

CHAPTER «ENGINEERING SCIENCES»

EFFECT OF PLANT EXTRACTS RICH IN POLYPHENOLS ON LIPID OXIDATION IN SMOKED SAUSAGES

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Abstract. Meat products are subject to degradation processes. Among them, the most important after microbial destruction are oxidative processes that affect lipids, pigments, proteins and vitamins. Lipid oxidation is a major non-microbial cause of deterioration in meat and meat products. *The purpose* of the study is a theoretical and experimental study of the content of bioflavonoids (phenolic substances) in berry extracts and their use in systems of biological origin to inhibit oxidative processes during storage. *Methodology* of the study is based on theoretical research of sources for the concentration of biologically active substances, including secondary metabolites of plants (bioflavonoid complex), in berries and on experimental studies of the dynamics of oxidative processes in food products with a high content of lipids according to indicators such as acid value, peroxide value, thiobarbiturate value using titrometric and colorimetric methods of analysis. *Results.* The work theoretically analyzes information on the high content of phenolic substances in black chokeberry (*Aronia melanocarpa*) and black currant (*Ribes nigrum L.*), which determines their high antioxidant properties and proves the prospect of using them as natural antioxidant additives in meat and meat technology meat products. The study experimentally proved the high antioxidant activity of black chokeberry and black currant extracts in smoked sausage technology. With the addition of black rowan and black

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currant extracts, inhibition of peroxidation is observed already after the first 5 days of storage. It was found that the addition of black chokeberry extract in the amount of 0.2-0.5% to the mass of minced meat significantly slows down the hydrolytic oxidation of lipids in the finished product, effectively suppresses the peroxidation of fat and stabilize the formation of secondary products of oxidation during the storage of smoked sausages with a high fat content. A microbiological safety study of experimental sausages during storage was conducted. *Practical implications.* Extracts of black chokeberry and black currant can be successfully used in the production technology of smoked meat and meat-containing sausages of a multicomponent composition for inhibition of lipid oxidation during the storage and preventing of spoilage of products. *Value/originality.* The effectiveness of this method is improving of smoked sausage quality with natural antioxidants such as black chokeberry (*Aronia melanocarpa*) and black currant (*Ribes nigrum L.*) extracts due to high content of phenolic compounds, which replace potentially harmful synthetic antioxidants.

1. Introduction

Meat and meat products are excellent sources of proteins and amino acids, fats, minerals (such as zinc, iron and phosphorus), vitamins and other essential nutrients. Therefore, they are an integral part of human nutrition [1, p. 15]. Despite the new dietary trends in Western societies, which contribute to the reduction and/or replacement of meat in diet [2, p. 104], global meat consumption is constantly increasing. It has increased by 58% over the past 20 years to reach 360 million tons annually, with 54% of this increase attributable to population growth and the remainder to increased per person consumption due to changes in consumer diets and incomes.

However, meat products are subject to degradation processes. Among them, the most important after microbial destruction are oxidative processes that affect lipids, pigments, proteins and vitamins. Lipid oxidation is a major non-microbial cause of deterioration in meat and meat products [3, p. 1].

Oxidation reactions not only reduce the nutritional value of meat due to the loss of essential fatty acids and vitamins. As a rule, the first observed change leads to a gradual decrease in sensory quality. These include changes in color, texture, and the appearance of rancid odors and flavors that affect consumer perception [3, p. 2]. In addition, numerous toxic

compounds are formed during lipid oxidation. One of the most important problems of lipid oxidation is the formation of harmful compounds that cause a number of human pathologies, including atherosclerosis, cancer, inflammation and aging processes [4, p. 2; 5, p. 432]. It has been established that lipid hydroperoxides contribute to cell cytotoxicity and that even low concentrations of hydroperoxides have a toxic effect on cells [6, p. 510]. Cholesterol oxidation products are also more dangerous to artery cells than cholesterol. They are associated with coronary disease, mutagenic activity and atherosclerosis [5, p. 433]. Recent studies have shown that aldehydes and oxysterols obtained as a result of lipid oxidation have pro-inflammatory, cytotoxic and mutagenic effects [7, p. 5]. Obviously, that oxidation products are involved in the development of many diseases.

One of the ways to prevent oxidative deterioration of meat products and to ensure the safety of finished products during production and storage can be the correction of lipid peroxidation of meat products with the help of natural antioxidants.

2. Natural plant antioxidants in the technology of meat and meat products

A major strategy used by the meat industry to inhibit lipid oxidation is the addition of antioxidants to meat and meat products. However, today's consumers are demanding more natural products, which limits the industry's use of currently permitted synthetic antioxidants in food products, leaving manufacturers with few options.

Compounds with antioxidant activity are used to mitigate the action of free radicals in meat systems. These compounds are characterized as "antioxidants". The term is now used to describe any substance that delays or prevents the oxidation of biomolecules in food, even when added at low concentrations [8, p. 440].

