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THE EFFECT OF ANTIOXIDANTS ON THE QUALITY OF SEMI-FINISHED MINCED RABBIT MEAT

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ABSTRACT

Under the current adverse environmental conditions, enrichment of the human diet with essential nutrients, including antioxidants, which exhibit immunostimulatory and adaptogenic properties and protect the body against the negative effects of free radicals, is extremely relevant. Their use in food production, including that of semi-finished meat products, improves the quality of such products and extends their shelf life. It is of scientific and practical interest to enrich rabbit meat with the antioxidant-containing plant raw materials and to study their influence on the quality parameters of semi-finished products. In this work, modern, standard, commonly accepted methods of research were used to fulfill the stated objectives. Statistical processing of the results obtained and evaluation of the reliability of the data was carried out by statistical methods using IBM SPSS Statistics for Windows. This study demonstrates the expediency of using grape-seed powder, green tea extract, and amaranth/flax flour in semi-finished rabbit meat products. The optimum component composition and amount of multiple supplements to add to the semi-finished product were determined. The total phenolic and flavonoid content and antioxidant characteristics of the test samples were studied. The highest antioxidant potential was observed in samples with flax-containing multiple supplements. This paper demonstrates that microbiological indicators in all samples throughout the storage period, in line with hygienic requirements, were lower than those in semi-finished products containing multiple supplements as compared with a reference sample, while organoleptic indicators of quality were more stable. The content of toxic elements indicates the sanitary reliability of semi-finished products. Determination of the acid number and peroxide number values during storage revealed high resistance of semi-finished products containing multiple supplements to the accumulation of free fatty acids and peroxide compounds. The obtained data indicate the effectiveness of using developed semi-finished products as antioxidant products in the diet of the population.

Keywords: minced rabbit meat; plant raw materials; antioxidant properties; oxidation processes; quality indicators

INTRODUCTION

The maintenance of population health and quality of life is a major task of the world today. Scientific evidence has established that 10% of human health depends on the health system, 20% on hereditary factors, 20% on environmental factors, and 50% on human lifestyle and nutrition. Under the current adverse environmental conditions, enrichment of the human diet with essential nutrients, whose deficiency in the body leads to decreased immunity and metabolic disorders, as well as cardiovascular, oncological, and other diseases, is extremely relevant. In this regard, along with proteins, vitamins, and minerals, antioxidants, which have a wide range of biological effects, also deserve attention: they protect the body against the negative effects of free radicals and have immunostimulant, cardioprotective, anti-tumor and adaptogenic properties. **(Lapin et al., 2007; Gichev and Gichev, 2012; Packer, 2001; Plavinsky and Plavinskaia, 2013; Tarakhovsky et al., 2013)**. It is believed that despite their high effectiveness, some synthetic antioxidants have adverse effects on human health; however, antioxidants of natural origin, including polyphenolic compounds found in plant raw materials, are minimally toxic and do not cause adverse reactions **(Carocho and Ferreira, 2013; Kumar et al., 2013; de Oliveira et al., 2018; Shahidi and Ho, 2005; Shebis et al., 2013)**. The use of plant supplements that contain functionally different antioxidants is very important in the production of healthy foods, including semi-finished meat products. Preventing the accumulation of harmful oxidative products, they reduce losses resulting from oxidative spoilage and extend their shelf life **(Denisovich, 2006; Maqsood et al., 2013; Nasonova, 2008; Nikitina et al., 2011; Stratil et al., 2006; Tomović et al., 2017)**. In the food industry, antioxidant activities are the result of interactions between air and oxygen; they are also used to increase the microbiological stability of foods and improve their quality indicators **(Banerjee et al., 2017; Kuzmina et al., 2017; Mandro et al., 2009; Poliakov et al., 2017)**.

