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ANALYSIS OF ANTIOXIDANT POTENTIAL AND STUDY OF THE FEATURES OF THE MICROSTRUCTURE IN CERTAIN TYPES OF SPICES AND HERBS USED IN THE MEAT PROCESSING INDUSTRY

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Keywords: *total antioxidant capacity, classes of antioxidants, methods of definition, histological analysis, adulteration*

Abstract

Spices and herbs are widely used in the meat processing industry to improve the taste and flavor of the food products. They contain a wide range of essential oils and biologically active components possessing antioxidant potential. Surge of spices consumption leads to their adulteration; at the same time, species identification is complex and requires increased knowledge about the peculiarities of their structure. This study researched the antioxidant potential (AOP) of six spices and three fragrant herbs, defined their structure and histological parameters of their identification. To assess AOP, total antioxidant capacity (TAC) was defined using the methods of Oxygen Radical Absorbance Capacity (ORAC) and free radical DPPH, and the main classes of AO were identified with the help of qualitative reactions, microstructure was analyzed via three staining methods. Among six classes of AO the flavonoids were found in all extracts. All of four AO classes were found in basil and allspice. Allspice extract showed the highest TAC_{DPPH} *(2,876.05 ± 19.83 μmol-eq.quercetin/l), the lowest value was found in parsley extract (157.97 ± 4.80 μmol-eq.quercetin/l). At the same time, the highest* TAC_{ORAC} *was found in the extract of dill greens and basil greens — 9,789.51 ± 433.22 μmol-eq.quercetin/l and 9,692.91 ± 203.42 μmol-eq.quercetin/l, respectively, and its lowest content was found in ginger — 956.98 ± 241.79 μmol-eq.quercetin/l. The microstructural features of cells peculiar for each sample were defined: external protective tissues, seed hulls, storage tissue, secretory and formative tissues, and their ability to perceive staining with general and specific dyes. The results obtained make it possible to test the composition of dry spices and herbs, to reveal their presence in the ready-to-consume meat products and to exclude cases of their adulteration.*

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Introduction

Spices are of great importance in nutrition and food technology all over the world, as they are natural components of plant origin that can embellish the food with an original and unique taste and flavor [1]. They have been an integral part of human diet and trade for thousands of years [2]. The wide application of spices in food products is primarily explained by their functional characteristics. Spices improve the taste and flavor of food products; they feature the bactericidal and antioxidant properties (they can be used for food preservation); benefit to the absorption of food as they are catalysts for a lot of enzymatic processes, and activate metabolism in general [3,4].

Spices are also important nutraceuticals. Recently the acknowledgement of the link between health and nutrition has increased their importance in the food industry and has attracted the interest of the researchers who attempt to define the mechanisms of the spices' action, find new bioactive compounds and the their beneficial properties with a view to apply the spices as modern functional food ingredients [5,6].

Now there are enough evidences that confirm that certain types of spices possess antioxidant, anti-inflammatory, anti-tumor, anti-carcinogenic, and glucose-lowering and cholesterol-lowering properties, as well as properties that beneficially influence cognitive function and raise vigor of mood [2,7,8]. A row of authors believe that spices are capable to protect from cardiovascular and neurodegenerative diseases, cancer and diabetes of the 2nd type [9–13].

More than 150 different types of spices are known, but not many of them are used. These are the so-called classic spices, which have the following characteristics as common [14]: they are consumed in dry form; they have specific well-pronounced aroma and pungency; they become bitter when heated strongly and when the amount of spices is increased above the recommended volume; they are used in wide scope of applications.

Depending on what part of the plant is used for production, the spices are divided into the following groups:

- seeds — mustard, nutmeg;
- fruits — pepper (black, white, allspice, red), cardamom;
- flowers, buds and their parts — cloves, saffron;

- leaves — bay leaf, rosemary;
- bark — cinnamon, cassia;
- rootstock — ginger, turmeric, galangal.

The researches over the past decade has shown the presence of essential oils and biologically active components in the spices, including sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes and vitamins, flavonoids and polyphenols [8,10,15]. Essential oils are the predominant and most important antiviral components of spices [16]. Natural antioxidants in spices help prevent oxidative stress [17].

Spicy herbs include dill, parsley, coriander, anise, mint, tarragon, fennel, rue, lemon balm mint, hyssop (blue St. John's wort), basil, sweet clover, oregano, thyme, wormwood, marjoram, lovage, etc. Usually the aboveground parts of the spicy plant (stem, leaves, flowers, fruits, seeds) are used as food; the root is used comparatively rare (only in calamus, angelica, coluria). Spicy herbs are also highly valued for the biologically active compounds they contain [18]. Potential cancer prevention substances such as anetepherone, carvone and limonene have been isolated from dill oil. Dill antioxidants prevent a number of diseases, such as diabetes, Parkinson's disease, atherosclerosis, and diseases of liver [19,20]. Basil contains a lot of flavonoids, including flavonols and flavone derivatives, or phenolic acids such as rosmarinic acid and caffeic acid [21]. Parsley contains up to 0.2% ascorbic acid, carotenoids, riboflavin, thiamine, nicotinic acid, flavonoids, phytoncides [22], and its essential oil features antioxidant, anti-inflammatory, antitumor and anti-apoptotic effects [23,24,25]. Consumption of spices and fragrant herbs can contribute a significant amount of antioxidants to human nutrition.

Over the past few years the global market for spices and herbs has been constantly growing. About 50 of the 86 items produced in the world are grown in India [26]. The Russian market of spices has already been formed long time ago, and in recent years it has faced very high rates of growth. According to research [27], both the number of consumers and the frequency of spices consumption have increased. As the trade in spices has exponentially increased around the world, they have become frequently subject to adulteration, which may happen deliberately or unintentionally. Deliberate adulteration usually has underlying economic motives; it is aimed at maximizing profits and is associated, first of all, with cheating in the quantity and quality of raw materials being used, sometimes they use the plants free of any biologically active substances [5]. Unintentional adulteration is associated with improper harvesting or processing of plant material [28]. This makes quality control and safety monitoring of these components an acute and pressing issue in the industry. Species identification of the spices is very difficult and requires the involvement of advanced analytical approaches [29,30]. It is promising to use microstructural analysis methods for these purposes, which are widely used to determine the components and technology of production of various types of semi-finished and finished products [31]. Microscopic identification is often used to verify the authenticity of medicinal plant materials and components, as it allows determining the botanical origin of the plant and to assess its quality [3,32,33].

The literature does not fully describe the issues of determining the quality and composition of spices and herbs used in the meat processing industry and widely distributed in Russia. The purpose of this article was to determine their antioxidant potential, define the most common classes of antioxidants and study the microstructural features of the structure and the main indicators of authentication.

Objects and methods

Objects

The objects of research were dry spices and fragrant herbs that are most often used in the meat processing industry: red pepper (*Capsicum annuum L.*), black pepper (*Piper nigrum L.*), allspice (*Pimenta officinalis L.*), turmeric (*Curcuma longa*), ginger (*Rhizoma zingiberis*), nutmeg (*Myristica fragrans*), dried chopped basil (*Ocimum basilicum L.*), dill (*Anethum graveolens L.*) and parsley (*Petroselinum crispum*). Each sample was purchased from three different manufacturing companies in the Moscow retail chain. The total number of samples under study was 27 pieces.