Antioxidants used in the meat industry are either natural or synthetic. Natural antioxidants can be further classified based on their origin (plant, animal, or bacteria) and chemical structure (phenols, tocopherols, or vitamin C). Natural antioxidants of plant origin are produced from fruits, teas and herbs, seeds, spices, vegetables, cereals and trees [9, p. 1]. Different plant organs, such as leaves, flowers, fruits, stems or roots, accumulate antioxidants in high concentrations that vary depending on the plant species and the

antioxidant substance itself. The above plant parts are used in food products either directly (e.g. fruit puree or juice) or after extraction and purification of the antioxidant substances they contain (e.g. rosemary extract).

The chemical structure of antioxidants is related to their properties and main mode of action, while the main groups of antioxidant substances based on their chemical structure are phenols, tannins, flavonoids and isoflavonoids, anthocyanins, lignans, stilbenes, tocopherols, carotenoids, and vitamin C.

Phenols are secondary products of plant metabolism, formed from the aromatic amino acid phenylalanine through the formation of shikimic acid (phenylpropanoids) and acetic acid (simple phenols); phenols contribute to the color, taste and astringency of plants. Phenolic compounds can be classified based on their (i) plant species origin, (ii) chemical structure (number and arrangement of hydroxyl moieties, double bonds in carbon rings, and type and degree of alkylation and/or glycosylation), and (iii) their solubility in water, which affects their nutritional, metabolic and physiological effect; their common feature is a hydroxy-substituted benzene ring in their structure [10, p. 5729]. Phenolic compounds act as hydrogen donors [10, p. 5729], thereby quenching free radicals or transferring individual electrons to restore chemical compounds with an oxidizing effect.

Tannins are water-soluble binding polyphenolic substances formed by polymerization of phenylpropanoid compounds [10, p. 5730]. Traditionally, tannins have been used for protein precipitation due to their ability to interact with at least two protein molecules and form insoluble cross-linked tannin-protein complexes [10, p. 5731]. Tannins are divided into (i) condensed tannins (proanthocyanidins), which are polymers of catechin, epicatechin, prodelphinidins, profisetinidines, and prorobinetidines, and (ii) hydrolyzed tannins, which can be hydrolyzed by weak acids or bases, the latter being a mixture of gallic and ellagic carbohydrates acids (halotannins and ellagitannins) [11, p. 317].

Proanthocyanidins donate hydrogen and electrons (primary antioxidant action), chelate iron and inhibit cyclooxygenase activity (secondary antioxidant action) [12, p. 5]. Consumption of small amounts of tannins has a positive effect on lipid metabolism and regulation of immune reactions, lowers blood pressure and has anti-carcinogenic and anti-mutagenic effects [11, p. 327].

Flavonoids and isoflavonoids can be found in various plants and are derived from the aromatic amino acids phenylalanine and tyrosine and malonate. The main structure of flavonoids is the flavone nucleus, which consists of three rings of carbon atoms (C6-C3-C6). The level of oxidation and the nature of substitution of the carbon atom in the ring are used to distinguish the classes of flavonoids (flavones, flavanones, isoflavones, flavonols, flavanols, flavan-3-ols, anthocyanidins, biflavones, chalcones, aurones, and coumarins) [13, p. 2]. The antioxidant effect of flavonoids includes (i) suppression of ROS formation by inhibition of enzymatic reactions and chelation of elements involved in the production of free radicals, and (ii) absorption of ROS [14, p. 1036]. Flavonoids have shown significant antioxidant capacity in *in vitro* experiments and are believed to be associated with a reduced risk of cardiovascular disease, hypertension, Alzheimer's disease and some cancers [13, p. 2; 15, p. 251].

3. Sources of natural antioxidants for meat production

Consumers are becoming increasingly aware of the unhealthy effects of high amounts of fat and saturated fatty acids in meat and meat products. Thus, there is a growing interest in the development of new products with a health concept, such as "high polyunsaturated fatty acid foods" or "rich ω -3" foods [16, p. 24; 17, p. 142]. For this, traditional products must be changed by replacing part of the animal fat with sources of polyunsaturated fatty acids. However, an increase in the content of unsaturated fats increases the risk of oxidative damage and the accumulation of free radicals, which can have a negative effect on the human body. Therefore, to prevent lipid peroxidation, natural antioxidants of various origins are used, including those from fruits and berries.

Fruits are considered to be excellent sources of antioxidants, vitamins, minerals and fiber, which is constantly increasing public interest in including them as healthy components in the human diet [18, p. 4].

The significant content of phenols and other antioxidants in fruits makes them an attractive alternative to synthetic antioxidants for food preservation [18, p. 4]. Whole, sliced or pureed fruits are used either raw (e.g. fruit purees) or processed (e.g. dried, powdered, extracts, etc.). In addition, fruit waste (peel, seed and pulp) has also been used to improve the antioxidant capacity of meat and meat products [18, p. 16–17]. Each of the fruits used exhibits different

antioxidant profiles and properties. Fruits and fruit products most commonly used in the meat industry for their antioxidant properties are grapes, plums, berries (currants, cranberries and strawberries) and pomegranates.

