effectiveness is related to activation energy, rate constants, oxidation–reduction potential, ease with which the antioxidant is lost or destroyed (volatility and heat susceptibility) and antioxidant solubility. The most effective antioxidants are those that interrupt the free radical chain reaction. Usually containing aromatic or phenolic rings, these antioxidants donate H to the free radicals formed during oxidation, so becoming a radical themselves. These radical intermediates are stabilised by the resonance delocalisation of the electron within the aromatic ring and formation of quinone structures [3]. Thus, antioxidants are compounds that are capable of donating hydrogen (H·) radicals for pairing with other available free radicals to prevent the propagation reaction during the oxidation process.

In human bodies, the antioxidant defence system includes enzymes (e.g., superoxide dismutase, glutathione peroxidases and catalase), iron and copper-binding extracellular proteins (e.g., albumin, transferrin, lactoferrin, haptoglobin and ceruloplasmin), antioxidant vitamins (e.g., vitamin C, vitamin E and β-carotene) and other cellular compounds (e.g., quinones, glutathione, uric acid and bilirubin) [9].

In the food industry, antioxidants can be divided into natural and synthetic antioxidants. BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), PG (propyl gallate) and TBHQ (tert-butylhydroquinone) are examples of synthetic antioxidants. Ingredients obtained from natural sources which exhibit antioxidative potential in a food model system are considered as natural antioxidants. These antioxidants play a very important role in the food industry. However, synthetic antioxidants have been identified as toxicological and carcinogenic agents in some studies [10-13]. For these reasons, some synthetic antioxidants (BHA, BHT, PG and EDTA) are regulated by the law as direct food additives. Thus, the food industry now chooses natural products over synthetic ones. Consequently, the food market is demanding natural antioxidants, free of synthetic additives and still capable of diminishing oxidation processes in high-fat meat and meat products [2].

Natural antioxidants are produced in living cells to maintain a delicate oxidation–reduction balance in the process of nutrient metabolism and immune function. Upon oxidative stress, antioxidants will react with radical and non-radical species to initiate defence mechanisms for the protection of both intracellular and extracellular components. The plant kingdom is the most abundant source of natural antioxidants, which are abundantly present in spices (seeds), herbs and essential oils used in meat products for sensory purposes. Certain fruits and vegetables are also good sources of antioxidants and other phytochemicals. Many tree leaves, although not used for flavouring, are also good sources of phenolic compounds, and tea is an excellent example of this plant antioxidant family. Some minerals (Se, Zn) and vitamins (vitamin A – β-carotene as precursor, vitamin C – ascorbic acid and vitamin E – α-tocopherol) function as co-factors for antioxidant enzymes and, therefore, are also considered natural antioxidants. Nature has also produced a number of multi-functional short peptides that are capable of neutralising free radicals and chelating prooxidative metal ions. The latter has led to the preparation of ‘natural’ antioxidant peptides through enzymatic hydrolysis of proteins [1].

Antioxidant activity is widely observed in plants (herbs and spices), for example, oregano, rosemary, thyme, cinnamon, pepper, nutmeg, liquorice, aniseed, cassia bark, fennel, prickly ash, round cardamom, basil, garlic, coriander and ginger. The activity is attributed to various phenolic compounds, which are structurally related but differ in quantity and type, depending on the specific source. The major phenolic constituents in herbs and spices are phenolic acids (gallic, protochatechuic, caffeic and rosmarinic acids), phenolic diterpenes (carnosol and carnosic acid), flavonoids – flavones, flavonols, flavanols and flavanones (quercetin, catechin, apigenin, kaempferol, naringenin and hesperetin) and volatile oils (eugenol, carvacrol, thymol and menthol) [14].

