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Review Article

Natural antioxidants – an alternative for reduction of nitrites in cooked meat products

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Abstract

Nature is a source of natural additives that can be incorporated into the meat products' matrix. Extracts from spices, herbs, nuts, fruits and vegetables are most common. The extracts can be from whole, individual parts, and from various waste products. Those extracts can be used in meat products for various purposes. They are rich in substances with antioxidant properties, such as anthocyanin, vitamins and polyphenols. Those substances are capable of inhibiting lipid and pigment oxidation, prolonging shelf life and at the same time having a positive effect on organoleptic characteristics. Sodium nitrite is almost essential ingredient in production of cooked meat products. Strict regulated use, the existing risk of carcinomas and even the direct toxicity of nitrites lead to increased interest in search of natural antioxidants. It is believed that they can maintain the quality characteristics of meat products with reduced nitrite content. In the past decade there is a growing interest in the search for, certification and implementation of safe natural additives. The growing demand for the so-called "clean label" foods is the driving force for many of the conducted researches in the last two decades.

Keywords

by-products, natural alternatives, meat pigments, valorisation

Abbreviations

PUFA – polyunsaturated fatty acid, LDL – Low density lipoprotein, MDA – malondialdehyde, ROS – Reactive oxygen species, RNS – Reactive nitrogen species, TBARS – Thiobarbituric acid reactive substances, MetMb – Metmyoglobin, NOMb – Nitrosomyoglobin, CLA – Conjugated linoleic acid, EMF – Extract from male flowers
DDRP – Dried distilled rose petals

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Introduction

In recent years, the attention of the food industry and consumers is focused on functional foods. Significant number of people uses diets with a limited number of foods for reducing the risk of metabolic diseases. A significant increase in metabolic diseases and cancer death are reported among consumers between the end of the 20th century and the beginning of the 21st century (Key et al. 2020). Possible reason is the unbalanced diet and stress. Most consumers would like to eat healthier without significantly changing their diet (Khajavi et al. 2020; Rodrigues et al. 2020). The accumulation of lipid radicals, malonic aldehyde and cholesterol oxides in various meat products, are the most likely a prerequisite for carcinogenesis (Estévez 2021). The initiation and development of lipid peroxidation takes place during the technological processing and storage of meat and fish. Definition for healthy diet is based on reducing food ingredients that could have a negative effect on the consumer. A number of studies changed the negative view of meat products by removing or greatly reducing the content of various additives or fats (Bulambaeva et al. 2014; Vlahova-Vangelova et al. 2014). Thus, newly created meat products have the characteristics of functional foods with a pronounced health effect (Tomović et al. 2020). Reducing the oxidative damage to human body can be achieved by consuming foods rich in antioxidants. The effect is directly related to the antioxidant activity of various chemical compounds (Lindsay 2011). Vitamin E (α -tocopherol), Vitamin C (L-Ascorbic acid), β -carotene and polyphenolic substances are some of the antioxidant compounds.

1.1. Meat composition. Effect on the quality of meat products

A number of endogenous antioxidants are found in the meat matrix, such as tocopherols, ubiquinone, carotenoids, glutathione, carnosine, anserine, lipoic acid, uric acid and spermine (Domínguez et al. 2019). Carnosine as well as anserine are histidyl dipeptides and are a major natural antioxidant in meat. Like any other food, meat and meat products contain substances that in inappropriate proportions have a negative impact on human health. Fats, cholesterol, contaminants in animal feed, pharmaceuticals etc. Others are added during the production proceses (salt, nitrites, phosphates, etc.).

Third group are the compounds which forms as a result of the technological processes (toxic compounds formed during heat treatment, smoking, etc.). The last can formed during storage of the final product due to the growth of pathogenic microorganisms and as products of lipid oxidation. The definition of functional food is broad and has been reformulated many times. In order to be classified as a functional food, it must contain ingredients that improve its' technological properties and quality, and at the same time be distinguished by specific benefits for human health (Lindsay 2011). Foods enriched with biologically active components are the most common functional foods (Balev et al. 2014; Bulambaeva et al. 2014; Abilmazhinova et al. 2020). Enriched with ω -3 polyunsaturated fatty acids (PUFAs), natural antioxidants, pre- and probiotics are only fraction of the functional foods (Toldrá and Reig 2011; Pogorzelska-Nowicka et al. 2018). The functional food can be with reduced content of substances prompting negative effects to human health. Such substances in meat products are the sodium chloride, residual nitrites and fats (Honikel 2008; Nicorescu et al. 2018). The formulation of new functional foods is an area under a development and various studies are carried out on a daily basis. The following approaches for a production of functional meat products have been significantly studied:

- 1) Modification of the proximate composition of carcasses. It is achieved by adding or replacing feed ingredients, which affects *in vivo* meat composition (Balev et al. 2015; Natalello et al. 2020).
- 2) Manipulation of meat raw materials. It is expressed in various preliminary physical or chemical treatments, aimed at improving the technological properties (Vlahova-Vangelova and Dragoev 2014).
- 3) Formulation of new meat products. It is achieved by reducing the content of sodium chloride, nitrites, fats, cholesterol, energy value of the product and incorporation of functional ingredients (Toldrá and Reig 2011; Tomović et al. 2020). First two approaches to the production of functional meat products are associated with a certain content of substances such as fats and cholesterol in raw material and their reduction. At the same time, the addition of antioxidants, vitamins and other biologically active substances of natural origin

would increase the positive health aspects. The proximate composition carcasses is directly dependent on the factors: sex, type of diet, age, etc. By breeding and creating new genetic lines, it is possible to reduce the fat content of the total weight. There is a growing interest in the production of carcasses with functional properties. The obtained meat is characterized by a modified proximate composition (change in the content of proteins, vitamins, as well as in the fatty acid composition), which is achieved through the use of nutrients added to feed (Balev et al. 2015; Vlahova-Vangelova et al. 2019; Natalello et al. 2020).

Second and third approaches for the production of functional meat products are based on the use of additives or processes aimed to improve the technological properties and quality of the final products. Marinating in combination with mechanical processing is the oldest method for improving the technological characteristics of meat (Vlahova-Vangelova and Dragoev 2014). Reducing the content of salt, residual nitrites, LDL-cholesterol, as well as increasing oxidative stability and enrichment with biologically active substances (antioxidants, vitamin C, E and K, etc.) are just some of the approaches for the production of functional meat products (Pogorzelska-Nowicka et al. 2018; Khajavi et al. 2020).

1.2. Use of antioxidants in cooked meat products

The first components of meat that undergo lipid oxidation are phospholipids. The main factors on which the rate and degree of development of lipid peroxidation depend, are:

- The nature of lipids
- The degree of their unsaturation.
- The presence of initiators and / or inhibitors of lipid peroxidation
- External factors - light, heat and ultraviolet radiation.

The susceptibility of phospholipids to oxidation is due to the content of long-chain polyunsaturated fatty acids, which are known to be easily involved in oxidative reactions. They are also located in cell membranes and are in direct contact with oxidation initiators located in the fluid part of cells (Schneider 2009). Before the discovery of the chain-radical theory were known substances with the ability to inhibit oxidative reactions (Santos-Sánchez et al.

2019). These substances are called antioxidants. Added in very small quantities in meat products, they have the ability to inhibit the initiation and spread of oxidative chain reactions (Shahidi and Ambigaipalan (2015).

1.2.1. Initiation of lipid peroxidation in the meat matrix

The oxidative processes with the transfer of electrons, are an integral part of metabolism in the aerobic organism. Deviations in electron exchange sometimes occur as a result of which free radicals and reactive oxygen species (ROS) are formed (Topal et al. 2016). First, the cell membranes composed primarily of two phospholipids and one protein layer, and characterized by low electron retention are under the impact of ROS. They are both free radicals and simple particles.

Free radicals:

- Superoxide ($O_2^{\cdot-}$)
- Hydroxyl radical (OH^{\cdot})
- Peroxyl radical (ROO^{\cdot})
- Protonated superoxide (HO_2^{\cdot})

and particles, which aren't radicals:

- Hydroperoxide (H_2O_2)
- Hydrochloric acid (HClO)
- Ozone (O_3)
- Singlet oxygen (1O_2)

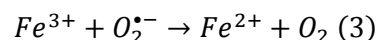
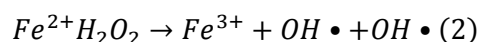
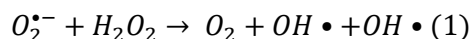
Lipid oxidation is a major factor in reducing shelf life, nutritional quality and products' safety. Meat products contain large amounts of polyunsaturated fatty acids that are susceptible to lipid oxidation. The International Agency for Research on Cancer classifies them in group 2A (probably carcinogenic to humans) (IARC 2018). Upon contact with oxygen from the air, meat ingredients in particular polyunsaturated fatty acids are oxidized, leading to generation and accumulation of lipid oxidation's products. End product of lipid oxidation is 4-hydroxynonenal (malondialdehyde - MDA), which exhibits cytotoxic and genotoxic properties and is a potential initiator in the formation of adenocarcinomas and colon cancer (Barbieri et al. 2021; Estévez 2021).

