Research Article

Biochemical Evaluation of Pumpkin Varieties

Lyudmila Yakovlevna Rodionova*, Irina Valeryevna Sobol, Lyudmila Vladimirovna Donchenko, Artem Vasilevich Stepovoy and Andrey Georgievich Koshchaev

Department of Plant Products Storage and Processing Technology, Kuban State Agrarian University, Krasnodar, Kalinina Street, 13, Krasnodar, 350044, Russian Federation.

Abstract | Pumpkins are used in the diet of different nations. However, depending on the region of cultivation, the amount of their nutrients and nutritional changes. The research aimed to study biochemical parameters (the content of dry substances, sugars, organic acids, vitamin C, carotene, pectin substances) of pumpkin varieties grown in the south of Russia. In the course of the study, standardized and modern methods of physical and chemical analysis of plant materials were used. The biochemical parameters of pumpkins were studied: the content of dry substances, sugars, titratable acidity, the content of ascorbic acid and carotene. Data on the content and fractional composition of pectin substances in different parts of pumpkins are presented. The kinetics of changes in the content of pectin substances in pumpkins after harvesting and storage for 90 days are shown. To assess the quality indicators of pectin substances, their analytical characteristics are determined – the content of free carboxyl groups, methoxyl, and acetyl components. The high content of galacturonic acid determines the technological significance of pumpkins as raw materials for the production of functional food products. The established etherification degree allows considering pumpkins as a raw source for the production of low-calorie food products. The data obtained confirm the high nutritional value of pumpkins and the possibility of their use in the production of functional food products for the prevention of diseases associated with metabolic disorders.

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***Correspondence** | Lyudmila Yakovlevna Rodionova, Department of Plant Products Storage and Processing Technology, Kuban State Agrarian University, Krasnodar, Kalinina Street, 13, Krasnodar, 350044, Russian Federation; **Email:** l.y.rodionova@mail.ru
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Introduction

The main trend in the development of modern society is an increase in the range and volume of production of specialized food products. These products are aimed at correcting the nutritional status, increasing stress resistance at a high rate of life, preventing social diseases, and increasing the active life expectancy of a person (Anisimova *et al.*, 2019; Koba *et al.*, 2017; Koshchaev *et al.*,2018). It is possible to solve these problems by adding physiologically active ingredients to food that help improve the functioning of systems and organs that are vital for humans (Semenov *et al.*, 2019; Kulikova *et al.*, 2019; Tuzov, 2018).

The main of these compounds, in our opinion, are

pectin substances (EU 432/2012, 2012; IPPA, 2016). Pectins are hetero-polysaccharides, have several functional properties *i.e.* the ability to detoxify, reduce cholesterol and glucose in the blood, regulate lipid metabolism, and increase immunity. Pectins are phytochemicals, found mainly as raw materials of fruits and vegetables (Sobol, 2016; Anisimova *et al.*, 2018; Donchenko *et al.*, 2006).

Pumpkin surpasses many vegetables in terms of carbohydrates, vitamins, and minerals. Pumpkins are a rich source of potassium salts (222 ± 5 mg per 100 g of raw mass), which support the alkaline reaction of the human body's blood and reduce the acidity of gastric juice; calcium and phosphorus contribute to the formation of bone tissue; iron stimulates the formation of red blood cells; iodine improves the functioning of the thyroid gland; zinc activates immune processes and improves the functioning of the brain and nervous system and memory; cobalt participates in the synthesis of enzymes and metabolism of sugars and accelerates the growth of bone tissue (IPPA, 2016; Troshin, 2018; Ratoshny and Soldatov 2018; Troshin and Onischuk, 2018).

Pumpkin pulp contains from 5 to 25% dry matter, 0.1-0.15% fat, 0.7-0.95% fiber, 15-20% starch, and 10-14% sugars. Pumpkin contains such vitamins as (in mg %): C – 15; B_1– 0.06; B_2– 4.4 ± 0.1; B_6; PP; E – 1.8. It contains more carotene than carrots (16-17 mg % and in some varieties – up to 30 mg %). Pumpkin is also rich in folic acid (vitamin B_9), which plays an important role in hematopoiesis, and pantothenic acid (vitamin B_5), the lack of which leads to metabolic disorders, since this vitamin is involved in the metabolism of fatty acids, normalizes lipid metabolism, and activates redox processes in the body (Sobol *et al.*, 2017; Kryukov *et al.*, 2018; Skvortsova *et al.*, 2018).