Plums are rich in vitamins, carotenoids, flavonoids and phenolic acids [19, p. 244]. After studying 20 plum genotypes, it was found that their antioxidant capacity ranges from 105 to 424 mg of ascorbic acid equivalents, and the total phenolic content ranges from 86 to 413 mg of gallic acid equivalents per 100 g of fresh fruit, with a high correlation coefficient between them ($r^2=0,96$). The pigmentation of plums is explained by the presence of anthocyanins (cyanidin-3-rutinoside, cyanidin-3-glucoside and peonidin-3-rutinoside), while the intensity of their color is related to the total content of phenols [19, p. 245]. Other phenolic compounds found in plums are hydroxycinnamic acid (chlorogenic acid and neochlorogenic acid) and quercetin derivatives, while plum skin is rich in anthocyanins and neochlorogenic acid [19, p. 246]. In the meat industry dried plums and dried plums puree are used for their antioxidant properties [20, p. 74]. Concentrated plum juice and spray-dried plum powder in beef liver, plum extract puree in irradiated turkey breast rolls, and low-fat plum puree in beef patties have been successfully used to prevent lipid oxidation.

Cranberries are rich in phenolic compounds such as phenolic acids, flavonoids, anthocyanins, p-hydroxybenzoic acid and their derivatives [21, p. 121; 22, p. 346]. Ripe cranberries have a total phenolic content of 4745 mg/kg in gallic acid equivalents and a total monomeric anthocyanin content of 111.0 mg/kg [23, p. 606]. The antioxidant capacity of cranberries is related to the content of phenols and anthocyanin-anthocyanidins [23, p. 606].

Thus, numerous in vitro studies have demonstrated the positive effects of natural antioxidants on lipid and protein oxidation, shelf life extension, antioxidant profiles of functional meat products, and corresponding potential health benefits. In addition, significant progress has been made in the analysis of antioxidant profiles and the action of food additives of plant origin, as well as in the study of the prospects for their use in the meat industry. The question of the effectiveness of certain antioxidants, their different forms and preparations for use in certain meat products, as well as meat-containing systems with a multicomponent composition of ingredients, especially with the inclusion of sources of unsaturated lipids that are prone to increased oxidation, remain unresolved.

4. Study of bioflavonoid composition and antioxidant properties of black chokeberry (*Aronia melanocarpa*) and black currant (*Ribes nigrum L.*)

The chemical composition of black chokeberry depends on a number of factors, such as variety, fertilizer, ripening of berries, date of harvest or place of cultivation [26, p. 774]. The chemical composition of berries or freshly squeezed juice is distinguished from other berries by the high content of polyphenols [27, p. 7]. The detailed composition of berries is given in Table 1. It was established that the content of dry matter in berries is 17-29%, while approximately 5-10% is determined as water-insoluble material.

The analysis of the table 1 showed that the total mineral content of fresh berries is 440 mg/100 g and 580 mg/100 g. The content of such vitamins as vitamin B₁, B₂, B₆, C, pantothenic acid and niacin was also found [24, p. 1627]. The content of vitamins in various chokeberry berries is described in [24, p. 1628; 27, p. 7]. In addition to these components, β -carotene and β -cryptoxanthin were also detected in relatively high amounts.

The most important components present in chokeberry, which are also responsible for many of its therapeutic and preventive properties, are phenolic compounds. Aronia berries have a high content of procyanidins, anthocyanins and phenolic acids, which have various physiological effects. In table 2 the results of studies of phenolic phytochemicals present in black chokeberry are presented.

Procyanidins have been identified as the major class of polyphenolic compounds in black chokeberry [29, p. 7852]. Aronia contains exclusively homogeneous B-type procyanidins with (-)-epicatechin as the main subunit monomer [29, p. 7854]. Varieties and genetic origin were found to be the main factors influencing procyanidin content and profile. The composition of procyanidins in chokeberry is as follows: monomers (0.78%), dimers (1.88%), trimers (1.55%), 4-6-mers (6.07%), 7-10-mers (7.96%) and > 10-mers (81.72%) [31, p. 56]. The total content of procyanidins in chokeberry was 5182 mg/100 g in dry matter [degree of polymerization 23] [29, p. 78520], 3992 mg/100 g dry matter (DP 14) [28, p. 812], as well as 664 mg/100 g in fresh berries [31, p. 56]. In the extracts, the content of polymeric procyanidins was 8192 mg/100 g of dry matter (DP=34) [29, p. 7852] and 5611 mg/100 g of dry water [28, p. 812], and in juice 1579 mg/100 g of dry matter (DP = 23) [29, p. 7850], as well as 3652 mg/100 g [28, p. 812].

Chemical composition of black chokeberry (*Aronia melanocarpa*)