As the auto-oxidation reactions take place, lipids and pigments become oxidized, which leads to a decrease in their quality. The color, taste and smell is changing, the biologically active components are

destroyed and carcinogenic and toxic substances are formed. At the same time, the formation of highly reactive radicals and ultimately various by-products of the sediment reaction such as ketones, aldehydes, etc. is observed. A representative of the secondary decomposition products that are subject to control is MDA (Barbieri et al. 2021). It is an indicator of the past oxidation of polyunsaturated fatty acids, and is one of the end products with a pronounced carcinogenic effect (Schneider 2009).

Free radicals are molecules that lack an electron in the last electron layer to form a stable compound with a corresponding number of system-stabilizing electron doubles. Their reactivity is due to their lack of electron and they seek to obtain it from other molecules in cells. The moment a radical takes an electron from a molecule, it becomes a radical looking for its electron. This process continues in the form of a chain autocatalytic reaction. (Schneider 2009). The process is catalyzed by enzymes such as peroxidases, peroxygenases, etc., as well as free ions of iron and copper, as well as substances called prooxidants. The lipid hydroperoxyl radical removes a hydrogen atom from the double bond of the nearest unsaturated fatty acid, thus forming a hydroperoxide and alkyl radical (Yavari et al. 2015). Later, the former binds to O₂ and a lipid hydroperoxyl radical is generated. He in turn has the potential to initiate another oxidation cycle. As a result of cellular activity (breakdown of ATP in mitochondria, inflammatory processes, etc.), ROS and reactive nitrogen species (RNS) are generated (Lin et al. 2016). These can be the superoxide radical (O₂^{•-}), the singlet oxygen (¹O₂) and the hydroxyl radical (OH[•]). Hydrogen peroxide and other hydroxyperoxides are not free radicals, but are also ROS with potentially high cytotoxicity. Most ROS exhibit strong prooxidant activity (Yavari et al. 2015). The most active in terms of oxidative action are hydroxyl radical (OH[•]) and singlet oxygen (¹O₂). Another highly reactive radical is protonated superoxide (HO₂[•]) (Schneider 2009) leading to peroxidation of PUFAs. Its amounts are severely limited under physiological pH conditions due to the short half-life of superoxide (O₂^{•-}). It is able to react only to the place where it is generated. In contrast, hydrogen hydroperoxide (H₂O₂) is a more moderate oxidizing agent, is relatively stable, and has the potential to cross through the cell membranes. No matter where

it is generated, it subsequently shows activity inside and outside of the cells (Santos-Sánchez et al. 2019). Enzymes containing thiols in their active centers can be inactivated by hydrogen hydroperoxide (H₂O₂). It is believed that H₂O₂ and O₂^{•-} exert their strongest effect through the formation of OH[•] by the reaction of Haber-Weiss presented by the following equations:



Cells have a defense system capable of fighting oxidative stress. The intake of exogenous antioxidants in the form of food supplements or foods rich in them is used as a preventive measure to treat various diseases caused by oxidative stress. Their antioxidant activity is due to the presence of a mixture of simple molecules, such as vitamin C, E and K (Sengul et al. 2017), pigments (carotenoids) (Alizadeh and Fattahi 2021), anthocyanins (Dinkova et al. 2014), also significantly more complex structures, such as those of polyphenols (flavonol and kaempferol glycosides, tannins, etc.) (Dragoev et al. 2021), and sometimes volatile oils (especially represented by terpenes) (Munekata et al. 2020). Their antioxidant action is expressed by the fact that they inhibit the formation of free radicals according to the following mechanisms:

- Capture compounds that cause peroxidation
 - Bind metal ions that catalyze oxidation reactions
 - Neutralizes superoxyl radicals and prevents the formation of process initiators - lipid peroxides.
- In this way, they stop the chain reactions of autoxidation (Perron et al. 2010).

The intake of various phytochemicals in animals and humans through enriched (functional) foods is a promising area. The prevention and treatment of cardiovascular and cerebrovascular disease, metabolic disorders and its complications in type 2 diabetes, respiratory diseases and cancer that are nowadays mainly provoked by oxidative stress (Lin et al. 2016; Key et al. 2020). Phenolic compounds are involved in a number of biological processes through their chemoprophylactic properties (antioxidant, anti-inflammatory, anticarcinogenic and antimutagenic) (Shahidi and Ambigaipalan 2015; Cory et al. 2018). They contribute to the delay of apoptosis (cell death) by slowing the cell cycle,

regulate carcinogenic metabolism and a number of other negative processes (Topal et al. 2016). The content of polyphenols can decrease significantly during food processing and storage. Some compounds are susceptible to oxidation and the addition of polyphenols to foods can reduce their shelf life. In the processing of meat products, the antioxidant properties of polyphenols are targeted. They are sacrificed to prevent the oxidation of other components of product, which extends the shelf life (Domínguez et al. 2019). The use of plant extracts as supplements is increasing, and the polyphenols can be used in production of meat products that are a source of dietary polyphenols, the so-called functional meat products (Cory et al. 2018).