The value of pumpkins is associated with the fact that they contain peptic enzymes that convert protein into a soluble form, which is important in dietary nutrition (Rodinova *et al.*, 2015; Rodinova, 2010).

With this in mind, the problem of ensuring the safety of the chemical composition when storing pumpkins is urgent. During storage, it is used for post-harvest maturation and other biochemical processes. In particular, change in the content of pectin substances may occur, which consequently changes the functional properties of pumpkin when considering it as an industrial raw material source (Rodinova *et al*, 2015; Rodinova, 2010).

In literature, studies related to the nutritional, medicinal, and biological potential of pumpkin. Food and other indicators present an overview in terms of growing pumpkins in the world and describe antidiabetic and antioxidant properties of pumpkin chemical compounds (Yadav *et al.*, 2010; Blessing *et al.*, 2011; Sharma and Rao, 2013; Balkaya and Kandemir, 2015; Rahman *et al.*, 2019). However, there are no data for a deep study of pectin substances, including their distribution and accumulation in various layers of pumpkins, fractional content of pectin substances, their analytical parameters that affect the properties of pectin substances and play an important role in human nutrition.

Scientists from different countries are engaged in the study of pumpkins, since pumpkin contains a large amount of biologically active substances and is used in the nutrition of different peoples. For example, Yadav *et al.* (2010), give a botanical description of pumpkin and study the biologically active components of pumpkins and their seeds: fatty oils, sterols, proteins, carotenoids, and minerals. The components of antibiotics and antifungal proteins are described. In connection with the research carried out, the use of pumpkin is recommended for diabetic conditions. Its hypoglycemic activity was shown and its antidiabetic potential was analyzed. The authors described the antioxidant activity of pumpkin fruit and seed extract, the anticarcinogenic effect of pumpkin juice and seed proteins, and the antimicrobial activity of pumpkin oil. The article by Balkaya and Kandemir (2015) provides an overview of pumpkin cultivation in the world and different provinces of Turkey. The area occupied by pumpkin cultivation is described, agrotechnological methods of pumpkin cultivation in Turkey are given. Rahman *et al.* (2019) studied three types of pumpkin (S.rero, C. moschata, C. maxima). The content of α- and γ-tocopherols, β-carotene, β-crypsoxanthin, proteins and amino acids, fats and fatty acids, vitamin and mineral composition, as well as the nutritional value and caloric content of pumpkins, were determined in the peel, pulp, and seeds. Sharma and Rao (2013) study the change in various physico-biochemical properties of pumpkins (C. maxima), such as pH, titratable acidity (TA), carbohydrates, free amino acids, total proteins, total phenols, carotenoids, ascorbic acid, and specific activity of enzymes (β-galactosidase, cellulase, pectin methyl esterase, polygalacturonase, and others).

However, in the studied literary sources, there are no data on the quantitative and qualitative composition of pectin substances in pumpkins, their accumulation and distribution in various layers of pumpkins, the fractional composition of pectin substances, and their analytical characteristics affecting the properties of pectin substances and playing an important role in human nutrition. There are no studies in the references on qualitative and quantitative changes in the pectin substances of pumpkins that occur during storage. These changes are an important factor, since pumpkins are used for processing not only immediately after harvesting but also after storage.

The chemical composition and nutritional and biological value of pumpkins grown in different regions differ significantly. This is due to different climatic conditions of cultivation, applied agricultural techniques, chemical composition of the soil, *etc*. (Sharma and Rao, 2013).

The aim of the article is to study the biochemical parameters (content of dry substances, sugars, organic acids, vitamin C, carotene, and pectin substances) of pumpkin varieties grown in the south of Russia. In accordance with the goal, the following research tasks were determined:

1. To study the chemical composition of pumpkins grown in the south of Russia.
2. To determine the content of pectin substances in pumpkins and to establish their change during storage.
3. To investigate the analytical characteristics of pectin substances that affect their properties and the possibility of processing pumpkins to obtain functional food products.

Materials and Methods

Study objects

The objects of the study were pumpkins of four varieties (Prikubanskaya, Mramornaya, Stolovaya zimnyaya, and Druzhelubnaya), zoned and most widespread in the Krasnodar Territory, located in the south of Russia. These varieties are highly resistant to diseases and possess high yield and excellent taste. The selected varieties of Pumpkins are stored for 5-7 months.