Among products containing natural antioxidants that can be used as an antioxidant supplement in semi-finished minced meat products, of particular importance are grape seed and green tea extract, which are characterized by a high polyphenol content **(Caleja et al., 2017; Higdon and Frei, 2003; Perumalla and Hettiarachchy, 2011; Shebis et al., 2013; Yashin et al., 2009)**. Together with natural antioxidants, we believe it is appropriate to use flax and amaranth as a functional supplement, as a source of plant protein enriching and additional biologically active ingredients **(Bernacchia et al., 2014; Kalač and Moudrý, 2000; Kidyaev et al., 2017; Oomah, 2001; Pastor and Acanski, 2018; Pękal and Pyrzynska, 2014; Perumalla et al., 2011)**. Therefore, it is of scientific and practical interest to enrich rabbit meat with the above-mentioned supplements and study their influence on the quality parameters of semi-finished products.

The work aims to develop technology to produce semi-finished rabbit meat products with antioxidant properties using a multiple plant supplement.

Scientific hypothesis

Scientific hypothesis

The addition of multiple supplements to semi-finished rabbit meat products will increase their resistance to the accumulation of oxidative products and improve quality indicators.

MATERIAL AND METHODOLOGY

The studies were carried out in the laboratories of the Department of Food of Akaki Tsereteli State University and the Department of chemical analysis and food safety of Shota Rustaveli State University. The study included natural semi-finished minced rabbit meat products made according to traditional recipes and model semi-finished and finished rabbit meat products containing plant supplements made according to recipes and technologies that we developed.

Test samples: 1 – Onion-containing semi-finished product; 2 – Fried onion-containing semi-finished product; 3 – Steamed onion-containing semi-finished product; 4 – Semi-finished product containing grape-seed and amaranth flour with multiple supplements; 5 – Fried semi-finished product containing grape-seed and amaranth flour with multiple supplements; 6 – A steamed semi-finished product containing grape-seed and amaranth flour with multiple supplements; 7– Semi-finished product containing grape-seed and flax flour with multiple supplements;

8 – Fried semi-finished product containing grape-seed and flax flour with aultiple supplements; 9 – A steamed semi-finished product containing grape-seed and flax flour with aultiple supplements.

Total phenolic compounds were defined using Folin-Ciocalteu spectral methods **(Stratil et al., 2006)**. The total flavonoid content (TFC) was determined by the aluminium chloride colorimetric method as previously described **(Pękal and Pyrzynska, 2014)**. Antioxidant activity was determined by using the DPPH (2,2-Diphenyl-1-pic-rylhydrazil) method **(Okawa et al., 2001)**.

During microbiological analysis, the quantities of mesophilic aerobic and facultative anaerobic microorganisms in rabbit meat were determined according to the state standard "Food products. Methods for determining the quantities of mesophilic aerobic and facultative anaerobic microorganisms" – State Standard **GOST 10444.15-94**. The number of intestinal bacilli was determined according to the state standard "Food products. Methods for the detection and determination of the number of bacteria of the *Escherichia coli* group (coliform bacteria)" – State Standard **GOST 30518-97/GOST P 50474-93**. *Salmonella* was determined according to state standard "Food products. Methods for the detection of bacteria of the genus Salmonella" – State Standard **GOST 30519-97**. The heavy metal content was determined on a SHIMADZU AA-6200 atomic adsorption spectrophotometer, the lead content by State Standard **GOST 26932-86**, the cadmium content by State Standard **GOST 26933-86**, the mercury content by State Standard **GOST 26927-86** and the arsenic content by State Standard **GOST 26930-86**.

The peroxide number value was determined according to State Standard **GOST 8285-91**. The method is based on the interaction of oxidative products of animal fats (peroxides and hydro-peroxides) with iodic potassium in a solution of acetic acid and isooctane or chloroform, with subsequent quantitative determination of iodine released by a solution of sodium thiosulfate using the titrimetric method.

The fatty acid value was determined according to the standard **GOST 13496.18-85**. The method is based on the neutralization of free fatty acids extracted from the product by a mixture of chloroform and ethyl alcohol with a 0.1 mol.L^{-1} solution of potassium hydroxide.

Organoleptic indices were determined on a scale of 1 to 9 according to the following characteristics: appearance, colour, smell, taste, consistency, and juiciness.