1.2.2. Antioxidants' action

According to their type of action, antioxidants are classified into the following groups:

- Strong antioxidants are those that interact directly with free radicals and bind them into stable inactive compounds, thus removing them from the system.
- Weak antioxidants prevent the formation of free radicals by reacting with hydroperoxides and neutralizing them, thus slowing down the rate of oxidative reactions.
- Synergists have the properties to enhance the action of antioxidants, but alone do not have antioxidant properties. Their properties are manifested in the binding of heavy metal ions, thus forming stable complexes and inhibiting their prooxidant action.

The amount and state in which antioxidants can be added to the matrix of meat products varies widely (Wang et al. 2015). As solutions, oil extracts or in dry powder form. By adding them to the meat during its processing, lipid peroxidation is slowed down, the color is stabilized for a longer time, the development of the microflora is inhibited, the taste and aromatic changes are limited and the shelf life is extended. On the other hand, muscle tissue contains natural antioxidant substances capable of inhibiting lipid peroxidation such as vitamin D and vitamin E, vitamin K, low molecular weight amino derivatives such as carnosine, anserine and carnitine (Domínguez et al. 2019). Recently, the highly-effective antioxidants represent, antioxidant blends of properly selected antioxidants and synergists. Instead of L-ascorbic acid (E 300) its salts - sodium

L-ascorbate (E 301) and potassium L-ascorbate (E 302) and erythorbic (D-isoascorbic) acid (E 315) and sodium erythorbate (E 316) were used.

Sepe et al. (2005) investigated the effect of various reducing agents on browning of minced beef. Sodium erythorbate, erythorbic acid, sodium ascorbate, ascorbic acid and ascorbyl palmitate at a concentration of 2.3 mM were used. The samples were stored at 4°C and -18°C and it was found that all tested reducing agents inhibited the lipid oxidation compared to the control sample. The effect is due to the ability of reducing agents to maintain Mb in a reduced state.

1.3. Use of nitrites in cooked meat products

The preservative effect of nitrates and nitrites on meat products has been known for a long time (Govari and Pexara, 2015). However along with the positive aspects of their use, there are also negative ones. The potential health risks by the addition of nitrites to meat products necessitate the search for natural alternatives (Nicorescu et al. 2018). Various sources of plant nitrites have been studied, which would help to reduce added nitrites or their overall substitutability. In accordance to Commission Regulation (EU) 1129/2011, nitrites (Sodium nitrite - E250) and nitrates (Potassium nitrate - E252) are listed as permitted food additives. The maximum dose allowed for use in raw dried meat products from the EU is 300 mg / kg nitrate (for some products 250 mg / kg nitrate) and 150 mg / kg K-nitrite (or 150 mg / kg Na-nitrite) EFSA Panel on Biological Hazards (BIOHAZ) 2004).

1.3.1. Nitrites' action

Role of nitrites in meat products is direct formation of the characteristic red color, inhibition of pathogenic microflora growth, especially the formation of botulinum neurotoxin produced by *Clostridium botulinum*. The indirection is due to increasing the stability of unsaturated fatty acids to oxidation, through a chelating effect and preventing the development of non-Fenton-type reactions. They are also responsible for the formation of the specific taste in the product (Lavado et al. 2021).

The color of meat and meat products is due to the presence of hemoglobin and myoglobin. They are easily oxidized complex chromoproteins. Their oxidized form Methemoglobin and Metmyoglobin have brown color, which is not inherent in meat and

meat products and repels the consumers (Sepe et al. 2005). Nitrates or nitrites, or a combination, are added to meat and meat products to prevent discoloration. At the time of addition of sodium nitrite to meat, it is converted into nitric acid, which is reduced to nitric oxide ($\bullet\text{NO}$), which interacts with myoglobin from muscle tissue to form nitrosomyoglobin (Fig 1.) by reaction 4:

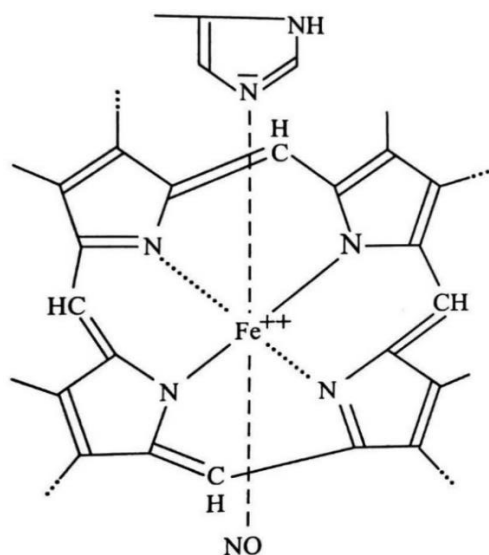
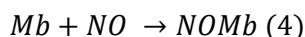


Figure 1. Nitrosomyoglobin (NOMb): The 6th coordination site of the Fe atom is occupied by NO

A fraction of added nitrite (≥ 25 ppm) forms the characteristic color (Govari and Pexara 2015). During heat treatment of sausages (roasting, cooking, hot smoking) due to denaturation of NOMb, a nitrosohemochromogen is formed, giving the characteristic pink-red color (Shpaizer et al. 2018). Nitrosohemochromogen is sensitive to the presence of oxygen and light. Exposure lead to a dissociation of nitric oxide from the chromoproteins and the color fades. A remarkable property of nitrites is the inhibition of lipid oxidation, which is largely initiated by the used salt (Govari and Pexara, 2015). Lipid oxidation is considered to be a major factor in the quality and nutritional value of meat and meat products. The antioxidant effect of nitrites has a similar mechanism of action to that in the formation of the specific color of meat. Through reactions of various proteins and metal ions, as well as chelation of free radicals, $\bullet\text{NO}$ forms nitroso- and nitrosyl compounds with antioxidant properties. The direct toxicity of nitrites comes from their

ability to oxidize respiratory proteins inhibiting the oxygen transport, leading to hypoxia of tissue cells. It's a huge problem in children under 3 years' old who can develop a "blue baby" syndrome. The adult body is able to fight this aggression, as it's equipped with an enzyme system that can transform methemoglobin into reduced hemoglobin. It is almost impossible to bind the entire amount of $\bullet\text{NO}$ formed during the reduction of nitric acid to the myoglobin. Non bounded amount is called "residual nitrites" (Honikel 2008). They are about 10-20% of the added nitrites. They are a major problem, the solution of which is the use of different technological approaches for partial or complete reduction. Trough production and storage of meat products, as well as in the digestive tract, residual nitrites step into reactions with secondary amines and form N-nitrosamines.

The International Agency for Research on Cancer classifies N-nitrosodimethylamine and N-nitrosodiethylamine as likely carcinogens, while N-nitrosopiperidine, N-nitrosodibutylamine and N-nitrosopyrrolidine (IARC 2018). Increased formation of N-nitrosamines is observed during heat treatment at temperatures above 130°C , such as frying or grilling (Nicorescu et al. 2018). Despite these significant dangers to the human health, their use in meat products is permitted. Sodium nitrite is used in meat products mainly due to its unique ability to inhibit the growth of *Clostridium botulinum* and anaerobic microorganisms responsible for both spoilage of meat products and food poisonings.

1.3.2. Natural antioxidants in cooked meat products

To reduce the amount of used and consequently the residual nitrites, various methods can be applied. Plant extract congaing substances with well-defined antioxidant properties is a subject to a number of studies. Polyphenols (particularly flavonol glycosides) and pigments (carotenoids) are secondary metabolic products that are not directly involved in plant and fruit development. Their main role is to protect plants from bacteria and viruses (Papuc et al. 2017). Polyphenols' group includes: phenolic acids, flavonoids, stilbenes and others. The structure is characterized by an aromatic ring having one or more hydroxyl groups (-OH). Their molecular structure varies ordinary phenolic

molecule to a complex high molecular weight polymer. Their antioxidant activity depends on structure, in particular on the location and number of -OH in the aromatic nucleus. The activity is also influenced by the other functional groups related to the aromatic nucleus. The flavonoid molecule, a three-ring structure of the C6-C3-C6 species (Fig. 2; Table 1), characterizes more than half (about 4000) of phenolic compounds (Lin et al. 2016).