Compounds	Content in raw berries
Dry matter, %	15,6 [24, p. 1627]; 16,7-28,8 [25, p. 267]
Glucose + Fructose	66-100 [24, p. 1627]; 130-176 g/kg [25, p. 267]
Food fibers	56 g/kg [24, p. 1627]
Pectins	3,4-5,8 g/kg [25, p. 267]
Fat	0,14% [24, p. 1627]
Protein	0,7% [24, p. 1627]
Organic acids	
Malonate	13,1 g/kg [24, p. 1627]
Citrate	2,1 g/kg [24, p. 1627]
Vitamins	
Vitamin C	137 mg/kg [24, p. 1627]
Folic acid	200 µg/kg [26, p. 774]
Vitamin B1	180 µg/kg [24, p. 1627]
Vitamin B2	200 µg/kg [24, p. 1627]
Vitamin B6	280 µg/kg [24, p. 1627]
Niacin	3000 µg/kg [24, p. 1627]
Pantothenic acid	2790 µg/kg [24, p. 1627]
Tocopherols	17,1 mg/kg [24, p. 1627]
Vitamin K	242 µg/kg [24, p. 1627]
Mineral substances	
Total mineral content	4400 [24, p.748]; 5800 mg/kg [25]
Na	26 mg/kg [24, p. 1627]
K	2180 mg/kg [24, p. 1627]
Ca	322 mg/kg [24, p. 1627]
Mg	162 mg/kg [24, p. 1627]
Fe	9,3 mg/kg [24, p. 1627]
Zn	1,47 mg/kg [24, p. 1627]
Phytochemicals	
Carotenoids	48,6 mg/kg [27, p. 7]
– β-carotene	7,7 [24, p. 1627], 16,7 mg/kg [27, p. 7]
– β-cryptoxanthin	4,63 [24, p. 1627], 12,2 mg/kg [27, p. 7]
– Violoxanthin	13,0 mg/kg [27]
Total Phenols	7849 mg/100 g dry matter [27, p. 7]
Amygdalin	201 mg/kg [27, p. 7]

The content of phenolic phytochemicals in black chokeberry

Phenolic compounds	Black chokeberry berries (mg/100 g)	References
Protocyanidins (total)	5182	[29, p. 7850]
	3992	[28, p. 811]
	664	[30, p. 3]
Anthocyanins (Total)	307-631; 428; 461	[30, p. 3; 31, p. 56; 33, p. 504]
	1480	[30, p. 3]
	770-970; 641, 1041	[31, p. 56; 32, p. 179; 33, p. 504]
	1959	[29, p. 7850]
Cyanidin-3-arabinoside	146; 142; 399, 582	[28, p. 811; 29, p. 7850; 30, p. 5; 32, p. 179]
Cyanidin3-O-galactoside	315; 237; 990, 1282	[28, p. 811; 29, p. 7850; 31, p. 56; 32, p. 179]
Cyanidin-3-glucoside	10; 1, 7; 37,6, 42	[28, p. 811; 29, p. 7850; 31, p. 56; 33, p. 504]
Cyanidin-3-xyloside	10; 47; 51,5, 53.	[28, p. 811; 29, p. 7850; 31, p. 56; 33, p. 504]
Pel-3-arabinoside	2,3	[30, p. 5]
Flavonols		
– quercetin-3-galactoside	30,2; 37	[29, p. 7850; 34, p. 4]
– quercetin-3-glucoside	27,3; 22	[29, p. 7850; 34, p. 4]
– quercetin-3-rutinoside	15	[29, p. 7854]
– other Quercetin derivatives	27	[29, p. 7854]
(–)-Epicatechin	15,4	[29, p. 7854]
Chlorogenic acid	302, 61	[29, p. 7852; 31, p. 56]
Neochlorogenic acid	291, 123	[29, p. 7852; 32, p. 179]

The content of flavonols and (-)-epicatechin in chokeberry is low compared to the other phenolic components described above. Flavonols make up only 1.3% of the total amount of phenols of black chokeberry [29, p. 7854]. Recently, five quercetin derivatives were identified: 3-O-(6'-O- β -arabinosyl- β -glucoside), 3-O-(6'- α -rhamnosyl- β -galactoside), 3-O-(6'- α -rhamnosyl- β -glucoside), 3-O- β -galactoside and 3-O- β -glucoside [32, p. 179]. Their content was estimated as approximately 71 mg/100 g [33, p. 504]. On the contrary, only three quercetin derivatives [3-O-(6'- α -rhamnosyl- β -glucoside), 3-O- β -galactoside and 3-O- β -glucoside,

their concentrations in the range of 13-27, 36-50 and 21-31 mg/100 g, respectively, were detected in fruits, squeezes and juice [29, p. 7854].

Many studies have been devoted to the study of antioxidant properties of chokeberry, chokeberry extract or its phenolic components using various well-known in vitro assays. Fresh black chokeberry berries have been shown to have the highest antioxidant capacity among berries and other fruits tested by ORAC [30, p. 5].

Thus, black chokeberry (*Aronia melanocarpa*) is one of the richest plant sources of very interesting phenolic phytochemicals, including procyanidins and anthocyanins. The high content and the structure of phenolic components are responsible for a wide range of its potential medicinal, therapeutic and antioxidant effects. A promising direction for food cleanliness and healthy nutrition is the wide use of black rowan and preparations from it for the production of food products for a healthy diet.

Blackcurrant (*Ribes nigrum* L.), native to central and northern Europe and northern Asia, is considered a rich source of vitamin C. Blackcurrant is particularly popular in Europe, where most of the fruit is consumed processed. form (juices, purees, syrups, jams, jellies, etc.) and only in small portions in the form of fresh products. In addition to the high content of vitamin C, black currant contains a large number of biologically active compounds with potentially useful properties (Table 3).

Phenolic compounds are secondary metabolites of plants and constitute one of the most common groups of natural products in plants. Phenolic compounds have an aromatic ring with one or more hydroxyl substituents. Phenolic compounds are formed from a common intermediate, phenylalanine, or its precursor shikimic acid [37, p. 1021]. In berries, including black currants, the main classes of phenolic compounds are phenolcarboxylic acids, flavonoids, stilbenes, tannins and lignans.

