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Antioxidant Food Supplement Fortified With Flavonoids.

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ABSTRACT

The aim of this research is to develop the technology of food supplement with preventional properties, made out of red grapes berries peel. The authors studied chemical constitution and molecular properties of red grapes berries peel flavonoids. On the basis of obtained results and planning matrix implementation the authors developed the antioxidant food supplement technology, consisting in extracting tartaric acids and sugars with the help of a polar solvent with subsequent dissociating of the solution and drying of prepared berries peel. The authors researched chemical constitution, functional technological properties and safety profile of the food antioxidant supplement.

Keywords: grape berries peel, antioxidants, flavonoids, molecular properties, food supplement.

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INTRODUCTION

Statistical data testify that more than 70% of people die untimely from diseases connected with insufficient antioxidant compensation of free radicals. In recent decades scientists revealed causes, factors and mechanisms of many pernicious processes in the human organism. The main reason of many illnesses is cells being damaged by free radicals. As a result all organs and tissues of the human body grow obsolete. Many researches showed that antioxidants, present in different natural foodstuffs, can substantially slow down destructive action of free radicals oxygen atom [1-4].

Long ago scientists found that the most forceful antioxidant properties belong to substances determining plants colouring. A good deal of antioxidants is contained in vegetables and fruit of red, orange, blue and black colours. Many researches revealed that red grapes, if they are regularly used for food, exercise a salutary influence over the human's cardiovascular system [5, 6]. Turns out that polyphenols (flavonoids), contained it them, substantially lower probability of thrombus formation. In whole red grapes and red dry wine (peel of a whole grape and the wine itself) one can find the most powerful antioxidants – resveratrol, quercetin, rutin, catechin, epicatechin, epicatechin gallate and others. Flavonoids have not only antioxidant, antibacterial and sun-protective properties, but also some other useful qualities, for example, we know a lot about high capillary vessels protective (P-vitamin) activity of grapes' flavonoids [7, 8]. Besides, nowadays scientists study antitumoral, anti-inflammatory and antimutagenic properties of flavonoid polyphenol compounds. Flavonoids are famous for their ability to reinforce fibers of collagen and elastin, protect the human's organism from sun ultraviolet. There are data on using flavonoids for radiation disease treatment too [8]. These red grapes flavonoids' properties are very important for development of the antioxidant food supplement production technology.

METHODS

As research object we used marc from red grapes of varieties "Levokumskiy" (harvest of 2010), grown by "Zarya" closed joint-stock company, in Stavropol region (Russia).

Grape berries peel chemical constitution was defined using standard practices. With the help of computer chemistry application HyperChem v. 8, based on usage of computer aided techniques and discrete mathematics, we studied molecular properties of the following flavonoids: resveratrol, quercetin, rutin, catechin, epicatechin and epicatechin gallate.

To obtain food supplement, fortified with flavonoids, we set optimal modes of extraction of tartaric acids and sugars from grapes marc. Experiment was performed in a laboratory reactor connected with a thermostat. We studied following parameters influencing extraction process: treatment temperature, extraction time, actual acidity (pH) and sodium chloride (NaCl) concentration in the solution. Sodium chloride was used for increasing ionic strength and increase in speed of extraction from hydrophilic components solution. The experiment was carried out according to a planning matrix based on Greco-Latin squares. Validity of obtained results was controlled according to permissible error, which was adequate for a significance value ($q \leq 0.05$). Research results analysis was carried out with the help of an application software package Statistic v. 7.0, 8.0, Statistic Neural Networks (SNN) v.4, Statistic Automatically Neural Networks Code Generator (SANN) v.8. To create data array we used algorithmic language Pascal.

With regard to the research results, we developed a model in the form of a multilayered perceptron and an array of input variables (t, τ, pH, C_{NaCl}), in which values of functional parameters were calculated with the help of a neural network.

MAIN PART

"Levokumskiy" grape belongs to main cultivated technical varieties, grown in Stavropol region; area of vinelands held by growing of this culture is 1326.33 ha (19.2%). Data analysis showed that marc output during processing of the investigated grape variety is 27.4%. Marc consists for 25% of seeds, for 50% of berries peel and for 25% of stalks. Results of the research of "Levokumskiy" grape berries peel chemical constitution are shown in table 1.