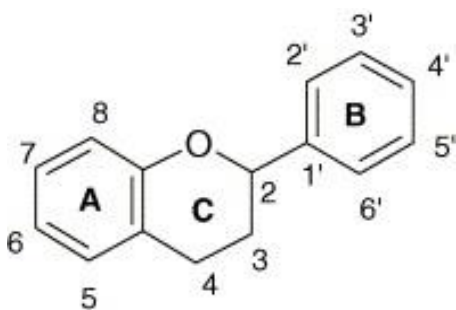


Figure 2. Generalized structure of the flavonoid molecule

Phenols have a strong ability to emit hydrogen atom, which determines their high radical-neutralizing activity. One part of the phenolic antioxidants neutralizes free radicals (Shevelev et al. 2020), and another part of them binds metal ions which have a prooxidant effect (Fe^{2+} , Fe^{3+} and Cu^{2+}). The best known flavonoids are: flavones, flavanones, flavonols, flavanonols, flavan-3-ol, isoflavones, neoflavonoids, chalcones, and anthocyanidins. The polyphenols most characteristic properties are: 1) detection and neutralization of superoxide, 2) capture and neutralization of hydroxyl and peroxy radicals. Due to these properties, they appear to be inhibitors of lipid peroxidation (Shevelev et al. 2020). They prevent the depletion of vitamin E and β -carotene, as they suppress metal-iron-mediated radical formation (Yavari et al. 2015). Polyphenols have the potential to form complexes with residual free ions of metals (Fe^{2+} , Fe^{3+} and Cu^{2+}), a process known as chelation (Lavado et al. 2021). They have been shown to affect iron-induced DNA damage. With the formation of complexes of compounds, polyphenols keep iron in a state (Fe^{3+}), as after its oxidation from a state (Fe^{2+}), in the presence of molecular oxygen (Perron et al. 2010).

Table 1. Classification of phenolic compounds of plant origin

Class	Structure
Basic phenols, Benzoquinone	C6
Hydroxybenzoic acid	C6-C1
Acetophenone, Phenylacetic acid	C6-C2
Hydroxy chanelic acid, Phenylpropene (Cumarin, Isocumarin, Chromone, Chromene)	C6-C3
Naphthoquinone	C6-C4
Xanthone	C6-C1-C6
Stilbene, Anthraquinone	C6-C2-C6
Flavonoids, Isoflavonoids	C6-C3-C6
Lignan, Neolignan	(C6-C3) ₂
Bioflavonoids	(C6-C3-C6) ₂
Lignins	(C6-C3) _n
Condensed Tannins (Proanthocyanidins)	(C6-C3-C6) _n

Many of the phytochemicals contained in plants, such as: phenolic acids, tocopherols, carotenoids, vitamin C etc. are characterized by pronounced biological activity, as well as significant antioxidant potential (Topal et al. 2016; Pogorzelska-Nowicka et al. 2018). A number of studies have consider a variety of sources of natural antioxidants, in the form of extracts, such as green tea (Wang et al. 2015), rosemary (Dragoev et al. 2016), sage (Šojić et al. 2018), savory (de Oliveira et al. 2012), also from different types of fruits, vegetables and nuts, such as: bananas (Rodrigues et al. 2020), pomegranate (Natalello et al. 2020), rosehip (Nicorescu et al. 2018), blueberries (Dinkova et al. 2014), blackberries (Lorenzo et al. 2018) etc.

Various parts of fruits and herbs can be used, such as: fruit peels, seeds as well as processing waste products (Bulambaeva et al. 2014; Natalello et al. 2020). Their extracts are rich in polyphenols and ascorbic acid with proven antioxidant action (Papuc et al. 2017). The fruits of dog-rose (*Rosa canina* L.), rosehips are rich in L-ascorbic acid and variety of polyphenols, carotenoid and anthocyanins with well-defined anti-inflammatory properties. Extract from rosehip is successfully incorporated in pork sausages with half reduced sodium nitrite. Nicorescu et al. (2018) reported that both lipid and

protein degradation was inhibited in the sausages with rosehip extract. As well as the rosehip all berries are among the richest in Vitamin C and polyphenols fruits (Dinkova et al. 2014; Lorenzo et al. 2018). The combination of plant polyphenols from green tea and α -tocopherol significantly reduces pH, lipid oxidation (formation of reactive substances with thiobarbituric acid), and the amount of residual nitrites at the end of ripening of raw-dried bacon (Wang et al. 2015).

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of octadecadienoic acid with conjugated double bonds. It is believed to be a stimulant of the immune system and has antioxidant, anticarcinogenic properties. CLA-rich vegetable oils are most often obtained from sunflower, soybean and saffron, whose fatty acid composition contains large amounts of alkaline isomers of linoleic acid (Pogorzelska-Nowicka et al. 2018). Replacement of some animal fats with conjugated linoleic acid is also common in practice. Significantly higher values of color brightness (L*) and red component (a*) were observed in emulsion-type sausages with 10% fat replacement by CLA for the storage period (Márquez-Ruiz et al. 2014). There is also a tendency to decrease the amount of residual nitrites by increasing the amount of substituted animal fat. However, due to the positive effects of the CLA addition in the studied samples, no difference in the values of the TBARS indicator associated with oxidative stability was observed (Márquez -Ruiz et al. 2014).