The objects of the study were pumpkins of four varieties recommended for cultivation in the southern regions of Russia, which include the Krasnodar Territory. Prikubanskaya variety (*C. muschata*) is distinguished by good transportability and keeping quality. During cultivation, it is slightly affected by powdery mildew. Productivity 35-40 t/ha. The pulp is juicy, tender, orange-red in color, and sweet.

Mramornaya variety (*S. maxima*) is characterized by high keeping quality and transportability. During storage, it is resistant to cracking and white rot. Recommended for cultivation only in the southern regions; otherwise, the sugar content in fruits decreases. Productivity 30-35 t/ha. The pulp is orange in color, firm, tender, and sweet.

Stolovaya zimnyaya variety (*C. maxima*), during cultivation, is slightly affected by anthracnose and powdery mildew. It is distinguished high keeping quality and transportability. Productivity 45-50 t/ha. The pulp is orange in color, firm, tender, and sweet.

Druzhelyubnaya variety (*C. muschata*) is characterized by high keeping quality and transportability. During storage, it is resistant to cracking. Productivity 50-60 t/ha. The pulp is red-orange in color, dense, tender, and sweet.

Studies were conducted on 240 samples of pumpkins in 2016, 2017, and 2018 in at least 6 replications. Samples of pumpkins were selected in the technical degree of maturity. Technical maturity is the maturity at which fruits or vegetables are best suited for processing or storage. Determination of pumpkin components and quality indicators was carried out immediately after collection and storage. The collection of pumpkins and their storage were carried out in September. Studies of indicators were carried out after 30, 60, and 90 days of storage. Storage was carried out at a temperature of + 9°C...+ 10°C with a relative humidity of 70% in a refrigerator.

Sample preparation

Pumpkin samples were prepared for the study as follows. Segments were cut out of ten fruits of each variety, which were crushed with subsequent selection of an average sample weighing 100 g. To study pectin substances, the prepared samples were subjected to hydrolysis-extraction with 0.25% hydrochloric acid solution at a temperature of 80°C for 2 hours. The pectin extracted at these parameters was precipitated with technical ethyl alcohol with a strength of at least 96%. The resulting coagulate was dried at a temperature of 40-45°C to the humidity of 10-12%.

Methods of analysis of plant raw materials

In the course of the study, standardized and modern methods of physicochemical analysis of plant materials were used. The refractometric method was applied using an EasyPlus refractometer to determine the mass fraction of soluble solids (METTLER TOLEDO, n.d.). When studying the content of total sugars and vitamin C, the method of capillary electrophoresis and appliance of a capillary electrophoresis system "Kapel-105M" were used. A titrimetric method was used when determining total acids. The determination of the carotene content was carried out using the spectrophotometric method (with a UNICO spectrophotometer). The content of pectin substances was considered using the calcium-pectate method. The study of the analytical characteristics of pectin was carried out using the conductometric method (with a conductometer manufactured by HANNA) (Sobol, 2016; Sobol *et al*., 2017; Azimova *et al*., 2017).

Statistical analysis

The data were processed using the Statistica7.0 program. The t-test was used to calculate the confidence interval, with the calculation of Student's criterion. To exclude experimental errors and assess the significance of the factor, Fisher's least significant difference test was used. The results are presented with allowance for acceptable deviations ($p<0.05$).

Results and Discussion

Description of varieties

Prikubanskaya variety is a medium-ripened variety of pumpkin for universal use. The fruit is cylindrical with a slight thickening at the flower end, weighing 2.3-4.6 kg. The color is orange. The bark is thin, leathery, and cream-colored when cut. The subcortical layer is not observed. The pulp is red-orange, tender, sweet, and juicy; the thickness of the pulp in the expanded part of the fruit is 3-4 cm, solid, dense to the fruit-stalk. Mass fraction of dry substances – on average 11.5%, total sugar – 6.3%, carotene – 12.2 mg/100 g. The commercial and taste qualities of the fruit are preserved for 90 days after removal.

Mramornaya variety refers to medium late. Fruits are formed largely with flattened shape and wrinkled surface with light gray inclusions. The color of the pumpkin is dark gray or gray, sometimes with a greenish tinge. The weight of the fruit ranges from 6 to 10 kg. At maturity, the pumpkin pulp is orange, dense, and tender. The mass fraction of sugars ranges from 11-12%, dry matter – up to 25 %, carotene – 12 mg/100 g, vitamin C – 25 mg/100 g.