Definition of antioxidant potential

Ethanol extracts were prepared to define the antioxidant potential. A weighed portion of the sample was mixed with 70% ethyl alcohol in a ratio of 1:15 (g: ml), obtaining extracts at a concentration of 66.67 g/l. Then it was infused at a temperature of 22 ± 2 °C for 24 hours. After that the extract was filtered through filter paper for quantitative analysis with a mass fraction of ash up to 0.03% (FB-III, GOST 12026–76). Before analysis, extracts were stored at 4 °C. To evaluate the AOP of the samples, the total antioxidant capacity was defined by the methods ORAC and DPPH.

Determination of the contribution to the TAC of the antioxidants acting via the mechanism of Hydrogen Atom Transfer (HAT) was defined by the ORAC method. This method was implemented on a Fluoroskan Ascent FL fluorimeter-luminometer (TermoLabsystems, Finland) with black 96-well plates (Greiner bio-one, USA). 30 µl of extract diluted with phosphate buffer depending on activity, or diluted with quercetin solution (Sigma-aldrich, India) in the concentration range 1–20 µM, was added to each well to create a calibration graph, and 75 mM phosphate buffer (pH 7.4) was used as a control sample. Then 200 µl of 0.5 µM freshly prepared sodium fluorescein (Sigma-aldrich, USA) was added to each well. After that, the plate was covered with a protective film (SSIbio, USA) and placed into an incubation device for 30 min at 37 °C, after which 30 µl of 153 µM AAPH (*2,2'-Azobis (2-methylpropionamidine) dihydrochloride*, Aldrich Chemistry, USA).

Fluorescence intensity was measured at 37 °C for 60 min with a reading interval of 5 min, while excitation and emission wavelengths were 485 nm and 535 nm, respectively. TAC_{ORAC} of the samples was calculated according to the calibration graph, which was calculated for each plate and expressed in μmol-eq.quercetin / l.

The total antioxidant capacity of the samples was determined by the DPPH radical method on the SF-2000 spectrophotometer (OKB Spektr, Russia) according to the method [34]. A stock 1 mM ethanol solution of the DPPH radical (Santa Cruz Biotechnology, USA) was prepared in a dark glass vial; the obtained solution was incubated in a special lightproof box at a temperature of 22 ± 2 °C for 12 hours. Before measurements, a DPPH working solution with a concentration of 100 μM was prepared, its optical density was not less than 1.00 optical units. To determine TAC, 1.52 ml of DPPH working solution and 80 μl of sample or 96% ethyl alcohol as a control sample, or quercetin solution (Sigma-aldrich, India) at concentrations of 100–275 μM were added to glass vials to create a calibration graph. The reaction mixture was shaken vigorously and incubated for 30 minutes in the dark at 22 ± 2 °C. The optical density of solutions was measured in cuvettes with a distance between the working faces of 1 cm at a wavelength of 517 nm. Each sample was measured in triplicate. The percentage of free radical scavenging activity (RSA,%) of DPPH was calculated by the following equation 1.

$$RSA = \frac{D_c - D_s}{D_c} \times 100\% \quad (1)$$

where D_c is the optical density of the control sample; D_s is the optical density of the sample.

TAC_{DPPH} of the samples was calculated from the calibration graph of RSA dependence of quercetin concentration ($R^2 = 0.9957$) and was expressed in μmol-eq.quercetin/l.

Analysis of the component composition of the studied samples was defined by phytochemical screening of extracts for the main classes of natural antioxidants (AO) like phenolic compounds, flavonoids, anthocyanins, coumarins, tannins and carotenoids, the reactions for which are presented below in the Table 1. The following reagents were used for the component analysis: anhydrous sodium carbonate (AppliChem, PanReac, Germany), sulfuric acid (Acros Organics, Belgium), sodium hydroxide (AppliChem, PanReac, Germany), iron (III) chloride (Sigma-Aldrich, Germany).

The analysis was run on the extracts diluted 11 times with 70% ethanol. The sample that showed a typical sign of the proceeding reaction was considered as positive; in color reactions, the color intensity of the reaction sample was associated with the quantitative content of the desired class of AO. The results of the analysis were assessed visually and recorded by photographing.

Microstructural studies

The microstructure of dry samples was analyzed on the preparations using the "crushed drop" method. The changes in cell structure during technological processing was determined and the indicators were established for identifying the spices included in the composition of finished meat products using model systems on minced beef with the addition of the samples being studied in amount of 1%. The integrity of cells was defined both in raw minced meat and after the heat processing (72 ± 2 °C).

Histological sections of model systems with a thickness of 14 μm were sliced on a microtome cryostat "MIKROM — HM525" (Thermo Scientific, USA), mounted on Menzel-Glaser glasses (Thermo Scientific, USA) and stained with Ehrlich hematoxylin and 1% aqueous-alcoholic solution of eosin (BioVitrum, Russia) according to the generally accepted method [35], Lugol's solution (for visualization of starch grains) and tolluidine blue O (for differentiation of the polysaccharide component of cells) according to the method [36].

The obtained histological preparations were studied and their photography were taken with a light microscope "AxioImaiger A1" (Carl Zeiss, Germany) using a connected video camera "AxioCam MRc 5" (Carl Zeiss, Germany). Images were processed on a computer image analysis system "AxioVision 4.7.1.0" (Carl Zeiss, Germany).

Statistical processing

To obtain reliable results, all analyzes were run in triplicate. The results of quantitative studies were presented as the mean value and standard deviation "*Mean* ± *SD*". Calculations were made in Microsoft Excel. Statistical significance was calculated in STATISTICA 10.0 by one-way analysis of variance followed by Tukey's test. A probability was assumed as significant at the level of 0.05.

Table 1. List of qualitative reactions for determining various classes of AO

Defined class of AO	Qualitative reaction	Sign of a positive reaction
Anthocyanins	1–2 ml of the sample +20 mcl Na_2CO_3 (2 M)	green staining
	1–2 ml of the sample +20 mcl H_2SO_4 (2 M)	pink-red staining
Carotenoids	1 ml of the sample +20 mcl H_2SO_4 (conc.)	dark blue staining
Coumarins	1 ml of the sample +200 mcl NaOH (10%) → (1)	yellowing (color changing) of the solution
	(1) + distilled water → (2)	Discoloration of the solution
	(2) + HCl (10%)	cloudiness / sediment precipitation
Phenols	1–2 ml of the sample +100 mcl $FeCl_3$ (1%)	violet staining
Flavonoids	1–2 ml of the sample +100 mcl $FeCl_3$ (1%)	green staining
Tannins	1–2 ml of the sample +100 mcl $K_2Cr_2O_7$ (4,4%)	Darkening of the solution

Results and discussion

At the first stage of the work the antioxidant potential of the studied samples was analyzed. The results of TAC determination by the ORAC and DPPH methods are presented below in the Table 2.

Table 2. Results of TAC analysis of spices and fragrant herbs extracts

Sample	TAC_{ORAC}, μmol-eq. quercetin/l	TAC_{DPPH} μmol-eq.quercetin/l
	Mean ± SD	Mean ± SD
Black pepper	3,629.51 ± 223.78 [a]	468.00 ± 4.88* [a]
Red pepper	2,476.18 ± 303.62 [b, c]	416.23 ± 6.87* [b, c]
Allspice	3,313.33 ± 198.83 [d, e]	2,876.05 ± 19.81* [b, d, e]
Parsley	6,265.13 ± 101.61 [b, d, f, g]	157.97 ± 4.80* [b, d, f, g]
Dill	9,789.51 ± 433.22 [b, d, f, h, k]	918.89 ± 2.02* [b, d, f, h, k]
Basil	9,692.91 ± 203.42 [b, d, f, h, m]	905.50 ± 6.35* [b, d, f, h, m]
Nutmeg	1,265.28 ± 136.92 [b, d, f, h, l, n, p]	425.29 ± 3.89* [b, f, h, l, n, p]
Ginger	956.98 ± 241.79 [b, d, f, h, l, n, s]	609.10 ± 6.53 [b, d, f, h, l, n, r, s]
Turmeric	7,757.39 ± 56.09 [b, d, f, h, l, n, r, t]	544.75 ± 5.06* [b, d, f, h, l, n, r, t]

* — statistically significant differences between TAC_{ORAC} and TAC_{DPPH} samples (P < 0,05);

a-b, c-d, e-f, g-h, k-l, m-n, p-r, s-t — statistically significant differences between TAC_{ORAC} or TAC_{DPPH} samples (P < 0,05).