The phenolics present in the natural antioxidants have strong H· donating activity [15] or have high radical-absorbance capacity. Some phenolics prevent the formation of free radicals and propagation of reactive oxygen species, whereas others scavenge free radicals and chelate prooxidants (transition metals) [16]. Phenolic acids trap free radicals; flavonoids scavenge free radicals and chelate metals
(Fe^{2+}, Fe^{3+} and Cu^{2+}) as well. The antioxidant potential of these natural compounds (phenolics) depends on their skeleton structure and pattern of functional groups on this skeleton [17]. For instance, the number and location of free hydroxyl (–OH) groups on flavonoid skeleton decide the free radical-scavenging potential [18]. The presence of multiple –OH groups and ortho-3,4-dihydroxy structures enhances the antioxidant potential of plant-based phenolics [19,20]. Polymeric structures (containing more –OH groups) possess more antioxidant potential [21], whereas glycosylation of functional groups (reduction of –OH groups) usually decreases antioxidant effectiveness. Plant-derived pigments (anthocyanins and their hydrolysed products, anthocyanidins) also contain –OH groups, which can donate H·, and thus, possess antioxidant properties. Some phenolics also contain vicinal –OH groups attached to aromatic ring. These phenolics donate H· as well as vicinal –OH groups that can chelate metals, thus preventing oxidation via more than one method. This type of natural antioxidant (for example, carnosol and carnosic acid) has several times the antioxidant activity as BHA and BHT because the latter do not have vicinal –OH groups, thus do not chelate metals and their antioxidant properties depend only on their H· donation mechanism [2]. The natural antioxidants ascorbic acid and α-tocopherol each have one aromatic ring as well.

Antioxidant (and flavour) components of herbs and spices can be removed/concentrated as extracts, essential oils or resins. Extracts are soluble fractions that can be removed from plant materials by solubilizing the component(s) of interest in an aqueous, lipid, alcohol, solvent or supercritical CO₂ phase, then removing it. Essential oils are the volatile oils and often contain isoprenoid compounds. Chemically, essential oils are extremely complex mixtures containing compounds of every major functional group class. Essential oils are isolated by steam distillation, extraction (solvent or CO₂), or mechanical expression from the plant material. Plants also contain resins that are nonvolatile, high molecular weight, amorphous solids, or semisolids that flow when subjected to heat or stress. Most resins are bicyclic terpenes (alpha and beta-pinene, delta-3 carene and sabinen), monocyclic terpenes (limonene and terpinolene) and tricyclic sesquiterpenes (longifolene, caryophyllene and delta-cadinene). They are soluble in most organic solvents but not in water. Resins can contain small amounts of volatile phenolic compounds [14].

It should be noted that antioxidant activity of food extracts can be determined using a variety of tests (stable free radical scavengers: galvinoxyl, diphenyl-b-picrylhydrazyl [DPPH]; lipid oxidation: peroxide oxygen, conjugated dienes, rancimat [measurements of oxygen consumption of a linoleic acid emulsion and oxidation induction period in lard at 100°C], oxygen radical absorbance capacity [ORAC] values), active oxygen method; iodine value (measure of the change in number of double bonds that bind I), anisidine value (reaction of acetic acid p-anisidine and aldehydes to produce a yellow colour that absorbs at 350 nm), measurement of absorbance at 234 nm (conjugated dienes) and 268 nm (conjugated trienes) to assess oxidation in the early stages and chromatographic methods; however, extraction procedures strongly influence the composition of the extracts and, therefore, also influence the antioxidant activity results [22-24]. In addition, the effect of the antioxidant compound in a food matrix can be significantly different than the activity of a purified extract [14].

3. Use of natural antioxidants for meat and meat products
The demonstrated efficacy of natural antioxidants, in the form of either a pure extract, a blend of active components, or a powder of the original seeds, leaves, etc., to retard lipid oxidation, colour and flavour deterioration in meat products has stimulated a broad interest within the meat industry to explore non-traditional food ingredient strategies [1]. Effects of natural antioxidants on lipid oxidation, development of rancidity and off-flavours and colour stability have been demonstrated in numerous studies (table 1). However, depending on the product type and added amount, it should be noted that various natural antioxidants could also have negative effect on the colour and sensory properties of the meat products.

Several studies have also shown the efficacy of natural antioxidants for preventing protein oxidation under meat processing conditions [27,78-80]. Besides antioxidant activities, natural
Antioxidants have potential to reduce numbers of bacteria and improve shelf-life of meat products [26,28,43,45,49,52,57,60,70,74-76,81-83].