Stolovaya zimnyaya variety also belongs to the mid-late ones. The fruit has a flattened shape. The surface is segmented and slightly bumpy, with depressions – strong at the fruit-stalk and weak at the top of the fruit. The average weight is 4.4-7.1 kg. The color is dark gray, gray at full maturity, with a pink pattern. The bark is leathery and thin. The pulp is orange, 5-6 cm thick, very dense.

Druzhelubnaya variety is medium-late. The shape of the fruit is ovoid, of medium length and diameter. The color is orange. The average weight of the fruit is 4.2-6.3 kg. The pulp is orange, crisp, dense, and medium juicy. Mass fraction of dry matter – 6.3-9.2%, total sugar – 4.4-5.1%, carotene – 8.4-31.7 mg per g of raw material.

At the first stage of the study, we determine the ratio of components of pumpkin in the selected varieties. The average numerical values of which are shown in Table 1.

From the data shown in Table 1, it is clear that almost all studied samples correspond to the characteristics of the varieties.

At the second stage, we studied the content of the main chemical compounds in pumpkin samples. The data obtained are presented in Table 2.

The given data show that in the studied varieties of pumpkin, the content of the mass fraction of dry substances varies depending on the varieties from 8.5 to 13.3%. The highest content is in the Stolovaya zimnyaya variety – 13.3%. The samples studied do not differ significantly in the mass fraction of total sugar. Titratable acidity is low and is less than 0.2% for all varieties. The carotenoid content of all varieties varies from 27.3 to 15.4 mg/100g. The highest content of carotene is seen in the Prikubanskaya and Druzhelubnaya varieties. The mass fraction of pectin substances ranges from 1.65-2.45 %. The highest content of total pectin is found in the Mramornaya and Stolovaya zimnyaya varieties – 2.45 and 2.20%, respectively; the lowest – in the Prikubanskaya variety (1.65%).

Additional studies to determine their amount in different parts of the fruit were conducted to solve the scientific problem of studying changes in the content

Table 1: *The ratio of the constituent parts of pumpkins.*

Indicator	Variety			
	Prikubanskaya	Mramornaya	Stolovaya zimnyaya	Druzhelubnaya
Fruit weight, kg	4.5±0.2	7.2±0.2	8.8±0.2	5.1±0.2
Mass fraction of the pulp, % by weight of the fruit	83.7±0.1	86.4±0.1	85.4±0.1	88.9±0.1
Mass fraction of the bark with subcortical layer, % by weight of the fruit	6.0±0.1	8.1±0.1	8.3±0.1	4.3±0.1
Mass fraction of seed chamber pulp, % by weight of the fruit	6.9±0.1	2.3±0.1	3.1±0.1	5.0±0.1

Table 2: *Chemical composition of the studied pumpkins.*

Indicator	Variety			
	Prikubanskaya	Mramornaya	Stolovaya zimnyaya	Druzhelubnaya
Mass fraction of soluble solids by refractometer, %	11.5±0.1	12.4±0.1	13.3±0.1	8.5±0.1
Mass fraction of total sugar, %	8.1±0.1	8.5±0.1	8.4±0.1	8.0±0.1
Mass fraction of total (titrated) acids, %	0.16±0.05	0.18±0.05	0.15±0.05	1.17±0.05
Mass fraction of vitamin C, mg/100 g	29.6±0.1	42.3 ±0.1	45.7±0.1	23.6±0.1
Mass fraction of carotene, mg/100 g	21.0±0.1	15.4 ±0.1	17.3±0.1	27.3±0.1
Mass fraction of pectin substances (per raw mass), %	1.65±0.05	2.45 ±0.05	2.2±0.05	1.84±0.05