Statistical analysis

To analyse the test parameters (total phenolic and total flavonoid content in the test samples, a correlation between total phenols and antioxidant activity) of natural semi-finished minced rabbit meat products, a statistical analysis was conducted of the obtained data, and the reliability of the obtained data was evaluated by statistical methods using the Windows IBM SPSS Statistics software program (version 20.0). To describe the continuous variables, we used statistical functions of the mean and standard deviation. Graphical interpretation of the results was carried out by using Microsoft Excel. The results of statistical analysis are presented in Tables 1 – 5 and Figures 1 – 4, and each value is an average of at least 10 determinations. Then, we computed the error of each measurement and calculated the squared errors to compute the absolute measurement error. We selected the value of reliability $p = 0.95$. Based on the number of measurements and the value of reliability, Student's coefficient equals $t = 3.77$ (Figure 2 and Figure 3) **(Romanov and Komarov, 2002)**.

RESULTS AND DISCUSSION

Pursuant to the stated goal, in the first stage of this work, we selected the main recipe components for the product. To prepare the reference samples, we added rabbit fat and spices to minced rabbit meat. The main ingredients in the reference sample recipes were minced rabbit meat, rabbit fat, onion, grape-seed powder, green tea extract, and amaranth/flax flour as multiple supplements. The multiple supplements were produced from grape-seed powder and hydrated amaranth/flax flour. Hydration was carried out in green tea extract - Hydromodulus 1:3 (1part of flour on 3 parts of extract). The multiple supplements were produced from grape-seed powder and hydrated amaranth/flax flour. Hydration was carried out in green tea extract - Hydromodulus 1:3 (1 part of flour on 3 parts of extract). The ratio of ingredients in multiple supplements was determined based on the optimum organoleptic parameters of semi-finished products under study.

The component and quantitative contents of multiple supplements are shown in Table 1. The amount of multiple supplements added to 100 g of semi-finished product was 30.2 g in the case of amaranth and 25.8 g in the case of flax flour.

In all samples except the one that did not contain a plant-based supplement, we determined the total phenolic content, flavonoid content, and antioxidant characteristics. To determine changes in these parameters as a result of heat treatment, meat was steamed. For this purpose, we took 9 test samples. The results obtained are illustrated in Figures 1 – 4. As can be seen from the diagrams, according to the total phenolic (Figure 1) and flavonoid (Figure 2) contents, samples with the flax supplements had the best characteristics. Since the contents of grape-seed and green tea extract in all test samples were almost identical, this difference can be explained by the chemical composition of flax, in particular, by the content of phenolic compounds and flavonoids.

Table 1 Components and quantitative composition of a multiple supplement.

No	Name of component	Content, %
1	Grape seed	0.7
2	Amaranth flour/flax flour	24.8
3	Green tea extract	74.5

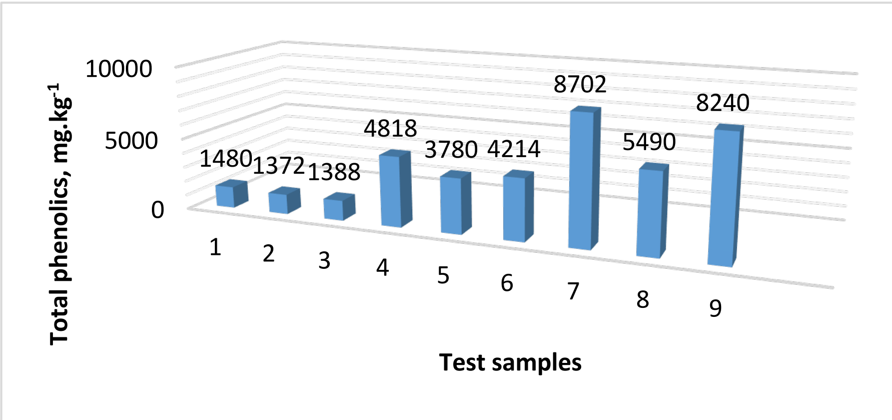

Figure 1 Total phenolic content in the test samples.