During the work, it was defined that the values of TAC, defined by the ORAC method, of almost all samples were statistically different (P < 0.05), except for black pepper and allspice, dill and basil, ginger and nutmeg. These samples featured quite similar values (P > 0.05). Similar to TAC_{ORAC}, almost all the spices under study had statistically significantly different DPPH capacities (P < 0.05). Red pepper and nutmeg, dill and basil were exception, as the exhibited similar TAC_{DPPH} (P > 0.05).

As a result of the analysis, it was defined that the highest TAC_{DPPH} was found in allspice extract (2,876.05 ± 19.83 μmol-eq.quercetin/l), while the lowest value was observed in parsley extract (157.97 ± 4.80 μmol eq.-quercetin equivalent/l). The TAC_{DPPH} values of red pepper and black pepper extracts were quite close and were equal to 416.23 ± 6.87 μmol-eq. quercetin/l and 468.00 ± 4.88 μmol-eq.quercetin/l, respectively, which is 6.9 and 6.2 times lower than the same value in allspice (P < 0.05). This may be due to the high content of phenols and terpenes in allspice, which possess pronounced antioxidant properties. Black pepper and red pepper showed similar AO composition, however, they contain less of terpenes [37].

Dill (918.89 ± 2.02 μmol-eq.quercetin/l) and basil (905.50 ± 6.35 μmol-eq.quercetin/l) showed similar content of TAC_{DPPH} (P > 0.05), which may be due to the similar composition of AO. Thus, in these plants the phenolic compounds of a number of flavonoids (rutin, quercetin), flavonols (kaempferol), terpenes (carvone, carvacrol), as well as phenolic acids (caffeic, rosmarinic) are represented at their fullest. Parsley showed TAC_{DPPH} values approximately 5.5 times lower (P < 0.05) than similar values of basil and dill, which is consistent with other studies and may be explained by the lack of terpenes in its composition [38].

The discrepancies between ORAC and DPPH methods of analysis are mainly caused by the greater sensitivity of the ORAC method. It is also worth considering the fundamental differences in methods and application of various radical-generating systems. In the case of the ORAC method, the total antioxidant capacity is measured in reference to the reactive oxygen species (ROS), and in the case of the DPPH method it is measured in reference to the reactive nitrogen species (RNS).

Thus, the highest values (above the average in comparison with other samples) of TAC_{ORAC} were found in extracts of dill and basil, which amounted to 9,789.51 ± 433.22 μmol-eq.quercetin/l and 9,692.91 ± 203.42 μmol-eq.quercetin/l, respectively. Significant differences were observed in parsley extract: while having the lowest TAC_{DPPH} value of 157.97 ± 4.80 μmol-eq.quercetin/l, it exhibited a rather high TAC_{ORAC} value — 6,265.13 ± 101.61 μmol-eq.quercetin/l. This can be explained by the high content of antioxidants in parsley that act via the HAT mechanism and are more inclined to reactions with ROS than with RNS [39,40].

Ginger and nutmeg featured the lowest TAC_{ORAC} values (956.98 ± 241.79 μmol-eq.quercetin/l and 1265.28 ± 136.92 μmol-eq.quercetin/l, respectively), while the samples showed average results for TAC_{DPPH} (609.10 ± 6.53 μmol-eq.quercetin/l and 425.29 ± 3.89 μmol-eq.quercetin/l, respectively).

The component composition of the extracts was assessed by qualitative reactions to such popular classes of AO-as phenols, flavonoids, tannins, carotenoids, anthocyanins and coumarins (Table 3, 4).

As a result of the analysis, none of the samples showed positive reaction for anthocyanins. The yellow staining is associated with the presence of flavones, flavonols, and flavanones. The rich red color in the turmeric extract is associated with the presence of the antioxidant curcumin in the plant — a polyphenol, which changes color to red in an alkaline pH and remains unchanged in an acidic pH [41].

As a result of the analysis of samples for coumarin content, positive results were registered in extracts of allspice, dill, basil, ginger, nutmeg and turmeric. Unchanged color intensity when adding distilled water may be caused by the presence of water-insoluble antioxidants that reacted with sodium hydroxide.

As can be seen from the obtained data, none of the samples showed a positive result for carotenoids. However, many studies have confirmed the high carotene content in turmeric [42], also red pepper is a rich source of bright representatives of carotenoids — lutein, carotene and capsanthin [43]. This indicates the inapplicability of this method of qualitative analysis to these extracts. Negative results may also be associated with poor solubility of carotenoids in alcohols, as a result of which the studied extracts either contain them in very small quantities or do not contain them at all.

Table 3. Results of qualitative analysis of the samples for classes of antioxidants: anthocyanins, phenols and flavonoids, tannins