The α - and β -carotene contained in pumpkin are one of the most widely studied antioxidants, with a proven antitumor effect. α -carotene slows down the aging of cells. Pumpkin contains smaller amounts of lutein, lycopene, alkaloids, flavonoids and palmitic, oleic and linolenic acids and has antidiabetic, antioxidant, anticarcinogenic, anti-inflammatory (Yadav et al. 2010). The pumpkin powder and goji berry fruits were used in formulation of a new functional meat products (Serikkaisai et al. 2014). Due to their polyphenolic composition the oxidative changes in cooked and smoked beef with reduced nitrite content were inhibited. The bioactive compounds contained in the extracts of different parts of banana inflorescences (bracts, male flowers, rachis and whole inflorescence) have different antioxidant activity. The extract from male flowers (EMF), has a high concentration of phenols and

flavonoids. The use of EMF in concentrations of 0.0, 0.5, 1, 1.5 and 2.0% in the production of sausages and subsequent 28-day refrigerated storage has a beneficial effect in terms of antioxidant activity. No major changes in pH, aw and color characteristics were observed. The sensory characteristics of the product didn't show a negative effect of the addition of up to 2% EMF (Rodrigues et al. 2020). Bee pollen is rich in substances with a pronounced antioxidant character. The addition of lyophilized bee pollen inhibits lipid oxidation and MDA formation during thirty days of refrigerated storage of raw sausages (Almeida et al. 2017). Dragoev et al. (2016) experimented with extract from cumin (*Cuminum cyminum*) and black pepper (*Piper nigrum* L.) in combination with rosemary (*Rosmarinus officinalis* L.) and dihydroquercetin (*Larix sibirica* Ledeb) extracts in Bulgarian type dry-fermented sausages. They found an inhibition in oxidative processes and lower accumulation of hydroperoxides and TBARS in studies sausages

Taxifoline (3,5,7,3,4-pentahydroxy flavanone, also known as dihydroquercetin) is common in citrus fruits and onions (Topal et al. 2016). Dihydroquercetin extracted from *Larix sibirica* Ledeb contains biologically active ingredients beneficial to human health. The main two bioflavonoids in Siberian larch's (*Larix sibirica* Ledeb) are dihydroquercetin and dihydrocampherol (Kolesnik et al. 2011) and small amounts of naringenin (Balev et al. 2010). Dihydroquercetin has the ability to inhibit the formation of free radicals and increases the oxidized stability of lipids in cell membranes (Rokaityte et al. 2019). It has studied anti-radical (Li et al. 2017), anti-inflammatory properties (Wan et al. 2021) and ability to inhibit the oxidation of LDL-cholesterol in blood serum (Weidmann 2012). Disadvantages of dihydroquercetin are: instability in light exposure, high temperature and variations in pH values (West and Mauer 2011; Bobolaki et al. 2018). There are a number of studies in which dihydroquercetin has been used as a natural antioxidant in the production of functional foods in which it also exhibits antimicrobial activity (Topal et al. 2016; Abilmazhinova et al. 2020). When feeding rats with a combination of dihydroquercetin and ascorbic acid Kobyalko et al. (2009) found a reduced

accumulation of MDA in the liver and kidneys in chronic cadmium poisoning.

With the growth of industrial processing of food and agricultural products, huge quantities of "waste" products are generated. They can be a dry type of dry rose petals, fruit peels (Natalello et al. 2020) seeds and / or grape must (Tayengwa et al. 2020) or wash waters from olive oil production (Barbieri et al. 2021). Those by-products are most often rich in biologically active substances (such as carotenoids, phenolic substances and essential oils) having antioxidant properties (Barbieri et al. 2021). Some of these by-products have been the subject of research over the years and have proven to be a good source of natural antioxidants. The production of natural antioxidants can easily be added to conventional foods such as meat products (Abilmazhinova et al. 2020; Munekata et al. 2020). The analysis of the available literature shows that in Europe there are very few functional meat products enriched with biologically active substances. Addition of volatile Sage oils and Sage extract from herbal dust (the processing by-product) in cooked pork sausages lead to positive effect. Both Sage oils and extract improved the oxidative stability and stabilize the color of the cross cut surface (Šojić et al. 2018). As an alternative of the sodium nitrite, Tomović et al. (2020) reported incorporation of *Juniperus communis* L. Essential Oils in fermented sausages. The incorporation stabilize the color and sensory characteristic of the sausages with reduced nitrite content. Those essential oil extracts are mixture of terpenes and phenylpropanones with strong antioxidant effect. The largest share of the extract is occupied by phenols, such as quercetin and thymol, which can be identified as major inhibitors of the radical chain reaction.