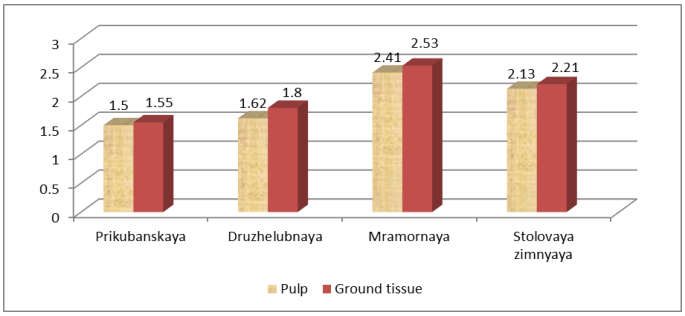

Figure 1: *The content of pectin substances in different parts of pumpkins, %.*

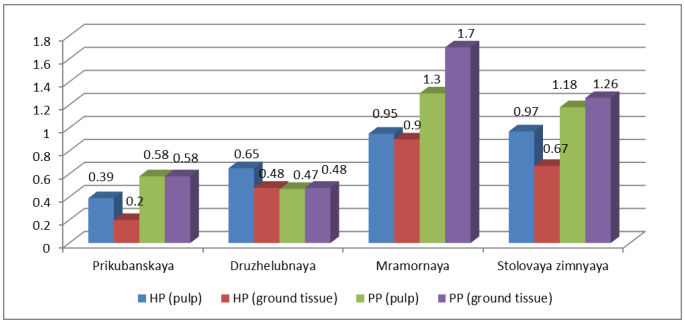

Figure 2: *Fractional composition of pectin substances in different parts of pumpkins, %.*

of pectin substances during storage. The results are shown in Figure 1.

Based on the results, the mass fraction of pectin substances located in the integumentary tissues is higher than the content of pectin substances in the pulp by 0.25-0.45%. Such experimental data are consistent with the general provisions of the chemistry of pectin substances. When studying the localization of pectin substances in plant tissue, they are mostly contained in the integumentary tissues of the fruit. This is confirmed, in turn, by experimental data on the fractional composition of pectin substances in various parts of the fruit (Figure 2).

From the presented data, it can be seen that in almost all samples of the studied varieties, the mass fraction of protopectin is higher in relation to the content of hydratopectin both in the pumpkin pulp and in the ground tissue. In this regard, the highest content of protopectin and hydratopectin is observed in the fruits of the Mramornaya variety.

At the third stage, we studied the kinetics of changes in the total content of pectin substances during storage of pumpkins.

To do this, all studied varieties of pumpkin were stored under active ventilation at a temperature of + 9°C...+ 10°C and relative humidity of 70% for 3 months. Storage of fruits of technical maturity was carried out after harvest in containers with a capacity of up to 400 kg. The results of the study are presented in Table 3.

These data indicate a decrease in the amount of pectin substances compared to their initial content. However, during the first storage period, such a decrease is

observed not in all varieties. In the Mramornaya and Stolovaya zimnyaya varieties, there is a slight increase in the total amount of pectin substances: by 2.85% (Mramornaya variety) and 5.0% (Stolovaya zimnyaya variety).

Table 3: *Change in the content of pectin substances during storage.*

Indicator	Variety			
	Prikubanskaya	Mramornaya	Stolovaya zimnyaya	Druzhelubnaya
Mass fraction of pectin substances, %	Storage for 30 days			
	1.47	2.52	2.31	1.72
	Storage for 60 days			
	1.01	1.88	1.74	1.21
	Storage for 90 days			
	0.98	1.48	1.39	1.00

The possibility of such accumulation of pectin substances during the first storage period is probably due to the maturation processes that take place in varieties of late maturation.

Such processes are not observed in varieties of early and medium maturity. They reach full maturation before the harvesting process. There is a steady decrease in the amount of pectin substances with further storage. By the end of storage, losses from the initial content reach 41-45% (for the Prikubanskaya and Druzhelubnaya varieties). Losses for late-maturing varieties are lower: 36.8% (Stolovaya zimnyaya) and 39.6% (Mramornaya). The amount of losses reached almost the same value by the end of the observed storage period.

The analytical characteristics of pectin substances contained in the studied samples were evaluated to assess the functional orientation of pumpkin for food production.

Applications of pectin are usually determined by such analytical characteristics as the content of galacturonic acid, free and esterified carboxyl groups, methoxyl and acetyl components, and the etherification degree.

Figure 3 shows the results of determining the content of galacturonic acid. This indicator determines the technological significance of pumpkins as raw materials for the production of functional food products. It was found that the high content of galacturonic acid was observed in the pectin in the Stolovaya zimnyaya variety (56%), lower – in the Mramornaya variety (48.4%), and the lowest – in the Druzhelubnaya variety (42.2%).