### Table 1. Natural antioxidants used to inhibit oxidation in processed meat products.

| Antioxidant category | Meat/Meat products | Reference |
|----------------------|--------------------|-----------|
| Ascorbic acid, α-tocopherol and sesamol | Irradiated ground beef rounds | [25] |
| Basil essential oil | Cooked sausage | [26] |
| Black currant extract | Raw pork patties | [27] |
| *Brassica nigra, Cinnamomum cassia, Origanum vulgare* and *Syzygium aromaticum* | Raw chicken meat | [28] |
| Butterbur and broccoli extracts | Ground beef patties | [29] |
| Caraway essential oil | Cooked sausage | [30,31] |
| Caraway essential oil | Dry fermented sausage | [32] |
| Caraway essential oil | Dry fermented sausage | [33] |
| Cinnamon extract | Chicken meatballs | [34] |
| Cinnamon stick, oregano, clove, pomegranate peel and grape seed extracts | Raw pork | [35] |
| Citrus extract | Cooked turkey meat | [36] |
| Combination of sage oregano and honey | Cooked chicken meat | [37] |
| Du-zhong extracts (leaf, roasted cortex and seed) | Raw pork patties | [38] |
| *Echinacea angustifolia* extracts | Cooked chicken meat | [39] |
| Grape seed and green tea extract | Cooked pork meatballs | [40] |
| Grape seed extract | Ground chicken thigh meat | [41] |
| Grape seed extract and pine bark extract | Cooked ground beef | [42] |
| Grape seed extract, oleoresin rosemary and oregano extract | Cooked beef and pork patties | [43] |
| Grape seed extract, oleoresin rosemary and oregano extract | Raw beef and pork patties | [44] |
| Grape seed extract, pine bark extract and oleoresin rosemary | Cooked ground beef | [45] |
| Green tea or commercial grape seed extract | Ground fresh goat meat | [46] |
| *Hypericum perforatum* L. extract | Heated pork meat batters | [47] |
| Isabel and Niagara grape seed and peel extracts | Raw and cooked processed chicken meat | [48] |
| *Juniperus communis* L. | Cooked sausages | [49] |
| Mushroom extract | Beef and fish meat | [50] |
| Mushroom extract | Bigeye tuna | [51] |
| Nutmeg | Cooked sausage | [52] |
| Oregano and sage essential oils | Porcine and bovine ground meat | [53] |
| Oregano essential oil | Dry fermented sausage | [54,55] |
| Oregano extract | Fresh beef steaks | [56] |
| Peel, pulp and seed from two avocado | Pork patties | [57] |
| Pomegranate rind powder extract, pomegranate juice and pomegranate seed powder extract | Raw ground pork | [58] |
| Pomegranate seed extract | Beef and chicken meatballs | [59] |
| Purple rice bran extract | Restructured patties formulated with minced channel catfish belly flap meat | [60] |
| Red grape pomace extracts | Pork burgers | [61] |
Rooibos forms (dried leaves, water extract and freeze-dried extract)  
Ostrich meat patties [62]
Rooibos tea extract  
Ostrich droewors [63]

| Table 1. Natural antioxidants used to inhibit oxidation in processed meat products (continued). |
|---------------------------------------------------------------|
| Antioxidant category | Meat/Meat products | Reference |
| Rosemary | Mechanically deboned turkey meat | [64] |
| Rosemary and lemon balm | Cooked pork meat patties | [65] |
| Rosemary and oregano extracts | Irradiated beefburger | [66] |
| Rosemary extract | Frozen and precooked-frozen pork sausage | [67] |
| Rosemary, carnosine and taurine, together with ascorbic acid | Fresh beef patties | [68] |
| Rosemary, green tea, coffee and grape skin extracts | Precooked pork patties | [69] |
| Rosemary powder, rosemary extract and α-tocopherol and their combinations | Turkish sucuk | [70] |
| Sage and garlic | Chicken meat | [71] |
| Spice extracts (n = 13) | Liposome system and cooked pork patties | [72] |
| Tamarillo | Cooked beef meat | [73] |
| Thyme and balm essential oils | Fresh chicken breast meat | [74] |
| Vegetables extracts (n = 10) | Raw beef patties | [75] |
| Vegetable powders | Patties | [76] |
| Winter savory essential oil | Mortadella-type sausages | [77] |

Finally, when used as antioxidants for product quality preservation, these natural compounds can also be regarded as nutraceutical ingredients or supplements for health promotion. Indeed, plant-derived antioxidants provide meat processors with the flexibility to develop novel products with enhanced nutritional value and health benefits and an attractive overall quality profile.

**4. Conclusion**

Animal lipids are especially susceptible to oxidation because of their electron-deficient double bonds. The breakdown products of primary and secondary oxidation can produce off-odours, new flavours, loss of nutrient content and colour deterioration. Synthetic phenolic antioxidants effectively inhibit oxidation. Spices and herbs, used in meat products for their flavour, often contain high concentrations of phenolic compounds (phenolic acids, phenolic diterpenes, flavonoids and volatile oils). The fact that they are natural and have antioxidative activity that is as good as or better than the synthetic antioxidants makes them particularly attractive for meat processors because of consumer demand for natural ingredients.

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