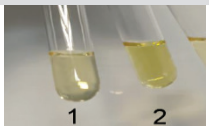
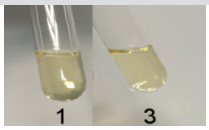
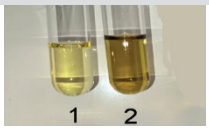
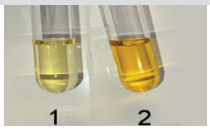
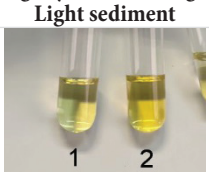
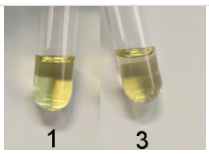
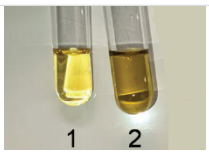
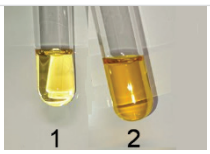
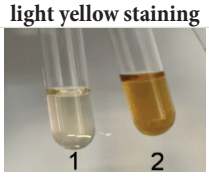
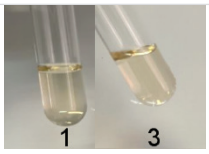
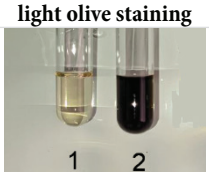
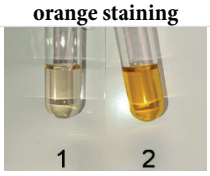
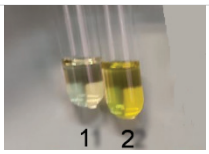
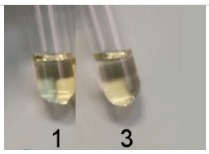
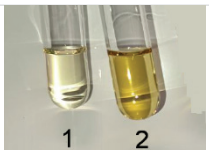
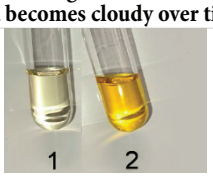
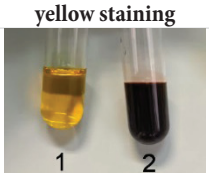
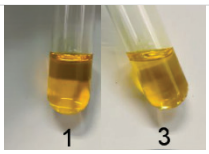
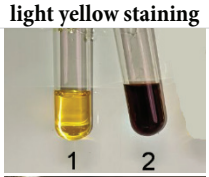
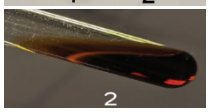
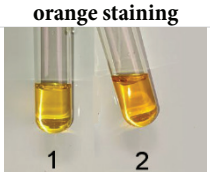
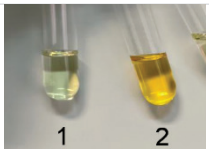
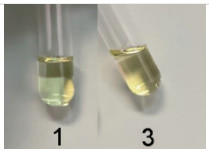
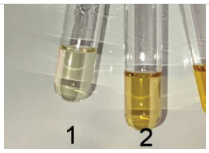
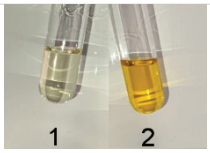
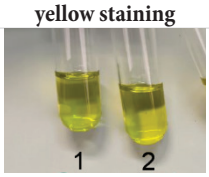
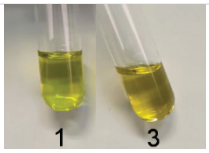
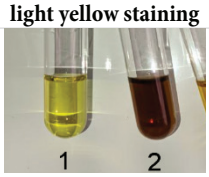
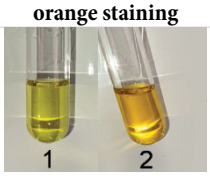
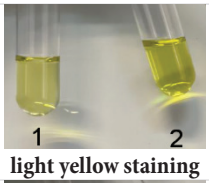
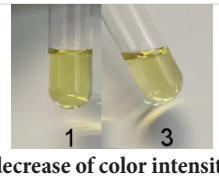
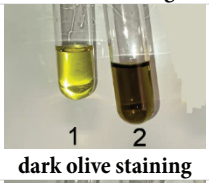
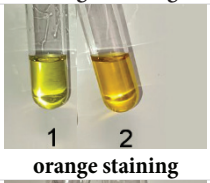
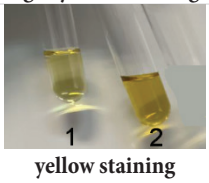
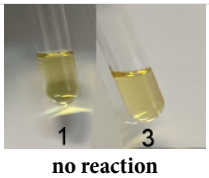
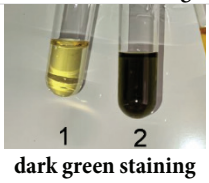
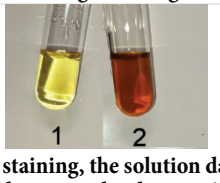
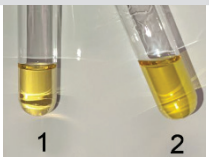
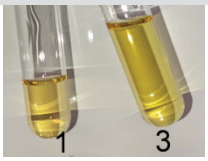
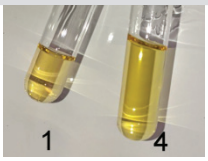
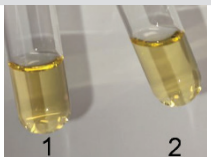
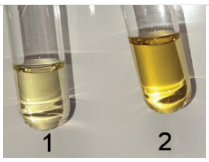
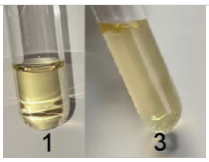
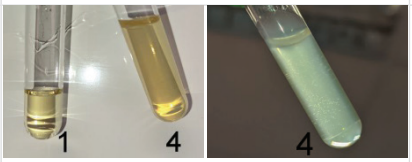
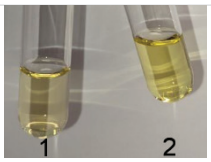
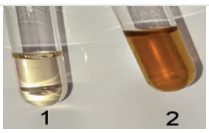
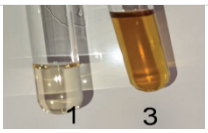
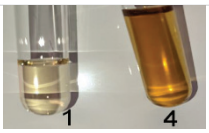
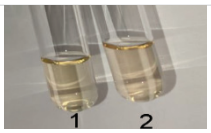
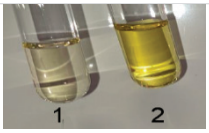
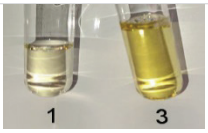
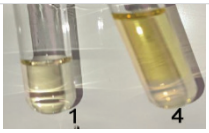
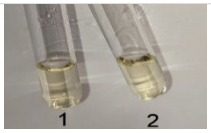
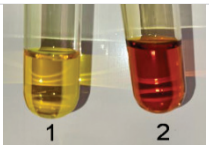
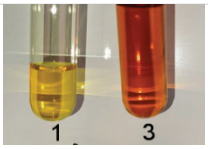
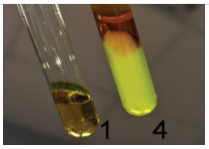
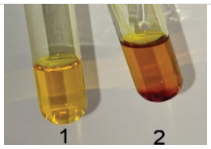
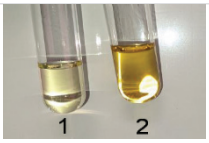
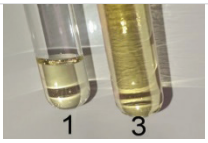
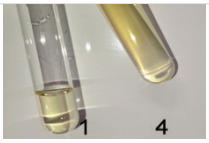
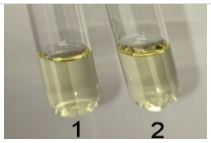
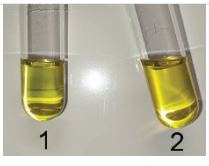
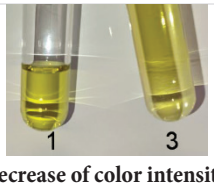
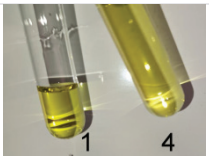
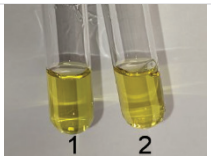
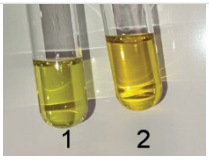
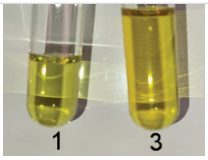
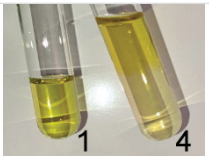
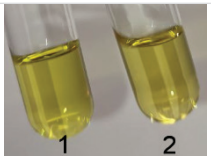
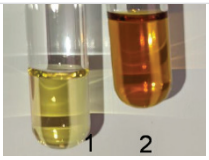
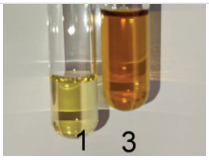
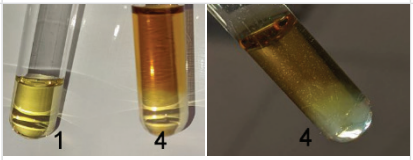
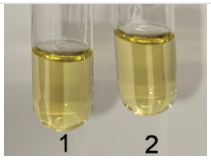
Sample	Classes of antioxidants			
	Anthocyanins	Anthocyanins	Phenols and flavonoids	Tannins
	1 — Sample 2 — Sample + Na_2CO_3	1 — Sample 3 — Sample + H_2SO_4	1 — Sample 2 — Sample + $FeCl_3$	1 — Sample 2 — Sample + $K_2Cr_2O_7$
1	2	3	4	5
Red pepper	 light yellow staining; Light sediment	 no reaction	 light olive staining	 orange staining
Black pepper	 light yellow staining	 no reaction	 light olive staining	 orange staining
Allspice	 dark yellow staining	 no reaction	 dark violet / black staining	 orange staining, the solution darkens and becomes cloudy over time
Nutmeg	 yellow staining	 no reaction	 light yellow staining	 orange staining
Turmeric	 dark red staining; cloudiness of the solution	 no reaction	  red and brown color; cloudiness of the solution	 orange staining
Ginger	 yellow staining	 no reaction	 light yellow staining	 orange staining
Parsley	 no reaction	 no reaction	 brown staining	 orange staining
Dill	 light yellow staining	 decrease of color intensity	 dark olive staining	 orange staining
Basil	 yellow staining	 no reaction	 dark green staining	 orange staining, the solution darkens and becomes cloudy over time