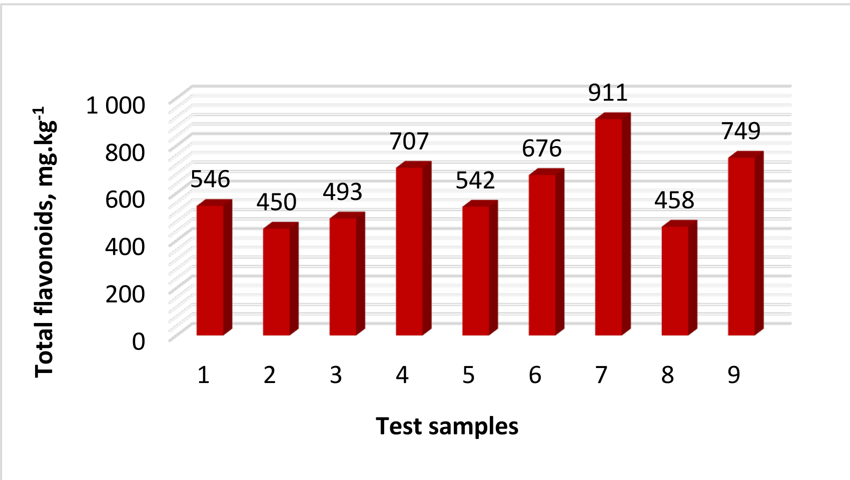

Figure 2 Total flavonoid content in the test samples.

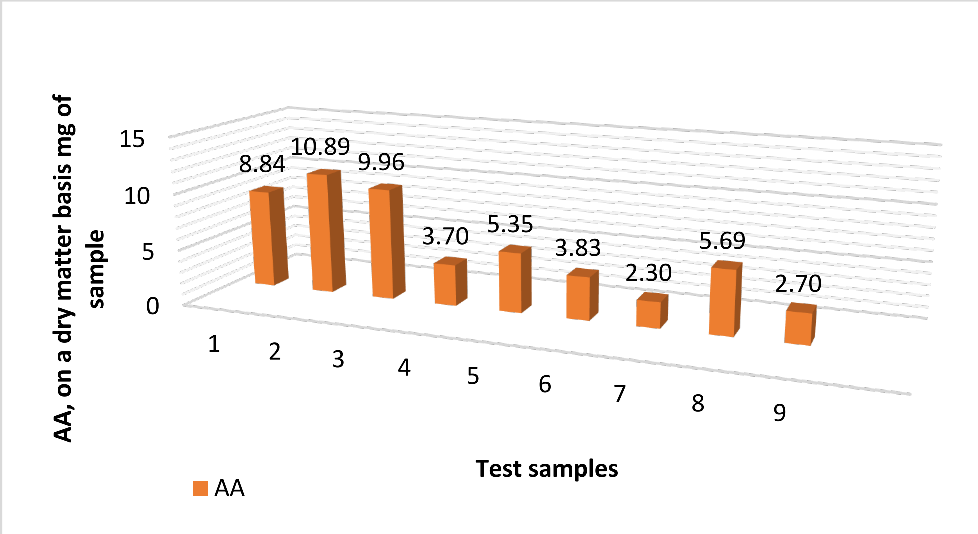

Figure 3 Antioxidant activity on a dry matter basis mg of sample.