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Table 1: "Levokumskiy" grape berries peel chemical constitution* (q≤0.05)

Parameters	Content	
	%	% of dry residue
Protein	5.8	12.7
Lipid	4.1	9.0
Fiber	13.3	29.0
Ash	4.1	9.0
Water	54.2	-
Saccharose weight percent	9.2	20.1
Titrated acids mass concentration (in equivalent to acetic acid)	0.75	1.6
Содержание флаваноидов	2,4	5,2

It should be noted that grape marc chemical constitution differs depending on the culture variety, climatic conditions, agricultural methods of raising, technological equipment used for grape processing, geographical zone, soil composition etc. However, qualitative chemical composition of marc components is permanent, which preconditions the possibility to develop the flavonoid-fortified food supplement production technology.

Study of molecular properties of grape marc flavonoids

Grape marc on contact with air spoils quickly and grow moldy, spirit turns into acetic acid, and tartrate compounds are broken up by bacteria of propionic fermentation. Therefore it is reasonable to process marc right after pressing via extracting sugars, tartrate and other compounds.

To select a solvent which provides extracting of tartaric acids and sugars and preserves polyphenol compounds in grape marc, it is necessary to study molecular properties of red grape flavonoids.

Charge density distribution surface map revealed generally hydrophobic characteristics of molecules under investigation with some insignificant hydrophilic zones. Fig. 1 presents rutin's charge density surface as an example (0.095 eV).

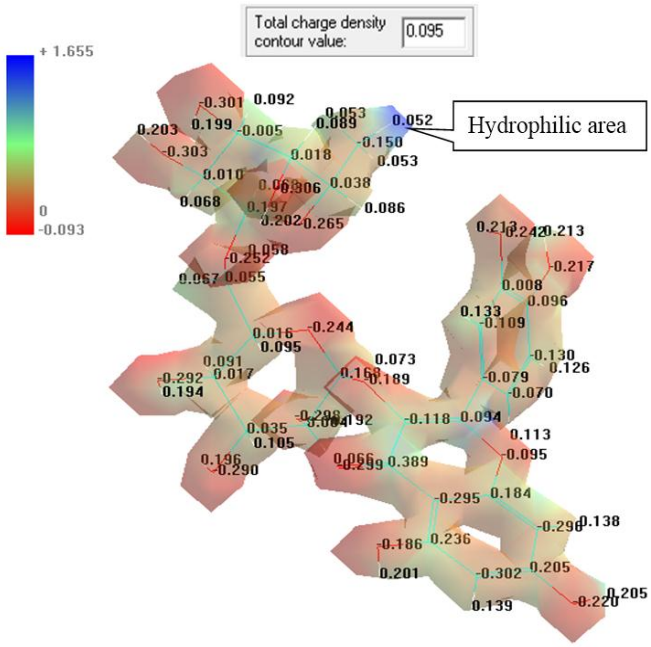

Figure 1: Study of rutin's molecule charge density distribution surface

* General content of pectin substances in grape marc was not defined.

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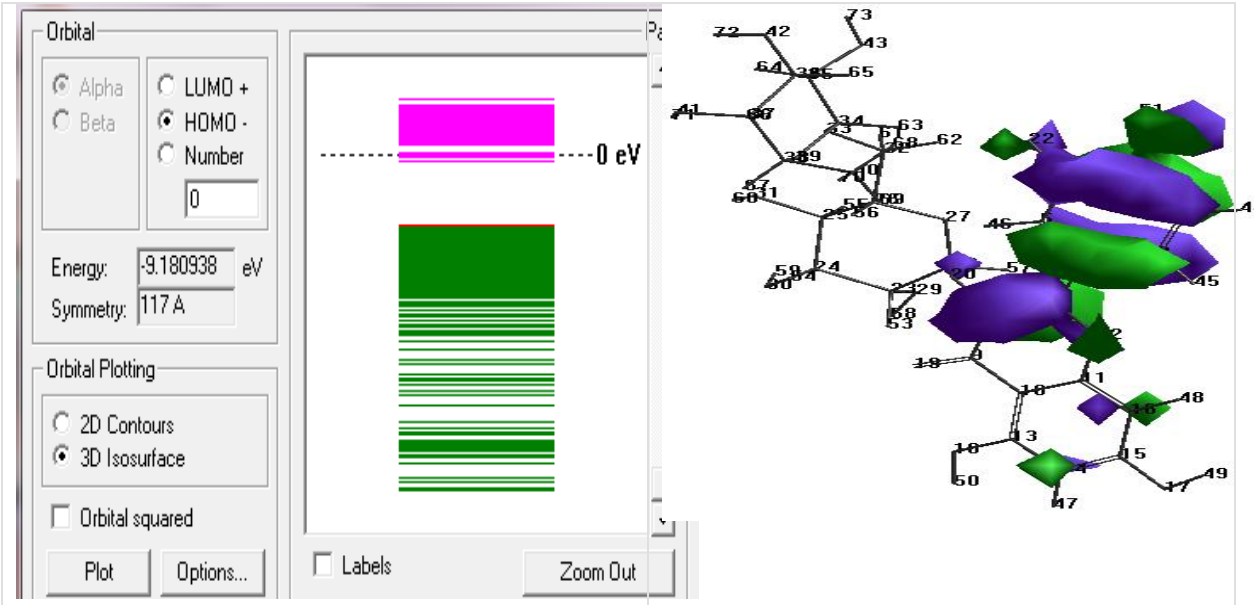
Similar results (0.010 – 0.095 eV) were obtained during investigation of other flavonoids: resveratrol, quercetin, catechin, epicatechin and epicatechin gallate (table 2). Therefore, flavonoids extraction with the help of polar solvents hardly can be done. However, it is known that polar solvents are able to extract tartaric acids and sugars well.