Table 4. Results of qualitative analysis of samples for classes of antioxidants: coumarins, carotenoids

Sample	Classes of antioxidants			
	Coumarines			Carotenoids
	1 — Sample 2 — Sample + NaOH	1 — Sample 3 — (2) + dist. water	1 — Sample 4 — (3) + HCl	1 — Sample 2 — Sample + H_2SO_4
1	2	3	4	5
Red pepper	 enhancing color intensity	 no reaction	 no reaction	 no reaction
Black pepper	 yellow staining	 decrease of color intensity	 White sediment; cloudiness of the solution	 no reaction
Allspice	 brown staining; cloudiness of the solution	 decrease of color intensity	 formation of brown flaky sediment	 no reaction
Nutmeg	 yellow staining	 decrease of color intensity	 formation of white cloudy sediment	 no reaction
Turmeric	 orange-red staining	 orange-red staining	 yellow cloudiness solution	 brown sediment formation
Ginger	 yellow staining	 decrease of color intensity	 discoloration and cloudiness of the solution	 no reaction
Parsley	 no reaction	 decrease of color intensity	 gas emission	 no reaction
Dill	 yellow staining	 no reaction	 discoloration and cloudiness of the solution	 no reaction
Basil	 orange staining	 orange staining	 discoloration and cloudiness of the solution	 no reaction

As a result of a qualitative reaction with iron (III) chloride, it was defined that allspice, parsley and turmeric contain large amounts of flavonoids, as evidenced by high color intensity. Brown coloration of parsley and turmeric extracts proved the presence of excess flavonoids with a 5-OH group. The remaining samples behaved negatively for phenols. Depending on the degree of color intensity, it is possible to conclude that the largest amount of phenolic compounds is found in the extracts of basil, turmeric and allspice. The staining of these samples featured a deep dark shade of indeterminable color, which also suggests the presence of a large amount of phenolic compounds. Black pepper and red pepper extracts showed approximately the same color intensity. Ginger and nutmeg demonstrated the weakest color changes. It cannot be stated though, that the samples with the greatest color intensity (allspice and basil) contain only one of the defined AO classes, since the possibility of color mixing should be also taken into consideration.

Analysis of the samples showed that tannins were found in basil and allspice extracts, which is consistent with several other studies [44]. The change in color of most solutions to orange can be explained by the bright orange color of the potassium bichromate solution which is the main reagent in this analysis.

Generalized data on defining of the components composition in the studied extracts for classes of antioxidants are presented below in the Table 5.

According to the authors, the amount of antioxidants in the purchased spices and herbs will be influenced by the integrity of plant cells. In addition, knowing the cells arrangement and structure of each spice, it is possible to identify it and test its purity both during the incoming control of raw materials of plant origin at the moment of their transfer to production, and during screening studies of products presented on the market. The use of light microscopy is widely used in China to identify Chinese herbal medicines according to their morphological structure [45]. In this regard, it was of interest to conduct microstructural studies of the samples and define the structural features of their cells.

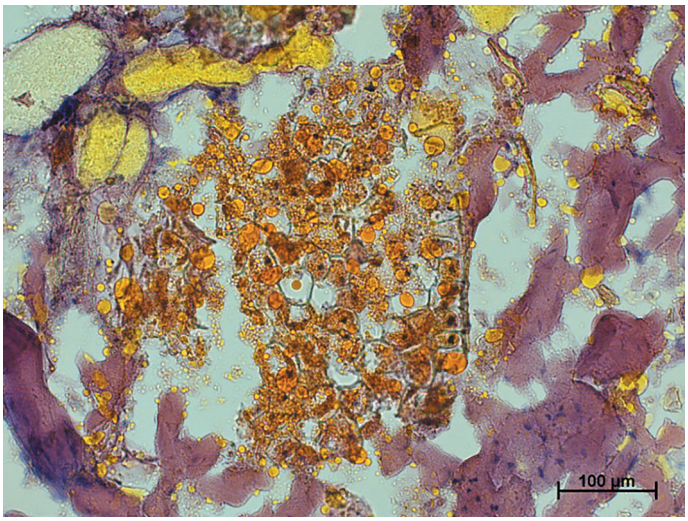
Table 5. Results of analysis of the components composition in the extracts

Sample	Phenols	Flavonoids	Tannins	Carotenoids	Anthocyanins	Coumarins
Red pepper	–	+	–	–	–	–
Black pepper	–	+	–	–	–	+
Allspice	+	+	+	–	–	+
Nutmeg	–	+	–	–	–	+
Turmeric	+	+	–	–	–	+
Ginger	–	+	–	–	–	+
Parsley	+	+	–	–	–	–
Dill	–	+	–	–	–	+
Basil	+	+	+	–	–	+

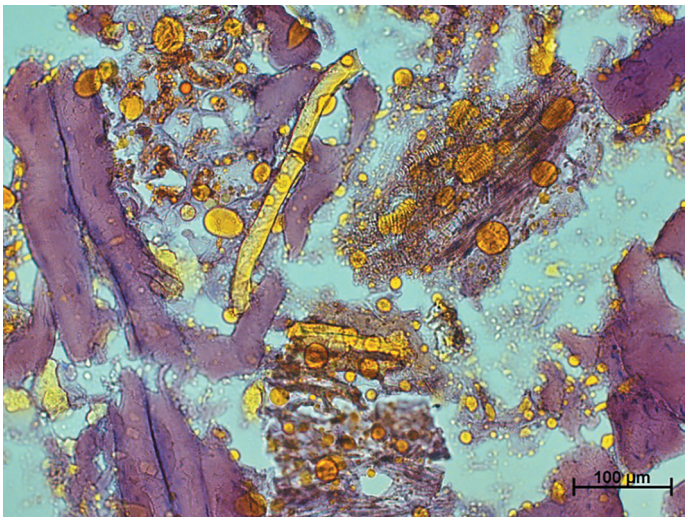
"+" — positive test for AO, "-" — negative test for AO

Histological preparations of red pepper demonstrated fragments of the external protective tissue (epidermis) with flattened, wrinkled cells. The main part consisted of colenchymatic cells of a pentagonal or hexagonal shape, with nuclei, acidophilic granules and yellow-orange oil droplets inside. The shape of large parenchyma cells looks like honeycomb. There are endocarp elements (cylindrical cells) and fragments of vascular bundles (Figure 1). After heat treatment, red pepper cells retain their shape (Figure 2).