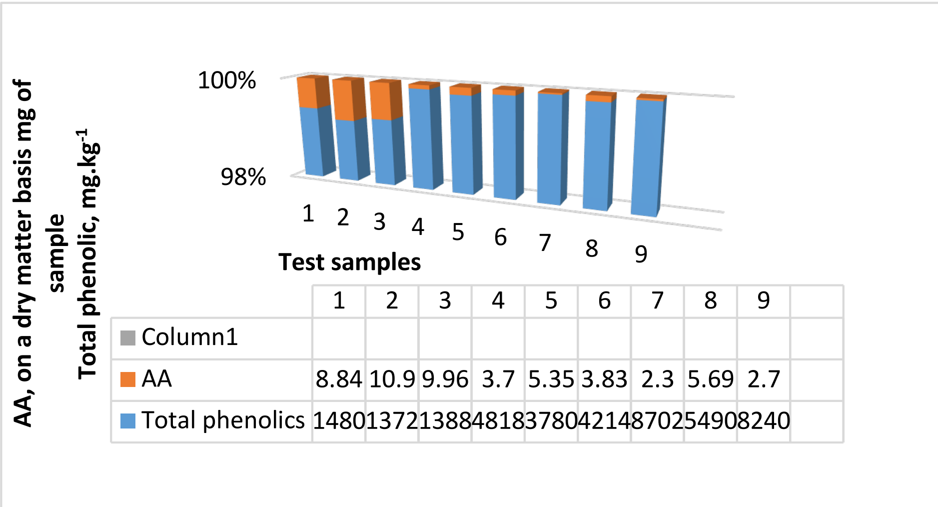

Figure 4 The correlation between total phenols and antioxidant aactivity.

The component and quantitative contents of multiple supplements are shown in Table 1. The amount of multiple supplements added to 100 g of semi-finished product was 30.2 g in the case of amaranth and 25.8 g in the case of flax flour. In all samples except the one that did not contain a plant-based supplement, we determined the total phenolic content, flavonoid content, and antioxidant characteristics. To determine changes in these parameters as a result of heat treatment, meat was steamed. For this purpose, we took 9 test samples. The results obtained are illustrated in Figures 1 – 4. As can be seen from the diagrams, according to the total phenolic (Figure 1) and flavonoid (Figure 2) contents, samples with the flax supplements had the best characteristics. Since the contents of grape-seed and green tea extract in all test samples were almost identical, this difference can be explained by the chemical composition of flax, in particular, by the content of phenolic compounds and flavonoids.

The total phenolic and flavonoid (Figure 2) contents were highest in semi-finished products (samples 1, 4 and 7), and after heat treatment, the value of phenolic compounds decreased by 5.3% in a steamed flax-containing sample (9) and by 36.9% in a fried flax-containing sample (8), and accordingly, the total flavonoid content decreased by 17.8% in the sample (9) and by 49.7% in the sample (8).

The phenolic compound content was 12.5% lower in the steamed amaranth-containing sample (6) and 21.5% lower in the fried sample (5). The total flavonoid content decreased by 4.4% and 23.3%, respectively. In the onion-containing samples, after their heat treatment, the phenolic compound content decreased by 7.3% in the fried sample (2) and by 6.2% in the steamed sample (3), while the flavonoid content decreased by 17.6% and 9.7%, respectively. The higher content of polyphenols in the steamed products was due to the use of the efficient mode of thermal treatment.

Figure 3 illustrates the antioxidant characteristics of a test sample. It shows that the highest antioxidant potential was observed in the sample containing the flax supplement. This would suggest that samples containing the flax supplement will be oxidized more slowly than other samples.

The diagram illustrating the correlation between total phenols and antioxidant activity (Figure 4) shows that the higher the total phenol phenolic content in samples, the higher their antioxidant activity (AA).

Table 2 Microbiological indicators of semi-finished products during storage.

Shelf-life, days	Mesophilic-aerobic and facultative anaerobic counts, cfu.g-1 (colony-forming unit per gram), less than			
	Control sample	Onion-containing semi-finished product	Amaranth-containing semi-finished product	Flax-containing semi-finished product
0	5.3×10^2	3.8×10^2	3.4×10^2	3.0×10^2
36	2.5×10^3	2.3×10^3	1.9×10^3	2.0×10^3
54	2.8×10^3	2.7×10^3	2.2×10^3	2.2×10^3
72	3.1×10^3	3.0×10^3	2.6×10^3	2.5×10^3
90	3.6×10^3	3.3×10^3	3.0×10^3	2.9×10^3

Table 3 Content of toxic substances in rabbit meat semi-finished products.