Among the various types of phenolic compounds in black currants, the highest amount of anthocyanins is observed, among which cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, delphinidin-3-O-glucoside and delphinidin-3-O-rutinoside were found [36, p. 280; 39, p. 536; 40, p. 533]. The authors [42] found that the total amount of anthocyanins in fresh berries of different varieties ranges from 1.81 to 5.48 mg/100 g.

The most common flavan-3-ols are catechin, epicatechin, epigallocatechin and their haloyl-substituted derivatives. Flavan-3-ols are present in many

Biologically active components of black currant berries

Phytochemicals	Concentration in blackcurrant berries	References
Ascorbic acid	130-200 mg/100 ml coky	[35, p. 5; 41]
Anthocyanins		
Cyanidin-3-O-glucoside	25,07 mg/100 g of raw berries	[36, p. 280; 37, p. 1021]
Cyanidin-3-O-rutinoside	160,78 mg/100 g	[39, p. 536; 40, p. 533]
Delphinidin-3-O-rutinoside	304,91 mg/100 g	[37, p. 1021; 40, p. 533]
Delphinidin-3-O-glycoside	86,68 mg/100 g	[36, p. 280; 39, p. 536]
Flavanols		
(+)-Catechin	0,70 mg/100 g	[41, p. 3903]
(-)-Epicatechin	0,47 mg/100 g	[41, p. 3903]
Flavonols		
Myricetin-3-O-glycoside	2,71 mg/100 g	[38, p. 23; 41, p. 3903]
Myricetin-3-O-rutinoside	3,14 mg/100 g	[38, p. 23; 41, p.3903]
Quercetin-3-O-glycoside	2,61 mg/100 g	[38, p. 23; 41, p. 3903]
Quercetin-3-O-rutinoside	4,65 mg/100 g	[38, p. 23; 41, p. 3903]
Phenolic acids		
3-caffeoylquinic acid	4,30 mg /100 g	[38, p. 23; 41, p. 3903]
Caffeoyl glucose	2,79 mg /100 g	[38, p. 23; 41, p. 3903]
3-p-coumaroylquinic acid	1,73 mg /100 g	[38, p. 23; 41, p. 3903]

foods, such as grapes and red wine. Common flava-3-ols present in various parts of blackcurrant plants are epigallocatechin, gallic acid, catechin, epicatechin, and epigallocatechin gallate [41, p. 3903]. A significant amount of proanthocyanidins is also found in black currants.

The most common flavonols are found in black currant berries. These are quercetin, myricetin, kaempferol and isorhamnetin [38, p. 23; 41, p. 3903], as well as their glycosides. Black currant berries contain myricetin and quercetin in the form of glycosides and rutinosides. The highest concentration in black currant berries is quercetin-3-O-rutinoside, which is 4.65 mg/100 g of fresh fruit [38, p. 23].

The authors experimentally determined that the total concentration of polyphenols in fresh blackcurrant berries is 39.70 ± 2.42 mg/100 g of gallic acid, which accounts for its high antioxidant properties [42, p. 5]. It has been proven that blackcurrant varieties are characterized by the

highest antioxidant capacity (499.26 ± 17.00 and 438.85 ± 67.26 $\mu\text{mol Trolox eq/100 g}$ of fresh berries), while a certain variety had one of the lowest antioxidant characteristics – 219.24 ± 53.18 $\mu\text{mol Trolox eq/100 g}$ of fresh berries (6.53 ± 0.71 $\mu\text{mol Trolox eq/ml juice}$) [42, p. 6].

It was also found [42, p. 5] that the darker-colored varieties have the highest concentrations of all four main anthocyanins in black currant, among which cyanidin-3-O-rutinoside is positively correlated with color, delphinidin-3-O-glycoside, delphinidin-3-O-rutinoside and general anthocyanins. It was experimentally established that blackcurrant juice inhibited the activity of the enzymes dipeptidyl peptidase-IV, α -amylase, α -glucosidase, nitric oxide synthase and cyclooxygenase-2, which are established biochemical markers of type 2 diabetes and inflammation [42, p. 5].

Thus, blackcurrant berries are rich in polyphenolic substances, which show high antioxidative activity, and can be proposed for use in the diet of various segments of the population for the treatment and prevention of diseases such as type 2 diabetes, chronic inflammation, etc. The antioxidative properties of blackcurrant berries also make it possible to include them and their processing products, such as extracts, as functional ingredients in the meat industry.

5. Effectiveness of correction of lipid oxidation in smoked sausages using extracts of black chokeberry and black currant

The subject of the study was smoked sausage with the following ratio of components: semi-fat pork – 30%, lean pork – 10%, duck meat (*Anas platyrhynchos*) – 30%, pork fat – 25%, hydrated bamboo fibers. Spices and additional materials were also used in the production. The use of semi-fat pork, duck meat and pork fat in the recipe caused an increase in the content of the lipid fraction.