Partial detachment of the epidermis is noticeable, expressed in an increase in interstructural spaces. In collenchyma cells, essential oils are clearly visible in the form of tiny yellow droplets, easily differentiated from each other. Drops of essential oil partially penetrate into the surrounding tissue under the influence of heat treatment and the previous process of mixing the components of the minced meat. Nuclear structures are represented by individual basophilic elements. In some cases, cell deformation and compression are observed.

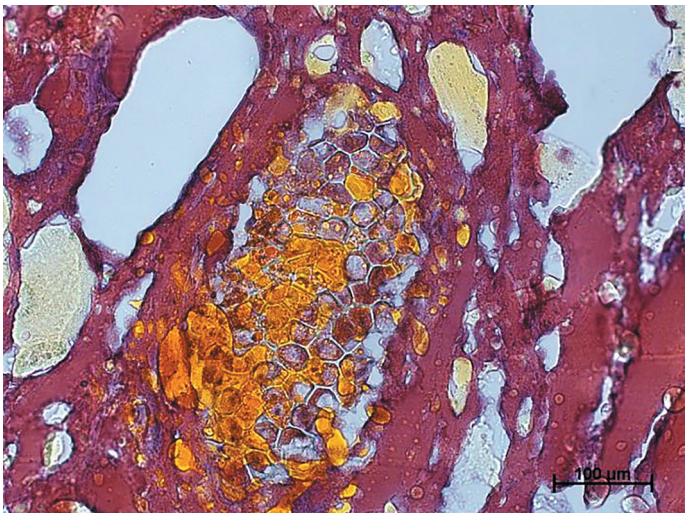


Parenchyma cells

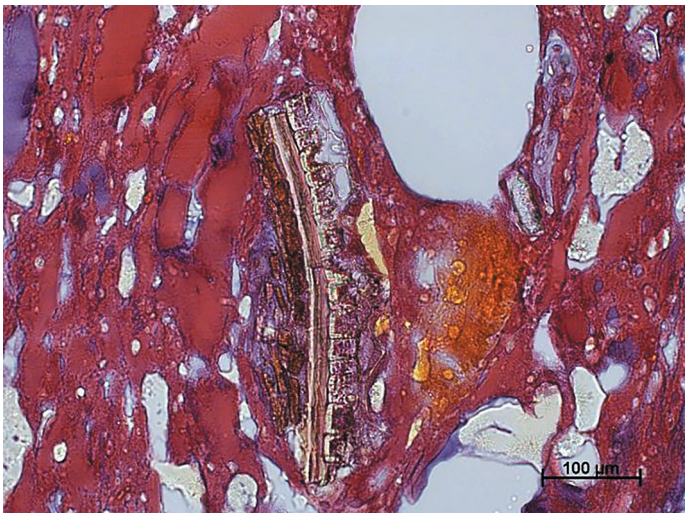


Fragments of vascular bundles

Figure 1. Red pepper. Hematoxylin and eosin staining (scale 100 µm)



Parenchyma cells



Epidermal fragment

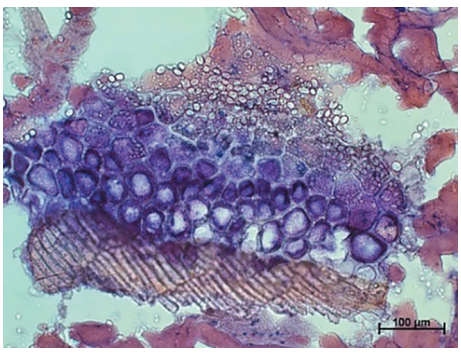
Figure 2. Red pepper after heat treatment. Hematoxylin and eosin staining (scale 100 µm)

Black pepper samples contain large fragments of the seed tissue with pronounced cell layers. Dense dark-colored fragments of the epidermis, sclereids (stony cells) layer and mesocarp parenchyma with large cells containing yellowish essential oil are visible. Multifaceted stony cells, voids and fragments of vascular bundles are clearly visible too. The elements of the seed hull include flattened prismatic epidermal cells, under which are located light-colored sclereids, almost similar in appearance to the sclereids of allspice. This is caused to the commonality of their taxonomy [46]. Moreover, in many cases these fragments are separated from the underlying tissues, possibly due to deformations during grinding of pepper seeds (Figure 3). The main part of the black pepper pea is occupied by perisperm, formed by large elongated hexagonal cells with numerous small starch grains (Figure 4), between which there are single cells with essential oil. There are small fragments of vascular bundles. After heat treatment, black pepper particles retain their microstructural characteristics and are well differentiated in histological preparations.

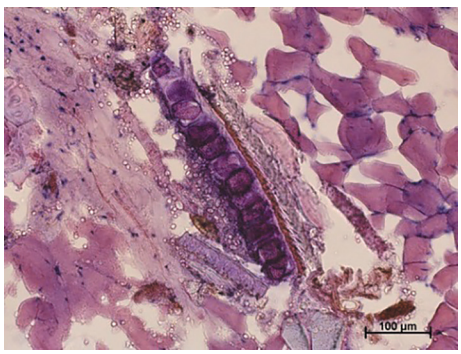
The peculiar particles of allspice samples contain variously sized, unstained "glassy" cells that form parenchymal tissue structures, as well as cylindrical, spiral-shaped conducting pathways. Fragments of the epidermis are clearly visible in the form of long strands of flattened cells. Sclereids are positioned under the epidermis, which are the unstained cells with concentrically located structural elements and a cavity in their center. Parenchyma cells are large, round, and have high content of small starch grains. In addition, secretory structures with inclusions of essential oil can be seen in the cellular elements of allspice peas (Figure 5). After heat treatment, cell complexes predominantly retain their structure (Figure 6). It can be noted that the essential oil is partially washed out during processing; on the histological specimen its drops were found only in the middle of the cell.

On histological preparations of nutmeg the fragments of the nutmeg endosperm are visible. The fragments consist of hexagonal-shaped cells containing storage substances: fats, oils, starch and aleurone (Figure 7). The brown perisperm cells are highly compressed. There are dense structures of dark brown color, consisting of small, closely spaced cells belonging to the epidermis (Figure 8). Large structural elements of external protective tissues are practically absent, since mature nutmegs have no outer pulp (the hull) and are usually dried. After heat treatment, the color of cells groups becomes lighter. Apparently, the heat treatment causes slight deformation of the cells. Within the cells small round starch grains and drops of essential oil are visible.

The special feature of turmeric is rounded conglomerates of large cells in the rootstock parenchyma, with inclu-

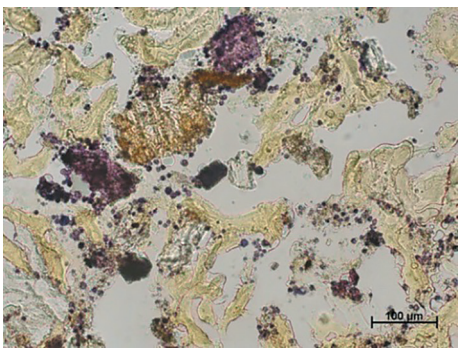


Perisperm fragment



Fragment of the epidermis

Figure 3. Black pepper. Hematoxylin and eosin staining (scale 100 µm)



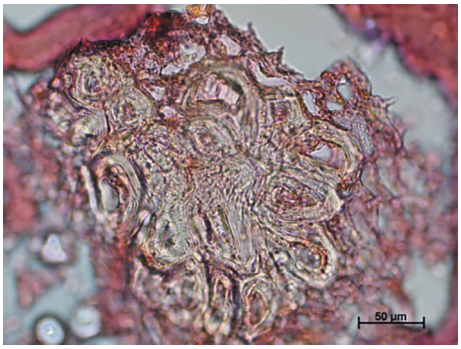
Starch grains

Figure 4. Black pepper. Staining with Lugol's solution (scale 100 µm)