Indicators	Acceptable norm, $mg.kg^{-1}$	Semi-finished product		
		Onion-containing semi-finished product	Amaranth-containing semi-finished product	Flax-containing semi-finished product
Lead	Up to 0.5	0.08 ±0.01	0.05 ±0.01	0.07 ±0.003
Cadmium	Up to 0.05	<0.01	-	-
Arsenic	Up to 0.1	<0.0025	-	-
Mercury	Up to 0.03	0.011 ±0.005	0.015 ±0.001	0.01 ±0.005

Table 4 Change in the peroxide number values of semi-finished products during storage at a temperature of -18°C.

Shelf life, full day	Peroxide number value, mmole($1/2O_2$)/kg			
	Reference sample	Onion-containing semi-finished product	Amaranth-containing semi-finished product	Flax-containing semi-finished product
0	0	0	0	0
12	0	0	0	0
24	1.45	0.65	0.58	0.52
36	1.76	0.83	0.72	0.67
48	2.68	1.56	1.03	0.96
60	4.5	1.75	1.67	1.43
72	5.28	2.12	1.93	1.74
90	7.34	2.96	2.32	2.15

Table 5 Change in the acid-number values of semi-finished products during storage at temperature of -18 °C.

Shelf life, days	Acid-number value, mg KOH/g			
	Reference sample	Onion-containing semi-finished product	Amaranth-containing semi-finished product	Flax-containing semi-finished product
0	0.47	0.39	0.18	0.21
12	0.83	0.76	0.33	0.31
24	0.98	0.87	0.52	0.37
36	1.36	1.15	0.54	0.46
48	1.43	1.21	0.57	0.51
60	1.59	1.42	0.65	0.62
72	1.78	1.59	0.74	0.71
90	2.89	2.38	1.22	1.11

Accordingly, the semi-finished rabbit meat products exhibit antioxidant properties due to the presence of a multiple supplement additive in the recipe.

When storing semi-finished meat products, it is essential to avoid bacteriological contamination. Thus, at the next stage of this work, we studied changes in microbiological indicators of minced semi-finished products of the developed rabbit meat under low temperature (-18 °C) storage conditions: the mesophilic-aerobic and facultative anaerobic count, colon bacillus group (Coliforms) bacteria, pathogenic microorganisms, including salmonellas (Table 2).

The data obtained indicate that throughout the storage period, the mesophilic-aerobic and facultative anaerobic count, in all samples, being in compliance with the hygienic requirements of microbiological safety, was less in semi-finished products containing multiple supplements than in the reference samples. This allowed us to conclude that the multiple supplements had an inhibitory effect on the development of microorganisms.

No colon *Bacillus* group bacteria or pathogenic microorganisms, including salmonellas, were found in samples, which also indicates the safety of semi-finished products.

Under today's difficult environmental conditions, food safety issues are of high relevance. Therefore, we identified the content of the toxic substances in the meat of rabbit breeds under study (Table 3). An analysis of Table 3 shows that the contents of lead, cadmium, arsenic, and mercury in different rabbit breeds did not exceed acceptable standards, which indicates their safety, as well as their sanitary and hygienic reliability.

It is known that lipid oxidation processes occur in meat products during storage, resulting in the accumulation of superoxide compounds. Therefore, we studied the dynamics of changes in the peroxide number reflecting the intensity of oxidative processes during storage at a low temperature (-18 °C). In parallel, we determined the acid-number value, which indicates the formation of free fatty acids in the products as a result of the hydrolytic spoilage of fats. As a reference sample, we took natural minced rabbit meat semi-finished products. The results are shown in Tables 4 and 5.

The analysis of these tables showed that the storage of rabbit meat semi-finished products was accompanied by an increase in acid-number and peroxide values. However, these processes were more intense in a reference sample than in the samples containing onions and multiple supplements. In particular, after 48 days of storage, the acid- number value was 15.4% lower than in a sample containing the onion supplement, 60.1% lower in a sample containing multiple supplements with amaranth, and 64.3% lower in the sample with flax-containing multiple supplements. After 90 days of storage, the acid- number values of samples with aultiple supplements containing onion, amaranth and flax flours were 17.6%, 57.8% and 61.6% lower, respectively, and remained within permitted limits.