Table 2: Main quantum chemical characteristics of molecules of grape berries peel flavonoids (q ≤ 0.05)

Characteristics	Flavonoids					
	resveratrol	rutin	catechin	quercetin	epicatechin gallate	epicatechin
Potential energy, kcal/mole	12.908	43.610	2.014	20.370	5.521	2.135
Dipole moment, Debye	1.565	2.526	1.495	4.321	3.997	2.018
Quadratic mean gradient, kcal/(Å×mole)	0.042	0.093	0.078	0.095	0.098	0.094
Total charge density, eV	0.050	0.095	0.010	0.010	0.010	0.010
Ionization energy, eV	8.807	9.181	8.856	8.906	8.879	9.040

We investigated molecular orbitals and main quantum chemical characteristics of flavonoids. During investigation of structure and molecular properties of rutin (fig. 1) we found sectors with low charge quantity on the highest occupied molecular orbital (HOMO = 0), and that proves the possibility of using this chemical compound as a proton donor. Fig. 2 shows results of analysis of the highest occupied molecular orbital of rutin.



a) b)

Figure 2: Research of rutin's molecular orbitals

a) – energy characteristics analysis (Energy);
b) – configuration of the highest occupied molecular orbital

Obtained results testify that rutin's ionization energy for HOMO = 0 is low and equals to 9.180938 eV (fig. 2 a). And this proves the possibility of abstraction of proton (H•) from oxygen atoms.

Defining extraction modes

Natural organic compounds are characterized by a great variety of physical and chemical properties. The most widespread way of extracting organic compounds from natural products is extraction with the help of solvents [9, 10]. Conditions of distribution of substances between two liquid phases and also extraction's dynamic conditions are substantially affected by the value of actual acidity (pH) of the aqueuos solution, ionic strength and process time.

The process of extracting marc is based on diffusion, which consists in that solutions having different concentration of solved substance interpenetrate on contacting. Extraction is the ratio of total concentration of substance in organic phase to its concentration in water phase under the conditions of established equilibrium. Ratio of amount of substance in the extract to its total amount in the system under the given conditions is called extraction degree [11].

After the end of extraction process we estimated the content of dry substances in the solution according to standard practice, and also their amount in percentage of the marc mass, and amount of flavonoids in the solution and their extraction degree. Results of the research are shown in table 3.

Table 3: Research of the process of grape marc extraction (q ≤ 0.05)

№ of experiment	Temperature (t), °C	Time (τ), min.	Actual acidity (pH)	Concentration of NaCl in solution (C_{NaCl}), %	Dry substances, % of marc mass	Flavonoids extraction degree, % of total amount
1	50	5	7.6	0	6.3	0.8
2	50	20	8.1	2	6.0	20.8
3	50	35	8.6	4	7.2	41.5
4	70	5	8.6	4	6.3	27.5
5	70	20	7.6	0	7.2	10.5
6	70	35	8.1	2	6.9	34.2
7	90	5	8.1	2	6.6	17.8
8	90	20	8.6	4	7.5	43.0
9	90	35	7.6	0	7.8	22.5

Obtained experimental data (table 3) speak for significant impact of temperature, extraction time, actual acidity (pH), concentration of sodium chloride on amount of extracted dry substances and degree of flavonoids extraction from grape marc.