To prepare sausages, the meat was ground on a laboratory meat grinder (Philips, Germany). Pork fat was cut by hand into 4×4 mm cubes. The chopped ingredients were mixed for 8 minutes. Minced meat was stuffed into lamb sausage casings. Sausages were deposited at a temperature of $4-8^\circ\text{C}$ for 2 hours, then dried in an oven at $90 \pm 10^\circ\text{C}$ for 30-40 minutes.

Smoking was carried out in a smoking chamber at an initial temperature of 43°C , every 30 min the temperature was increased by $8-10^\circ\text{C}$ until the

temperature in the center of the sausage was $70\pm 2^{\circ}\text{C}$. After smoking, the sausages were cooled to a temperature no higher than 8°C .

Berry extracts of "Food Ingredients Mega Trade" (USA) were added to the test samples of minced meat in the following concentrations: sample C – control, without antioxidants, EBC 1 – 0.2%, EBC 2 – 0.3% EBC 3 – 0.5% extract of black chokeberry (*Aronia melanocarpa*) to the mass of raw minced meat; BCE 1 – 0.2%, BCE 2 – 0.3%, BCE 3 – 0.5% blackcurrant extract (*Ribes nigrum L.*). Doses of natural antioxidants in the technology of meat products were used according to the recommendation [43, p. 169; 44, p. 181].

Boiled sausages were stored for 25 days at a temperature of $+4^{\circ}\text{C}$ and a relative humidity of 75-78%. During the storage of sausages, the controlled parameters were acid value (AV), peroxide value (PV), thiobarbituric acid reactive substances (TBARS), microbiological indicators [45, p. 525; 46, p. 41; 47, p. 35].

Statistical analysis data were processed using Microsoft Excel software (USA). All experiments were performed in triplicate. The results reported are the results of these replicate determinations with standard deviations. The Student's t-test was used for statistical analysis of the obtained results. Data are presented as mean \pm standard deviation of the mean (M \pm m). The smallest acceptable difference for samples from one sample was specified at the level of 5%.

The results of research on the change in acid value in experimental samples of smoked sausages are shown in Figure 1.

Analysis of Figure 1 shows that at the start of storage of sausages, the AV in all samples was almost the same and amounted to 0.019-0.021 mg/KOH. It could be explained by a small amount of free fatty acids and a low intensity of hydrolysis of triacylglycerides.

The analysis of the first oxidation process phase in the experimental samples showed that the difference between the AV in the control sample and experimental sausages was observed on the 5th day of product storage. Thus, the AV in the control was 0.417 ± 0.02 mg/KOH, while in the experimental samples this value ranged from 0.247-0.378 mg/KOH, which is 9.35-59.23% lower. This dynamic was observed til the end of the storage, when the difference increased significantly.

The AV of experimental sausage had the lowest value in sample EBC3 on the 25th day of storage and was 0.391 ± 0.06 mg/KOH. It is 39%

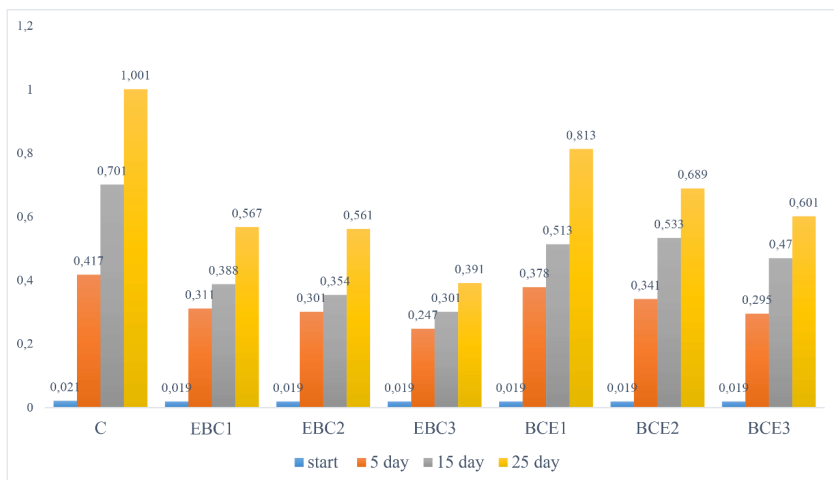


Figure 1. Results of acid value studies in sausage samples using berry extracts, mg KOH

less compared to the control. At the end of storage, the concentration of free fatty acids in all studied samples was significantly lower than in the sample without the addition of antioxidants.

It was established [48, p. 798; 49, p. 27] that artificially synthesized antioxidants are potentially harmful, so the use of natural preparations is a profitable alternative in meat processing technology. It is known that berry extracts contain polyphenol compounds that have high antioxidant activity [50, p. 199]. Moreover, due to the antioxidant and antibacterial effect of phenolic substances, plant extracts are a safe alternative to chemical preservatives used in the meat industry. They can suppress the growth of pathogenic microflora, oxidation of meat constituents (lipids and proteins) and prevent color change [51, p. 3; 52, p. 4; 53, p. 3]. The effectiveness of the antioxidant preparation is determined by its ability to suppress the rate of hydrolytic breakdown of fat, peroxidation and the formation of secondary products of lipid oxidation of meat during storage [53, p. 3].