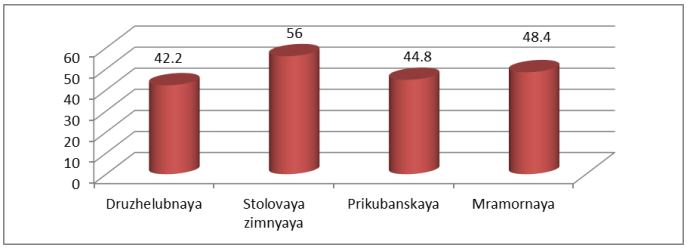

Figure 3: *The content of galacturonic acid in pectin substances isolated from the varieties, %.*

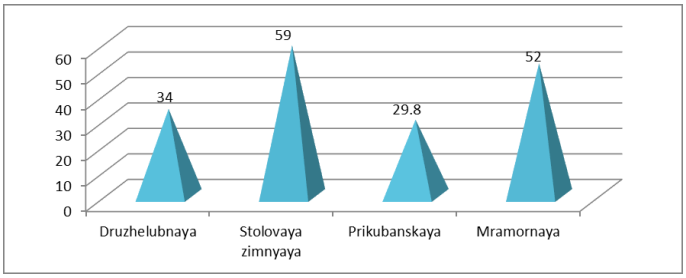

Figure 4: *The etherification degree of pectin substances isolated from pumpkins of different varieties, %.*

Thus, the technological value of pumpkins of the Stolovaya zimnyaya and Mramornaya varieties for the production of functional food products is higher in comparison with other studied varieties.

Such an indicator as the etherification degree of pectin substances also has a significant impact on the range of functional food products. A low degree of etherification of less than 50% makes it possible to reduce the sugar content of food.

Figure 4 shows data on determining the etherification degree of pectin substances in the studied varieties.

The etherification degree of the isolated pectin substances is within 34.0-59.0%, which makes it possible to consider the fruits of the Prikubanskaya, Druzhelubnaya, and Mramornaya varieties as a raw material source for the production of low-calorie food products.

The ability of pectin substances to detoxify is determined by the content of free carboxyl groups (Figure 5).

Research results show that the pectin substances of

pumpkin grown in the Krasnodar region differ in the average content of free carboxyl groups. Their number is in the range of 3.70-4.27 for pumpkins with thinner ground tissue and early maturation and 7.21-7.65 for pumpkins with thick ground tissue and late maturation, characterized by better shelf life.

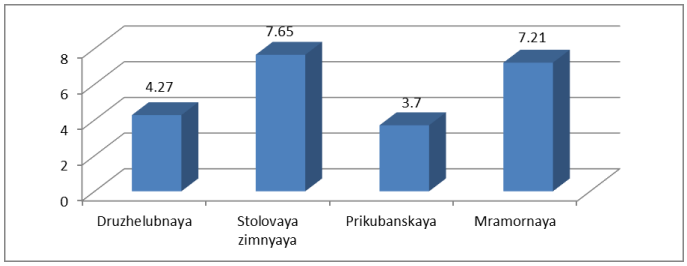

Figure 5: *Content of free carboxyl groups in pectin substances isolated from the varieties, %.*

An increase in the number of carboxyl groups in pectin leads to the rapid formation of larger flocules and, consequently, an increase in the complexing ability. This makes it advisable to consider pumpkins of the Stolovaya zimnyaya and Mramornaya varieties as a raw material source for the production of specialized products with high detoxification properties.

One of the important characteristics of pectin substances, which determines their wide range of applications in the food industry, is the ability to gelatinize. Gelation depends on the polyuronide component, the etherification degree of the pectin molecule, and the content of functional groups – methoxyl and acetyl. In this regard, additional research was conducted. The content of methoxyl groups in pectin substances is shown in Figure 6.

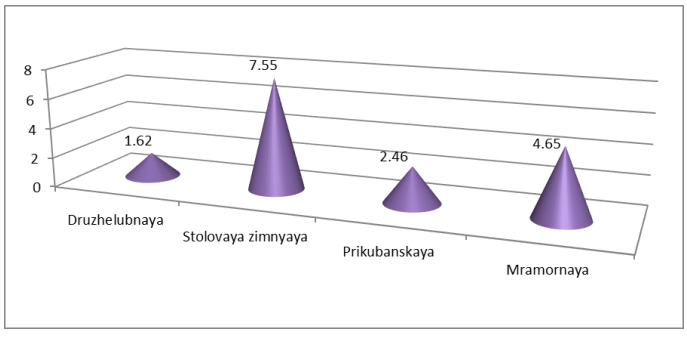

Figure 6: *Content of methoxyl groups in pectin substances isolated from the varieties, %.*

Experimental data show that the pectin substances of pumpkins of the Stolovaya zimnyaya variety are characterized by a sufficiently high value of the methoxyl component, which suggests that they can be used in the production of food systems with a gelatinous consistency.