With regard to the research we created an input variables (t, τ, pH, C_{NaCl}) array, for which values of functional parameters were calculated with the help of the neural network (table 4).

It is evident that the task of optimization of grape marc processing technological parameters is multicriteria, that is it is necessary to set such modes when the maximum amount of dry substances is extracted and when the flavonoids extraction degree is minimum. In this case for optimization of extraction modes we used the method of multidimensional scaling of variables.

As a result, in laboratory conditions we set and tested optimal modes of extraction of compounds, which are soluble in polar solvents (9.9 – 11.2% of marc mass), from grape marc, and flavonoids extraction degree under the established parameters of processing was minimum and equaled to 0.5 – 2.3% of their total amount in starting material.

After the extraction end grape marc was separated from the solution, homogenized to particle sizes 30 – 50 micron and dried at a temperature of 75 – 80^{o}C, up to moisture content not more than 8%. As a result

we obtained powdered supplement without pronounced flavour, of purplish dark cherry colour, fortified with flavonoids.

Table 4: The slice of array of variables on studying grape marc extraction process

№ of experiment	Temperature (t), °C	Time (τ), min.	Actual acidity (pH)	Concentration of NaCl in solution (C_{NaCl}), %	Dry substances, % of marc mass	Flavonoids extraction degree, % of total amount
1	60.3	6.4	8.2	0.6	5.8	13.2
2	56.4	11.9	8.2	0.5	5.8	16.4
3	52.3	17.9	8.4	0.4	5.8	20.4
….	….	….	….	….	….	….
942	62.3	13.0	8.2	2.8	6.5	29.9
943	59.5	23.7	7.8	1.6	6.5	13.4
944	81.3	12.5	8.2	0.9	6.5	18.4
….	….	….	….	….	….	….
1295	50.8	9.5	8.2	0.1	5.6	12.3
1296	50.6	8.5	8.4	0.2	5.5	14.7
1297	53.5	5.9	8.5	0.1	5.5	14.8

Content and properties of the antioxidant food supplement. Results of the research of the antioxidant food supplement chemical constitution are shown in table 5.

Table 5: Chemical constitution of the antioxidant food supplement (q ≤ 0.05)

Title	Value	
	%	% of dry residue
Moisture content	7.8	-
Protein content	8.1	8.7
Fiber content	27.7	29.8
Lipid content	1.7	1.8
Ash content	8.1	8.7
Saccharose mass content	4.8	5.2
Mass concentration of titrated acids (in equivalent to acetic acid)	not found	-
Flavonoids content	4.9	5.3

Obtained results spoke for decrease of mass fraction of protein (8.7 and 12.7), lipid (1.8 and 9.0) and saccharose (5.2 versus 20.1% of dry residue) in the antioxidant food supplement in comparison with the starting material (table 1). Titrated acids were not found in the food supplement. We estimated sensory characteristics in dried and refined samples (table 6).

Table 6: Sensory characteristics of the antioxidant food supplement

Title	Data
Colour	Purplish dark cherry
Smell, taste	Neutral taste, slight smell of grape, without foreign smells and flavours
Appearance	Dry powder, may be few compact clumps which easily break up under mechanical influence
Structure	Friable

Since oxidation products quantity index is in inverse ratio to antioxidant activity index, lipids oxidation rate was estimated with the help of changing of peroxide value, which characterizes accumulation of triglycerides desintegration primary products. As a model lipid system we used dairy butter (unsalted with lipid weight fraction 72.5%). In course of the research it was found that the sample with antioxidant supplement had a higher peroxide value than the test sample (without antioxidant supplement) (0.033 against 0.055 mmole of active oxygen / kg).

Microbiological parameters of the antioxidant supplement spoke for its high tenacity for storing: amount of mesophilic aerobic and facultative anaerobic microorganisms after 6 months was 4.1×10^3 CFU/g. In the course of researching of the antioxidant food supplement mineral composition it was found that no limited element exceeded maximum allowed norms specified in Sanitary Regulations and Standards (No 2.3.2.2401-08).

We studied the antioxidant food supplement's functional technological properties (table 7).