The analysis of the dynamics of the acid value showed that during storage there is a gradual accumulation of the final metabolites of the breakdown

of triglycerides. The concentration of triglycerides reaches a maximum by the end of the shelf life, which is confirmed by the data [54, p. 5]. A comparative analysis of the effectiveness of various antioxidants showed that the black chokeberry extract at a concentration of 0.5% has the greatest positive effect on the inhibition of the primary phase of lipid oxidation in meat products. This is confirmed by the results of the authors' research [55, p. 27], who established the antioxidant and antimicrobial effectiveness of black chokeberry extract when used in pork products.

Figure 2 shows the results of the study of the secondary peroxides accumulation in smoked sausages.

As can be seen from Figure 2, at the end of the 25-day storage period, the PV in the control sample reached $0.046 \pm 0.003\% J_2$, while in the experimental samples this indicator was within 0.017-0.027% J_2 . The smallest amount of peroxides accumulated in sample EBC3 with a concentration of black chokeberry extract of 0.5% and was

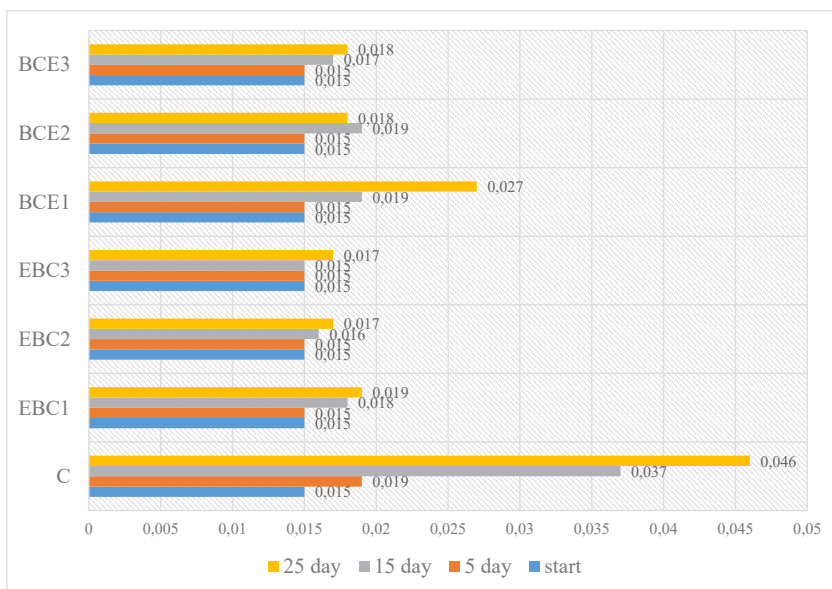


Figure 2. Results of the peroxide value studies in sausage samples using berry extracts, % J_2

0.017±0.003% J₂, which is 36.95% less than in the control. With the addition of blackcurrant extract, a decrease in the intensity of peroxidation was also observed, but at a lower rate. In the second phase of oxidative damage, further oxidation of free fatty acids occurs. Lipid hydroperoxides are known not to alter food quality because they are odorless and tasteless [56, p. 473]. However, hydroperoxides are unstable compounds, so they can be degraded into alkyl and peroxide radicals [57, p. 237].

These radicals are further broken down into secondary compounds responsible for sensory disturbances, such as odors and tastes associated with lipid oxidation. In the early stages of oxidation, an increase in hydroperoxides is observed, since the level of formation is higher than the level of decomposition. Nevertheless, since these compounds are unstable, at deeper stages of oxidation, the process of decomposition of hydroperoxides is more intensive than the process of formation [58, p. 1098].

The analysis of the dynamics of PV in the experimental sausages shows that with the addition of black chokeberry and black currant extracts, inhibition of peroxidation is observed already after the first week of storage. This could be explained by the fact that the components of the extracts make it impossible to attach active oxygen to fatty acid radicals and thus interrupt free radical oxidation [59, p. 1255]. Black chokeberry contains high concentrations of phenolic compounds, proanthocyanidins, anthocyanins and phenolic acids with high antioxidant activity [60, p. 5; 61, p. 48; 62, p. 3710].

In the early stages of oxidation, the use of peroxides as an indicator of oxidative deterioration leads to an underestimation of the degree of oxidation [63, p. 21], so this parameter is not guaranteed to be reliable in meat with a high degree of oxidation. In this regard, although the amount of peroxide is a widely used parameter for determining the degree of oxidation, it is effective only in the initial stages of oxidation processes [64, p. 406].

In order to establish the degree of accumulation of secondary oxidation products on the last day of storage of sausage samples, the TBARS was detected, the results of which are presented in Figure 3.