In Bulgaria, work on this topic began to joint projects with scientists from Kazakhstan only in 2014 (Bulumbaeva et al. 2014; Serikkaisai et al. 2014). In 2015, an experiment was conducted with broilers to determine the changes in the composition of the meat obtained after feed enrichment with dried distilled rose petals and dihydroquercetin (Balev et al. 2015). Particular attention should be paid to the fact that naturally enriched meat with functional properties is not available on the market! Functional meat products enriched with dihydroquercetin and DDRP extract have not been developed worldwide.

Rosa damascena Mill. from the family *Rosaceae* also known as damask rose, oil rose or queen of flowers is both an ornamental and important herb used in modern pharmacology. It has antioxidant, antimicrobial and anti-inflammatory properties (Afsari et al. 2019; Alizadeh and Fattahi 2021). The available literature mentions its' use in the treatment of constipation, erectile dysfunction, and cardiovascular disorders (Mahboubi 2016; Akram et al. 2020). About 18,000 different varieties and over 200 species can be found worldwide. Except as the perfume effect of *Rosa damascena* Mill. of particular importance is the *in vitro* inhibitory potential for lipid peroxidation. *Rosa damascena* essential oil has been extracted in Iran since ancient times. Its use is associated with healing properties, as well as various religious beliefs. Today, the main production of rose oil is carried out in Bulgaria and Turkey (Akram et al. 2020). *Rosa damascena* oil is an expensive product, due to the huge amounts of rose petals needed to produce a single milliliter of it. From approximately 242,000 rose petals, only 5 ml of rose oil is obtained (Baydar and Baydar 2013). Currently, an average of 10-15 thousand tons of oil-bearing rose are harvested in Bulgaria each year (MAFF 2020). Water, hexane or ethanol can be used to obtain rose petal extracts. Hexane is not appropriate, due to the extract use as a food supplement, solvent. Both water and ethanol extracts showed antimicrobial activity against most common pathogenic microorganism, responsible for foodborne diseases (Shohayeb et al. 2014). Dragoev et al. (2021) found the presence of polyphenols in the extract obtained from the dry distilled rose petals (DDRP). In the 30% water-ethanol (v/v) extract flavonol glucosides were detected, mainly quercetin and kaempferol. They exert high radical scavenging activity evaluated by DDPH test, as well as metal-reducing ability – FRAP. The same extract was used successfully to stabilize the color of canned fruits (Shikov et al. 2012). Baydar and Baydar (2013) studied the properties of methanol extracts obtained by different methods from *Rosa damascena* Mill. The total phenolic amount by the Folin-Ciocalteu method, antiradical activity against DPPH radical and antioxidant capacity by the metal-reducing method of the methanol extracts were evaluated. Both in hot and cold extraction yielded the most significant amounts of polyphenols read as gallic acid equivalents per gram (GAE/g), but the

antioxidant activity recorded by the FRAP assay was higher in the extract obtained by cold extraction. Different extraction techniques were studied by Sengul et al. (2017). They found catechin, rutin and gallic acid in the extract from *Rosa damascena* Mill. Vlahova-Vangelova et al. (2014) found reduced formation and accumulation of end products of protein (protein carbonyls) and lipid (TBARS) oxidation in cooked sausages with added dried distilled rose petals' extract and 50% reduced nitrite content. When using DDRP extract in concentration up to 0.05%, no changes in sensory characteristics (odor, taste, color, texture) are observed. There is also a stabilization of the color of the cross cut surface of sausages with 50% reduced nitrite content (Balev et al. 2014).

Conclusions

The literature review over the past 20 years resources show an growing interest in natural antioxidants and their use in food industry. Their properties to inhibit oxidative processes preserve quality and extend the shelf life of products. The combination of antioxidants in blends is appropriate due to the emerging synergism and the smaller amount needed to achieve the same effect. The formulation of new functional meat products with natural antioxidant properties have the potential health benefits and should help in the fight against oxidative stress. Last but not least, they can be an alternative to part of added sodium nitrite, thereby reducing or completely eliminating residual nitrites in meat products. The reduction of sodium nitrite in combination with natural antioxidants has the potential to increase the oxidative stability of lipid and protein fractions and to protect meat pigments from oxidation.

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