Table 7: Values of the antioxidant food supplement's functional technological properties (q≤0.05)

Title	Data
Actual acidity, pH (10% suspension)	7.3
Water-absorption capacity, %	147
Lipophagic capacity, g of lipid / g	7.2
Water-holding capacity, g of water / g	13.8
Swelling ability, %	182

It was found that values of water-absorption (147%), lipophagic (7.2 g of lipid / g of food supplement), water –holding (13.8 g of water / g of food supplement) capacities and swelling ability (182%) of the food supplement were quite high, and this allows to recommend it for using in food technology.

SPECULATION

In the course of study of "Levokumskiy" grape berries peel chemical constitution we found that the peel contains generous amount of protein (12.7), lipid (9.0), flavonoids (5.2%) and saccharose (20.1% of dry residue). Grape marc actual acidity (pH) was equal to 3.86, and that proves the presence of titrated acids, mass concentration of which was equal to 1.6% of dry residue.

We studied molecular properties of red grape flavonoids and configuration of the highest occupied orbital; we found that for electron's passing to the lowest unoccupied orbital it is necessary to spend minimal energy of activation. We proved antioxidant properties of resveratrol, quercetin, catechin, epicatechin and epicatechin gallate. We found that it is reasonable to use polar solvents for extraction of sugars and tartaric acids.

We found such technological modes of extraction of compounds, which are soluble in polar solvents (9.9 – 11.2% of raw material weight), from grape berries peel, that provide minimal extraction of flavonoids (0.5 – 2.3% of total amount in the starting material).

Dried end product appeared to be a powder supplement without pronounced flavour, of dark cherry colour. The antioxidant food supplement chemical constitution analysis revealed that suggested technological parameters allow to extract 74.1% of sugars, 80.0% of lipids and 3.3% of mineral substances of total amount in the starting material. Titrated acids were not found in the food supplement, apparently it was due to using an extragent with high actual acidity (pH > 7.0). Perhaps extraction of soluble pectin substances takes place too, but their amount was not controlled.

In the course of studying of antioxidant activity on model lipid samples we found that the test sample after storing had a much lower peroxide value than the control one (0.033 against 0.055 mmole of active oxygen / kg), and that proved the food supplement antioxidant properties.

Organoleptic, microbiological and functional technological parameters make it possible to recommend using of the antioxidant food supplement in food technology.

CONCLUSION

Thus, on the basis of studying of "Levokumskiy" grape berries peel chemical constitution and research of flavonoids molecular properties we proved the expedience of polar solvents using and developed the technology of extraction of sugars and tartaric acids from the starting material, which provides minimal losses of flavonoids (0.5 – 2.3% of total amount). Researches with the help of model lipid samples showed the dry food supplement's high antioxidant activity. Due to functional technological properties and safety indicators, the developed antioxidant supplement is appropriate for using for meal

REFERENCES

[1] Nesterenko AA et al. Newsletter of Nizhny Novgorod State Engineering Economics Institute 2012;8: 30-36.
[2] Timoshenko NV et al. Works of Kuban State Agrarian University 2008;1(15):176-179.
[3] Khamitsaeva, AS and Britaev BB. J. The commodities of food products, Krasnodar 2010;7:45-48.
[4] Omarov RS et al. Food Industry 2011;12:56-57.
[5] Selimov MA. Development of technology of meat products with an antioxidant food supplement [Text]: synopsis of thesis of an applicant for a degree of PhD in Technical Sciences, Stavropol, 2013. – 22p.
[6] Vallverdu-Queralt A, Medina-Remyn A, Andres-Lacueva C, & Lamuela-Raventos RM. Food Chem 2011; 126:1700–1707.
[7] Bagchi D, et al. Annals of New York Academy of Science 2002;957:260–270.
[8] Saito M, Hosoyama H, Ariga T, Kataoka S, & Yamaji N. J Agr Food Chem 1998;46:1460–1464.
[9] Murcia M, et al. J Agr Food Chem 2004;52:1872–1881.
[10] Limareva NS, Donchenko LV. http://ej.kubagro.ru/2014/01/pdf/12.pdf.
[11] Khamitsaeva AS, Kolodyaznay VS, and Kairov VR. 2010, "Scientific-practical bases of the use of bioactive regional plants in production of sausage and bread", Vladikavkaz, Mountain state agrarian university, p.140.