The difference between the acid-number values in the amaranth- and flax-containing samples, in our opinion, was due to the fact that flax flour contains more phenolic compounds than amaranth **(Bernacchia et al., 2014; Kalač and Moudrý, 2000; Kidyaev et al., 2017; Oomah, 2001; Pastor and Acanski, 2018; Pękal and Pyrzynska, 2014)**.

The dynamics of change in peroxide number values during the storage of semifinished products were approximately similar. Particularly, after 48 days of storage, the mean peroxide number values as compared with reference samples were 41.8%, 61.6%, and 64.2% lower in samples with the onion, amaranth and flax supplements, while after 90 days of storage, these values were 59.7%, 68.4% and 70.7% lower, respectively.

The higher stability of the test samples as compared with the reference samples to the accumulation of free fatty acids and peroxide compounds is explained by the presence of plant supplements with antioxidant activity in their composition that slows the rate of these processes.

The results obtained confirm similar data available in the literature. For example, the addition of grape-seed powder, green tea extract **(Perumalla et al., 2011; Reshetnik et al., 2014)**, dihydroquercetin **(Kuzmina et al., 2017; Nasonova, 2008)**, pignoli nut extract nut **(Litvinova, 2012)** and rosemary **(Sharigina, 2011)** to semi-finished meat products prevents the accumulation of oxidative products and protects semi-finished products against oxidative spoilage. According to **Sharigina (2011)**, this is caused by the impact of the supplement's active components (phenolic diterpenes, including carnosic acid, essential oils, bioflavanoids, catechins) on chain reactions.

We studied organoleptic indicators of the quality of semi-finished and finished products. After 60 days of storage of the semi-finished products, the reference sample had a more pronounced foreign flavour as compared with samples containing multiple supplements, which perhaps was due to faster accumulation of oxidative products in the reference sample. As to colour stability, after 60 days of storage, it was more stable in samples containing multiple supplements, which was due to the presence of ingredients with antioxidant properties.

The finished product had better consistency and juiciness than the reference sample, which is explained by the high water-retention and water-binding ability of amaranth and flax flour **(Carocho, Morales and Ferreira, 2018; Denisovich, 2006; Higdon and Frei, 2003; Tarakhovsky et al., 2013; Tavdidishvili et al., 2018; Vaitanis and Khodyreva, 2017)**.

Based on the above, samples containing multiple supplements, both semi-finished and finished products had better organoleptic indicators of quality during storage.

Thus, a multiple plant supplement that protects products against microbiological and oxidative spoilage and improves their quality imparts the antioxidant properties to the developed semi-finished products, demonstrating the effectiveness of their use in the production of healthy semi-finished meat products.

CONCLUSION

1. To impart antioxidant properties to minced semi-finished rabbit meat products, we have proven the effectiveness of using grape-seed powder, green tea extract, and amaranth/flax flour as multiple supplements in the recipe.
2. We have determined the optimum component composition of multiple supplements to added to semi-finished rabbit meat products: 0.7% grape-seed powder, 24.8% amaranth flour/flax flour, and 74.5% green tea extract. The amount of multiple supplements to be added to 100 g of semi-finished product is 30.2 in the case of amaranth flour and 25.8 g in the case of flax flour.
3. In the test samples, we studied the total phenolic and flavonoid content. It has been established that the highest antioxidant potential was observed in samples with flax-containing multiple supplements.
4. Microbiological indicators of semi-finished products with multiple supplements throughout storage at low temperature were in line with the hygienic safety requirements, and organoleptic indicators of quality were more stable as compared with a reference sample. The content of toxic elements indicates the sanitary reliability of semi-finished products.
5. The higher stability of semi-finished rabbit meat products with a control sample to the accumulation of free fatty acids and peroxide compounds has been established.

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