However, acetyl groups play a negative role in the strength of the gelatinous skeleton during the formation of the gelatinous frame. The results of the research show that the pectins of the Stolovaya zimnyaya and Druzhelubnaya varieties have the highest content of acetyl groups (Figure 7). Such data make it expedient to consider the studied varieties of pumpkin as a raw source for the production of food products with a "soft" gelatinous consistency.

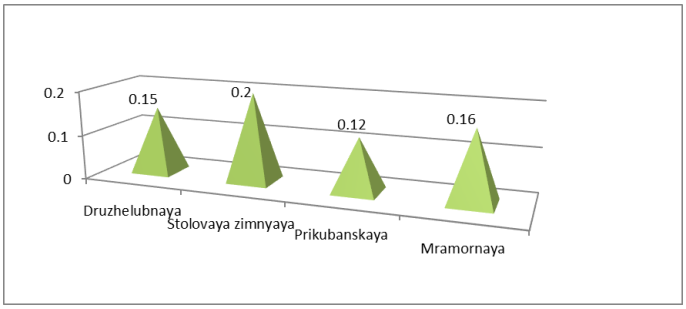

Figure 7: *Content of acetyl groups in pectin substances isolated from the studied varieties, %.*

The results of the research show that pumpkins grown in the south of Russia are characterized by a higher content of sugars, vitamin C, and carotenoids in comparison with the literature data (Blessing *et al.*, 2011; Balkaya and Kandemir, 2015; Rahman *et al.*, (2019); Sharma and Rao, 2013), as well as a high content of soluble solids and low acidity. Pumpkin pulp and ground tissue (skin) contain a high amount of pectin substances, which are natural detoxifiers.

The research results of the effect of storage time on the kinetics of changes in the content of pectin substances showed that in the first 30 days of storage, a decrease in pectin substances was observed not in all varieties. Thus, in the Mramornaya and Stolovaya zimnyaya varieties, there was a slight increase in the total amount of pectin substances: by 2.85% (Mramornaya variety) and 5.0% (Stolovaya zimnyaya variety). There was a steady decrease in the number of pectin substances with further storage. After 90 days, the content of pectin substances, depending on the variety of pumpkin, decreased by 36.8-45%. At the same time, the kinetics of changes in the pectin content was almost identical by the end of the observed storage period.

Evaluation of the analytical characteristics of pectin substances showed that the pectin substances of

pumpkins were distinguished by a high content of galacturonic acid, a low etherification degree, and a high content of free carboxyl groups, which determines the high detoxifying properties of pectin substances and allows for the use of pumpkins as a raw material for the production of food products of therapeutic and prophylactic direction.

Despite a slight decrease, the content of pectin substances after 90 days of storage remained at a sufficiently high level, which allows using the high nutritional and biological potential of pumpkins for the production of functional food products during the entire storage period.

Conclusions and Recommendations

The results of the research showed that pumpkins grown in the south of Russia are characterized by a higher content of sugars, ascorbic acid, and carotenoids, as well as a high content of soluble solids and low acidity. Pumpkin pulp and ground tissue (skin) contain a high amount of pectin substances, which are natural detoxifiers. The data obtained confirm the high nutritional value of pumpkins and the possibility of their use in the production of functional food products for the prevention of diseases associated with metabolic disorders.

Novelty Statement

The novelty of the work lies in the fact that for the first time the quantitative and qualitative composition of pectin substances contained in pumpkin fruits has been studied in detail. The fractional composition of pectin substances was established, analytical characteristics affecting the properties of pectin substances were studied in detail, changes in the pectin substances of pumpkin fruits during storage were revealed.

Author's Contribution

Lyudmila Yakovlevna Rodionova: Formulating the problem, writing a major portion of the paper.
Irina Valeryevna Sobol: Literature review, collecting the data, interpreting the results.
Lyudmila Vladimirovna Donchenko: Structuring the experimental design, formulating the problem.
Artem Vasilevich Stepovoy: Organizing and conducting the statistical analysis.
Andrey Georgievich Koshchaev: Structuring the experimental design, building the apparatus.

Conflict of interest
The authors have declared no conflict of interest.

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