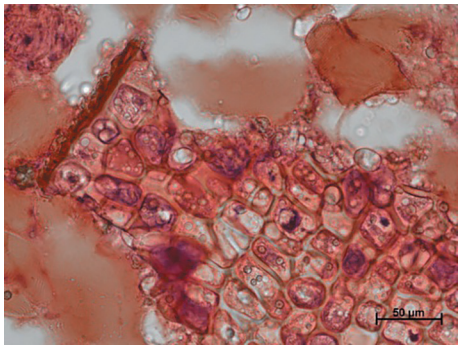
sion of elongated starch grains and a granular mass of yellowish-orange color (due to the presence of a yellow dye, curcumin, and inclusions of essential oil). The groups of epidermal type cells (elongated cells look like "columns"), fragments of vascular bundles (Figure 9) were observed. After heat treatment, inclusions of essential oils become practically indistinguishable, apparently due to their washing out during processing. The presence of parenchyma particles with wrinkled cells, sometimes with yellowish inclusions (essential oils, curcumin) should be noted. Large round cells are stained less intensely (Figure 10).

Unlike turmeric, ginger features the clusters of flattened cells in the surface layer of the rootstock, and large polyhedral parenchyma cells weakly sensitive to hematoxylin and eosin staining. These cells are characterized by a thin cellulose membrane and the high content of starch grains in the cytoplasm (Figure 11). The sample is also characterized by the presence of "columnar" tissue fragments. There are fragments of wide and thick-walled vessels bundles (Figure 12). Also on the preparation there are scattered starch grains created by grinding, which presence is a distinctive feature of ground ginger. The sizes of starch grains vary from 20 to 40 microns, they have peculiar flattened shape, narrowed at the ends. Particles of essential oils from yellow to dark yellow are observed, which are located in special cellular "secretory" structures of the parenchyma — idioblasts. The type and peculiar feature of these structural elements is one of the characteristics of this plant family.

After heat treatment, large fragments of ginger tissue are destroyed. Cover cells in the form of "columns", flattened spirals of vascular bundles, sometimes scattered, characterized by an increase in intercellular spaces, remain visible on the preparation. There are individual cells of storage tissue with the presence of yellow essential oil,

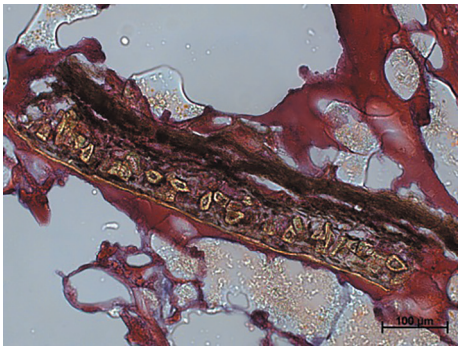


Sclereids



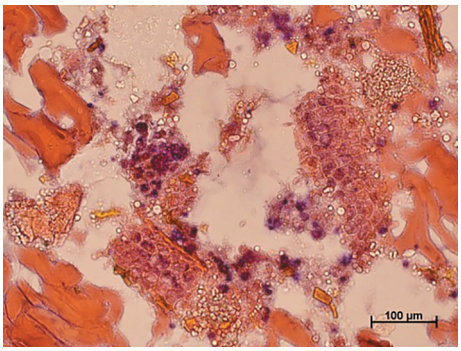
Parenchyma cells

Figure 5. Allspice. Hematoxylin and eosin staining (scale 50 µm)

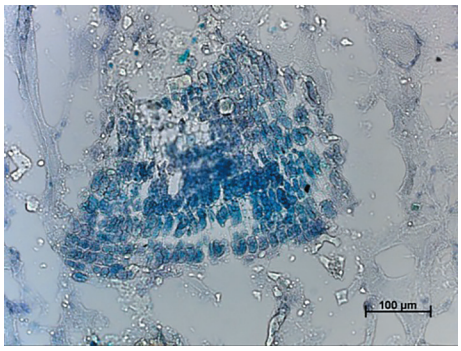


Fragment of the epidermis

Figure 6. Allspice after heat treatment. Hematoxylin and eosin staining (scale 100 µm)



Hematoxylin and eosin staining



Toluidine blue O staining

Figure 7. Nutmeg, endosperm fragments (scale 100 µm)

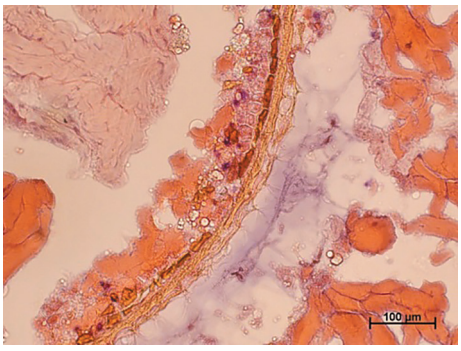
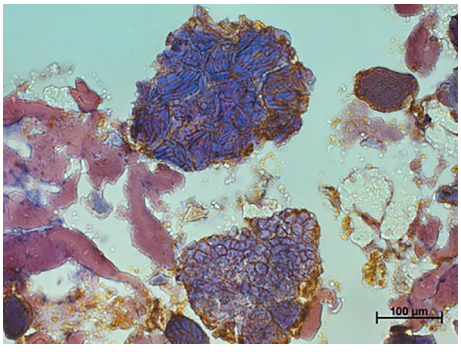
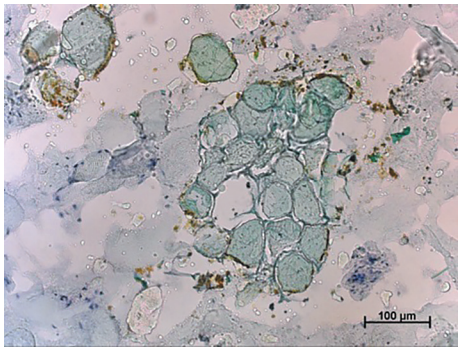


Figure 8. Nutmeg, epidermal fragment. Hematoxylin and eosin staining (scale 100 µm)



Hematoxylin and eosin staining



Toluidine blue O staining

Figure 9. Turmeric. Fragment of parenchyma. (scale 100 µm)

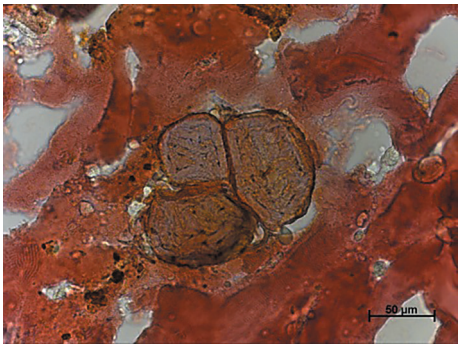
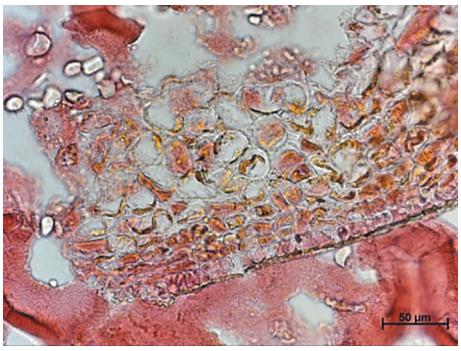
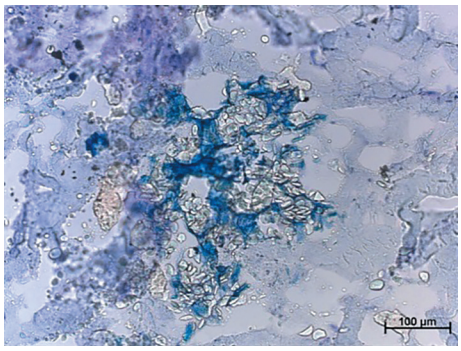