As we can see from Figure 3, the use of berry extracts slows down the accumulation of secondary oxidation products that react with thiobarbituric acid. After storage of smoked sausage, the concentration of malonaldehyde in the control sample was the highest and has value 0.738 mg/kg. Sausages

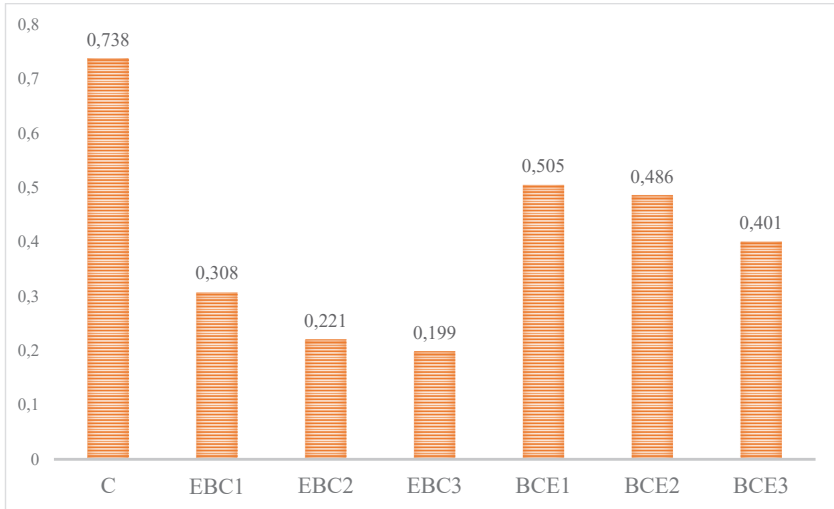


Figure 3. The effect of the bioflavonoid complex of berry extracts on the TBARS of smoked sausage, mg MA/kg

with black chokeberry extract had this indicator at 0.199-0.308 mg/kg, which is 58.27-73.0% lower than in sausage without antioxidants. The most effective was extract of black rowner in a concentration of 0.5% in the sample EBC3, where the amount of malon aldehyde in the sausages at the end of the storage period was the lowest and was 0.197 ± 0.001 mg MA.

The addition of blackcurrant extract also had a positive effect. The lowest MA content in sausages with blackcurrant extract was found in sample BCE3. TBARS in this sample was 0.401 ± 0.003 mg/kg, which is 45.66% less compared to the control.

The study of the content of secondary oxidation products made it possible to estimate the depth of oxidation processes occurring in samples of smoked sausages when stored for 25 days at a temperature of 0-6°C. The concentration of secondary oxidation products was the highest in the control sample, and in the experimental samples it decreased in proportion to the concentration of the added antioxidant additive.

It is known that the secondary products of oxidation are carriers of the unpleasant taste and smell of oxidized fats [65, p. 305; 66, p. 381;

67, p. 29]. As a result of the conducted research, it was established that the introduction of berry extracts helps to slow down the accumulation of secondary oxidation products. Of particular importance is the effectiveness of berry extracts in inhibiting the accumulation of secondary oxidation products when using them as part of multi-component meat-containing products that include ingredients of different origins [68, p. 360].

A microbiological safety study of experimental sausages during storage was also conducted. It was established that microbiological indicators of all samples corresponded to the norm or State Standard for smoked sausages. The difference between the test samples and the control sample was observed in the number of mesophilic aerobic and facultative anaerobic microorganisms.

The least number of mesophilic aerobic and facultative anaerobic microorganisms was recorded in the sample with the highest concentration of black *срщлуиуккн* extract – 0.5% in sample ECB3. The number of mesophilic aerobic and facultative anaerobic microorganisms of the test samples ECB3 was 48.48% lower than in the control sample. The tendency to decrease the number of mesophilic aerobic and facultative anaerobic microorganisms was noted in all experimental samples. The intensity of this decrease depended on the concentration of the extracts.

6. Conclusion

1. The black chokeberry (*Aronia melanocarpa*) has been found to be one of the richest plant sources of phenolic phytochemicals, including procyanidins and anthocyanins. The high content of phenolic components is responsible for a wide range of potential medicinal, therapeutic and antioxidant effects of mountain ash, which makes it a promising raw material for food purity and healthy nutrition.

2. It has been theoretically proven that blackcurrant berries (*Ribes nigrum L.*) are rich in polyphenolic substances that demonstrate high antioxidant activity. Therefore, they can be proposed for use in the nutrition of different segments of the population for therapeutic and preventive purposes. The antioxidative properties of black currant berries also allow the inclusion of fruits and their processing products, such as extracts, as functional ingredients in the meat industry.

3. Studies have confirmed the high antioxidant activity of black chokeberry and black currant extracts when used in smoked sausage technology. It has been established that the addition of black chokeberry extract in the amount of 0.2-0.5% to the mass of minced meat allows to significantly slow down the hydrolytic oxidation of lipids in finished products, and effectively suppress the peroxidation of fat. The addition of blackcurrant extract also has an antioxidant effect, but is weaker.

4. It has been confirmed that the stabilization of lipid peroxidation in smoked sausages leads to inhibition of the primary oxidation products formation. At the end of the storage period, the peroxide value of the experimental sausages was at least 0.017 mg/KOH, which is 63.04% less than in the control.

5. Sausages with black chokeberry extract had TBARS at 0.199-0.308 mg/kg, which is 58.27-73.0% lower than in sausage without antioxidants. The most effective was extract of black chokeberry in a concentration of 0.5%. The addition of blackcurrant extract also had a positive effect. The lowest MA content in sausages with blackcurrant extract was found in sample with concentration of antioxidant 0.5%. TBARS in this sample was 45.66% less compared to the control.

6. A microbiological safety study of experimental sausages during storage was conducted. The number of mesophilic aerobic and facultative anaerobic microorganisms of the test samples was 48.48% lower than in the control sample.

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