Figure 10. Turmeric. A fragment of parenchyma after heat treatment. Hematoxylin and eosin staining (scale 50 µm)



Staining with hematoxylin and eosin (scale 50 µm)



Tolluidine blue O staining (scale 100 µm)

Figure 11. Ginger. Parenchyma fragment

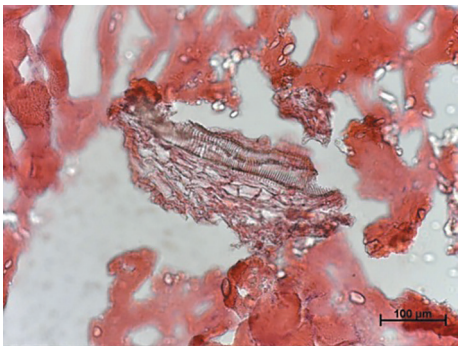


Figure 12. Ginger, fragment of a vascular bundle. Hematoxylin and eosin staining (scale 100 µm)

as well as "secretory" structures that do not perceive color well and are deformed as a result of the treatment. The membranes of parenchyma cells fit tightly to each other and are sometimes difficult to differentiate.

The samples of fragrant herbs contain a large number of particles attributed to plant leaves and having corresponding general morphological features. On the outside there are unstained closely spaced cells of the epidermis (leaf skin). The cuticle and stomata are poorly distinguishable in preparations. Mesophyll cells are located between the upper and lower epidermis. Under the top layer there are large rectangular cells of the palisade (columnar) parenchyma with numerous chloroplasts. Underneath them are several layers of cells of loose (spongy) parenchyma of irregular shape with large intercellular spaces. There are clearly visible elements of vascular-fibrous bundles: vessels, sieve-shaped tubes and collenchymal cords of mechanical tissue. On preparations of parsley and basil samples, a clear boundary is visible between the upper and lower sides of the leaf fragments (Figures 13, 14). Parenchyma cells contain starch grains, chloroplasts and small drops of essential oils. Papillary glandular hairs (essential oil) on the surface of parsley [22] and basil [47] leaves are not found.

Dill samples contain fragments of leaves in both transverse (rounded) and longitudinal sections (elongated horseshoe-shaped fragments of leaves). The epidermal cells are predominantly irregular in shape, with serpentine walls; beneath them there is a single layer of palisade tissue, which surrounds densely arranged spongy parenchyma cells. Dill does not have a clear border between the upper and lower sides of the leaf (Figure 15).

After heat treatment, fragments of fragrant herbs retain their microstructural characteristics, and their identification is not difficult in real practice. The main changes can be noted only in regard to the washing out of the essential oil: its drops remain only in the middle of the cell, mainly in the deeper layers of tissue fragments.

In a result of the studies it was found that spices and fragrant herbs at the microstructural level are characterized by the presence of cells that have a cellulose hull, peculiar for each type, due to which they are "expressively outlined" on the preparations. In this case, "transparency" of the structures can be observed due to the low density and specific tinctorial properties of plant protoplasm. Depending on the part of the plant used for production of the spice, corresponding cellular complexes and organ fragments are identified [48]. The microstructural features that play a significant role in identification include the following: characteristic parameters of the cells of the external protective tissue, seed hull, storage tissue (location and ratio of layers — endosperm and perisperm, cell color, presence of cells containing starch, essential oils, aleurone, etc.), secretory and formative tissues, and their ability to perceive staining with general and specific dyes.

The obtained results are consistent with the works [31,33], and confirm that microscopy is a practical approach for authenticating of the plant components, both in the dry mixtures composition and within the structure of ready-to-consume meat products. Currently, it is promising to combine microscopy with antioxidant analysis to determine the AOP of various plant tissues and establish a correlation between microscopic features and the active components they contain.

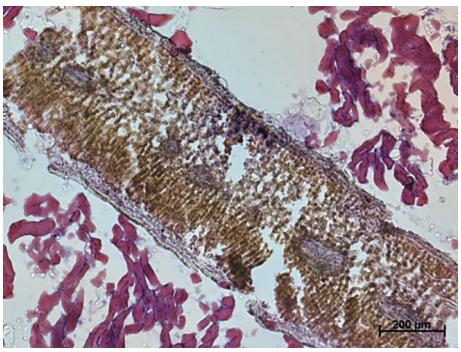


Figure 13. Basil. Hematoxylin and eosin staining (scale 200 µm)

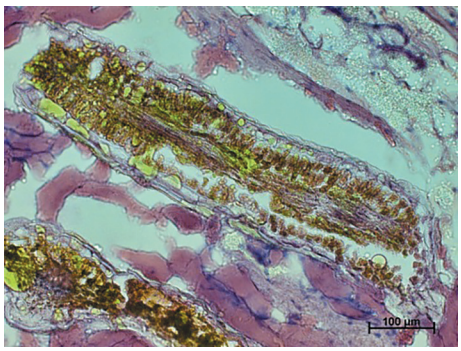


Figure 14. Parsley. Hematoxylin and eosin staining (scale 100 µm)

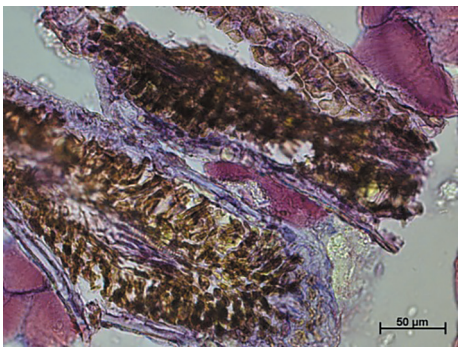


Figure 15. Dill. Hematoxylin and eosin staining (scale 50 µm)

Conclusion

The study examined the antioxidant potential of spices and herbs widespread in Russia, defined their structure and histological parameters of identification. Flavonoids were the most popular class of AO found in all samples. They were the only class of AO found in red peppers. Coumarins, which are unsaturated aromatic lactones, turned out to be quite common. Tannins and phenols were also detected in samples of allspice and basil. To expand the list of identified biologically active compounds, it is feasible to use other extraction methods and solvents.

The microstructural characteristics of the samples were studied and the main characteristic features of their cellular structure were defined. The studies have shown that accurate identification of all included plants components is a sophisticated task due to the variety of raw materials used and the complexity of cellular elements. However, in most practical cases, a significant part of them can be defined qualitatively. It should be noted that in addition to the light microscope and the general method of staining (hematoxylin and eosin), in order to increase the accuracy of spice identification it is promising to use specific staining methods, as well as polarizing microscopes and fluorescent microscopes, which allow expanding the number of morphological indicators.

The obtained results of histological analysis allow running incoming control of the dry spices and fragrant herbs compositions supplied to the meat processing plants in order to authenticate them and eliminate cases of raw materials adulteration. The established features of changes in cellular structures, that take place during technological processing, do not have fundamental nature, so it is possible to identify the spices and fragrant herbs in the ready-to-consume meat products.

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